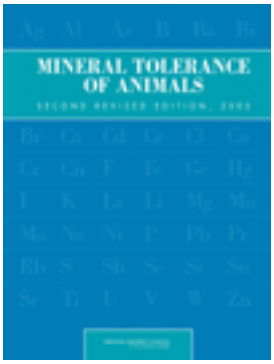

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Committee on Minerals and Toxic Substances in Diets and Water for Animals, National Research Council

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MINERAL TOLERANCE OF ANIMALS

SECOND REVISED EDITION, 2005

Committee on Minerals and Toxic Substances in Diets and Water for Animals

Board on Agriculture and Natural Resources

Division on Earth and Life Studies

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published reports as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Gary L. Cromwell, University of Kentucky. Appointed by the National Research Council, he was responsible for making certain that an independent examination of the report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Preface

In 2003, the National Research Council's Committee on Animal Nutrition convened an ad hoc committee to conduct a thorough review of the scientific literature related to minerals and toxic substances in the diets and water for animals and to update the 1980 edition of *Mineral Tolerance of Domestic Animals.* In particular, the committee was asked to provide recommendations on animal tolerances and toxic dietary levels, and identify minerals that pose potential human health concerns. A nine-person committee of scientists specializing in nutrition, toxicology, and veterinary medicine accepted this task. Individual members brought to this project diverse expertise and perspective on the impact of nutrition on the health of fish, poultry, livestock, companion animals, and humans. The committee met three times and had monthly teleconferences over a period of more than a year.

The challenge of making recommendations on the tolerable and toxic levels of close to 40 elements for a variety of species is daunting. Each of these elements exists in many chemical forms, each with differing properties and toxicity profiles, further complicating the task. The toxicity profile of some minerals is very well described, whereas the toxicity of other minerals, especially to livestock and companion animals, has received little attention. A wide variety of informational sources, ranging from government surveys to professional experiences, was considered for this report. Expert reports on several minerals were also solicited. However, the backbone of this report is the primary literature of peer-reviewed journal publications. The committee recognized that much of the information in the 1980 publication was still relevant, but that this historic foundation needed to be reevaluated in the context of newer information on the methods of mineral analysis, mechanisms of homeostasis and toxicity, and appropriate indices of animal health and well-being. Consequently, a reanalysis of the historic literature is synthesized with the recent literature to form the recommendations in this report. This edition considers a greater breadth of animal species than the past edition and expands the coverage on the metabolism and mechanisms of toxicity of minerals, methods and problems in mineral analysis, and the relationships between mineral exposure of animals and the mineral levels in animal products destined for human consumption. New chapters provide additional focus on acid-base balance, nitrates, and water quality. Finally, this edition has placed increased emphasis on the safety of animal products in the human diet as criteria for setting maximum tolerable levels of minerals in the feed and water of farm animals.

The recommendations in the 1980 report have been widely cited and served as the basis of decisions made by regulatory agencies and by practicing nutritionists responsible for the formulation of animal diets. The previous report has also been used extensively in teaching, research, and veterinary practice. We hope that the utility of this new edition equals or surpasses its predecessor.

KIRK C. KLASING, *Chair*
Committee on Minerals and Toxic Substances in Diets and Water for Animals

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BACKGROUND

Inorganic elements found in the Earth's crust are often referred to as minerals. Some minerals are essential for health and productivity of animals and have well-defined nutritional and biochemical roles. Many other minerals naturally occur at trace levels in the foods and tissues of animals but are not typically suspected to play a useful nutritional purpose and are considered incidental contaminants. However, all minerals, whether essential or nonessential, can adversely affect an animal when amounts in the diet and water become excessive, so the prevention of mineral toxicosis is a fundamental part of animal nutrition and management. Establishing the levels at which each mineral becomes toxic to animals aids nutritionists, veterinarians, toxicologists, government regulators, and ranchers and other animal owners to safeguard the feed that animals consume and the water that they drink to optimize animal health and minimize tissue residues.

In 1980, the National Research Council published *Mineral Tolerance of Domestic Animals*, which reviewed and evaluated the literature relating to mineral tolerance of domestic animals and set maximum tolerable levels of dietary minerals in feeds. Since that time, there have been important developments in nutrition and toxicology that compel a reevaluation of mineral tolerances of animals. These developments include the following:

- Improvements in the sensitivity and specificity of analysis of mineral concentrations in feeds and animal tissues;
- New information on the bioavailability, homeostasis, and mechanism of toxicity of minerals;
- New understandings of appropriate indices of animal health and well-being;
- Increased disposal of municipal and animal wastes on crop lands and pastures, potentially resulting in greater exposure of animals to certain minerals;
- Increased feeding of recycled animal by-products and wastes that contain high levels of certain minerals;
- Emergence of the aquaculture industry;
- Increased evidence for potentially toxic levels of mercury, cadmium, lead, and other minerals in the diet of humans and companion animals;
- Refinements in the Recommended Dietary Allowances and the Tolerable Upper Intake Levels of minerals for humans that permit more precise evaluation of potentially toxic mineral levels in foods of animal origin;
- New appreciation of the impact of minerals on environmental quality.

Preventing adverse effects of minerals on the health of animals, consumers, and the environment requires the application of appropriate nutritional and toxicological principles to set limits on mineral exposure to animals. To address this need, the Food and Drug Administration of the U.S. Department of Health and Human Services asked the National Academy of Sciences to convene a committee of scientific experts to make recommendations on animal tolerances and toxic dietary levels. A subcommittee of the Committee on Animal Nutrition undertook this task. The subcommittee consisted of nine scientists specializing in nutrition, toxicology, and veterinary medicine, with diverse expertise and perspectives on the impact of nutrition on the health of fish, poultry, livestock, companion animals, and humans.

COMMITTEE CHARGE AND APPROACH

The committee was given the following task: "An ad hoc committee of the standing Committee on Animal Nutrition will be convened to conduct a thorough review of the scientific literature on trace elements and macro minerals, including an analysis of the effects of exposure and toxic levels in animal diets; provide recommendations on animal tolerances and toxic dietary levels, and identify elements that pose po-

tential human health concerns. The report will address recent research on tolerance and toxicity of minerals in animal diets including the following areas: general considerations; mineral sources, discrepancies and difficulties in methods of analyses and evaluation of biological status; metabolic mineral interactions; assessments of form and species interactions; supplementation considerations; bioavailability of different mineral forms and sources; maximal tolerable levels; and effects of diet composition, stressors, and animal physiological status on mineral utilization; and environmental exposure considerations. The report will include all species for which adequate information is available—updating the previous report, greatly expanding the topics covered, and increasing the usefulness of the report. Recommendations will be provided on maximum tolerable and toxic dietary levels of minerals in animal diets. Potential for toxic exposure, toxicosis, factors affecting toxicity, and essentiality of dietary minerals in various animal species will be discussed."

This report is based mostly on the primary literature of peer-reviewed journal publications. The committee recognized that much of the information in the 1980 publication was still relevant, but that this historic foundation needed to be reevaluated in the context of newer information on the methods of mineral analysis, mechanisms of homeostasis and toxicity, and appropriate indices of animal health and well-being. Consequently, a reanalysis of the historic literature is synthesized with the recent literature to form the recommendations in this report. In addition, this report considers a greater breadth of animal species than the past edition and expands the coverage on the metabolism and mechanisms of toxicity of minerals, methods and problems in mineral analysis, and the relationships between mineral exposure of animals and the mineral levels in animal products destined for human consumption. New chapters provide additional focus on acid-base balance, nitrates, water quality, and rare earth metals.

ESSENTIALITY

The essential minerals are those that have well-defined biochemical roles and must be in the diet of vertebrates for optimum health and productivity. Minerals play many vital roles: for example, they activate many proteins including enzymes, maintain the ionic and pH balance, provide for the structural rigidity of bones and teeth, and serve as regulatory signals in metabolic homeostasis. Inadequate dietary concentrations of essential minerals compromise animal growth, reproduction, and health. As shown in Table S-1, the committee identified

- Seventeen minerals that are required by vertebrates: calcium, chlorine, chromium, cobalt, copper, fluorine, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodium, sulfur, and zinc;
- Six additional minerals that may be required based on experiments that indicate beneficial effects when supplemented to the diet: arsenic, boron, nickel, rubidium, silicon, and vanadium. However, specific biochemical functions have not been identified for these six, and there is not a consensus among nutritionists that these minerals are essential.

EXPOSURE

Animals may be exposed to toxic levels of minerals from a wide variety of sources. Feedstuffs, especially those derived from plants, are a common source of potentially toxic levels of minerals. Molybdenum and selenium occur naturally in soils of some regions at concentrations sufficient to cause certain plants to accumulate levels that can be toxic for animals. High soil, and consequently plant, concentrations of cadmium, lead, molybdenum, copper, and zinc are the primary minerals of concern from the application of municipal wastes and other biosolids to the land. Mining, smelting, and other industries are often associated with local areas of mineral contamination to the water, soil, and air, and, ultimately, the plants grown in that area. Feedstuffs of animal origin may also be sources of toxic levels of minerals. For example, some types of fishmeals may be high in mercury because mercury bioconcentrates through the aquatic food chain.

Mineral supplements are commonly added to animal diets to correct deficiencies found in pastures, forages, and other dietary ingredients. Some mineral supplements may contain potentially toxic levels of contaminating minerals, depending upon the source of the supplement and the method of its processing. Toxic levels of minerals may accidentally occur due to mistakes in feed formulation and manufacturing, or from contamination during storage or transportation. Such accidental administration can result in very high mineral levels and cause acute toxicosis and death, whereas most other modes of introduction typically cause toxicosis only after chronic exposure. Surface water and occasionally even deep-well or domestic water supplies may contain excessive levels of certain minerals due to naturally high levels in the ground. Sulfur, sodium, manganese, selenium, and fluorine are among the minerals most likely to reach toxic levels in natural water supplies. Minerals may also be introduced into water supplies from industrial wastes, pesticide contamination, and other sources of pollution. Finally, minerals such as arsenic, bromine, bismuth, copper, lithium, magnesium, silver, zinc, and some of the rare earths are sometimes added to feed or water as therapeutics or for growth promotion. Mistakes in use of these minerals have occasionally resulted in toxicoses.

TOXICITY

The committee looked at two main aspects of the effects of toxicity on animals: the mechanisms of toxicity of each mineral and the maximum tolerable level that will not impair

animal health or performance. The individual chapters describe findings in each of these areas. Some general conclusions are summarized below.

Mechanisms of Toxicity

The adverse effects of minerals depend on the dietary concentration and length of exposure and range from subtle effects on homeostatic processes, to impairments in animal growth or reproductive rates, to specific pathologies and death. An understanding of the biochemical or physiological mechanism by which a mineral exerts its detrimental effect is useful in diagnosing toxicity problems and in designing research to identify the level at which that mineral becomes toxic. Although the means by which minerals cause their toxic effects are diverse, several general mechanisms are common. Some minerals cause oxidative damage to cellular macromolecules, either by their propensity to undergo redox reactions or by binding to and deactivating antioxidant molecules or enzymes. This property contributes to the toxicity of arsenic, cadmium, chromium, copper, cobalt, lead, iron, mercury, nickel, selenium, and vanadium.

Many minerals antagonize chemically related minerals that are nutritionally essential by impairing their absorption, transport, excretion, or incorporation into active sites of molecules. Aluminum, arsenic, bromide, cadmium, calcium, copper, lead, manganese, mercury, molybdenum, nickel, phosphorus, selenium, silver, strontium, sulfur, tin, tungsten, and zinc antagonize the homeostasis of at least one other mineral. Minerals that exert toxic effects by disturbing acid-base homeostasis include calcium, chloride, magnesium, phosphorus, potassium, sodium, and sulfur. Electrolyte balance can be disrupted by magnesium, potassium, sodium, bromine, and chloride. The toxic effects of chromium and vanadium are at least partly due to their ability to mimic or potentiate the action of hormones.

Individuals are often most sensitive to toxicity during embryonic development, growth, and periods of stress such as infections or trauma. Tolerance to minerals usually increases with age. Healthy, mature animals are often most resistant to mineral toxicoses because they have passed important developmental events, their homeostatic mechanisms are well developed, and their relative rates of feed intake are low. However, cadmium and mercury are not easily excreted, and they can accumulate during an animal's lifetime resulting in toxic effects in older animals.

Maximum Tolerable Levels

The "maximum tolerable level" (MTL) of a mineral is defined as the dietary level that, when fed for a defined period of time, will not impair animal health or performance). Tolerable mineral levels are typically distinguished from toxic levels in experiments that use incremental additions of the mineral of concern to the diet or the water and measure the impact on performance and pathological signs of toxicosis. The duration of exposure to the test mineral markedly influences the level that causes toxicosis. The committee considered three exposure durations: a single dose, acute exposure, and chronic exposure. A single dose is defined as exposure due to the consumption of a single meal or by a single gavage of the mineral. Acute exposure is defined as an intake of 10 days or less. Chronic exposure is set as an exposure of 10 days or more but emphasis is given to the studies that had the longest durations of exposure. In this report, MTL recommendations for 38 minerals are provided, which is seven more than included in the 1980 *Mineral Tolerance of Domestic Animals*. When information is available, MTL are given for fish, rodents, and companion animals, in addition to the poultry and livestock species considered in the previous report. Research on mineral toxicities in domestic animals conducted during the past 25 years has resulted in adjustments to many of the MTL provided in the 1980 report. The previous report adjusted the MTL for lead and mercury in order to decrease tissue residues, but those recommendations were not based on animal health. Furthermore, the rationale for those adjustments was not provided. The current MTL are based solely on considerations of animal health. As compared to the 1980 report, the recommended MTL based on indices of animal health were

- Not changed appreciably for 8 minerals: boron, bromine, iodine, silicon, silver, sulfur, tungsten, and vanadium;
- Increased for 13 minerals: aluminum (nonruminants), barium, bismuth, cadmium, calcium (poultry), cobalt, fluorine (cattle), lead (ruminants), magnesium, manganese (ruminants and swine), molybdenum (swine), nickel (ruminants and swine), and selenium;
- Decreased for sodium chloride and 12 minerals: arsenic, calcium (ruminants), chromium, copper, iron (cattle and poultry), lead (nonruminants), mercury, molybdenum (ruminants), phosphorus (cattle and swine), potassium, strontium (swine and poultry), and zinc (poultry).

The recommended MTL in this report do not include a built-in safety factor. Consequently, the recommended values should be adjusted according to their intended use, and each chapter contains relevant information on modifying factors for each mineral. Animals that are very young, old, reproducing, sick, exposed to stressful environments, or consuming nutritionally imbalanced diets may be especially sensitive to toxicoses, and each chapter provides additional information on the impact of these factors. In practice, the MTL is highly dependent upon the form of the mineral to which the animal is exposed. Important chemical factors that determine the bioavailability of the mineral sources include the solubility of a mineral compound in the digestive tract, its valence state, and whether the mineral is in an organic,

metallic, or other inorganic form. For some minerals, such as silica, chromium, iron, tin, lead, aluminum, and barium, the MTL may vary by several orders of magnitude depending upon the chemical form of the mineral. The individual chapters provide further information on the bioavailabilities of common sources of minerals. Each chapter contains a table that summarizes the details on the levels, chemical form, duration, animal ages, and signs of toxicosis used in the studies reviewed to establish the recommendations. In some cases, there is a wide interval between the MTL and the levels that are toxic because research is insufficient to make finer discrimination. The summary tables provided in each chapter give insight into the level of uncertainty.

While all minerals can become toxic when exposure levels are sufficiently high, the frequency that animals are exposed to excessively high levels differs greatly for each mineral. As shown in Table S-1, the committee identified

- Some minerals where toxicosis is not normally of concern because levels in feed and water are unlikely to be excessive: aluminum, antimony, barium, bismuth, chromium, cobalt, germanium, iodone, lithium, magnesium, manganese, nickel, rare earth elements, rubidium, silicon, silver, strontium, tin, titanium, tungsten, and uranium;
- Eight minerals as being of occasional concern for animal toxicosis: arsenic, boron, bromine, calcium, iron, potassium, phosphorus, and zinc;
- Sodium chloride and nine minerals as being of frequent concern for animal toxicosis: cadmium, copper, fluorine, lead, mercury, molybdenum (ruminants), selenium, sulfur (ruminants), and vanadium (poultry).

ENVIRONMENTAL HEALTH

Although this report focuses predominantly on levels of minerals that are toxic for animals, there are some cases where environmental factors may be the primary considerations that limit the acceptable levels of minerals in the feed and water of animals. The concentration of some minerals in excreta is greater than that in the feed consumed, and this relationship is magnified by high dietary levels. In some situations, application of animal wastes to the land as fertilizer can reduce crop yields, result in high residue levels in crops, or cause environmental or human health concerns.

The committee identified 10 minerals that could be of concern because of their potential effects on crop yields or the environment: cadmium, copper, iron, mercury, phosphorus, potassium, sodium, selenium, sulfur, and zinc. Of these, phosphorus is often the primary concern.

Depending upon manure management, location of disposal, and climate factors, environmental considerations may limit the levels of these minerals that can appropriately be fed to animals. In some regions, environmental issues should be considered along with levels that are tolerated by animals in regulation of the concentrations of these minerals in the feed and water of animals. However, the maximum tolerable levels recommended in this report are based solely on indices of animal health and productivity.

HUMAN HEALTH

Meat, milk, and eggs are an important part of the human diet, in part, because they supply highly bioavailable forms of minerals. Often animals can serve as an important buffer for the high mineral concentrations found in some plants or supplements, thereby reducing human exposure to potentially toxic minerals. However, levels of some minerals may accumulate in animal tissues intended for human consumption to concentrations that might adversely affect human health, even when animals are exposed to safe levels (i.e., levels at or below their respective MTL). Consequently, acceptable concentrations of minerals in feeds and water of animals raised for food must consider the health of the human consumer as well as the health of the animal itself.

The committee identified minerals of concern for human health by a three-step process. First, the amount of a mineral that accumulates in meat, milk, bone, and eggs in animals fed their MTL was estimated. Second, acceptable safety standards for mineral intake by humans were identified. Third, the maximum concentrations in animal tissues were compared to levels known to be safe for humans. Individual chapters in this report provide information on the dose–response relationship between feed mineral levels and tissue levels. The committee relied on recent recommendations by the Food and Nutrition Board of the National Academies and other national or international organizations. Recent data on food consumption trends in the United States were used to estimate daily intake of animal meat, milk, and eggs. It was assumed that all of the protein-rich foods consumed by an individual came from animals consuming minerals at their MTL. Using this process, the committee identified minerals for which levels that are tolerated by animals could result in unacceptably high mineral concentrations in tissues used for human foods.

- Cadmium, lead, mercury, and selenium could accumulate to excessive levels in skeletal muscle. If it is assumed that 5 percent of meat was bone fragments (due to inappropriate processing), barium, and fluorine might also be excessive in some cases.
- Cadmium, iodine, lead, and mercury might, in some cases, become excessive in milk.
- Arsenic, cadmium, copper, lead, mercury, selenium, and possibly iron could become excessive in liver.
- Arsenic, bismuth, cadmium, chromium, cobalt, fluoride, lead, mercury, and selenium could become excessive in kidney.

Establishing specific recommendations for mineral levels in animal feeds that are safe for human health was be-

yond the charge of the committee. However, for arsenic, barium, bismuth, cadmium, chromium, cobalt, copper, fluoride, iron, lead, mercury, and selenium, the MTL in this report, while safe for animals, could result in unacceptably high levels of the mineral in some types of foods derived from these animals.

REMAINING QUESTIONS AND RESEARCH NEEDED

Finally, the committee was charged with identifying gaps in knowledge that would benefit from further research. The individual chapters fairly consistently highlight three main areas across the minerals considered:

- The bioavailability of minerals that animals commonly encounter in feedstuffs is not well characterized, especially when fed at concentrations near the MTL. Identifying the bioavailability of minerals in the form that animals would likely be exposed to and at moderately toxic levels is needed for nutritionists and veterinarians to use the MTL recommendations in this report. The MTL are often based on reagent-grade, highly available forms. Research on the bioavailability of minerals at dietary levels near the MTL would also permit refinement of the recommended MTL.
- The relationship between mineral concentrations in feed and water and the levels in meat, milk, and eggs is not well characterized for most minerals, particularly at levels that are near the MTL of the animal. For most minerals, the available information was determined during relatively short-term studies using unrealistically high levels of exposure. Information on mineral accumulation in tissues following lifetime exposure to minerals is needed to evaluate tissue residue levels and impacts on human health.
- Relevant information for predicting the MTL of minerals for aquatic and companion animals is relatively incomplete. Mineral absorption and excretion in aquatic animals is often considerably different from that in terrestrial animals. Studies designed specifically to determine the MTL in aquatic species are needed. Companion animals have long life spans and there are few studies on chronic mineral toxicoses in these species.

TABLE S-1 Summary of Minerals Reviewed in This Report

Element	Required Nutrient[a]	Concern for Animal Health	MTL Relative to 1980 Recommendations[b]
Aluminum	No	Low	Increased (nonruminants) [c]
Antimony	No	Low	New
Arsenic	Possibly	Medium	Decreased[c]
Barium	No	Low	Increased[c]
Bismuth	No	Low	Increased[c]
Boron	Possibly	Medium	Similar
Bromine	No	Medium	Similar
Cadmium	No	High	Increased[d]
Calcium	Yes	Medium	Decreased (ruminants)[c] Increased (poultry)[c]
Chromium	Yes	Low	Decreased[c]
Cobalt	Yes	Low	Increased[c]
Copper	Yes	High	Decreased[c]
Fluorine	Yes	High	Increased (cattle)[c]
Germanium	No	Low	New
Iodine	Yes	Low	Similar
Iron	Yes	Medium	Decreased (cattle and poultry)[c]
Lead	No	High	Increased (ruminants)[d] Decreased (nonruminants)[c]
Lithium	No	Low	New
Magnesium	Yes	Low	Increased[c]
Manganese	Yes	Low	Increased (ruminants, swine)[c]
Mercury	No	High	Decreased[c]
Molybdenum	Yes	High[e]	Decreased (ruminants)[c] Increased (swine)[c]
Nickel	Possibly	Low	Increased (ruminants, swine)[c]
Phosphorus	Yes	Medium	Decreased (cattle, swine)[c]
Potassium	Yes	Medium	Decreased[c]
Rare earths	Possibly	Low	New
Rubidium	Possibly	Low	New
Selenium	Yes	High	Increased[c]
Silicon	Possibly	Low	Similar
Silver	No	Low	Similar
Sodium Chloride	Yes	High	Decreased[c]
Strontium	No	Low	Decreased (swine, poultry)[c]
Sulfur	Yes	High[e]	Similar
Tin	No	Low	New
Titanium	No	Low	New
Tungsten	No	Low	Similar
Uranium	No	Low	New
Vanadium	Probably	High[f]	Similar
Zinc	Yes	Medium	Decreased (poultry)[c]

[a]Possibly: Indicates that circumstantial data indicate the possibility that the mineral is essential but mechanistic information is lacking. See specific chapters for supportive information.
[b]Similar: Recommended Maximum Tolerable Levels for poultry and livestock in this report are not appreciably different than in the 1980 report; Decreased: Recommendations in this report are lower than the previous recommendations; Increased: Recommendations in this report are higher than the previous recommendations; New: Mineral was not reviewed in 1980 or no recommendation was provided.
[c]MTL changed due to new information.
[d]MTL changed because the 1980 report was based on human health concerns and not on toxicity to animals.
[e]Ruminants.
[f]Poultry.

1

Introduction

Inorganic elements found in the Earth's crust are often referred to as minerals. The essential minerals are those that have well-defined biochemical roles and must be in the diet of vertebrates for optimum health and productivity. There are more than a dozen essential minerals and, for each, the amount needed for optimal health and productivity is referred to as the dietary requirement. Close to a dozen other minerals are suspected to be essential because they increase weight gain or efficiency of feed use but definitive biochemical or physiological roles have yet to be determined. Dozens of other minerals naturally occur at trace levels in foods and tissues of animals but are not typically suspected to play a useful nutritional purpose and are considered incidental contaminants. However, all minerals, whether essential or nonessential, can adversely affect an animal when amounts in the diet and water become excessive (Figure 1-1); so the prevention of mineral toxicoses is a fundamental part of animal nutrition and management. The concentration at which a specific mineral becomes toxic varies greatly. The most highly toxic minerals, such as mercury, can cause impairments in health and productivity of animals when present in the diet at concentrations of less than a few milligrams per kilogram, whereas such low levels would be well below the dietary requirement of most essential minerals. It is generally thought that those minerals that were enriched in the aquatic environment of early evolution became essential and are tolerated at much higher levels than those that are nonessential and were historically present at very low concentrations.

EXPOSURE

Animals may be exposed to toxic levels of minerals from a wide variety of sources. Feedstuffs derived from plants are a common source of minerals. Levels of minerals in plant tissues vary greatly due to the natural variation in soil minerals. Molybdenum and selenium may naturally occur in soils of some regions at levels sufficient to cause certain plants to accumulate levels that can be toxic for animals. High soil and consequently plant concentrations of cadmium, lead, copper, and zinc can occur from the application of municipal wastes and other biosolids to the land. Mining, smelting, and other industries are often associated with local areas of mineral contamination to the water, soil, and air, and ultimately the plants that grow in the area. Plants can accumulate toxic levels of minerals by deposition into their tissues or as the result of surface contamination with soil or dust. Some aquatic plants accumulate iodine from seawater to toxic levels. Feedstuffs of animal origin may also be sources of toxic levels of minerals. For example, some types of fish meal may be high in mercury because it bioconcentrates through the aquatic food chain. Mineral supplements are commonly added to animal diets to correct deficiencies found in pastures, forages, and feed ingredients. Some sources of mineral supplements may contain potentially toxic levels of contaminating minerals, depending upon the source of the supplement and the method of its processing. For example, some rock phosphate deposits may be naturally high in fluoride or vanadium and cause toxicosis when supplemented in animal diets to meet their phosphorus requirement. Toxic levels of minerals may accidentally occur due to mistakes in feed formulation and manufacturing, or from contamination during storage or transportation. Such accidental administration can potentially result in very high mineral levels and cause acute toxicosis and death, whereas most other modes of introduction typically cause toxicosis only after chronic exposure. Surface water and occasionally even deep-well or domestic water supplies may contain excessive levels of some minerals due to naturally high levels in the ground. Sulfur, sodium, iron, magnesium, selenium, and fluoride are among the minerals most likely to reach toxic levels in natural water supplies. Minerals may also be introduced into water supplies from industrial wastes, pesticide contamination, and other sources of pollution. Finally, minerals such as copper, zinc, bromine, bismuth, and some of the rare earths are sometimes added to feeds or water as therapeutics or growth promoters and their potential for toxicity is accentuated.

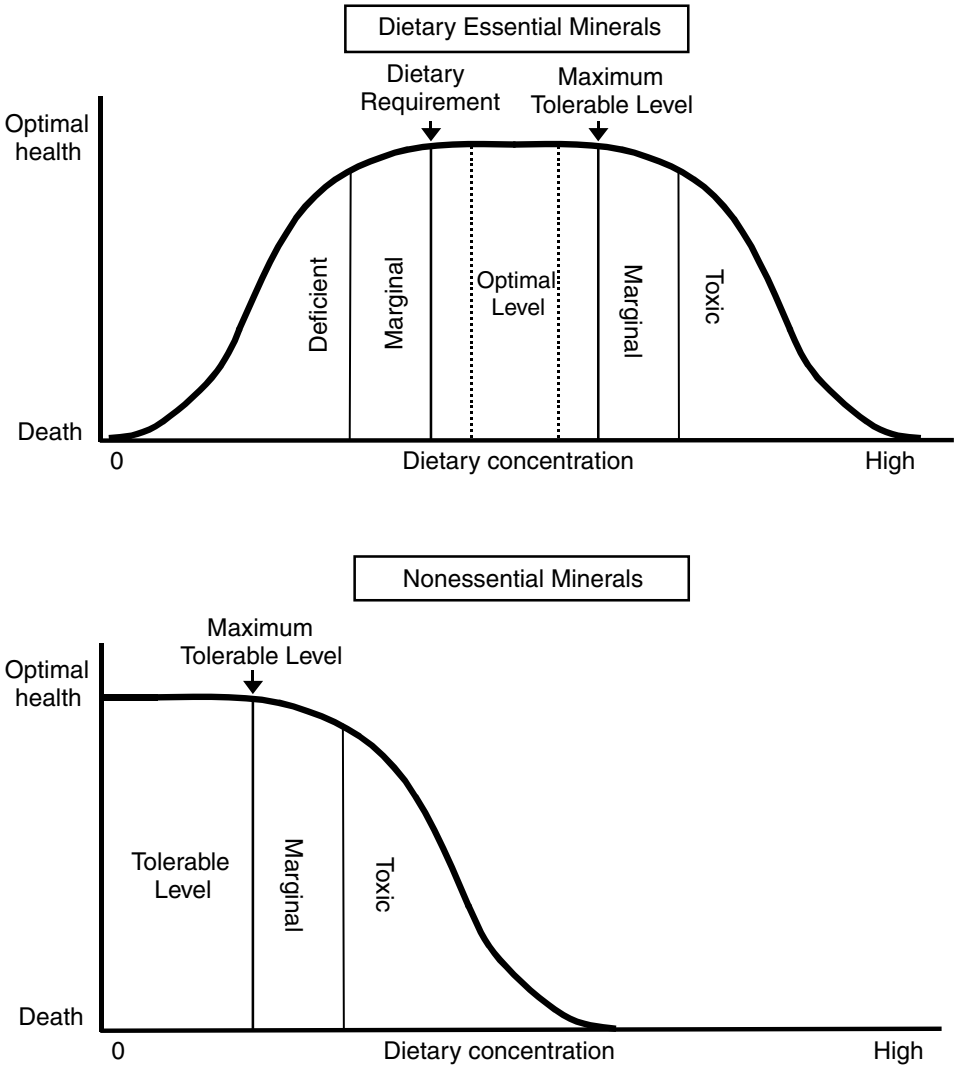

FIGURE 1-1 Mineral concentrations and animal health. The relationship between dietary mineral concentrations and animal health is very different for essential versus nonessential minerals. For essential minerals (top graph), increasing amounts of a mineral in the diet are highly beneficial up to a point (requirement), beyond which additional amounts have little additional value. At some point (maximum tolerable level), higher dietary concentrations become detrimental to the animal's health. In the case of nonessential minerals (bottom graph), low levels are tolerated without detrimental effects on the health of the animal. At some point, higher concentrations become detrimental to the animal.

Mineral exposure is commonly reported in several different ways. Expressing exposure as an amount per day (e.g., mg mineral consumed/day or mg/kg BW/day) is very precise and especially useful when intake is variable, such as the case when the mineral is delivered in water or in a free-choice supplement. Expressing exposure as a proportion of the feed or water (e.g., percent or mg/kg of feed) has many obvious practical advantages in feed formulation and government regulation. These two methods of expression can be interconverted when the rate of consumption is known. To the extent possible, tables in this report express exposure as the concentration in feed or water. Feeds that contain forages or fresh feedstuffs have extremely variable moisture contents, so their mineral contents are best expressed on a dry matter (DM) basis whenever possible.

TOXICITY

The adverse effects of minerals are dependent on the intensity of exposure, and they range from subtle effects on homeostatic processes, to impairments in animal growth or reproductive rates, to specific pathologies and death. An understanding of the biochemical or physiological mechanism by which a mineral exerts its detrimental effect is use-

ful in diagnosing toxicosis problems and in designing research to identify the level at which that mineral becomes toxic. For example, some minerals owe their toxicities to displacing a nutritionally essential mineral from its normal biochemical and physiological role. This is illustrated by bromine, which has physical characteristics that are very similar to those of chlorine, and which displaces chlorine from its important functions as an electrolyte. Other examples of this phenomenon include the displacement of copper by molybdenum, the displacement of sulfur by selenium, and the ability of lead to displace zinc and calcium. Toxicity experiments designed to exploit these relationships are most informative.

The toxicity threshold of each mineral is dependent on its chemical form, enteric absorption, metabolism, duration of consumption by the animal, levels of other minerals in the diet or water, and the species and physiological state of the animal. Important chemical factors that determine the bioavailability of the mineral sources include the solubility of a mineral compound in the digestive tract, its valence state, and whether the mineral is in an organic, metallic, or other inorganic form. Although extensive research has described the bioavailability of minerals in feedstuffs and supplements when fed at concentrations below the dietary requirement, much less research has been directed toward distinguishing the bioavailability of different mineral sources at toxic dietary concentrations.

Characteristics of different animal species that are important modifiers of toxicosis include the efficiency of homeostatic mechanisms such as absorption or excretion pathways, presence of a rumen containing microflora that modify the chemical form of the mineral, and the rate of feed or water consumption. For example, fish use their gills to both absorb and excrete minerals and often have very different toxicosis profiles than terrestrial animals. Among domestic animals, certain ruminants are sometimes more tolerant of high levels of dietary minerals than nonruminants. This has been attributed to their ruminal microflora as well as their relatively low rate of feed intake.

Individuals are often most sensitive to toxicity during embryonic development, growth, and periods of stress such as infections or trauma. Some minerals, such as mercury and lead, affect development of complex biological systems of the brain and the immune system. Development of these organs occurs in the late embryonic and early neonatal period, which is when toxicoses of these minerals are often most evident. Tolerance to minerals usually increases with age, and healthy, mature animals are often most resistant to mineral toxicoses because they have passed important developmental events, their homeostatic mechanisms are well developed, and their relative rates of feed intakes are low. However, some minerals are not easily excreted and they accumulate during an animal's lifetime, resulting in toxic levels in older animals. Cadmium and mercury are two such minerals, and their accumulation in tissues over time has important implications for the safety of foods derived from animals that have consumed high levels of these minerals.

ENVIRONMENTAL AND HUMAN HEALTH

Although this report focuses predominantly on levels of minerals that are toxic for animals, there are some cases where environmental factors or the wholesomeness of foods for human consumption derived from animals are the primary considerations that limit the acceptable levels of minerals in the feed and water of animals. High levels of copper, zinc, phosphorus, and heavy metals in animal excreta can cause environmental damage. Depending upon manure management, location of disposal, and climate factors, environmental considerations may limit the levels of these minerals that can appropriately be fed to animals. The text of this report provides additional information on environmental issues and key references to allow a detailed assessment of these concerns. However, the maximum tolerable levels recommended in this report are based solely on indexes of animal health and productivity.

Even at dietary levels that are apparently safe for animals, some minerals accumulate in their tissues to concentrations that are unsafe for human consumption. Liver, kidney, and spleen are the tissues of maximal accumulation of cadmium, lead, and mercury and limit the dietary concentrations that can be safely fed to animals destined for human consumption. Organic forms of selenium and mercury accumulate in all tissues, including muscle. Health issues related to high levels of these minerals in human foods have been considered in detail by many national and international committees, and their recommendations are referenced in this report. Levels of cadmium, lead, and mercury in the feeds consumed by animals are normally regulated based on concerns related to human health. The recommendations for maximum tolerable levels of these minerals in this report are based solely on indexes of animal health and productivity and would likely result in food products that exceed recommendations for humans.

2

Maximum Tolerable Levels

The "maximum tolerable level" (MTL) of a mineral is defined as the dietary level that, when fed for a defined period of time, will not impair animal health and performance. Tolerable mineral levels are typically distinguished from toxic levels by experiments that add incremental amounts of a mineral to the diet or the water and measure the impact on performance and pathological signs of toxicosis. The duration of exposure to the test mineral markedly influences the level that causes toxicosis. Long-term and multigenerational studies are usually most informative, but, in practice, short-term studies are most commonly conducted. The committee considered three exposure durations: a single dose, acute exposure, and chronic exposure. A single dose is defined as exposure due to the consumption of a single meal or by a single gavage of the mineral. Acute exposure is defined as an intake of 10 days or less. Chronic exposure is set as an exposure of 10 days or more. However, in making recommendations, emphasis was given to the studies that had the longest durations of exposure. A "toxic level" is defined as the minimal level that, when fed for a defined period, impairs animal health or performance (rate of production of milk, meat, or eggs; growth rate; reproductive capacity; disease resistance). Table 2-1 summarizes the committee's recommendations for MTL of dietary minerals for domestic animals following chronic consumption. Prior to applying these recommendations, the accompanying text should be consulted for exceptions and for additional information on the ages of animals, their physiological state, and the length of the studies used to establish these values. Each chapter also provides information on the MTL following single and acute exposures to minerals as well as exposure through water. Information on the toxicity of minerals to dogs and cats is generally lacking. In cases where information is available, it is often a single study using only a single toxic level. Providing a summary of the MTL for dogs and cats in Table 2-1 without accompanying text would be misleading. Individual chapters should be consulted for specific information on mineral toxicities to dogs and cats, as well as additional species.

The MTL in Table 2-1 and in each individual chapter do not include built-in safety factors because the magnitude of the safety factor varies with each specific situation. Consequently, the recommended values should be adjusted according to their intended use, and each chapter contains relevant information on modifying factors for each mineral. Animals that are very young, old, reproducing, sick, exposed to stressful environments, or consuming nutritionally imbalanced diets may be especially sensitive to toxicoses, and individual chapters provide additional information on the impact of these factors. In practice, the MTL is highly dependent upon the form of the mineral to which the animal is exposed. For some minerals, such as silica, chromium, iron, tin, lead, and barium, the MTL may vary by more than an order of magnitude depending upon the chemical form of the mineral, and the individual chapters provide further information on the relative toxicities (i.e., bioavailabilities) of common sources of minerals. Each chapter contains a table that summarizes the details on the levels, chemical form, duration, animal ages, and signs of toxicosis used in the studies reviewed to establish the recommendations. In some cases, there is a wide interval between the MTL and levels that are toxic because research is insufficient to make finer discrimination. Inspection of the summary tables presented in each chapter provides insight into critical experiments and the level of uncertainty.

Research on mineral toxicoses in domestic animals conducted during the past 25 years has resulted in adjustments to many of the MTL provided in the NRC report *Mineral Tolerance of Domestic Animals*, which was published in 1980. Also, an appreciation for the limitations and pitfalls inherent in mineral analysis has changed interpretation of some older studies. In some cases (e.g., aluminum, boron, mercury, and vanadium), the dietary concentrations of minerals reported in older literature are suspect because of inadequacies in sample preparation or analytical procedures; thus greater reliance is placed on more recent publications. For some minerals, the previous report necessarily relied on in vitro experiments or studies in which animals were injected

with minerals, bypassing homeostatic digestive processes. Newer information from feeding studies has eliminated reliance on injection studies and often resulted in marked changes in recommendations.

HUMAN HEALTH

Meat, milk, and eggs are an important part of the human diet because they supply highly bioavailable forms of minerals. Animals, via homeostatic control of gastrointestinal absorption, can often serve to diminish the high mineral concentrations found in some plants or supplements, thereby reducing human exposure to potentially toxic minerals. However, levels of some minerals may accumulate in animal tissues intended for human consumption to concentrations that might adversely affect human health even when animals are exposed to safe levels (i.e., levels at or below their respective MTL). Consequently, in the case of animals raised to supply human food, acceptable concentrations of minerals in feeds and water must consider the health of those consuming food products derived from these animals as well as the health of the animal itself.

The committee identified minerals of concern for human health by a three-step process. First, the amount of a mineral that accumulates in meat, milk, bone, and eggs when animals are fed their respective MTL was estimated. Second, acceptable safety standards for mineral intake by humans were identified. Third, the maximum concentrations in animal tissues were compared to levels known to be safe for humans. Individual chapters in this report provide information on the dose–response relationship between feed mineral levels and tissue levels. Recently the NRC (2000; 2001a,b) has extensively reviewed the scientific literature on mineral toxicosis in humans due to the ingestion of high levels of minerals in foods. The human toxicological standards based on these reviews and recent data on food consumption trends in the United States were used to estimate the potential for mineral toxicosis among humans consuming tissues and fluids from animals exposed to mineral levels at their respective MTL based on animal health.

Toxicological Standards for Minerals in Humans

The NRC (2000; 2001a,b) set Tolerable Upper Intake Levels (UL) for minerals that are nutritionally required for humans (Table 2-2). The UL was defined as "the highest average daily nutrient intake that is likely to pose no risk of adverse effects to almost all (human) individuals in the general population." The UL were based on lowest-observed-adverse-effect levels (LOAEL) and no-observed-adverse-effect levels (NOAEL) that were calculated using primarily data from studies with humans.

In several cases, the NRC could not identify studies in humans or sometimes even laboratory animals in which the mineral as it occurred naturally in the diet was toxic. Thus, the NRC did not set UL for several minerals (i.e., arsenic, chromium, silicon), explicitly stated that the ULs applied only to supplemental (not dietary) forms of two minerals (i.e., magnesium, nickel), and based UL on data from only laboratory animal studies for other minerals (i.e., boron, molybdenum, nickel, vanadium). Furthermore, the NRC noted in the text that the UL for iron and zinc were based on studies with humans in which the primary source of the mineral was from supplements.

These decisions by the NRC (2000; 2001a,b) reflect a number of factors. One is that the form of minerals in food is sometimes different from that in supplements (e.g., heme-iron from meat versus supplemental inorganic iron). Another is that the total milieu of factors (especially protein, phytate, fiber, and other minerals) in foods can reduce the potential of toxicosis of high levels of dietary minerals in those foods. For example, there are no reports of adverse effects from consuming high levels of calcium and phosphorus in milk or manganese from cereal products.

The NRC did not set unacceptable levels of several other minerals (aluminum, barium, bismuth, bromine, cobalt, and tin). Generally, diet is a minor source of these elements as compared to pharmaceuticals. The Food and Agriculture Organization/World Health Organization (FAO/WHO) Pesticide committees (1967) set the acceptable daily intake of 1 mg bromide/kg BW/day. Minimal effects (i.e., reductions in the absorption of essential minerals) were the major signs when human diets were supplemented with aluminum salts (125 mg Al/day; Greger and Baier, 1983) or tin salts (50 mg Sn/day; Johnson et al., 1982). Cadmium, mercury, and lead are generally considered the elements most apt to produce toxic effects when ingested. The minimum risk level for mercury, which is the dose that can be ingested daily for a lifetime without a significant risk of adverse effects, was set at 0.0003 mg Hg/kg BW/day for a 70-kg person, based on neuro-developmental outcomes in children exposed in utero to methylmercury from maternal fish ingestion (ATSDR, 1999). For pregnant women the suggestion is not to consume fish containing greater than 0.25 mg Hg/kg fresh weight. Research has demonstrated that blood lead levels >10 µg/dL whole blood were associated with adverse neurological and hematological effects in children and that blood lead levels of two-year olds declined when dietary intake of lead was reduced from 0.053 to 0.013 mg of lead per child daily (NRC, 1993). The individual mineral chapters in this report contain additional information on toxic levels of specific minerals in human diets.

Maximum Exposure to Minerals from Consumption of Animal Products

Americans consumed an average of 105.7 kg of meat, fish, and poultry; 228.6 kg of milk and milk-derived products (on a calcium equivalent weight basis, which converts cheese and other products into an equivalent amount of

milk); 14 kg of eggs; and 9.3 kg of legumes, nuts, and soy (high-protein alternative to meat) per year during 1990–1999 (Gerrior and Bente, 2002). These data are based on USDA figures on the disappearance of food available for human consumption. Accordingly, the average American potentially consumes 0.95 kg, fresh weight, of animal products per day or 0.98 kg of "protein-rich" foods per day. The actual average consumption is likely to be considerably less than 0.98 kg because this estimate is based on disappearance values and not actual intakes. However, for the purposes of identifying minerals that might be of concern for human health, consumption data provide a reasonable margin of safety. A more extensive risk analysis should be conducted for those minerals that are identified below as being of concern for human health.

The committee used this information to establish relative risks. The first question asked was this: How much of a mineral would an individual ingest if the individual consumed that entire kilogram of "protein-rich" food daily as the muscles from an animal fed at the MTL for that mineral? Based on the highest concentrations of minerals found in muscles of livestock and fish fed at the respective MTL (see individual mineral chapters for data on tissue levels), we would *not* expect humans ingesting these muscles to consume excessive amounts of minerals, except for cadmium, lead, mercury, and selenium. If it is assumed that 5 percent of the kilogram of "protein-rich" food were bone fragments (due to incorrect processing), intake of barium and fluoride might be excessive in some cases.

If the kilogram of "protein-rich" food consumed daily was milk, intake of cadmium, iodine, lead, and mercury might in some cases be excessive. The calculated exposures become of greater concern if the kilogram of "protein-rich" food consumed daily is from the livers or kidneys of livestock and fish fed toxic levels of minerals. Potentially, humans ingesting 1 kg of liver daily could ingest excessive amounts of arsenic, cadmium, copper, iron (see below), lead, mercury, and selenium. Potentially, humans ingesting 1 kg of kidney daily could ingest excessive amounts of arsenic, bismuth, cadmium, chromium, cobalt, fluoride, lead, mercury, and selenium. Generally, the organic forms of arsenic and iron in tissues are assumed to be relatively less toxic.

These calculations underscore the concerns of the FDA about certain elements in animal products, namely iodine in milk (Hemken, 1980), mercury in fish, cadmium in kidneys, and lead and fluoride in bone. Establishing specific recommendations for mineral levels in animal feeds that are safe for human health was beyond the charge of the committee. However, for arsenic, barium, bismuth, cadmium, chromium, cobalt, copper, fluoride, iron, lead, mercury, and selenium, the MTL in Table 2-1, while safe for animals, could result in unacceptably high levels of the mineral in some types of foods derived from these animals

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TABLE 2-1 Maximum Tolerable Levels of Minerals in the Feed (mg/kg or % of the DM) of Animals Based on Indexes of Animal Health[a,b]

Element	Rodents	Poultry	Swine	Horse	Cattle	Sheep	Fish
Aluminum, mg/kg	200	1,000	(1,000)	(1,000)	1,000	1,000	—
Antimony, mg/kg	70–150	—	—	—	—	—	—
Arsenic, mg/kg[c]	30	(30)	(30)	(30)	(30)	(30)	5
Barium, mg/kg[c]	250	100	(100)	(100)	—	—	—
Bismuth, mg/kg[c]	(500)	1,000	500	(500)	—	—	—
Boron, mg/kg	150	(150)	(150)	(150)	150	(150)	—
Bromine, mg/kg	300	2,500	200	(200)	200	(200)	—
Cadmium, mg/kg[c]	10	10	10	10	10	10	10
Calcium, %[d]	2	1.5, growing birds; 5, laying hens	1	2	1.5	1.5	0.9
Chromium, mg/kg[c]							
soluble Cr^{+++}	100	500	(100)	(100)	(100)	(100)	
CrO	30,000	3,000	(3,000)	(3,000)	(3,000)	(3,000)	3,000
Cobalt, mg/kg[c]	25	25	100	(25)	25	25	
Copper, mg/kg[c]	500	250[e]	250	250	40[h]	15[h]	100
Fluorine, mg/kg[c]	150	150	150	(40)	40	60	—
Germanium, mg/kg	30	—	—	—	—	—	—
Iodine, mg/kg[c]	—	300	400	5	50	50	—
Iron, mg/kg[c]	(500)	500	3,000	(500)	500	500	—
Lead, mg/kg[c]	10	10	10	10	100	100	10
Lithium, mg/kg	(25)	25	25	(25)	25	25	—
Magnesium, %	0.5	0.5, growing birds; 0.75, laying hens	0.24	0.8	0.6	0.6	0.3
Manganese, mg/kg	2,000	2,000	1,000	(400)	2,000	2,000	—
Mercury, mg/kg[c]							
inorganic	0.2	0.2	(0.2)	(0.2)	—	—	—
organic[f]	1	1	(2)	(1)	2	2	1
Molybdenum, mg/kg	7	100	(150)	(5)	5	5	10
Nickel, mg/kg	50	250	250	(50)	100	(100)	50
Phosphorus, %[d]	0.6	1, growing birds; 0.8, laying hens	1.0	1	0.7	0.6	1
Potassium, %	1	1	1	1	2	2	—
Rare earth mineral, mg/kg	100	—	—	—	—	—	—
Rubidium, mg/kg	200	—	—	—	—	—	—
Selenium, mg/kg[c]	(5)	3	4	(5)	5	5	(2)
Silicon, %	—	—	—	—	(0.2)	0.2	—
Silver, mg/kg	—	100	(100)	—	—	—	> 3
Sodium chloride, %	4	1.7	3	6	4.5, growing animals 3.0, lactating cows	4	—
Strontium, mg/kg	1,000	2,000	2,000	(2,000)	2,000	(2,000)	—
Sulfur, %	(0.5)	0.4	0.4	(0.5)	0.30, high-concentrate diet; 0.50, high-forage diet	0.30, high-concentrate diet; 0.50, high-forage diet	—
Tin, mg/kg	100	(100)	(100)	(100)	(100)	(100)	—
Titanium, mg/kg	—	—	—	—	—	—	—
Tungsten, mg/kg	(20)	20	(20)	(20)	(20)	(20)	—
Uranium, mg/kg	100	—	—	—	—	—	< 100

continued

TABLE 2-1 Continued

Element	Rodents	Poultry	Swine	Horse	Cattle	Sheep	Fish
Vanadium, mg/kg	—	25, growing birds; <5, laying hens	(10)	(10)	50	50	—
Zinc, mg/kg	(500)	500	1,000[g]	(500)	500	300	250

[a]The accompanying text must be consulted for additional details about factors that might increase or decrease the tolerable levels. The levels in parentheses were derived from interspecies extrapolation. Dashes indicate that data were insufficient to set a maximum tolerable level (MTL). Individual chapters provide further information on human safety and environmental problems related to feeding high levels of the mineral.
[b]The MTL are based on highly soluble forms of the mineral and other forms may have lower bioavailabilities. MTL are also based on the assumption that exposure of the animal to minerals from water and other environmental sources is minimal and that the diet is nutritionally complete, especially in regard to other minerals that might be antagonized.
[c]The MTL provided for this nutrient is based on animal health and not human health. Lower levels are necessary to avoid excessive accumulation in edible tissues.
[d]The MTL are based on diets with phosphorus levels at, but not above, the animal's requirement. Considerably higher levels can be tolerated if phosphorus levels are increased sufficiently to maintain an appropriate calcium:phosphorus ratio.
[e]For ducks the MTL for copper is 100 mg/kg diet.
[f]A margin of safety should be added for pregnant animals to assure normal neurodevelopment of the fetus.
[g]Higher levels of zinc as zinc oxide (2,000 to 3,000 mg/kg diet) are tolerated for several weeks and may provide growth promotion in weanling piglets.
[h]Assuming normal concentrations of molybdenum (1–2 mg/kg diet) and sulfur (0.15–0.25%). At molybdenum and sulfur concentrations below these, copper may become toxic at lower levels.

TABLE 2-2 Toxicity Assessments of Nutrients for Humans Based on Food and Nutrition Board Assessments (NRC, 2000; NRC, 2001a,b)

	Lowest (or No)-Observed-Adverse-Effect Level (LOAEL/NOAEL)	Tolerable Upper Intake Level (UL)
Arseni[a]	Not set	Not set
Boron[b]	9.6 mg/kg/d	20 mg/d
Calcium[c]	5 g/d	2,500 mg/d
Chromium[d]	Not set	Not set
Copper[e]	10 mg/d	10 mg/d
Fluorid[f]	0.10 mg/kg/d	10 mg/d
Iodine[g]	1.7 mg/d	1.1 mg/d
Iron[g]	70 mg/d (primarily for inorganic salt supplement)	45 mg/d
Magnesium[h]	360 mg from non food sources/d	350 mg/d from supplement
Manganese[e]	11 mg/d	11 mg/d
Molybdenum[b]	0.9 mg/kg/d	2.0 mg/d
Nickel[b]	5 mg/kg/d	1.0 mg/d of soluble nickel salts
Phosphorus[i]	10.2 g/d	4.0 g/d
Selenium[b]	800 µg/d	400 µg/d
Silicon[j]	Not set	Not set
Vanadium[k]	7.7 mg/kg/d	1.8 mg/d
Zinc[g]	60 mg/d (primarily for inorganic salt supplement)	40 mg/d

[a]Chronic intake of 10 µg/kg/d of inorganic arsenic produces arsenicism with skin changes in humans (ATSDR, 2000).
[b]NOAEL based on data in rodents or humans (selenium); UL for >19 years of age.
[c]LOAEL for adults: UL for 19–70 years of age.
[d]Insufficient data to establish a UL for soluble chromium III salts. Concern only for Cr in supplements.
[e]NOAEL for adults: UL for >19 years of age.
[f]LOAEL for children through 8 years: UL for >8 years of age.
[g]LOAEL for adults: UL >19 years of age.
[h]LOAEL for adults: UL >8 years of age.
[i]NOAEL for adults: UL for 19–70 years of age.
[j]No evidence that Si naturally occurring in food or water produced adverse health effects.
[k]LOAEL based on data in rats: UL for >19 years of age.

3

Aluminum

INTRODUCTION

Aluminum (Al) has an atomic number of 13 and is one of the lightest metals. It has two oxidation states: Al (0) and Al (+3). However, aluminum does not occur naturally in the elemental state, but rather in combination with oxygen, silicon, and fluorine primarily. The most important raw material for the production of aluminum metal is bauxite, which contains 40 to 60 percent alumina (aluminum oxide) (ATSDR, 1999).

Aluminum is the third most abundant element in the Earth's crust after oxygen and silicon. Although it has been produced in commercial quantities for just over 100 years, it is used more today than any other metal, except iron. In 2000, the United States' estimated consumption of aluminum was 7.5 million metric tons (Plunkert, 2002). Aluminum recovered from discarded aluminum products accounts for about 20 percent of estimated consumption of aluminum in the United States (Plunkert, 2002).

Aluminum is used industrially because it is malleable, ductile, durable, and easily machined and cast, and has excellent corrosion resistance. The following areas constitute the major uses of aluminum in the United States: transportation, 35 percent; packaging, 25 percent; building, 15 percent; consumer durables, 8 percent; electrical, 7 percent; and other, 10 percent (Plunkert, 2002).

ESSENTIALITY

Horecker et al. (1939) suggested that aluminum promoted the reaction between cytochrome C and succinic dehydrogenase in vitro. The activation of purified guanine nucleotide binding protein, the regulatory component of adenylate cyclase, by fluoride was shown to require the presence of Al^{+3} in vitro (Sternweis and Gilman, 1982; Kahn, 1991). The significance of these observations in vivo is not known. No conclusive evidence exists to suggest that aluminum is essential for growth, reproduction, or survival of animals (Alfrey, 1986; Ganrot, 1986; Greger, 1993). However, it may be a cariostatic agent by itself and in combination with fluoride (Kleber and Putt, 1984).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Monitoring the tissue levels of aluminum and the absorption and excretion of aluminum is very difficult for a variety of methodological reasons (Kostyniak, 1983; Ganrot, 1986; Savory and Wills, 1988; Greger and Sutherland, 1997; ATSDR, 1999). Estimated amounts of aluminum in plasma decreased by more than 50-fold between 1950 and 1980 because of improvements in methodology (Versieck and Cornelis, 1980). Accordingly, this review emphasizes literature published after 1980.

One reason for the analytical problems is that aluminum is ubiquitous in the environment and contamination of biological samples is difficult to prevent. When contamination from anticoagulants and glassware was prevented, serum aluminum concentrations were between 1 to 5 µg/L (Kostyniak, 1983; Ganrot, 1986; Savory and Wills, 1988).

A second limitation is that flame atomic absorption spectroscopy is not sensitive enough for analyses of biological samples. Matrix effects decrease the sensitivity of several methods and hence preparation of samples is often time-consuming (ATSDR, 1999). The most commonly used method is graphite furnace atomic absorption spectrometry. Researchers must correct for interfering interaction with phosphorus and silicon when using neutron activation analysis to determine aluminum because all three produce ^{28}Al when activated. A major problem with inductively coupled plasma-atomic emission spectrometry is that calcium in the sample raises the limit of detection for aluminum. Two other techniques have improved sensitivity, but are expensive and require highly trained personnel. Inductively coupled plasma-mass spectrometry has sensitivity down to 1 mg/L of aluminum in blood. Laser ablation microprobe mass

analysis (LAMMA) has been used for analyses of aluminum in brains where sensitivity and location are important.

A third methodological limitation is the lack of usefulness of aluminum isotopes for metabolic studies (Greger and Sutherland, 1997). Most aluminum isotopes have very short half-lives; ^{23}Al, ^{24}Al, ^{25}Al, ^{28}Al, and ^{30}Al have half-lives of less than 6.6 minutes (Bureau of Radiological Health and Training Institute, 1970). Thus they cannot be used in vivo to monitor absorption and excretion processes. The only aluminum isotope with a biologically usable half-life is ^{26}Al (7.2×10^5 years), but it is very scarce and hence the study designs and replicates are often limited (Jouhanneau et al., 1993; Priest et al., 1995; Schönholzer et al., 1997; Yokel et al., 2001). Other investigators attempted to use ^{67}Ga (a type IIIa element like aluminum) as a marker for aluminum (Greger et al., 1994, 1995; Priest et al., 1995). These investigators concluded that ^{67}Ga was an unacceptable marker for aluminum because ^{67}Ga did not distribute in tissues like aluminum and was more sensitive to iron intake and status than was aluminum.

Finally, absorption of aluminum is difficult to quantitate because absorption is generally <1 percent (Alfrey 1989; Greger and Sutherland, 1997). In response to this methodological quandary, Ganrot (1986) suggested that urinary aluminum excretion could be assumed to equal aluminum absorption. This assumption is faulty because animals accumulate aluminum in tissue with continued oral exposure to aluminum; this would not occur if absorption and urinary excretion were equal. Moreover, the relative percentage of absorbed aluminum retained (not excreted in urine) has been found to vary with a variety of factors, including renal function, age, and disease states (Ecelbarger et al., 1994a; Greger and Radzanowski, 1995). Thus one alternate (but time-consuming and difficult) way to estimate relative aluminum absorption from diet is to compare tissue accumulation of aluminum in relation to the dose in animals fed aluminum and in animals matched for age and weight that are injected with aluminum (Greger and Powers, 1992). Another alternate (but also time-consuming and difficult) way to estimate absorption from a single large dose is to sum all aluminum in the carcass, urine, and bile (Sutherland and Greger, 1998).

REGULATION AND METABOLISM

Absorption

The gastrointestinal tract is the most important body system that protects mammals against accumulating aluminum in tissues. As noted in the previous section on Difficulty in Methods of Analysis and Evaluation, absorption of aluminum is difficult to determine, partially because fecal losses approximate intakes. Using a variety of alternate methodologies (measurement of aluminum in urine, usage of isotopes, comparison of tissue aluminum accumulation when equal doses of aluminum were ingested and injected, and summation of the increase in aluminum excretion and tissue retention after a dose), aluminum absorption has been estimated to be 0.01 to 0.8 percent of intake when aluminum was ingested in diet (Ganrot, 1986; Greger and Powers, 1992; Jouhanneau et al. 1993) and 0.3 percent from water (Yokel et al., 2001). However, fasted rats absorbed 2 to 5 percent of the small amounts of aluminum administered by gavage in a citrate solution (Froment et al., 1989a; Schönholzer et al., 1997; Sutherland and Greger, 1998).

Aluminum absorption occurs to the greatest extent in the proximal intestine. Plasma aluminum and labeled plasma glucose levels peaked simultaneously 45 minutes and 30 minutes after aluminum citrate and glucose were administered together by gavage to rats (Froment et al., 1989a,b) and humans (Nagy and Jobst, 1994), respectively.

The mechanisms of aluminum absorption include both paracellular passage of aluminum through tight junctions by passive processes (Provan and Yokel, 1988b; van der Voet, 1992) and transcellular passage through enterocytes, involving passive, facilitated, and active transport processes (van der Voet, 1992). The active transcellular processes involved in aluminum absorption are saturable (Feinroth et al., 1982). At least some of the active processes (particularly the transport of aluminum into the serosal fluid) are shared with processes for active absorption of calcium (Feinroth et al., 1982; Provan and Yokel, 1988a). Some of these processes are dependent on vitamin D metabolites (Ittel et al., 1988, 1990). Aluminum did not appear to affect the ability of calbindin-D9K (the diffusional translocator of calcium across mucosal cells) to bind calcium in rats (Adler et al., 1991) but reduced the transcription and/or stability of calbindin-D28K in chicks (Cox and Dunn, 2001).

Adler and Berlyne (1985) perfused in situ guts with aluminum chloride at pH 2 and estimated that 23 percent of aluminum absorption in rats was due to non-saturable processes and the rest was due to active processes. However, the relative importance of absorptive processes varies with the concentration and speciation of aluminum in the gut and other dietary factors (van der Voet, 1992; Greger and Sutherland, 1997). These factors are discussed in the section further below on Bioavailability.

Urinary Excretion

Urinary excretion of aluminum is proportional to aluminum intake under controlled conditions (Ganrot, 1986; Greger and Powers, 1992). Approximately half of the absorbed aluminum is estimated to be excreted in urine (Jouhanneau et al., 1993). Thus humans with normal renal function who consume typical diets with no medications excrete less than 50 µg/day of aluminum in urine (Ganrot, 1986; Alfrey, 1986; Greger, 1993). This primarily reflects the protective effect of the gut. When patients were infused with parenteral solutions contaminated with aluminum, sub-

jects excreted 0.1 to 3.8 mg/day of aluminum (Klein et al., 1982).

The importance of renal excretion of aluminum has been demonstrated many times in humans with reduced renal function, even in the absence of parenteral exposure to aluminum (King et al., 1981; Committee on Nutrition, 1986; Hewitt et al., 1990; Strong et al., 1996; ATSDR, 1999). Alfrey et al. (1980) found that patients with undialyzed uremia had 12-fold more aluminum in bone, and more than 6-fold more aluminum in liver and spleen than renally competent patients. Rats given unilateral nephrectomies (that reduced renal excretion only moderately as might occur naturally with age) accumulated more aluminum in bone and liver than sham-operated rats (Ecelbarger and Greger, 1991; Ecelbarger et al., 1994a). Some of this increased accumulation of aluminum by uremic animals could reflect increased gut permeability to aluminum in uremic animals (Ittel et al., 1988, 1992).

Biliary Excretion

Biliary excretion is less important than urinary excretion in preventing accumulation of aluminum in tissues. Dogs and rats given intravenous doses of aluminum excreted from 0.1 to 7 percent of the doses in bile (Kovalchik et al., 1978; Klein et al., 1988; Xu et al., 1991). In contrast, biliary aluminum excretion sometimes exceeded urinary excretion of aluminum in humans whose main source of aluminum exposure was diet (Ishihara and Matsushiro, 1986). Although rats exposed to low (6.9 to 10.3 μg/g diet of aluminum) levels of aluminum in diet excreted more aluminum in bile than in urine, rats given moderate (0.25 to 1 mmole/kg BW of aluminum) amounts of aluminum by gavage excreted more aluminum in urine than bile (Sutherland and Greger, 1998). This reflected that the biliary excretion of aluminum was rapid (within 15 minutes after a gavage dose in rats) and saturated by doses as low as 0.2 mmole of aluminum (Sutherland et al., 1996; Sutherland and Greger, 1998).

SOURCES AND BIOAVAILABILITY

Dietary Sources

Grain and vegetable products contain more aluminum naturally than animal products (Schlettwein-Gsell and Mommsen-Straub, 1973; Sorenson et al., 1974; Greger, 1985; Greger et al., 1985b; Pennington and Jones, 1989). A few plants, such as tea and herbs (e.g., bay, oregano, and thyme) often "accumulate" more than 500 mg/kg dry weight of aluminum. The aluminum content of vegetable and grain products varies with plant varieties and soil conditions, including pH (Hopkins and Eisen, 1959; Eden, 1976).

Water is generally not a major source of aluminum. The amount of aluminum in surface and ground water varies from <12 mg to 2.25 mg/L of aluminum in the rivers of North America (Jones and Bennett, 1986). Flaten (1995) estimated that average aluminum concentrations of water supplies in Europe ranged from 6 to 60 μg/L of aluminum. Miller et al. (1984) estimated that there was a 40–50 percent chance that aluminum containing flocculants added to clarify municipal water supplies increased the aluminum concentration of finished water above that naturally present in water. They reported that the median concentration of aluminum in "finished" water supplied by water utilities was 17 μg/L of aluminum (range <14 μg/L to 2.67 mg/L of aluminum). However, very high concentrations (30–620 mg/L) of aluminum have been found in "finished" water when processing plants made serious errors in England (Eastwood et al., 1990).

The biggest source of aluminum in the diets of most Americans is food additives, although aluminum-containing additives are present in only a limited number of foods (i.e., chemically leavened baked goods and processed cheese) (NRC, 1984; Greger, 1985; Pennington and Jones, 1989). It is unlikely that aluminum-containing food additives are intentionally added to the diets of livestock and pets. However, aluminum contamination of some additives used in livestock and pet feed is possible. For example, aluminum concentrations were found to be 3- to 10-fold higher in soy-based infant formulas than in milk-based infant formulas (Committee on Nutrition, 1986). Calcium and phosphate additives (with unintentional aluminum contamination) were likely sources of the aluminum in the soy formulas (Burgoin, 1992).

Packaging and utensils can also be a source of dietary aluminum to humans. Most foods stored or cooked in aluminum pans, trays, or foil accumulated less than 2 mg/kg food of aluminum when frozen, refrigerated, and stored at ambient temperature (Ondreička et al., 1971; Greger et al., 1985b; Liukkonen-Lilja and Piepponen, 1992). A few acidic foods (e.g., tomato products, applesauce), when cooked for long periods of time, accumulated as much as 17 mg/100 g serving of aluminum. However, packaging is probably not an important source of aluminum in the diets of livestock or even pets, as aluminum cans are lined to prevent seepage of aluminum into foods.

Pharmaceutical and Other Sources

Lione (1983, 1985) estimated that humans could ingest as much as 5 g/day of aluminum in pharmaceuticals such as antacids, buffered analgesics, antidiarrheals, and certain antiulcer drugs. Only the antidiarrheals (e.g., kaolin, attapulgite, and aluminum magnesium silicate) are apt to be used in veterinary practice.

Aluminum hydroxide, aluminum phosphate, aluminum potassium sulfate (alum), and aluminum silicates (zeolite) are used in the preparation of a number of vaccines and other injectants to adsorb antigenic components and to serve as adjuvants that enhance immune response (Lione, 1985). The

importance of adjuvants as a source of aluminum is debatable (Malakoff, 2000). Keith et al. (2002) estimated that the body burden of aluminum for human infants is greater from vaccinations than from dietary sources, but is still at a safe level. More aluminum was absorbed into blood by rabbits injected intramuscularly with aluminum phosphate than with aluminum hydroxide adjuvant (Hem, 2002). It is unlikely that injectables are a major source of aluminum exposure to livestock or even most pets.

Feed or water for livestock could be contaminated with aluminum when aluminum sulfate and zeolite are applied to litter and waste lagoons to reduce phosphorus run-off from lands fertilized with these wastes and to reduce ammonia volatilization in facilities (Moore et al., 1999, 2000; Codling et al., 2002). Alum has also been added to dairy slurry to reduce ammonia emissions (Lefcourt and Mesinger, 2001).

Bioavailability

A variety of dietary factors affect absorption of aluminum.

Speciation and Solubility

Speciation of aluminum in water is complex and changes dramatically with pH (Martin, 1991). The net result is that aluminum is less soluble between pH 5 to 7 than at higher or lower pHs. Accordingly, rats absorbed more aluminum from in situ perfusions at pH 4 than at pH 7 (van der Voet, 1992).

The solubility of aluminum compounds also affects their efficiency of absorption. Patients' serum aluminum levels tended to be higher after they were dosed with sucralfate in suspension rather than in tablet or granular form (Conway et al., 1994; Schütze et al., 1995). Berthon and Daydé (1992) noted that aluminum phosphate was less toxic than aluminum hydroxide because aluminum phosphate is virtually insoluble at acidic pHs. However, Yokel and McNamara (1988) found no correlation between the percent bioavailability of aluminum from eight aluminum-containing compounds to rabbits and the compounds' solubility.

Citric Acid

Citrate in diet and pharmaceuticals has been repeatedly found to increase aluminum absorption in humans (Slanina et al., 1986; Greger and Sutherland, 1997). Citrate has been found to increase aluminum retention in tissues of laboratory animals when administered in drinking water (Fulton et al., 1989; Fulton and Jeffery, 1990), diet (Ecelbarger et al., 1991, 1994b; Greger and Powers, 1992; Owen et al., 1994), or gavage solutions (Slanina et al., 1984, 1985; Yokel and McNamara, 1988; Froment et al., 1989 a,b; Quartley et al., 1993). Generally animals fed aluminum and citrate in diets retained more aluminum than animals given citrate with aluminum in drinking water, but less aluminum than animals dosed with only citrate and aluminum by gavage. The presence of other substances (i.e., fluoride, phosphorus, calcium) in the gut, as occurs when aluminum and citrate were added to feed and water, may have moderated the effect of citrate on aluminum absorption (Greger, 1993; Glynn et al., 1995).

Other Organic Acids and Anions

Although the interaction of citrate and aluminum is practically important, the uniqueness of the interaction has been overstated. A variety of organic acids found in food beside citric acid (including ascorbic, gluconic, lactic, malic, oxalic, and tartaric acids) have been found to increase the solubility of aluminum and to increase tissue retention of aluminum in rats orally dosed with aluminum (Domingo et al., 1991, 1994). Limited data indicate that dietary factors (e.g., phytate and polyphenols) that reduced the absorption of other minerals also reduced the absorption of aluminum (Powell and Thompson, 1993; Powell et al., 1993).

Several investigators have compared the biological effects of various aluminum salts. Large (aluminum as 0.5 percent of diet) oral doses of aluminum as acetate, chloride, nitrate, and sulfate salts were more toxic to chicks than similar doses of aluminum as phosphate salt (Storer and Nelson, 1968). Similarly, human subjects had less aluminum in serum and urine after being dosed with 2.2 g aluminum daily as aluminum phosphate than after being dosed with aluminum hydroxide, aluminum carbonate, or dihydroxyaluminum aminoacetate (Kaehny et al., 1977). Rabbits dosed with aluminum nitrate or citrate experienced greater increases in serum aluminum than those dosed with aluminum as borate, hydroxide, chloride, glycinate, sucralfate, and acetate salts (Yokel and McNamara, 1988). No differences in tissue aluminum concentrations were noted when rats were dosed with moderate levels (205 to 278 mg/kg diet of aluminum) of various aluminum hydroxide compounds (reagent and dessicated gel) and aluminum as lactate, palmitate, and phosphate (Greger et al., 1985a). However, bone phosphorus levels were only affected by aluminum hydroxide.

Silicates

Dietary silicates may reduce the absorption of aluminum because silicates replace phosphates as the primary complexors of aluminum in solutions that are more alkaline than pH 6.6 (Birchall and Chappell, 1988; Birchall, 1992). Edwardson et al. (1993) noted that plasma ^{26}Al concentrations one hour after ingestion of ^{26}Al and silicon in orange juice were only 15 percent of those observed when silicon was not consumed with aluminum. Quartley et al. (1993) demonstrated that oral consumption of silicic acid previously to and concurrently with aluminum reduced tissue aluminum concentrations in rats four hours after dosing. Jugdaohsingh et al. (2000) demonstrated that oligomeric silica, but not monomeric silica, reduced dietary aluminum

bioavailability by 67 percent in humans. The oligomeric silica was not absorbed; the monomeric silica was. Addition of silicon (6.5 mg/L of silicon) to water containing 527 µgl/L of aluminum resulted in the formation of hydroxyl-aluminosilicates and decreased lysosomal accumulation of aluminum by freshwater snails, presumably due to poorer absorption of aluminum (Desouky et al., 2002).

Desouky et al. (2002) hypothesized that the interaction of aluminum and silica may extend beyond absorption because snails exposed to aluminum (without supplemental silicon) accumulated more silica in their lysosomes as hydroxyl-aluminosilicates.

Calcium and Magnesium

Aluminum may share some of the active processes involved in calcium absorption (Feinroth et al., 1982; Provan and Yokel, 1988a). Accordingly, several investigators have attempted to determine whether calcium- and magnesium-deficient animals were more sensitive to oral aluminum exposure. Yasui et al. (1991) found that rats deficient in calcium and magnesium retained more aluminum in tissues. Ecelbarger and Greger (1991) found that increasing the dietary calcium levels 4-fold (67 to 250 mmol/g diet of calcium) resulted in reduced bone aluminum concentrations in rats. However, Yokel et al. (2001) suggested that the presence of calcium and magnesium in the gut delayed but did not reduce the absorption of aluminum.

Iron

Interactions between aluminum and iron theoretically occur in a variety of tissues because aluminum is at least partially transported in plasma by transferrin. The importance and the mechanism(s) of the interactions are controversial. The stability constants for transferrin binding to Al^{+3} are 10 log scales lower than for iron (i.e., log K of 22 versus 12, respectively) (Martin et al., 1987). Fatemi et al. (1991) estimated that 60 percent of the aluminum in human plasma (at pH 7.4 with 5 µM aluminum and 25 mM HCO_3^-) would be bound to transferrin, 34 percent to albumin, and the remainder to citrate.

It is unlikely that aluminum and iron compete directly for absorption. The addition of equimolar quantities of iron to a gavage dose of aluminum did not affect tissue aluminum levels of rats (Greger et al., 1995). The presence of Fe III in in situ perfusion systems did not affect the luminal disappearance or the intestinal absorption of aluminum (van der Voet and de Wolff, 1987). However, the presence of Fe II decreased the intestinal absorption of aluminum by decreasing the release of aluminum into the blood from mucosal cells.

Fernández-Menéndez et al. (1991) proposed that in iron deficiency the decreased saturation of transferrin resulted in greater aluminum absorption. In two studies, anemic rats orally administered aluminum for 30 to 42 days excreted more aluminum in urine and retained more aluminum in tissues than normal rats (Cannata et al., 1991; Brown and Schwartz, 1992). However, the ingestion of ferrofumarate with aluminum aminoacetate for 90 days by renal patients did not decrease plasma aluminum levels (Andersen et al., 1987). Moreover, transferrin receptors are not present on the apical surfaces of mucosal cells (Anderson et al., 1990).

An alternate explanation for the effect of dietary iron on tissue aluminum levels involves the effect of iron deficiency on the tissue turnover of aluminum. The half-lives of aluminum in liver (56 versus 17 days), muscle (33 versus 16 days), spleen (29 versus 24 days), and serum (12 versus 8 days) were longer in iron-deficient anemic rats than in controls following a single oral aluminum dose (Greger et al., 1994).

TOXICOSIS

There are no practically important studies reporting the toxic effects of a single oral dose of aluminum (ATSDR, 1999). The toxic effects of chronic (>2 weeks) oral exposure to aluminum in livestock appear to be related to aluminum effects on general growth and longevity or on the utilization of essential elements (NRC, 1980). Hence the studies are presented accordingly. Table 3-1 summarizes their findings.

Growth and Longevity

Few gross changes have been observed in normal animals exposed to aluminum orally in long-term (6 months) studies in which nothing was done to enhance aluminum absorption. The longevity of rats exposed to aluminum (5 mg/L of aluminum) in their drinking water throughout their life was unaffected (Schroeder and Mitchener, 1975). Rabbits administered aluminum in water (5 mg/L of aluminum) for 12 months gained weight more slowly than controls but demonstrated no histological changes except for elevation of tissue aluminum concentrations (Wills et al., 1993). The food intake and weights of dogs were not adversely affected when sodium aluminum phosphate (up to 3 percent of diet was sodium aluminum phosphate) was added to their diets for six months (Katz et al., 1984).

Similarly, Berlyne et al. (1972) observed that adding 1 percent or 2 percent aluminum chloride or aluminum sulfate to the drinking water or administering aluminum hydroxide (150 mg/kg BW/day of aluminum) by gavage to rats had no adverse effects on the growth or organs of normal rats, except to elevate tissue aluminum concentrations. However, the administration of the same doses of aluminum to rats that had five-sixths nephrectomies increased the death rate of animals (Berlyne et al., 1972).

Neonatal animals may be more sensitive to aluminum. Administration of aluminum chloride or aluminum lactate (100 and 200 mg/kg BW/day of aluminum) by gastric

intubation to young (5- to 14-day-old) rats reduced body weight and decreased plasma protein and albumin but increased α1 globulins (Cherroret et al., 1995).

Bone Metabolism

More than 100 papers have been published on the importance of aluminum in the etiology of dialysis osteodystrophy in human patients with uremia. There are two etiologies for the osteodystrophy: contamination of dialysates with aluminum and the use of pharmaceutical levels of aluminum to treat the hyperphosphatemia associated with renal failure, especially in children (Alfrey, 1986; Committee on Nutrition, 1986; Hewitt et al., 1990; Jeffery et al., 1996; ATSDR, 1999). Neither of these practices is important in the treatment of uremic livestock, and references to the studies in humans will not be included here.

Animal models (e.g., rats, dogs, mice) for renal osteodystrophy were generally created by injecting aluminum into animals. In those cases, aluminum was deposited at osteoid bone interfaces (Quarles et al., 1985) and inhibited osteoblast and osteoclast activities (Jeffery et al., 1996). Feeding aluminum (37.6 μmol/g diet of aluminum) to normal rats for 6 or 9 months did not affect urinary excretion of hydroxyproline (a marker of bone resorption), but feeding aluminum to rats that had one kidney removed elevated hydroxyproline excretion (Ecelbarger et al., 1994a,b). This suggests that reductions in kidney function (as occurs with age) may cause animals to be more sensitive to aluminum exposure in terms of bone metabolism.

Phosphorus Metabolism

Many of the symptoms associated with aluminum toxicity in bone and the occasional effects of aluminum on growth reflect the effects of oral aluminum exposure on phosphorus utilization. A series of case studies demonstrated that the chronic ingestion of aluminum in antacids (as aluminum hydroxide gels or magnesium aluminum hydroxide) caused phosphorus depletion (with hypophosphatemia, hypophosphaturia, hypercalciuria) and osteomalacia (bone loss of calcium and phosphorus, fractures, bone pain, elevated alkaline phosphatase levels) in non-uremic human patients (Bloom and Flinchum, 1960; Lotz et al., 1968; Insogna et al., 1980). The symptoms appeared in only a small number of subjects ingesting pharmaceutical levels of antacids (0.5 to 9.6 g/day of aluminum) chronically (more than 6 months). Moreover, healthy adult males fed 125 mg/day of aluminum as aluminum lactate experienced only a transient (first 12 days) decrease in phosphorus absorption with no other changes in phosphorus or calcium utilization (Greger and Baier, 1983a).

Aluminum (>200 mg/kg of aluminum diet as aluminum sulfate or hydroxide salts) has been shown to depress phosphorus utilization by rats (Ondreička et al., 1966, 1971; Greger et al., 1985). Ingestion of 2,000 mg/kg of aluminum diet as aluminum chloride depressed growth, feed intake, plasma phosphorus levels, and phosphorus absorption in lambs, especially those fed low levels of phosphorus (Valdivia et al., 1982). Chicks fed 0.15 or 0.3 percent aluminum had depressed growth, reduced feed efficiency, and decreased bone ash. Only the higher level of aluminum reduced plasma phosphorus levels and reduced egg production (Wisser et al., 1990).

Another source of exposure to aluminum is when alum is applied to poultry litter or dairy slurry. Moore et al. (1999, 2000) reported that broilers grown on alum-treated poultry litter were significantly heavier than control (1.73 versus 1.66 kg) and had less mortality than controls (3.9 versus 4.2 percent). Not only did the application of the alum not adversely affect the livestock, but also it had minimal negative effects on crops grown on the land. Tall fescue yields on fertilized fields were improved due to increased nitrogen availability presumably because of less ammonia volatilization (Moore et al., 1999). Soluble reactive phosphorus runoff from treated pastures were greatly reduced (Codling et al., 2002; Moore et al., 2000) without severely impacting soil fertility, although phosphorus concentrations in plants were progressively reduced during the three-year study (Codling et al., 2002).

Fluoride Utilization

The oral administration of high (1.8 g/day of aluminum as antacids) and moderate (125 mg/day of aluminum as aluminum lactate) amounts of aluminum has been found to depress serum and urinary fluoride levels in healthy adult humans (Spencer et al., 1981; Greger and Baier, 1983a). Similarly rabbits given aluminum in their drinking water (100 and 500 mg/g water of aluminum as aluminum chloride) accumulated less fluoride in their tissues (Ahn et al., 1995). However, rabbits accumulated more aluminum in bone when the fluoride content of the drinking water was increased.

Animal scientists have used knowledge of the interaction between aluminum and fluoride to reduce fluoride toxicity. Aluminum has been intentionally added to the diets of sheep (3.3 g aluminum chloride/day) to reduce the symptoms of fluorosis (mottling of teeth) in animals provided with 30 mg/kg water of fluoride (Said et al., 1977). Although the ingestion of aluminum increased fecal fluoride and reduced urinary and tissue fluoride levels, the incidence and severity of mottling of teeth was not reduced. Similarly, aluminum (295 or 550 mg/kg diet of aluminum) was added to the diets of turkeys fed fluoride-contaminated phosphate fertilizers as a phosphorus source (Cakir et al., 1977). The aluminum partially prevented the toxic effects of the fluoride (i.e., reduced bone fluoride concentrations and increased weight gain). The addition of aluminum (1,040 mg/kg diet of aluminum as aluminum sulfate) to diets reduced the toxic effects (i.e.,

reduced feed intake, reduced egg production, and increased tissue fluoride levels) of dietary fluoride (1,300 mg/kg of fluoride) in laying hens (Hahn and Guenter, 1986).

Calcium and Other Minerals

The effect of dietary aluminum on the utilization of other minerals is less consistent in livestock. Lambs fed aluminum (>1,450 mg/kg diet aluminum as aluminum chloride) had reduced calcium absorption, but no changes in tissue calcium concentrations; lower concentrations of magnesium in some tissues; and minor changes in tissue iron and copper concentrations in two studies (Valdivia et al., 1982; Rosa et al., 1982). Although cows with clinical grass tetany had elevated concentrations of aluminum in their ruminal dry matter than did control cows or asymptomatic, hypomagnesemic cows, the administration of aluminum orally to lactating cows did not reduce plasma magnesium concentrations (Kappel et al., 1983). Similarly, dietary aluminum had small inconsistent effects on tissue concentrations of calcium, magnesium, and trace elements in rats fed generally balanced diets (Greger et al., 1985; Ecelbarger and Greger, 1991; Ecelbarger et al., 1994b; Belles et al., 2001; Yasui et al., 1990).

Neurological Symptoms

Aluminum is known to be part of the etiology of dialysis dementia and hypothesized to be part of the etiology of a progressive dementing encephalopathy reported in occasional industrial workers and in patients with amyotrophic lateral sclerosis with Parkinson's dementia in Guam. Brain accumulation of aluminum has also been associated with the development of Alzheimer's disease in some patients (King et al., 1981; Committee on Nutrition, 1986; Ganrot, 1986: Strong et al., 1996; Savory et al., 1996). Injection of aluminum, especially intracisternally, will induce neurological changes in animal models (Wisniewski et al., 1980; ATSDR, 1999). The effects of orally administered aluminum on the brain are more debatable. Only those studies in which aluminum was provided orally are cited here.

Administration of aluminum (0.3 percent Al as aluminum sulfate) in drinking water for 30 days resulted in impairment of both consolidation and extinction of passive avoidance tasks in rats with a slight increase in hippocampal muscarinic receptor numbers (Connor et al., 1988). Rats orally administered aluminum (100 mg/ kg BW/day of aluminum) for 90 days accumulated more aluminum in their brains, were slower to learn a labyrinth, had increased brain acetylcholinesterase activity, and had decreased brain choline-acetyltransferase activity (Bilkei-Gorzó, 1993).

Mice fed high aluminum levels (1,000 mg/kg diet of aluminum as aluminum lactate) were less active than controls after 6 weeks (Golub et al., 1989) and were less active, had decreased grip strength, and increased startle responses after 90 days (Golub et al., 1992). When mice were fed aluminum (500 or 1,000 mg/kg diet of aluminum as aluminum lactate to dam and then offspring) from conception to weaning or to adulthood, mice attained training faster but had reduced grip strength (Golub et al., 1995). The effects were not greater in mice fed aluminum to adulthood than just to weaning.

Other research groups fed animals diets that maximized the bioavailability of aluminum to potentially increase the absorption and accordingly the neurological toxicity of dietary aluminum. For example, Oteiza et al. (1993) observed that mice fed diets containing 1,000 mg/kg diet of aluminum as aluminum chloride with sodium citrate accumulated more aluminum in the brain nuclear fraction and spinal cord, had lower grip strength, and greater startle responsiveness after 5 and 7 weeks. Old (18 months of age) rats exposed to aluminum (100 mg/kg BW/day of aluminum) in drinking water with citrate (356 mg/kg BW/day of citrate) had decreased numbers of synapses and a greater percentage of perforated synapses than controls, but no changes in behavior (Colomina et al., 2002).

Several groups fed low calcium levels to increase animals' sensitivity to aluminum. Garruto et al. (1989) observed that cynomolgus monkeys fed a low calcium (3,200 mg/kg diet) diet with aluminum (125 mg/day of aluminum) and manganese (50 mg/day of manganese) for 41 to 46 months had more degenerative changes (that were consistent with early Alzheimer's disease or Parkinson's dementia in Guam) in the central nervous system than control monkeys. Golub and Germann (2001) observed growth depression and poorer performance on standardized motor tests in mice as adults when dams were exposed to aluminum (1,000 mg/kg diet of aluminum as aluminum lactate) with marginal levels of calcium and magnesium during pregnancy and lactation. Kihira et al. (2002) observed that mice fed a low rather than recommended levels of calcium (2,500 versus 5,000 mg/kg diet of calcium) with aluminum (15,600 mg/kg diet of aluminum hydroxide) for 11 to 25 months accumulated more hyperphosphorylated tau protein in the cortical neurons and had more atrophic neurons in the central nervous system.

Pratico et al. (2002) reported that transgenic mice that over-expressed human amyloid precursor protein (but not wild-type littermates) had increased brain isoprostane levels and more amyloid b peptide formation and deposition when aluminum was added to their diets. The effects of aluminum were reversed by additional dietary vitamin E.

In general, experimental animals did not exhibit large behavioral changes because of oral aluminum exposure even when fed diets that augmented the bioavailability of aluminum. It is unlikely that aluminum is part of any neurological syndromes in livestock.

Fish and Mollusks

The effects of aluminum on fish and mollusks appear to be unique and worthy of a separate discussion. Acidification of the aquatic environment often causes aluminum to be

mobilized into the aqueous environment. Atlantic salmon (*Salmo salar*) exposed to acidified water (pH 5.2) with aluminum (50 µg/L of aluminum as aluminum chloride) for 36 days reduced their feed consumption at first but lost weight and increased their swimming activity throughout the test period (Brodeur et al., 2001). Rainbow trout fry avoided aluminum at concentrations as low as 1 µmole/L of aluminum at pH 5 but were insensitive to aluminum at pH 6 (Exley, 2000).

Temperature may also affect the sensitivity of fish to aluminum. Graylings (*Thymallus thymallus*) were exposed to water containing 1 mg/L of iron and 100 µg/L of aluminum as aluminum sulfate at pH 5.5 and at pH 6.9 (control). Fifty percent of the fish exposed to the aluminum at pH 5.5 died after 2 weeks when the temperature was 13°C but none died at 3°C. Surviving fish at both test temperatures suffered gill damage (adherence of lamellae), increased hematocrits, reduced plasma chloride levels, and reduced oxygen consumption. The fish exposed to aluminum at the higher temperature recovered more completely than those exposed at the lower temperature (Peuranen et al., 2003).

Acclimation to aluminum may also affect the severity of symptoms when fish are exposed to toxic levels of aluminum. Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to a pulse of aluminum (36 µg/L of aluminum at pH 5.2) for 4 days. One group was exposed to a lower level of aluminum (24 µg/L of aluminum) for 16 days before and 10 days after the pulse; another was acclimated only to the pH. Fish became hypoactive upon exposure to the lower level of aluminum but recovered after 4 days. Exposure to the higher level of aluminum by the acclimated fish resulted in hypoactivity, but they quickly recovered normal swimming behavior during the 4-day pulse; mortality was 4 percent. In contrast, the fish not acclimated to the lower level of aluminum became hypoactive when exposed to 36 µg/L of aluminum and did not recover in the 6 days after the pulse; mortality was 26 percent (Allin and Wilson, 2000).

Mollusks appear to have several ways to avoid exposure to aluminum. Freshwater mussels (*Anodonta cygnea L*) reduced their filtering activity by reducing shell opening by 50 percent when exposed to 500 µg/L of aluminum as aluminum nitrate at neutral pH for 15 days (Kadar et al., 2001). Freshwater snails (*Lymnaea stagnalis*) accumulated aluminum, silicon, phosphorus, and sulfur in non-absorbable granules in their digestive cells when exposed to aluminum (Elangovan et al., 2000).

TISSUE LEVELS

Humans do not accumulate large body burdens of aluminum because of oral exposure to dietary aluminum from a variety of undefined sources. Total body aluminum loads for healthy adults have been estimated to range from 30 to 300 mg aluminum (Skalsky and Carchman, 1983; Ganrot, 1986). Alfrey (1986) estimated that normal tissue (bone and soft tissue, except lungs) levels are 1 to 4 mg/kg dry weight of aluminum. Concentrations of aluminum in serum are lower than in other tissues; 1 to 5 µg/L of aluminum are typical for serum samples drawn from fasted, normal human subjects (Versieck and Cornelis, 1980; Greger and Baier, 1983b; Savory and Wills, 1988).

Rodents exposed to aluminum (as aluminum sulfate, chloride, hydroxide, palmitate, lactate, phosphate, or citrate) orally accumulated more aluminum in bone than soft tissues (Ondreička et al., 1966, 1971; Greger et al., 1985, 1986; Slanina et al., 1984, 1985). Rats fed purified control diets (that contained less than 15 mg/kg diet of aluminum) retained less than 0.3 µg/g wet tissue of aluminum in their soft tissues (kidneys, livers, brains, and muscles) and less than 0.9 µg/g wet tissue of aluminum in bone (Greger and Powers, 1992; Ecelbarger et al., 1994a,b; Sutherland and Greger, 1998). When rats were fed 1,000 mg/kg diet of aluminum in these studies, the concentrations of aluminum in soft tissues sometimes increased significantly but were generally less than 0.7 µg/g wet tissue of aluminum and in bone were less than 2.0 µg/g wet tissue of aluminum.

Similarly steers (Valdivia et al., 1978), sheep (Valdivia et al., 1982), and chickens (Wisser et al., 1990) fed 800–3,000 mg/kg diet of aluminum accumulated aluminum in their tissues, but the concentrations of aluminum in their tissues were still <11 µg/g dry tissue of aluminum.

The amounts of aluminum in tissues were much greater if kidney function of the rats had been reduced or aluminum had been given orally by gavage to fasted animals. In those cases, soft tissues and bone concentrations of aluminum were as great as 6 and 27 µg/g wet tissue of aluminum, respectively (Greger and Powers, 1992; Domingo et al., 1994; Greger et al., 1994).

Table 3-2 summarizes the effect of aluminum on selected tissues.

MAXIMUM TOLERABLE LEVELS

The Agency for Toxic Substances and Disease Registry (ATSDR, 1999) noted that toxicity to aluminum had been reported many times in patients with reduced kidney function, but limited data were available about toxic responses to aluminum in normal humans. They suggested a LOAEL (lowest-observed-adverse-effect level) of 130 mg/kg/day of aluminum on the basis of the observation that adult mice exposed to dietary aluminum lactate for 6 weeks decreased their spontaneous motor activity (Golub et al., 1989). This would mean a 70-kg human should be unaffected by ingestion of 9.1 g of aluminum daily (18,000 mg/kg dry diet of aluminum if 0.5 kg dry diet is consumed daily). The occasional case histories of adverse responses to aluminum-containing antacids among adults with normal kidney function suggest that this LOAEL is too high.

Ingestion of 2,000 and 1,450 mg/kg diet of aluminum depressed growth, feed intake, plasma phosphorus levels,

and/or phosphorus absorption in lambs (Valdivia et al., 1982; Rosa et al., 1982, respectively). Laying hens (White Leghorn) fed 1,500 mg/kg diet of aluminum had depressed growth production (Wisser et al., 1990). Thus, a cautious maximum tolerable level of aluminum in the diets of livestock would be 1,000 mg/kg diet of aluminum.

The NRC (1982) considered the minimum toxic effect dose of aluminum in water for rats to be 1,000 mg/kg/day of aluminum. If 200-g rats consumed 20 ml water daily, the maximum toxic level would be 10,000 mg/L water of aluminum (which exceeds the solubility of aluminum in non-acidic solutions). Brodeur et al. (2001) observed toxic effects in fish exposed to 36 µg/L water of aluminum at pH 5.2. These data suggest that fish are more sensitive to aluminum exposure than are mammals.

FUTURE RESEARCH NEEDS

Research on the role of aluminum in the etiology of disease syndromes in humans has little significance to livestock production industry but could be of interest to veterinarians treating old and/or diseased pets. The relationship of tissue aluminum concentration and neurological problems in pets, especially those with reduced renal function, deserves consideration.

The effects of acid rain and the resulting aluminum contamination of aquatic environments will continue to deserve further study. In both cases, the mediation of the effects of aluminum exposure by other dietary elements is likely to be important and worthy of further study.

SUMMARY

Aluminum is a ubiquitous element that is not essential to mammals. Livestock are occasionally exposed to high levels of aluminum, but toxicity to orally administered aluminum is not a problem as long as gut and kidney functions are normal. The first signs of aluminum toxicity in normal animals reflect the adverse effect of dietary aluminum on phosphorus utilization. On the other hand, fish are very sensitive to aluminum in acidic water.

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TABLE 3-1 Effects of Aluminum Exposure in Animals

Animals	N[a]	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Human (case studies)	6	0.5–9.6 g Al/d	Aluminum hydroxide	6 mo–12 yrs	Oral antacid	Osteomalacia, bone pain, bone loss, hypophosphatenice, hypercalciuria, elevated alkaline phosphatase activity	Insogna et al., 1980
Human	8	125 mg Al/d	Aluminum lactate	20 d	Diet	Transient decrease of phosphorus absorption; decreased urine fluoride; increased serum aluminum	Greger and Baier, 1983a,b
Monkeys	3	150 mg Al/d and 50 mg Mn/d	Aluminum salt not defined	41–46 mo	Diet with low Ca (0.32%)	Increased histological lesions in CNS; increased phosphorylated neurofilaments	Garruto et al., 1989
Dogs	6	3,000–30,000 mg/kg diet	Sodium aluminum phosphate	6 mo	Diet	No effect	Katz et al., 1984
Mice	10	19.3 mg Al/kg/d	Aluminum chloride	3 generations	Water	Doubling Al intake reduced growth in second and third generations	Ondreička et al., 1966
Mice	12	1,000 mg Al/kg diet	Aluminum lactate	90 d	Diet	Decreased motor activity, grip strength, and startle response	Golub et al., 1992
Mice	10	1,000 mg Al/kg diet	Aluminum chloride	5–7 wks	Diet	Increased Al levels in liver, spinal cord, and brain nuclear fraction. Decreased lower grip strength and greater startle response	Oteiza et al., 1993
Mice	8–20	500 and 1,000 mg Al/kg diet	Aluminum lactate	Conception to weaning or adulthood	Diet	Both levels reduced grip, strength and faster training. No greater response when mice treated until adulthood	Golub et al., 1995
Mice	16–20	100, 500, 1,000 mg Al/kg diet	Aluminum lactate	Conception to 35 d	Diet of dam	Growth retardation and poorer performance on standardized motor tests as adults by progency of mice exposed to 1,000 ug Al/kg diet with marginal calcium and magnesium intakes	Golub and Germann, 2001
Rabbits	12	5 mg Al/L	Aluminium citrate	12 mo	Water	Depress growth, accumulate Al in tissues; no histological changes	Wills et al., 1993
Rabbits	3	100 and 500 mg/L Al in water	Aluminum chloride	10 wks	Water	Al reduced tissue F retention; F in water increased Al retention	Ahn et al., 1995
Rats	8	2,665 mg Al/kg diet	Aluminum sulfate	8 d	Diet	Increased Al retention in tissues, increased fecal P; decreased P retention	Ondreička et al., 1966

continued

TABLE 3-1 Continued

Animals	N[a]	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Rats	5	10,000 and 20,000 mg Al/kg diet	Aluminum sulfate and chloride	8 d	Drinking water	No effect in normal rats except increased aluminum in tissues; increased mortality in rats with 5/6 nephrectomy	Berlyne et al., 1972
Rats	52	5 mg Al/L	Aluminum sulfate	Natural lifespan up to 1,464 days	Drinking water	No effect on longevity; slight increase in weight gain and tumor incidence in males	Schroeder and Mitchener, 1975
Rats	6	205–272 mg Al/day	Aluminum as hydroxide (reagent and dried gel), palmitate, lactate, and phosphate	18 d	Diet	Slight Al accumulation in tissues; Al hydroxide slightly depressed bone phosphorous	Greger et al., 1985
Rats	7	39 mmole Al/kg diet	Aluminum hydroxide	28 d	Diet	Al accumulated in tissues; rats with 1 kidney and fed Al had elevated blood urea nitrogen	Ecelbarger and Greger, 1991
Rats	24-Nov	37.6 mmole Al/kg	Aluminum hydroxide	6 and 9 mo	Diet	Al accumulation in tissue, greater in rats with 1 kidney removed; increased urinary hydroxyproline excretion in urine and increased blood urea nitrogen in rats fed Al with 1 kidney	Ecelbarger et al., 1994a
Rats	10	100 and 200 mg Al/kg BW/d	Aluminum chloride and lactate	9 d (5–14 d postnatal)	Oral	Reduced body weight, food intake, decreased plasma protein and albumin but increased plasma α-1-globulin	Cherroret et al., 1995
Chickens	10	0.5% of diet	Aluminum as acetate, chloride, nitrate, sulfate and phosphate	3 weeks	Diet	High mortality (>80%) in chicks fed aluminum salts (except aluminum phosphate); decreased weight gain and bone ash for all aluminum salts but aluminum phosphate	Storer and Nelson, 1968
Chickens	30	40, 80, 520, 1,040 mg Al/kg diet	Aluminum sulfate	112 d	Diet	Al reduced toxic effect of F on feed intake, egg production, and tissue F retention	Hahn and Guenter, 1986
Chickens	20	1,500 and 3,000 mg Al/kg diet	Aluminum sulfate	22 wk	Diet	Both levels depressed growth, feed efficiency, and bone ash; higher level depressed egg production and plasma P	Wisser et al., 1990
Chickens	3 houses	0.091 kg alum/bird or 1,816 kg alum/house	Alum	3 yr	Alum-treated poultry litter	Improved weight gain and decreased mortality among broilers when litter treated with alum	Moore et al., 1999, 2000

Turkeys	6 and 25	295 and 590 mg Al/kg diet	Aluminum sulfate	4 and 20 wk	Diet	Aluminum reduced effect of F-contaminated phosphate fertilizer on weight gain and bone F levels	Cakir et al., 1977
Sheep	4	3.3 g $AlCl_3$/d	Aluminum chloride	25 mo	Diet	Aluminum increased fecal F; decreased urinary loss and tissue retention of F; decreased mottling of teeth of animals given 30 mg F in water	Said et al., 1977
Sheep	5	2,000 mg Al/kg diet	Aluminum chloride	56 d	Diet	Al reduced weight gain, feed intake, P and Ca absorption, plasma P and Mg	Valdivia et al., 1982
Sheep	3	1,450 mg Al/kg diet	Aluminum chloride	76 d	Diet	Al reduced feed intake, weight gain, serum P	Rosa et al., 1982
Freshwater bivalve	24	250 and 500 µg Al/L	Aluminum nitrate	15 d	Water	At higher Al level, bivalve reduced shell opening by 50%; lysosomes increased in all organs at both Al levels	Kadar et al., 2001
Fish, graylings	24	100 µg Al/L and 1 mg Fe/L	Aluminum sulfate at pH 5.5 or 6.9 (control)	1 wk exposure	Water	50% of fish diet after exposure at 13°, none at 3°C; surviving fish had increased hematocrit, gill damage	Peuranen et al., 2003
Fish, salmon	115 for activity; 10 or 15 (analyses)	50 µg Al/L at pH5.2	Aluminum chloride	36 d	Water	Weight loss and elevated swimming activity in fish exposed to Al; elevated plasma glucose and K; elevated hematocrits; depressed Na and Cl	Brodeur et al., 2001
Fish, trout	Triplicate 24	Pulse: 36 µg Al/L acclimation: 24 µg Al/L	Aluminum at pH 5.2; salt added not defined	Pulse: 4 d acclimation: 16 d before, 10 d after	Water	Hypoactive in 36 µg Al/L; 26% mortality in non-acclimated fish; 4% mortality in acclimated fish	Allin and Wilson, 2000

[a]Number of animals per treatment group.
[b]Quantity of aluminum dosed. SI conversion: 1 mg aluminum equals 37.1 µmoles aluminum.

TABLE 3-2 Aluminum Concentrations in Fluids and Tissues of Animals (mg/kg)

Species	Quantity[a]	Source	Duration	Route	Tissue Concentrations (mg/kg)[b]	Reference
Humans	Unknown	Unknown	Lifetime	Diet	All tissues: 1–4 (dry weight)	Alfrey, 1986
Humans	5 mg/d	Unknown	20 d	Diet	Serum: 4 µg/L	Greger and Baier, 1983b
	125 mg/d	Al lactate	20 d	Diet	Serum: 7 µg/L	
Mice	25	Unknown	≈50 d	Diet	Liver: 0.01; bone: 3	Donald et al., 1989
	500	Al lactate	≈50 d	Diet	Liver: 0.03; bone: 5	
	1,000	Al lactate	≈50 d	Diet	Liver: 0.05; bone: 6	
Rats	11	Unknown	6–7 mo	Diet	Liver: <0.3; muscle: <0.4; bone: <0.8	Ecelbarger and Greger, 1994b
	1,005	Al hydroxide with and without citrate	6–7 mo	Diet	Liver: <0.3; muscle: <0.7; bone: 1–1.4	
Chickens	160	Unknown	17 wk	Diet	Bone: <2 (dry weight)	Wisser et al., 1990
	1,500	Al sulfate	17 wk	Diet	Bone: 6 (dry weight)	
	3,000	Al sulfate	17 wk	Diet	Bone: 10 (dry weight)	
Steers	210	Unknown	84 d	Diet	Soft tissues: 4–8 (dry weight)	Valdivia et al., 1978
	510	Al chloride	84 d	Diet	Soft tissues: 4–8 (dry weight)	
	810	Al chloride	84 d	Diet	Soft tissues: 5–10 (dry weight)	
	1,410	Al chloride	84 d	Diet	Soft tissues: 5–11(dry weight)	
Sheep	168	Unknown	56 d	Diet	Soft tissues: 2–5 (dry weight)	Valdivia et al, 1982
	2,168	Al chloride	56 d	Diet	Soft tissues: 4–6 (dry weight)	

[a]Quantity of exposure reported as mg/kg diet of aluminum unless noted otherwise.
[b]Concentrations reported as mg/kg wet tissue unless noted otherwise.

4

Arsenic

INTRODUCTION

Arsenic (As) is a solid, brittle metalloid and thus has both metallic and nonmetallic properties. The most common stable form of arsenic at room temperature is metallic or gray arsenic. Another form of elemental arsenic, yellow arsenic, occurs when arsenic vapors are cooled suddenly to below 0°C (Stoeppler, 2004). It is unstable and more volatile than gray arsenic. Arsenic is the 20th most common element in the Earth's crust with an average natural abundance of about 1.5–3 mg/kg (Mandal and Suzuki, 2002). Arsenic naturally occurs in over 200 different forms with approximately 60 percent as arsenates, 20 percent as sulfides and sulfursalts, and the remainder as arsenides, arsenites, oxides, silicates, and the elemental form (Mandal and Suzuki, 2002). The most common arsenic mineral is arsenopyrite. Other common minerals of arsenic are orpiment and realgar (natural sulfides) and arsenolite. However, arsenic in its most recoverable form is found in various types of metalliferous minerals (Mandal and Suzuki, 2002) such as iron pyrite, galena, chalcopyrite, and sphalerite. Arsenic trioxide (As_2O_3), the common commercial form of arsenic, is produced as a by-product of roasting various ores.

For more than 50 years, chromated copper arsenate (CCA) has been the main preservative for wood products used outdoors. In response to consumer concerns about arsenic toxicity, a voluntary phase-out of CCA-treated wood for certain residential uses, such as play structures, picnic tables, decks, and fencing, was to be completed before 2004. Industrial-use wood products, such as marine pilings, utility poles, roofing shakes, and shingles, still can be treated with CCA. Because of reduced use of CCA, the demand for arsenic, as As_2O_3, has markedly dropped. Still, about 90 percent of arsenic trioxide produced is used to make CCA. The balance of arsenic trioxide is mainly used in agricultural chemicals such as insecticides, herbicides, and coccidiostats. Uses of other arsenic compounds and metallic arsenic include electronics, pigments, metal alloys, and as a bubble dispersant or decoloring agent in glassmaking (Brooks, 2002).

Arsenic exists predominantly in nature as an oxyanion with an oxidation state of either 3^+ or 5^+, but arsenic also forms compounds where it has an oxidation state of 3^- (Hindmarsh et al., 2002). Arsenic binds covalently with most metals and nonmetals, and forms stable organic compounds. In animals, arsenic occurs mainly as inorganic arsenate [$O{=}As(OH)_3$; $H_2AsO_4^{1-}$; $HAsO_4^{2-}$; AsO_4^{3-}] and arsenite [$OH\text{-}As(OH)_2$; $H_2AsO_3^{1-}$; $HAsO_3^{2-}$; AsO_3^{3-}], and in the methylated form, mainly dimethylarsinic acid [$(CH_3)_2AsO(OH)$] and monomethylarsonic acid [$CH_3AsO(OH_2)$]. The major arsenic species in fish, crustaceans, and mollusks apparently have the tetraalkylarsonium structure (R_4As^+), and in marine algae and bivalves the major arsenic species have the trialkylarsine oxide structure (R_3AsO) (McSheehy et al., 2003). Thus, arsenobetaine [$(CH_3)_3As^+CH_2\text{-}COO^-$] and arsenocholine [$(CH_3)_3As^+CH_2\text{-}CH_2\text{-}OH$] are found in sea animals and arsenosugars are found in marine algae and seaweeds.

ESSENTIALITY

Arsenic is generally not accepted as an essential nutrient for higher animals. However, the large number of responses to apparent arsenic deprivation (e.g., <12 µg/kg diet for rats and chicks; <35 µg/kg diet for goats) reported for a variety of animal species by more than one research group suggests that it may have an essential or beneficial function in ultra trace amounts (Anke, 1986; Uthus, 1994; Nielsen, 1998). In the goat, pig and rat, the most consistent signs of apparent arsenic deprivation have been depressed growth and abnormal reproduction characterized by impaired fertility and increased perinatal mortality. Other notable signs include depressed serum triglyceride concentrations and death with myocardial damage during lactation in goats (Anke, 1986), and depressed hepatic S-adenosylmethionine and elevated S-adenosylhomocysteine in rats and hamsters (Uthus, 1994).

Many of the responses of experimental animals to arsenic deprivation and other in vitro or cell culture findings suggest that arsenic affects the utilization of labile methyl groups arising from methionine in higher animals. Thus, arsenic may affect the methylation of metabolically or genetically important molecules, whose functions are dependent on or influenced by methyl incorporation. It has been suggested that rats and chicks have an arsenic requirement that is greater than 12 but less than 50 µg/kg diet (Nielsen, 1998).

In vitro findings suggest that arsenic also has an essential or beneficial action in low amounts. For example, Snow et al. (2001) found that sub-toxic arsenite induces a multi-component protective response against oxidative stress in cultured human keratinocytes and fibroblasts.

Some forms of arsenic have beneficial effects in supranutritional amounts. Some organic arsenicals, because of their antibiotic and anticoccidial properties, were used extensively in the past as growth promoters for swine and poultry (Anderson, 1983); this use has been largely supplanted by newer antibiotics. However, roxarsone (3-nitro-4-hydroxyphenylarsonic acid; $C_6H_6AsNO_6$) is still used extensively in the feed of broiler poultry to control coccidial intestinal parasites, improve feed efficiency, and promote rapid growth. Also, melarsoprol {2-[4-[(4,6-diamino-1,3,5-triazin-2-yl)amino]phenyl]-1,3,2dithiarsolane-4-methanol; $C_{12}H_{15}AsN_6OS_2$} is still used to treat trypanosomiasis in humans. High doses of arsenic trioxide recently have been found to be an effective treatment for acute promyelocytic leukemia through apototic, not necrotic, mechanisms (Chen et al., 2001). Arsenic trioxide also apparently is an effective treatment for other malignancies including megakaryocytic leukemia (Tallman, 2001) and lymphoma (Dai et al., 1999). Organic arsenicals such as melarsomine dihydrochloride given by intramuscular or intravenous injection are used to treat heartworm infection in dogs.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

The most common methods for determining total arsenic in biological samples after decomposition to mineral ash in solution are hydride atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry, and graphite furnace AAS (Stoeppler, 2004). However, the silver diethyldithiocarbamate colorimetric method, which results in a very stable red color, is still in use to determine total arsenic, after conversion into arsine, in polluted ground water (Tareq et al., 2003). Converting biological samples to ash that can be solubilized has been accomplished by a number of different methods including dry ashing, wet or microwave-assisted ashing with acid mixtures, or ashing with nitric acid alone in open and closed (often pressurized) systems (Stoeppler, 2004). An optimal sample decomposition method that avoids loss of analyte is high-pressure ashing. This method uses concentrated nitric acid in closed quartz vessels at temperatures up to 320° C (Würfels, 1989; Knapp, 1990). A microwave-assisted procedure attaining >300° C also has been found to result in complete sample decomposition (Goessler and Pavkov, 2003). Complete decomposition is of utmost importance when the hydride AAS method of analysis is used because traces of organic compounds interfere with the analysis.

Because of the widely varying toxicities of different arsenic species, the development of analytical methods to determine the chemical forms of arsenic and their concentrations in biological and environmental samples have become of interest. Collection of samples for arsenic speciation must be done with care and kept at a low temperature to prevent the modification of arsenic species by contaminating bacteria or inherent biological activity of the sample. The methods used to determine arsenic species require an extraction step. Tissues with high fat content may need to be defatted with a solvent, such as ether or acetone, before the extraction of arsenic. Most arsenic species in biological tissues are water soluble and thus can be extracted with water alone or with a mixture of water and methanol (McSheehy et al., 2003). After extraction, arsenic species are determined by a combination of separation techniques such as high-performance liquid chromatography and capillary zone electrophoresis, and detection techniques that are specific and highly sensitive for arsenic such as inductively-coupled plasma mass spectrometry and electrospray mass spectrometry with tandem mass spectrometry (McSheehy et al., 2003). The difficulties encountered in the determination of arsenic species in biological samples have been reviewed (McSheehy et al., 2003) and include incomplete extraction, retention time irreproducibility, co-elution of species, presence of unidentified species, the lack of standards, and detection interference. An example of arsenic species determination in the domestic animal situation is that of Pavkov and Goessler (2001), who identified and quantified organoarsenic compounds in finishing chicken feed and chicken litter.

REGULATION AND METABOLISM

Absorption and Metabolism

There apparently are two components to the absorption of arsenic (Fullmer and Wasserman, 1985). Initially, arsenate becomes sequestered primarily in or on the mucosal tissue. Eventually the sites of sequestration become filled, with concomitant movement down a concentration gradient into the body. In rats, some forms of organic arsenic are absorbed at rates directly proportional to their intestinal concentration over a 100-fold range (Hwang and Schanker, 1973). This finding suggests that organic arsenicals are absorbed mainly by simple diffusion. The absorption and metabolism of arsenic may be influenced by intestinal bacteria that can methylate arsenic or metabolize methylated arsenic (Hall et al., 1997; Kuroda et al., 2001).

Once absorbed, inorganic arsenic is transferred to various tissues including the liver and testis where arsenic is methylated with S-adenosylmethionine as the methyl donor (Healy et al., 1999). Before arsenate is methylated, it is reduced to arsenite; this reduction is facilitated by glutathione (Vahter, 1994). The conversion of arsenate into arsenite may also be accomplished in the animal gastrointestinal tract by bacteria with arsenate reductase activity (Herbel et al., 2002). Arsenite methyltransferase methylates arsenite to form monomethylarsonic acid, which is then reduced to monomethylarsonous acid [$CH_3As(OH)_2$]. Monomethylarsonous acid, a relatively toxic form of arsenic, is rapidly methylated by a methyltransferase to form dimethylarsinic acid; in humans, monomethylarsonous acid is found in the urine only when excessive inorganic arsenic is consumed (Aposhian et al., 2001). Dimethylarsinic acid can be reduced to dimethylarsinous acid [$(CH_3)_2AsOH$], which is a relatively toxic form of arsenic. However, the formation of dimethylarsinic acid usually is the final step in the metabolism of arsenic in humans and most animals, and thus is the major form of arsenic in urine. Inorganic and methyl arsenic compounds apparently are protein-bound when they are substrates for enzymatic biomethylation (Styblo and Thomas, 1997). Pregnancy increases arsenic methylation, especially late in gestation; dimethylarsinic acid is the main form of arsenic transferred to the fetus (Vahter et al., 2000). In addition to the methylated forms, inorganic arsenic bound to transferrin is found in plasma (Zhang et al., 1998). Trimethylated organic forms of arsenic stay trimethylated after absorption. Arsenobetaine passes through the body into urine without biotransformation. Some orally ingested arsenocholine appears in the urine, and some can be incorporated into body phospholipids in a manner similar to choline; however, most is transformed into arsenobetaine before being excreted in the urine. Absorbed tetramethylarsonium also is not biotransformed before excretion (Yoshida et al., 1998). Unlike arsenobetaine and tetramethylarsonium, arsenosugars are transformed to many different arsenic species after absorption. Francesconi et al. (2002) found at least 12 arsenic metabolites in the urine of humans after they ingested an oral dose of a synthetic arsenosugar. The metabolism of arsenic in some animal species is quite unusual. For example, unlike other mammals, rats concentrate arsenic in their erythrocytes. Chimpanzees, marmosets, squirrel monkeys, tamarins, and guinea pigs are unable to methylate arsenic. These species apparently have other mechanisms for facilitating arsenic excretion (Vahter, 1994; Healy et al., 1999; Vahter et al., 2000).

Excretion of ingested arsenic is rapid, principally in the urine. In some species, significant amounts of arsenic are excreted in the bile in association with glutathione (Vahter, 1994; Kala et al., 2000). The usual proportions of the forms of arsenic in human urine are about 20 percent inorganic arsenic, 15 percent monomethylarsonic acid, and 65 percent dimethylarsinic acid (Vahter et al., 2000). The proportions are quite different, however, with consumption of organic arsenic in forms found in seafood (mostly trimethylated arsenic, e.g., arsenobetaine). For example, the urine of Japanese students who consumed high amounts of organic arsenic in seafood contained 9.4 percent inorganic arsenic, 3.0 percent monomethylarsonic acid, 28.9 percent dimethylarsinic acid, and 58.2 percent trimethylated arsenic compounds (Yamato, 1988).

Metabolic Interactions

Both arsenic deprivation and toxicity are influenced or affected by factors that change labile methyl metabolism. Generally, factors that reduce the availability of labile methyl groups exacerbate arsenic deprivation and toxicity. For example, methionine or choline deficiency, excessive dietary arginine, and dietary guanidoacetic acid supplementation enhanced the response to arsenic deprivation in chicks and rats (Uthus, 2003). Treatment with periodate-oxidized adenosine, a methylation inhibitor, exacerbated the toxic effects of inorganic arsenic on development in mice (Lammon et al., 2003). Arsenic toxicity is also influenced by factors that affect reactive oxygen metabolism. For example, ascorbic acid and α-tocopherol alleviated arsenic toxicosis signs in rats of lipid peroxidation and enzyme inhibition in mitochondria of rats fed 100 mg As/L drinking water as sodium arsenite (Ramanathan et al., 2003). Other nutrient deficiencies that can affect arsenic toxicity or deficiency through affecting labile methyl or oxidative metabolism include cysteine (Siewicki and Leffel, 1980; Czarnecki et al., 1984), folic acid (McDorman et al., 2002), pyridoxine (Uthus and Poellot, 1991–1992), and zinc (Uthus, 1994).

A well-established metabolic interaction is the multifaceted and complex interaction between arsenic and selenium. Both methylation and oxidation could be involved in the interaction, possibly through competition for the methyl donor, S-adenosylmethionine, and competition for the antioxidant, glutathione (Kenyon et al., 2001). Interaction at the level of methylation is plausible because arsenite inhibits selenium methylation both in vivo and in vitro, and selenium is a potent inhibitor of arsenic methylation in vitro (Kenyon et al., 2001). However, most findings indicate that selenium deficiency (not excess) exacerbates arsenic toxicity and impairs arsenic metabolism. Also, supplementing with arsenic as arsenite or arsenate alleviates the toxicity of most forms of selenium (e.g., selenate, selenite, selenocysteine, selenomethionine) (Levander, 1977), and selenium supplementation as sodium selenate apparently can alleviate the toxicity of arsenic as arsenate (Biswas et al., 1999). These actions are opposite of those expected if there was a competition for factors involved in the metabolism and excretion of methylated arsenic and selenium. Possible mechanisms for the apparent dichotomy involve metabolism and distribution antagonism and effects on oxidative metabolism. Levander (1977) suggested that arsenic enhances the biliary excretion of selenium via the formation of a detoxification conjugate.

The identification of the seleno-bis(S-glutathionyl) arsinium ion in the bile of rabbits injected with selenite followed by arsenite (Gailer et al., 2000) supports this suggestion. Also, arsenic and selenium can combine directly to form As_2Se, which becomes concentrated and precipitated in renal lysosomes (Berry and Galle, 1994). The oxidative metabolism suggestion is based on selenium, in the form of selenocysteine, being a critical component of several enzymes that maintain intracellular redox balance, including thioredoxin reductase and glutathione peroxidase. Selenium deficiency would decrease the antioxidant capacity to antagonize the oxidative stress induced by excessive arsenic, and thus exacerbate arsenic toxicity (Kenyon et al., 2001).

Another established interaction is between arsenic and copper. Pharmacologic or toxicological amounts of arsenic 50 mg/kg diet as sodium arsenate and 100 mg/kg diet as sodium arsenite induce copper accumulation in the kidney of rats (Mahaffey et al., 1981; Uthus, 2001; Yu and Beynen, 2001), chicks (100 mg roxarsone/kg diet) (Czarnecki and Baker, 1985), and guinea pigs, but not in mice (100 mg/kg diet as sodium arsenate) (Hunder et al., 1999). High dietary arsenic does not increase liver copper, but may slightly reduce it (Yu and Beyen, 2001). High dietary arsenic decreases plasma copper concentrations (Yu and Beynen, 2001; Schmolke et al., 1992). High dietary arsenic also exacerbates copper deficiency in rats (Uthus, 2001), and high dietary roxarsone (3-nitro-4-hydroxyphenylarsonic acid) enhances copper toxicity in pigs (Edmonds and Baker, 1986). On the other hand, low-dose roxarsone administration apparently ameliorates copper toxicity (Edmonds and Baker, 1986). The mechanism through which arsenic affects copper distribution in the body has not been established. However, Yu and Beynen (2001) suggested that arsenic toxicosis decreases the excretion of copper through the predominant site of excretion, the bile, by reducing hepatic copper. Biliary copper excretion is determined by the copper concentration in liver. As a result of the change in biliary excretion, copper is directed to the renal cortex where it accumulates.

Mechanisms of Toxicity

As indicated above, three mechanisms of action through which arsenic may be toxic are oxidative stress, altered methylation, and altered metabolism of other essential minerals. Oxidative stress and altered methylation are the bases for arsenic being categorized as a human carcinogen (Kitchin, 2001; Thomas et al., 2001; Hughes, 2002). Other mechanisms of toxicity are more specific to pentavalent and trivalent forms of arsenic. Arsenate apparently can replace phosphate in some biochemical reactions because they have similar structure and properties (Hughes, 2002). This replacement uncouples oxidative phosphorylation and thus can result in the depletion of adenosine triphosphate (ATP). However, the toxicity of arsenate may be mainly the result of its conversion into arsenite. Trivalent arsenic (e.g., arsenite) readily binds with thiols and vicinal sulfhydryls that are specific functional groups within enzymes, receptors, and co-enzymes (Thomas et al., 2001; Hughes, 2002). This binding can result in the inhibition of critical biochemical functions or reactions. It should be noted, however, that the binding of arsenite at nonessential sites in proteins may be a detoxification mechanism (Aposhian, 1989).

SOURCES AND BIOAVAILABILITY

Sea plant and fish products and supplemental minerals supply most of the arsenic found in animal feeds. The concentration of arsenic has been found to range between 1 and 180 mg/kg dry weight for various marine macro algae, <2 and 170 mg/kg fresh weight in marine fish and bivalves, and <0.1 and 3 mg/kg fresh weight for freshwater fish (Stoeppler, 2004). The arsenic content of commercial fishmeals used for livestock was found to range between 2.9 and 9.1 mg/kg dry weight (Lunde, 1968). Most arsenic in fish and algae is in the relatively nontoxic organic form (e.g., arsenobetaine, arsenosugars). Grains have measurable amounts of arsenic; reported mean concentrations (in μg/kg fresh weight) include oats, 189; barley, 67; and wheat, 45 (Wiersma et al., 1986). Grass species contain about 100 μg/kg dry weight (Stoeppler, 2004). Grass from areas close to industrial sites, or grown on high-arsenic soils, can be markedly higher (up to 62 mg/kg dry weight near a lead smelter) (Woolson, 1983). Some of the arsenic in plants growing near mining and smelting operations comes from aerial deposition, but much comes from root uptake (Woolson, 1983). Straw from rice grown in a greenhouse in pots flooded with water containing 8 mg As/L accumulated about 100 mg As/kg, mostly in the inorganic form (Abedin et al., 2002). Interestingly, arsenic increased only slightly in the rice grain (from 0.15 mg/kg to 0.42 mg/kg) with the arsenic treatment, and thus did not exceed the food hygiene limit of 1 mg/kg. Sheep and cattle do not find arsenic distasteful and actually may develop a taste for it (Clarke and Clarke, 1975). Ruminants apparently will graze selectively on contaminated forage. The arsenic content in muscle of terrestrial animals (e.g., cattle, swine, and poultry) is generally below 20 μg/kg fresh weight (Michels, 1986). Drinking water can be a major source of arsenic, especially in the inorganic form. Arsenic concentrations in unpolluted fresh waters, mainly as arsenate, generally range between 1–10 μg/L. However, the arsenic content can be much higher in waters in some geochemical environments. These include aquifers under strongly reducing conditions; aquifers under oxidizing, high-pH (>8) conditions; areas of sulfide mineralization and mining; and geothermal areas (Smedley et al., 2001). Waters that may be used for drinking purposes have been found to be as high as 0.1–5 mg As/L (Smedley et al., 2001; Mandal and Suzuki, 2002).

The bioavailability of inorganic arsenic from the gastrointestinal tract correlates well with the solubility of the compound ingested (Vahter, 1983; Marafante and Vahter,

1987). In humans and most laboratory animals, >90 percent of inorganic arsenate and arsenite in a water solution is absorbed. However, only 20–30 percent of arsenic in arsenic trioxide and lead arsenate, which are only slightly soluble in water, is absorbed by hamsters, rats, and rabbits. In a dog study, the bioavailability, or the amount absorbed, of inorganic arsenic from bog ore-containing soil was found to be only 8.3 percent (Groen et al., 1994). Other studies found similar low bioavailability of soil arsenic. Rabbits absorbed 24 percent and cynomolgus monkeys absorbed 19 percent of the arsenic in soils near smelters (Freeman et al., 1993, 1995). About 60–75 percent of inorganic arsenic ingested with food is absorbed by humans (Hopenhayn-Rich et al., 1993).

The form of organic arsenic also determines it bioavailability. When orally dosed, >90 percent of arsenobetaine was recovered in the urine of hamsters; 70–80 percent of arsenocholine was recovered in the urine of mice, rats, and rabbits; and 45 percent of dimethylarsinic acid was recovered in the urine of hamsters (Marafante et al., 1984; Yamauchi and Yamamura, 1984; Yamauchi et al., 1986). In contrast, >90 percent of an oral dose of sodium-*p*-N-glycolylarsenilate was recovered in the feces of rats or humans within 3 days of administration; urinary excretion accounted for only 4–5 percent of the dose (McChesney et al., 1962). The bioavailability of organic arsenic as found in fish is highly available. Rats absorbed 98–99 percent of the arsenic in flounder (Siewicki and Sydlowski, 1981), and humans absorbed 66–86 percent of arsenic in fish cakes and flounder (Freeman et al., 1979; Tam et al., 1982). The bioavailability of arsenosugars as found in seaweeds has not been well established. Shiomi (1994) found that in mice, orally administered partially purified arsenosugars from the red alga *Porphyra yezoensis* were excreted mainly through the feces; this suggests that the arsenosugars were not very bioavailable. In contrast, Francesconi et al. (2002) found that 80 percent of an orally administered synthetic arsenosugar to humans was excreted in the urine within 4 days. Further evidence that arsenosugars in seaweed are absorbed and metabolized is that sheep living largely on seaweed have elevated wool, blood, and urinary arsenic concentrations and no arsenosugars in the urine (Feldmann, 2000).

TOXICOSIS

Considering its reputation as a poison, it may be surprising to some individuals that arsenic has a low order of toxicity, especially when it is in the pentavalent oxidation state. The ratio of the toxic to the apparent nutritional dose of inorganic arsenic for rats is near 1,250 (Nielsen, 1996). The lethal dose in domestic animals ranges from 1 to 25 mg/kg body weight as sodium arsenite, which is 3-fold to 10-fold more toxic than arsenic trioxide (Stoeppler, 2004). Some forms of organic arsenic, particularly those found in seafood, are virtually nontoxic; for example, a 10 g/kg BW dose of arsenobetaine depressed spontaneous motility and respiration in male mice, but these signs disappeared within one hour (Kaise et al., 1985). Arsenocholine is slightly more toxic than arsenobetaine; a dose of 5.8 g/kg BW caused death in some rats, but a dose of 4.8 g/kg BW did not (Kaise et al., 1992).

Acute

The acute toxicity of arsenic is determined by its chemical form and oxidation state. Generally, the acute toxicity of trivalent arsenic is greater than pentavalent arsenic (Thomas et al., 2001). Most pentavalent organic arsenic species are relatively nontoxic. In contrast, some organic forms of trivalent arsenic (e.g., monomethylarsonous acid) are more toxic than inorganic arsenite, and the toxicity of some others (e.g., dimethylarsinous acid) is similar to inorganic arsenite (Thomas et al., 2001; Hughes, 2002). As a result, providing one LD_{50} value for arsenic cannot be done. This is demonstrated by the reported LD_{50} for various forms of arsenic in mice (in mg As/kg body weight): arsenic trioxide, 26; monomethyarsonic acid, 916; dimethylarsinc acid, 648; trimethylarsine oxide, 5,500; arsenocholine, 6,500; and arsenobetaine, >4,260 (Kaise et al., 1985, 1989, 1992). The reported LD_{50} of arsenic trioxide for the rat is 15 mg/kg body weight (Harrison et al., 1958). The lethal range of inorganic arsenic (i.e., arsenic trioxide) for humans has been estimated to be 1–3 mg/kg body weight (Ellenhorn, 1997). The signs of acute arsenic toxicosis include intense abdominal pain, vomiting, diarrhea, weakness, staggering gait, hypothermia, and death (Stoeppler, 2004).

Fish also have a fairly high tolerance to arsenic in their environment. In a study testing seven fish species, the 96-hr LC_{50} values ranged from 13.3 mg/L for rainbow trout to 41.5 mg/L for bluegill (National Research Council of Canada, 1978). The LC_{50} values for tilapia were determined to be (in mg/L pond water) at 24 hr, 69; 48 hr, 51; 72 hr, 38; and 96 hr, 28 (Liao et al., 2003).

Chronic

Chronic oral arsenic toxicosis in domestic animals is seldom reported. The reason for this may be the fact that arsenic is relatively nontoxic to domestic animals. For example, sheep chronically consuming about 35 mg daily in the form of seaweed (mostly dimethylated arsenoribosides) exhibited no abnormalities or signs of arsenic toxicosis (Hansen et al., 2002). The fatal dose of arsanilic acid for horses and cows was reported to be 40 mg/kg BW and 6–12 mg/kg BW for sheep. Horses and cattle ingested 2.66 to 4 mg arsanilic acid per kg BW daily for 18 months without any discernible injury (Reeves, 1925). The National Toxicology Program (1989) reported no significant toxic effects in mice fed 200 mg roxarsone (57 mg As)/kg diet for two years. Table 4-1 summarizes some reported doses that cause

arsenic toxicosis and the effects of chronic consumption of these high amounts of arsenic by various animals. Signs of chronic arsenic intoxication usually reported were depressed growth, feed efficiency, feed intake, and, for some species, convulsions, uncoordinated gait, and decreased hemoglobin. Chronic high exposure to arsenic can induce numerous biochemical changes. For example, in rats and guinea pigs, dosing the drinking water with 10 or 25 mg of As^{3+}/L for 16 weeks resulted in reduced blood δ-amino-levulinic acid dehydratase activity, increased urinary δ-aminolevulinic acid, and significant changes in whole-brain concentrations of neurotransmitters, 5-hydroxytryptamine, dopamine, and norepinephrine (Kannan et al., 2001). In humans, chronic exposure to arsenic has been associated with various forms of cancer (Wang et al., 2002). Animal models showing that arsenic promotes or induces cancer have been difficult to establish; special strains of experimental rodents, non-oral routes of administration, and stressors such as a carcinogen generally have to be employed (Wang et al., 2002). Because arsenic carcinogenicity apparently is not an issue for domestic animals, it will not be reviewed here.

Prenatal death, malformation, and inhibition of growth can result from exposure to arsenite, arsenate, dimethylarsinate, and monomethylarsonate in hamsters, mice, and rats (Hood, 1983). Such effects generally are seen only at doses causing maternal toxicity including death. To induce malformation in animals, the arsenic typically has to be administered intraperitoneally or intravenously rather than orally (Hood, 1983; Thomas et al., 2001; DeSesso, 2001). A few studies have shown that extremely high single oral doses of inorganic arsenic (40–125 mg/kg BW as sodium arsenate) will cause some fetal stunting and malformations (Hood, 1983). Male mice fed 40 mg As/L of drinking water as sodium arsenite for 35 days exhibited decreased sperm count and motility and increased abnormal sperm; no effect was seen at 20 mg/L (Pant et al., 2001). Published reproductive findings do not appear practically relevant; thus, reproductive toxicity of arsenic under natural conditions is unlikely.

Factors Influencing Toxicity

In addition to those indicated in the metabolic interactions section, other factors that may affect arsenic toxicity include vitamin B_{12} status (Zakharyan and Aposhian, 1999) and nutritional factors affecting the *S*-adenosylmethionine-mediated transmethylation/transulfuration pathway, including niacin, riboflavin, folate, and iron (Donohue and Abernathy, 2001). Age, sex, and pregnancy apparently affect arsenic toxicity. Vahter et al. (2000) reported findings indicating that children are more sensitive to arsenic toxicity than adults, and that pregnancy may increase the ability to metabolize or detoxify arsenic. Even when not pregnant, women may be less susceptible to arsenic toxicity. Dose level, ethnicity, and genetic polymorphism also are factors influencing arsenic toxicity (Vahter et al., 2000).

TISSUE LEVELS

If the ingestion of arsenic is low, no tissue significantly accumulates arsenic; most tissues contain less than 50 µg/kg wet weight. However, high intakes of arsenic can markedly increase the arsenic content of tissues; this is indicated by the data in Table 4-2. Arsenic is widely distributed in the body with the highest amounts in skin, hair, and nails, probably the result of arsenite binding to SH groups of proteins such as keratin that are relatively plentiful in these tissue. Organs highest in arsenic are kidney and liver; these organs also accumulate the highest amount of arsenic when excessive amounts are ingested (see Table 4-2). Although tissue arsenic can be increased to relatively high amounts in seafood (e.g., shrimp) and fish, the toxicity of the organic forms of arsenic found in the edible parts of these animals is essentially nontoxic. Even with high intakes of arsenic, the data in Table 4-2 suggest that no domestic animal tissue, except perhaps liver and kidney, used as a food will contain enough arsenic to be of toxicological concern for humans.

MAXIMUM TOLERABLE LEVELS

The highest dietary level at which arsenic has no adverse effect, and the lowest level that induces toxicosis, varies with species. The data in Table 4-1 indicate that, in the inorganic form, 50 mg As/kg diet is toxic to rats and 100 mg As/kg diet is toxic to chicks; other animals are apparently more tolerant to inorganic arsenic. Pigs fed roxarsone may be the best indicator of a species sensitive to organic arsenic; 187.5 mg/kg diet of roxarsone (53.4 mg As/kg) was toxic to pigs (Rice et al., 1985; Kennedy et al., 1986). The preceding indicates that the maximum tolerable levels set for domestic animals in the previous edition of this book (NRC, 1980) of 50 mg/kg diet for inorganic arsenic and 100 mg/kg diet for organic arsenic may be too high. Because a 12.5 mg As/kg diet apparently is not toxic to rats (Hisanaga, 1982), a more appropriate maximum tolerable limit for arsenic would be between 12.5 and 50 mg/kg diet; the midpoint, or 30 mg/kg diet, may be a reasonable maximum tolerable level for domestic animals. Based on the data in Table 4-1, fish are less tolerant to dietary inorganic arsenic than mammals; diets as low as 10 mg As/kg induced toxicosis in rainbow trout. Thus, a maximum tolerable level for inorganic arsenic of 30 mg/kg diet for fish is inappropriate. A maximum tolerable limit for fish may be in the range of 5 mg As/kg diet.

The European Communities (2002) issued a directive that set the maximum arsenic contents allowable in products intended for animal feed with a moisture content of 12 percent at much lower levels than 30 mg/kg diet. The scientific basis for setting these low values was not given in the directive. The level set for animal feeds was 2 mg/kg, with the exception of meals made from grass, dried lucerne, dried clover, dried sugar beet pulp, and dried molasses sugar beet pulp (set at 4 mg/kg), as well as phosphates and feedstuffs ob-

tained from the processing of fish or other marine animals (set at 10 mg/kg). The maximum for complete feedstuffs for fish was set at 4 mg/kg, which is near the maximum tolerable limit of 5 mg/kg suggested above. The maximum for complementary feedstuffs for fish was set at 4 mg/kg with the exception of complementary mineral feedstuffs (set at 12 mg/kg).

HUMAN HEALTH

Generally, in human health, arsenic is considered only as a toxicant and it is classified as a carcinogen. Thus, recent efforts have been directed towards decreasing the ingestion of arsenic, particularly through drinking water. Under the authority of the Safe Drinking Water Act, the U.S. Environmental Protection Agency (EPA) in 2001 reduced the drinking water standard from 50 μg/L to 10 μg/L; public water systems have until January 2006 to comply (Brooks, 2002). There are epidemiological findings showing that some cancer is correlated with markedly decreased serum arsenic concentrations (Mayer et al., 1993). Also, it has been noted that the incidence of some forms of cancer were higher when drinking water contained very low amounts of arsenic than when it contained reasonable amounts of arsenic (Guo et al., 1995). These findings are consistent with the report that an exposure to drinking water containing 20 to 50 μg/L apparently does not affect mortality (Buchet and Lison, 1998). Biochemical evidence suggesting that low dietary arsenic can increase cancer susceptibility includes the finding that low and excessive amounts of arsenic, compared to control amounts, significantly decreased global methylation of DNA in cultured Caco-2 cells (Davis et al., 2000). DNA hypomethylation is associated with some types of cancer (Dizik et al., 1991; Zapisek et al., 1992). This finding, in addition to those described in the essentiality and metabolic interactions sections above, suggests that an inadequate arsenic status may cause hypomethylation because of a depressed methylation function and excessive arsenic may cause hypomethylation through an increased need for methyl groups for its elimination, and thus making less available for DNA methylation. In both cases, the hypomethylation could result in an increased susceptibility to cancer.

FUTURE RESEARCH NEEDS

Although the toxicity of arsenic, especially its carcinogenic properties, for humans has been extensively studied, there are some research needs. Establishing the basis for the apparent essential or beneficial action of arsenic, and the intake below which this action is compromised, would be helpful in setting the lower limits of toxicity standards for arsenic. Mechanisms through which the various forms of arsenic are absorbed need to established. Speciation of arsenic found in animal feedstuffs would help in determining the toxicity potential of those containing relatively high amounts of arsenic. The toxicity of arsenic to some domestic animals (e.g., cattle, goats) need to be better defined. The basis for the apparent difference in arsenic toxicity for humans (cancer, heart disease, diabetes, etc., thought to occur at relatively low intakes) and domestic animals (limited signs of toxicosis at relatively high intakes) should be determined.

SUMMARY

Arsenic is widely distributed in the biosphere and exists predominantly as an oxyanion in an oxidation state of 3^+ or 5^+. In animals, arsenic occurs mainly as inorganic arsenate or arsenite, monmethylarsonic acid, and dimethylarsinic acid. The major form of arsenic in sea animals is arsenobetaine. In seaweeds, arsenic is found in arsenosugars. Arsenic generally is not accepted as an essential element, but studies with goats, chicks, hamsters, and rats suggest that it may have an essential or a beneficial function in ultra trace amounts (micrograms per kg diet). Some organic arsenicals (e.g., roxarsone) are still used for antibiotic or anti-coccidial purposes in poultry. Arsenic trioxide has been found to be an effective treatment for some forms of cancer in humans. Soluble inorganic arsenic and organic arsenic as found in sea foods are highly absorbed and excreted mainly in the urine. Most inorganic arsenic is enzymatically methylated before being excreted. Arsensobetaine is not, but arsenosugars are transformed before being excreted. Sea plant and fish products are sources of arsenic for animal feeds; this arsenic is relatively nontoxic. Contaminated drinking water and foliage are major sources of inorganic arsenic. Mechanisms through which arsenic may be toxic are altered methyl metabolism, oxidative stress, altered metabolism of other essential minerals, replacement of phosphate in biochemical reactions, and inhibition of critical biochemical functions by binding thiols and vicinal sulfhydryls. Arsenic is relatively nontoxic to domestic animals. The toxic dietary concentrations of arsenic are generally between 500 and 1,000 times greater than the concentrations normally found in animal feeds. Signs of chronic arsenic intoxication include depressed growth, feed intake, feed efficiency, and, for some species, convulsions, uncoordinated gait, and decreased hemoglobin. The suggested maximum tolerable level for domestic animals is 30 mg/kg diet. The suggested maximum tolerable level for fish is 5 mg/kg diet. Thus, except for localized areas where arsenic is extremely high in drinking water, major arsenic contamination by mining and smelting industries has occurred, or old arsenic pesticides or ashes of CCA lumber are accessible, arsenic toxicosis is not a concern for domestic animals.

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TABLE 4-1 Effects of Arsenic Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	30	7–8 mo	2.3 and 4.6 mg/d/kg body weight	Sodium arsenite 4 and 8 mg/d/kg body weight	183 d	Diet	Decreased weight gain and food intake; elevated serum aspartate and alanine aminotranserases	Neiger and Osweiler, 1989
Mice	30	25–30 g	250 mg/L	Sodium arsenite	32 d	Water	Depressed growth	Benes and Bencko, 1981
Mice			3.2 mg/L		15 mo	Water	Fatty infiltration of liver at 12 mo and liver fibrosis at 15 mo	Santra et al., 2000
Rabbits	16		28.8 mg/L	Sodium arsenite (50 mg/L)	10 mo	Water	Reduced stroke volume and cardiac output; increased vascular resistance	Carmignani et al., 1985
Rats	15	Young adult	50 mg/kg	Sodium arsenate	10 wk	Diet	Decreased growth and hemoglobin	Mahaffey et al., 1981
Rats	10		28.8 mg/L	Sodium arsenite (50 mg/L)	18 mo	Water	Reduced stroke volume and cardiac output; increased vascular resistance	Carmignani et al., 1985
	10		20.2 mg/L	Sodium arsenate (50 mg/L)	18 mo	Water	No cardiovascular effects	Carmignani et al., 1985
Rats	10	6 wk	100 mg/kg	Sodium arsenite	2 wk	Diet	Decreased growth and feed and water intake	Yu and Beynen, 2001
Chickens	4 4	22 wk	100 and 1,000 mg/kg	Arsenic pentoxide	56 d	Diet	Decreased body weight, feed intake, and egg production	Hermayer et al., 1977
Chickens	15	8 d	85.4 mg/kg	Roxarsone (300 mg/kg)	14 d	Diet	Decreased weight gain and feed efficiency	Czarnecki et al., 1984
			183, 281 and 379 mg/kg	Arsenic pentoxide (281,431 and 581 mg/kg diet)				
			426 mg/kg	Arsenic trioxide (563 mg/kg diet)				

Ducks	15	1 d	200 mg/kg	Sodium arsenate	4 wk	Diet	22% protein diet—decreased growth; 7% protein—diet decreased growth, liver histopathology and mortality	Hoffman et al., 1992
Pigs	27 6	17 kg	1,000 and 10,000 mg/kg	Arsanilic acid	18 d	Diet	Quadriplegia	Ledet et al., 1973
Pigs	54	4 wk	114 mg/kg	Roxarsone (400 mg/kg diet)	28 d	Diet	Depressed growth and feed efficiency; muscle trembling and posterior paralysis	Edmonds and Baker, 1986
Pigs	24	20 kg	53.4 mg/kg	Roxarsone (187.5 mg/kg diet)	30 d	Diet	Muscle tremors; clonic convulsions induced by exercise; paraparesis progressing to paraplegia; spinal cord myelin and axonal degeneration	Rice et al., 1985; Kennedy et al., 1986
Sheep	1	34 kg	570 mg/kg	Arsanilic acid	56 d	Diet	Convulsions by 66 d, weight loss	Bucy et al., 1955
	1	30 kg	1,139 mg/kg				Convulsions by 28 d	
Sheep	1	29 kg	570 mg/kg	Postassium arsenite	56 d	Diet	Weight loss, less feed consumption	Bucy et al., 1955
	1	32 kg	1,139 mg/kg				Weight loss, less feed consumption	
Fish, rainbow trout	50	Juvenile	200 mg/kg	Arsenic trioxide	8 wk	Diet	All-intakes—depressed growth and feed intake	Cockell and Hilton, 1988
			400 mg/kg	Disodium arsenate			All intakes—depressed growth and feed intake	
			800 mg/kg	Dimethyl arsenate			No toxicosis	
			1,600 mg/kg	Arsanilic acid			No toxicosis	
Fish, rainbow trout	50	Juvenile	8 mg/kg	Disodium arsenate	16 wk	Diet	No toxicosis	Cockell et al., 1991
			44 mg/kg				Depressed growth and feed intake	
			100 mg/kg				No growth and feed refusal	
			174 mg/kg				No growth, feed refusal, and decreased hemoglobin	
Fish, rainbow trout	25	Juvenile	49 and 182 mg/kg	Disodium arsenate	12 wk	Diet	Depressed growth, feed intake, and gall bladder wall inflammation	Cockell et al., 1991

continued

TABLE 4-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Fish, rainbow trout	50	Juvenile	12 mg/kg	Disodium arsenate	24 wk	Diet	No adverse effects	Cockell et al., 1991
			33 mg/kg				Depressed growth and gallbladder inflammation	
			65 mg/kg				Depressed growth, feed intake, and gallbladder inflammation	
Fish, rainbow trout	96	29 g	10 mg/kg	Sodium arsenite	8 wk	Diet	Depressed hemoglobin	Oladimeji et al., 1984
			20 mg/kg				Depressed hemoglobin	
			30 mg/kg				Depressed growth and hemoglobin	
Fish, whitefish	72	4 yr	1, 10, and 100 mg/kg		10, 30, and 64 d	Diet	Liver and gallbladder histopathology	Pedlar et al., 2002

[a]Number of animals per treatment group.
[b]Quantity of arsenic dosed. SI conversion: 1 mg arsenic equals 13.3 μmoles of arsenic.

TABLE 4-2 Arsenic Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L on a dry weight basis unless indicated to be the fresh weight basis (fw) of animals and humans)

Animal	Arsenic Intake[a]	Plasma/Serum/ Blood	Skeletal Muscle	Liver	Heart	Kidney	Bone	Reference
Humans	Not given	0.0041 fw		0.014.5 fw		0.012.4 fw		Dang et al., 1983
	Not given		0. 106 fw	0. 129 fw		0. 129 fw		Yamauchi and Yamamura, 1983
Rats[h]	Not given	15.34 fw		1.13 fw		1.20 fw		Hisanaga, 1982
	0.125[g]	30.85 fw		1.45 fw		1.80 fw		
	12.5[g]	277.63 fw		14.93 fw		22.20 fw		
				19.09 fw		47.67 fw		
	62.5[g]	302.16 fw						
Rats[h]	Not given	5.02 fw		0.0004		0.0002		Valkonen et al., 1983
	58[g]	253.45 fw		0.0087		0.0121		
Pigs	Not given		0.61	0.25		0.53		Morrison and Chavez, 1983
	100		3.07	1.90		2.55		
Chickens	Not given	0.0023 fw	0.0009 fw	0.002 fw		0.003 fw		Stibilj et al., 1997
	30	0.0463 fw		0.083 fw		0.208 fw		NRC, 1977
	50[c]		0.083 fw	1.26 fw		0.640 fw		
			0.040 fw					
Ducks	Not given			0.10 fw				Hoffman et al., 1992
	200			2.30 fw				
Cattle	Not given		0.008 fw	0.063 fw				NRC, 1977
	300[b]		8.8	27.0				Dickinson, 1972
Goats, adult	<0.01[f]		0.011	0.0048	0.026	0.0058		Anke et al., 1996
	0.35		0.029	0.025	0.032	0.028		
Goats, kid	<0.01[f]		0.036		0.015	0.016	0.039	Anke et al., 1996
	0.35		0.077		0.130	0.737	0.090	
Fish, Rainbow trout[d]	1			0.09	0.05	0.11		Cockell et al., 1991
	8			0.025	0.67	1.06		
	44		1.12	3.78		7.61		
	92		2.43	9.15		21.03		
	104		4.33	16.25		28.55		
	174		5.69	34.39		31.88		
Fish, tilapia	17 .8[e]		3.96	3.00				Liao et al., 2003
	49.0[e]		3.13	7.70				

[a]Intake values are mg/kg diet.
[b]Dose is mg/day as monomethylarsonate.
[c]Roxarsone.
[d]Amount in environment (pond water).
[e]Although not clearly indicated in reference, all values in this row are probably on a dry weight basis.
[f]Considered deficient.
[g]Provided in drinking water (mg/L).
[h]Rats accumulate arsenic in their red blood cells.

5

Barium

INTRODUCTION

Barium (Ba) is a dense alkaline metal in Group IIA of the periodic table and is the 16th most abundant element of the Earth's crust, constituting about 0.04 percent of it. Barium occurs as a divalent cation in combination with other elements and is chiefly found in underground ore deposits of barite and witherite. Barite is predominantly $BaSO_4$ and witherite is predominantly $BaCO_3$. Barite occurs in abundance in Alaska, Arkansas, California, Georgia, Missouri, Nevada, and Tennessee, as well as in Canada and Mexico (USGS, 2000). Worldwide, China leads in barite ore mining followed by the United States, with production predominating in Nevada and Georgia. $BaSO_4$ is used as raw material for producing $BaCl_2$, BaOH, and other compounds.

The primary use of $BaSO_4$ is by the oil and gas industries as drilling muds. Additionally, barium compounds are used to make ceramics, brake linings, paints, bricks, tiles, glass, rubber, inks, adhesives, additives for fuels and oils, and rodenticides. Barium nitrite and sulfide are used in fireworks and depilatories, respectively. Medically, $BaSO_4$ is used as a radiocontrast medium for x-rays of the gastrointestinal tract. The natural forms of barium have poor water solubility: 0.001 g/L for $BaSO_4$ and 0.025 g/L for $BaCO_3$. Commercially produced salts are usually much more soluble in water. For example, $BaCl_2$ has a solubility of 375 g/L and consequently exhibits a very different toxicological profile than $BaSO_4$.

ESSENTIALITY

Barium is not considered as an essential nutrient for plants or animals. The primary beneficial action attributed to barium is reduction of dental carries (Zdanowicz et al., 1989). Several thorough reviews are available on barium and its toxicological profile in human and environmental health (ATSDR, 1992; NTP, 1994; EPA, 1998; IPCS, 2001).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Total barium concentrations in water, feed, and tissues can be determined by atomic absorption spectrophotometry (AAS) after preparing samples by digestion with acid (Sharp and Knevel, 1971; EPA, 1974). Graphite furnace AAS has detection limits below one part per billion, but flame AAS is sufficiently sensitive for biological samples that have toxicity potential. Inductively coupled plasma-atomic emission spectrometry is also a sensitive method for measuring barium in biological samples; however there is potential for interference from spectral bands from other compounds, such as boric acid or sodium borate (IPCS, 2001). Standard techniques for barium determination give total barium concentration in the sample. However, measuring soluble barium concentrations is more meaningful for toxicological evaluation because the common but insoluble $BaSO_4$ form is relatively nontoxic compared to soluble forms such as $BaCl_2$ or barium acetate. Unfortunately, standardized techniques for extracting soluble compounds have yet to be developed.

REGULATION AND METABOLISM

Due to its insolubility, $BaSO_4$ is poorly absorbed from the gastrointestinal tract. In chickens with free access to feed and given a single oral dose of $BaSO_4$, 97.8 percent of the dose was recovered from the feces within 48 hours (Vohra and Kratzer, 1967). Healthy humans absorb a similarly small proportion of $BaSO_4$ even when a high dose is given (Mauras et al., 1983). A very wide range (0.7 to 85 percent) of absorption efficiencies of soluble barium compounds have been reported in animals dosed orally, depending upon the chemical form, species, age, and fasting status of the animal. In rats, young animals (22 days old) absorb about 10 times more barium chloride than older animals. Consumption of barium following fasting increases absorption by about 20 percent (ATSDR, 1992; IPCS, 2001).

Barium in blood is quickly transported into cells where it blocks potassium channels and in some systems has Ca^{++} agonist properties. When barium is transported into muscle cells, it blocks the exit of potassium, causes depolarization, and stimulates muscle contraction. Barium can substitute for calcium ions in stimulating nitric oxide production in the endothelium of blood vessels and changes vascular tone (Yamazaki et al., 1995). These effects on calcium and potassium metabolism appear to mediate the toxic effects of barium.

About 90 percent of total body barium is found in bone and teeth. The efficiency of bone uptake of barium is 1.5 to 5 times higher than uptake of calcium or strontium (IPCS, 2001). Barium is primarily excreted in the feces after oral, inhalation, or parenteral exposure. Bile is not an important mechanism of excretion and other routes of loss into the intestines must be important in elimination of systemic barium (Edel et al., 1991). In humans, sweat accounts for a greater proportion of the daily loss than urine (Schroeder et al., 1972). The biological half-time of $BaCl_2$ in beagle dogs was estimated to be 12.8 days (Cuddihy and Griffith, 1972).

SOURCES AND BIOAVAILABILITY

Barium is not typically supplemented to the diets of animals, and intake is due to background levels in feed ingredients and water. Some commercially available sources of calcium phosphate used in animal production may have high levels of barium (Fernandes et al., 1999), but levels of this mineral are not typically monitored because of its low toxicity profile. The form of barium in calcium phosphate sources has not been determined, but would likely be present as $BaSO_4$ and $BaCO_3$.

Barium concentrations in surface waters are extremely variable and depend on local geography, depth that the sample was taken, and water hardness (NRC, 1977). Concentrations between 43 and 58 mg/L are typical in U.S. surface waters although levels as high as 1,000 mg/L have been reported. Water from deep rock and drift wells may have barium levels that exceed 20 mg/L. Seawater contains between 0.02 and 25 mg/L depending upon the ocean, latitude, and depth. High levels of barium in water are often due to the presence of insoluble barium attached to suspended particles, which are typically removed during water purification. Domestic drinking water supplies normally contain less than 1 mg/L barium. In seawater, the limit of soluble barium is about 1 mg/L because above this level $BaSO_4$ forms and precipitates out of solution (Spangenberg and Cherr, 1996).

Barium levels in soils range from 50 to 3,000 mg/kg (IPCS, 2001). Soils formed from limestone, feldspar, and biotite are highest in barium, which is mostly in the barium sulfate form. In general, the relationship between soil barium level and plant barium level is not very strong, but the correlation is greatly increased if soil levels are expressed as exchangeable barium (Robinson et al., 1950). An average bioconcentration factor (BCF = barium in organism/barium in soil) of 0.4 was found for a large variety of plant species living at a site with a soil barium concentration of 104 mg/kg (Hope et al., 1996). In the same environment, insects and rodents had a BCF factor of 0.02. Wheat stalks, wheat grain, corn grain, and alfalfa have BCFs of 0.31, 0.01, 0.03, and 0.93 of the extractable barium, respectively (Robinson et al., 1950). Leaves from red ash (*Fraxinus pennsylvanica*), black walnut (*Juglans nigra*), and hickory (*Carya sp.*) have BCFs above 1.0. Brazil nuts from regions with high soil levels of barium have the highest plant barium concentrations measured at 1,500 to 3,000 mg/kg (Smith, 1971).

Few studies have examined the bioavailability of barium in foodstuffs or water. In one study, rats fed a diet containing Brazil nuts supplying 249 mg/kg barium for 29 days had bone barium levels that were 78 percent of control rats fed an equal level of $BaCl_2$ (Stoewsand et al., 1988). This suggests that most of the barium in Brazil nuts is considerably more bioavailable than $BaSO_4$. Nutritional bioavailability tests have not been done for any other food or water source.

TOXICOSIS

The toxicity of barium is dependent upon its solubility. Barium sulfate is practically insoluble and virtually nontoxic so it has been used for decades in human and animal medicine as a radiocontrast medium. Toxicity of more soluble barium salts is due to their calcium agonist properties and their ability to block potassium transport. Consequently, therapy includes administration of calcium antagonists and potassium. Other toxic mechanisms have not been found and barium salts are not currently considered genotoxic, carcinogenic, or teratogenic, though extensive research has yet to be conducted (NTP, 1994; IPCS, 2001).

Single Dose

Barium sulfate is routinely dosed at up to 8 g/kg BW to adult humans prior to gastrointestinal x-rays and is generally considered safe. In humans, ingestion of soluble barium compounds (e.g., $BaCl_2$) causes vomiting, diarrhea, abdominal pain, hypokalemia, cardiac arrhythmias, respiratory weakness, renal failure, and skeletal muscle paralysis (ATSDR, 1992; IPCS, 2001). In dogs, intravenous infusion of $BaCl_2$ causes salivation, diarrhea, hypertension, ventricular tachycardia, skeletal muscle paralysis, and, finally, respiratory arrest and ventricular fibrillation (Roza and Berman, 1971). Potassium administration prevents all of these effects except hypertension. In rats, a single gavage of 300 mg Ba/kg BW as $BaCl_2$ causes ocular discharge, fluid in the trachea, darkened liver, and intestinal inflammation (Borzelleca et al., 1988). The LD_{50} of $BaCl_2$ in adult rats after a single dose was 132 mg Ba/kg BW in one experiment, but about twice this level in two other experiments (Table 5-1).

The relative toxicity of different soluble barium compounds has received little attention. $BaCO_3$ is considerably less soluble than $BaCl_2$, but is only slightly less toxic when administered in water to rats (Schwartze, 1920). However, toxicity of $BaCO_3$ is decreased considerably if it is provided in alkaline bicarbonate buffer (McCauley and Washington, 1983) or in feed (Schwartze, 1920).

Acute

In a 10-day acute toxicity experiment using rats, daily oral gavage of 209 mg Ba/kg BW as $BaCl_2$ in water did not cause changes in body weight, tissue weights, histopathology, or clinical chemistry except a decrease in blood urea nitrogen (Borzelleca et al., 1988). Barium at 300 mg/kg BW resulted in increased mortality in females, but not in males. The acute LD_{50} of $BaCO_3$ in the diet of rats is about 10,000 mg/kg (Schwartze, 1920).

Chronic

Rats and mice given water with up to 1,318 mg/L of barium as $BaCl_2$ for 13 weeks were clinically, behaviorally, and histopathologically normal, but those fed 2,636 mg/L exhibited increased mortality (Dietz et al., 1992; NTP, 1994). This indicates that the NOAEL of highly soluble barium from $BaCl_2$ in rodents is 1,318 mg/L, although a reanalysis of the organ weight data suggest a NOAEL of 659 mg/L (EPA, 1998). Similar results were found in a 2-year study in rats and mice (NTP, 1994).

The primary clinical signs of chronic barium intake at high levels are cardiomyopathies and hypertension that mimic the effect of digitalis. Rats chronically exposed via the water to 100 mg/L of barium from $BaCl_2$ for 1 month or 10 mg/L for 8 months develop a modest increase in systolic pressure (Perry et al., 1989). The lower level did not have functional consequences on the heart but 100 mg/L resulted in depressed rates of cardiac contraction and electrical excitability. The kidney is also sensitive to barium and very high levels of $BaCl_2$ (2,636 mg Ba/L in water) cause nephrosis in rodents characterized by tubular dilation and deposition of refractile crystals in the lumen of the tubules (Dietz et al., 1992).

Chronic toxicity studies using barium delivered in the diet at toxic levels are not available. Rats fed diets containing 249 mg/kg of barium from $BaCl_2$ or the same amount from Brazil nuts did not cause observable effects (Stoewsand et al., 1988), but a toxic level has not been established.

The toxicity of barium in livestock and poultry has received little attention and the few studies that have been conducted used insufficient dosage levels to establish a toxicity threshold (Table 5-1). Similarly, aquatic systems have had little study. The 30-day LC_{50} values for two species of crayfish (*Austropotamobius pallipes pallipes* and *Orconectes limosus*) were 46 and 78 mg/L of barium as $BaCl_2$, respectively (Boutet and Chaisemartin, 1973). Highly soluble barium acetate interferes with calcification of the shell and delayed development of marine mussel larvae (*Mytilus californianus*). Interestingly, this developmental defect was observed only at levels between 200 and 800 µg/L because additions of 1 mg/L and above caused precipitation of $BaSO_4$, which apparently resulted in the formation of crystal seeds that rapidly precipitated virtually all of the barium from the seawater (Spangenberg and Cherr, 1996). Exposure of unspecified species of marine fish, crustaceans, and mollusks to drilling mud containing 7,500 mg/kg of barium as $BaSO_4$ did not cause mortality (Daugherty, 1951).

Factors Influencing Toxicity

The toxicity of barium is dependent upon its solubility. It has been hypothesized that insoluble $BaSO_4$ can be solubilized by the acid conditions of the stomach, especially if given in unbuffered water to fasted animals (McCauley and Washington, 1983). The water solubility of $BaCO_3$ and its toxicity when dosed in buffered water depend on the pH.

Barium competes with calcium for uptake into bone, but it is not clear if high dietary levels of calcium decrease the toxicity of barium. Barium blocks potassium transport across cell membranes, and potassium administration decreases many of the signs of barium toxicosis (Roza and Berman, 1971).

TISSUE LEVELS

Barium is found predominantly in the bone and teeth, where it substitutes for calcium. Muscle levels of barium are below dietary levels regardless of the level or type of barium salt consumed (Table 5-2), and bioaccumulation of barium through the food chain is not expected to be a problem. Barium levels in meat, milk, and eggs of poultry and livestock exposed to high levels of barium by diet or water have not been determined. However levels in soft tissues (e.g., muscle, kidney, heart, liver) of rats fed high levels of $BaCl_2$ are low (<1 mg/kg fresh weight) (Perry et al., 1989; Tardiff et al., 1980).

There has been concern that the buildup of barium in the aquatic environment from use of oil drilling muds high in $BaSO_4$ could result in high levels of barium in aquatic organisms harvested for human consumption. However, barium in clams (*Maritrix maritrix*) collected from oil fields in the Arabian Gulf averaged only 0.99 mg/kg wet weight and the concentration was not significantly correlated with the level in the sediment (Sadiq et al., 1990). Presumably, this is because of the very low solubility of barium in seawater due to its high sulfate content.

MAXIMUM TOLERABLE LEVELS

The Maximum Tolerable Level (MTL) of barium is defined as the dietary level that, when fed for a defined period

of time, will not impair accepted indices of animal health or performance. The MTL of barium is highly dependent upon its chemical form, which is not known for most foodstuffs or water supplies. The most common form in the environment, $BaSO_4$, is very poorly absorbed and very high levels (1 g/kg BW/d) in the diet or drinking water are tolerated by rodents and humans. Soluble forms of barium, such as barium chloride, acetate, or nitrate have a much different toxicity profile. In rats, the level at which $BaCL_2$ becomes toxic is similar for acute and chronic exposure. The cardiovascular system is the most sensitive indicator of barium toxicosis in rats, and modest but measurable functional changes occur at barium levels of 100 mg/L water as $BaCl_2$ (Perry et al., 1989). However giving 1,250 mg/L for 2 years does not result in observable pathology to any organ system (NTP, 1994). In rats and mice, organ pathology occurs at barium levels of 2,500 mg/L. A dietary barium level of 249 mg/kg diet from $BaCl_2$ does not cause an observable effect on rats (Stoewsand et al., 1988), but 218 mg/kg decreases the rate of weight gain of chicks (Taucins et al., 1969). As $BaCl_2$ does not occur naturally in foods or water, these levels are most applicable to barium arising from accidental contamination of water or feed with soluble forms of barium. Estimates of the bioavailability of intrinsic barium in feedstuffs and water are needed in order to determine the MTL arising from normal dietary sources. Based on only one study in rats, a dietary barium level of 249 mg/kg diet from Brazil nuts does not cause observable toxic effects (Stoewsand et al., 1988). The NOAEL for barium salts or natural forms of barium is not known for poultry or livestock but, based on rodent studies, it is unlikely that barium naturally found in feedstuffs reaches sufficiently high levels to be toxic. Based primarily on rodent studies, the MTL for poultry, swine, and horses is set at 100 mg/kg diet.

HUMAN HEALTH

The relationship between dietary barium levels and the levels in edible tissues of animals is not known. However, the levels in soft tissues (e.g., muscle, kidney, heart, liver) of rats fed high levels of $BaCl_2$, which is a highly bioavailable form of barium, are low (<1 mg/kg fresh weight) (Tardiff et al., 1980; Perry et al., 1989). This level would not be expected to be of concern for human health (ATSDR, 1992; NTP, 1994; EPA, 1998; IPCS, 2001). However, barium accumulates in bone, and bones from animals fed at their MTL could have excessive levels of barium.

FUTURE RESEARCH NEEDS

Future research on the toxicity of barium should focus on two areas. First, the bioavailability of barium that naturally contaminates water and feedstuffs should be determined because it is not known if the bioavailability in these sources is similar to that of insoluble salts (e.g., $BaSO_4$), soluble salts (e.g., $BaCl_2$), or is intermediate to these salts. Second, the NOAEL of barium to poultry, livestock, companion animals, and aquatic species needs to be determined. At this time recommendations must be based on toxicity experiments done in rats and mice that use salts not normally found in nature.

SUMMARY

There are no known essential biochemical functions of barium, and it is not usually considered as an essential nutrient. The natural forms of barium have poor water solubility and have very low toxicity. Commercially produced barium salts, such as $BaCl_2$, are usually much more soluble in water and are considerably more bioavailable and toxic. Once absorbed, barium is deposited predominantly in bone and teeth. Toxic properties of barium are attributed to blocking potassium channels and Ca^{++} agonist properties. Barium is not typically supplemented to the diets of animals. Dietary levels result from background levels in feed ingredients and by accidental contamination.

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TABLE 5-1 Effects of Barium Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
				Single Dose Exposure				
Rats	10	29–37 d	Variable	$BaCl_2$	1 dose	Oral in drinking water	LD_{50} of 273 mg/kg BW	Borzelleca et al., 1988
Rats	10	21–25 d	Variable	$BaCl_2$	1 dose	Oral in drinking water	LD_{50} of 220 mg/kg BW	Tardiff et al., 1980
Rats	10	60–70 d	Variable	$BaCl_2$	1 dose	Oral in drinking water	LD_{50} of 132 mg/kg BW	Tardiff et al., 1980
				Acute Exposure				
Rats	44 23 26	NA	5,000 mg/kg diet 10,000 mg/kg diet 20,000 mg/kg diet	$BaCO_3$	4 d	Diet	27% death 57% death 88% death	Schwartze, 1920
Rats	20	29–37 d	100 mg/kg BW 145 mg/kg BW 209 mg/kg BW 300 mg/kg BW	$BaCl_2$	10 d	Oral, by gavage	No adverse effects No adverse effects No adverse effects Decreased survival	Borzelleca et al., 1988
Chickens	NA	NA	218 mg/kg BW 3,954 mg/kg BW	$BaCl_2$	Acute	Diet	Reduced weight gain Death	Taucins et al., 1969
Swine	NA[c]	NA	733 mg/kg BW	$BaCO_3$	Acute	NA	Death	Esser, 1935
Horses	NA	NA	450–675 mg/kg BW	$BaCl_2$	Acute	NA	Death	Esser, 1935

continued

TABLE 5-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
				Chronic Exposure				
Mice	20	43 d	82 mg/L 330 mg/L 659 mg/L 1,318 mg/L 2,636 mg/L	$BaCl_2$	92 d	Oral in drinking water	No adverse effects Increased serum P Increased serum P Decreased liver weight, Increased mortality, decreased body weight	Dietz et al., 1992
Mice	60	Weanling	500 mg/L 1,250 mg/L 2,500 mg/L	$BaCl_2$	2 yr	Oral in drinking water	No adverse effects No adverse effects Increased mortality, renal pathology, lymphoid depletion of spleen, thymus, and lymph nodes	NTP, 1994
Rats	20	43 d	82 mg/L 330 mg/L 659 mg/L 1,318 mg/L 2,636 mg/L	$BaCl_2$	92 d	Oral in drinking water	No adverse effects No adverse effects Increased kidney weight, serum P Increased kidney weight, serum P Increased mortality, decreased body weight	Dietz et al., 1992
Rats	60	28 d	10 mg/L 50 mg/L 250 mg/L	$BaCl_2$	13 wk	Oral in drinking water	No adverse effects	Tardiff et al., 1980
Rats	60	Weanling	500 mg/L 1,250 mg/L 2,500 mg/L	$BaCl_2$	2 yr	Oral in drinking water	No adverse effects No adverse effects Increased kidney weights	NTP, 1994
Rats	4	Weanling	249 mg/kg diet 249 mg/kg diet	$BaCl_2$ Brazil nuts	29 d	Diet	No adverse effects No adverse effects	Stoewsand et al., 1988

[a]Number of animals per treatment group.
[b]Quantity of barium dosed. SI conversion: 1 mg barium equals 7.28 μmoles barium.
[c]Not available, NA.

TABLE 5-2 Barium Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)

Animal	Quantity	Source	Duration	Route	Muscle	Kidney	Bone	Reference
Rat	0 mg/L 10 mg/L 50 mg/L 250 mg/L	$BaCl_2$	13 wk	Drinking water	0.19[a] 0.48 0.58 0.44	—	9. 1[a] 14.5 50.5 220	Tardiff et al., 1980
Sheep (wethers)	7 mg/kg	Pasture	Weaning to 30 months	Feed	—	0.15[a]	60[b]	Healy and Ludwig, 1968
	35 mg/kg	Pasture				0.61	250	

[a]Data are on a fresh tissue basis.
[b]Data are on a dry tissue basis.

6

Bismuth

INTRODUCTION

Bismuth (Bi) is a pinkish-white, lustrous, soft mineral and is widely distributed in small quantities throughout the world. Although it has the crystal structure of a semi-metal, bismuth is often considered a metal. Of all the metals, bismuth has the widest range between melting and boiling points, the lowest thermal and heat conductivity, the highest diamagnetic index, and the lowest absorption for neutrons. Its crystal structure along with several of its other physical properties makes it a substitute for lead in some applications. Bismuth forms compounds in oxidation states +3 and +5, and its chemistry is diverse and poorly understood (Briand and Burford, 1999). Bismuth nitrate is the initial material used for the production of many bismuth compounds used in industry and medicine.

World reserves of bismuth are usually associated with lead deposits, except in Asia, where economically recoverable bismuth is found with tungsten ores and some copper ores, and in Australia, where bismuth is found with copper-gold ores. Currently, bismuth is not refined in the United States. Worldwide production is dominated by China, Mexico, Peru, and Canada (USGS, 2003).

In 2002 about 42 percent of the bismuth used in the United States was in fusible alloys, solders, and cartridges; 37 percent in pharmaceuticals and chemicals; 19 percent in metallurgical additives; and 2 percent in other uses (USGS, 2003). Bismuth is used in several applications designed to provide nontoxic substitutes for lead, including shot for waterfowl hunting, fishing weights, plumbing fixtures, solders, and lubricating greases. Other applications of bismuth chemicals and compounds include uses in superconductors and pearl-colored pigments for cosmetics and paints. Pharmaceuticals based on trivalent bismuth have been used for more than a century and include a variety of compounds to treat gastrointestinal ailments including diarrhea, upset stomach (bismuth subsalicylate, bismuth subnitrate, and bismuth subcarbonate), and ulcers and gastritis caused by *Helicobacter pylori* (bismuth subcitrate).

ESSENTIALITY

Bismuth is not considered an essential nutrient for plants or animals and has no known essential biochemical functions in normal metabolism.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Bismuth can be measured by atomic absorption spectrophotometry (AAS). Graphite furnace AAS has greater sensitivity than flame AAS. The primary difficulty in analysis is volatilization, but this can be prevented by use of a platinum matrix modifier (Slikkerveer et al., 1993). Sample preparation by acid digestion is necessary due to evaporation of bismuth during ashing. Other methods of analysis include particle-induced x-ray emission (Canena et al., 1998), inductively coupled plasma-mass spectrometry, and atomic fluorescence spectrophotometry (Cava-Montesinos et al., 2003). These other methods are rarely used because expensive equipment is needed.

REGULATION AND METABOLISM

Bismuth compounds are very poorly absorbed in the intestinal tract. In rats and humans, greater than 99 percent of an oral dose of a variety of bismuth compounds is lost in the feces without absorption (Dresow et al., 1992; Slikkerveer et al., 1995). This poor absorption is probably due to poor solubility in both gastric juice (pH 1.2) and duodenal juice (pH 6.8). Bismuth citrate is more soluble at neutral pH (35 μmol/L) than bismuth salicylate, gallate, aluminate, chloride, or nitrate (<2 μmol/L) and is slightly better absorbed. In dogs, absorption in the small intestine appears to be greater than absorption from the stomach (Hespe et al., 1993).

Once absorbed, bismuth binds to transferrin at specific iron binding sites (70 percent) and to albumin (30 percent). Presumably, these transport proteins deliver bismuth to tissues (Sun and Szeto Ka, 2003). The tissue distribution of

absorbed bismuth is kidney>>bone>red blood cells>>liver, heart, spleen, muscle >> serum, and fat. Because of this distribution, bismuth concentrations in whole blood are a much better indicator of bismuth exposure than levels in serum. Bismuth is excreted relatively quickly by the kidney and to a lesser extent in bile (Dresow et al., 1991).

SOURCES AND BIOAVAILABILITY

Bismuth is a rare element, occurring at about 200 to 350 µg/kg in soils and 20 µg/L in fresh water and below 1 µg/L in seawater (Lee, 1982). Bismuth levels in plants grown on uncontaminated land are usually less than 60 µg/kg dry weight, and levels in plants grown on contaminated soils do not usually exceed 200 µg/kg dry weight (Bowen, 1979). Levels in leaves are higher than in fruits or seeds (Jung et al., 2002).

Bismuth is not normally added to the diet of animals and is not typically a quantitatively important contaminant of feedstuffs or additives. Consequently, the primary exposure of animals is use of bismuth-based medications or accidental contamination. A variety of over-the-counter and prescribed preparations of bismuth compounds (e.g., Pepto Bismol®) are used to treat animals for intestinal disorders ranging from ulcers, gastritis, irritable colon, and constipation, to diarrhea (Lambert, 1991; Roussel and Brumbaugh, 1991). They are also used to improve the consistency and odor of stools.

TOXICOSIS

A characteristic sign that high levels of trivalent bismuth compounds have been consumed is dark- or black-colored feces, which is due to bismuth sulfide produced in the large intestine. Toxic levels of bismuth may affect several target organs depending upon dose, duration, and type of compound (Table 6-1). In humans, encephalopathy is the most life-threatening consequence of long-term exposure to bismuth subnitrate or subgallate, whereas bismuth subcitrate results in nephrotoxicity. The nephrotoxicity is characterized by necrotic death of proximal tubular epithelial cells, apparently because bismuth destabilizes the cell membrane (Leussink et al., 2002). Bismuth compounds are not thought to be carcinogens (Preussmann and Ivankovic, 1975).

Bismuth toxicities due to high levels in drinking water have not been reported. Most bismuth compounds are not sufficiently soluble in water to result in toxic levels. Suspensions or colloids of bismuth compounds administered as pharmaceuticals can be toxic if consumed at relatively high levels.

Single Dose

Clinical signs of acute bismuth toxicosis in rats consist of erect hairs and hunched posture within 6 hours of administration of 627 mg/kg BW of bismuth as bismuth subcitrate; at 48 hours rats have swollen ceca filled with gas and black fluid (Leussink et al., 2001). Rats gavaged with 314 mg/kg as bismuth subcitrate appeared visually normal but had impaired kidney function, which returned to normal within 48 hours. Absorption and toxicity of bismuth is enhanced when it is complexed with cysteine. Such complexes have an LD_{50} of 156 mg Bi/kg BW in rats (Chaleil et al., 1981).

Shot designed for waterfowl hunting given as a single oral dose of 460 mg of bismuth metal to adult mallards did not adversely affect food intake, body weight gain, hematology, clinical chemistry, and histopathology (Ringelman et al., 1993). Furthermore, bismuth levels in tissues were low and not different from control birds.

Chronic

In humans the primary behavioral symptoms of chronic consumption of bismuth subgallate or subnitrate is apathy, mild ataxia, and headaches. More severe cases present with dysarthria, confusion, hallucinations, epileptic seizures, and occasionally death (Tillman et al., 1996). In the majority of cases of bismuth toxicosis in humans, symptoms occurred after consumption of doses that often exceeded 10 g of bismuth per day for 2 to 20 years. The pathogenesis of bismuth-induced neurotoxicity is unknown, although it has been suggested that the conversion of the original compounds into more soluble or more toxic forms by intestinal bacteria is involved (Bader, 1987). This is supported by the observation that patients taking bismuth because of intestinal bacterial overgrowth were most likely to suffer toxicosis. Bismuth subcitrate and subsalicylate do not usually result in severe neurological symptoms, but cause nephrotoxicity (Gorbach, 1990).

Pigs fed 333 mg/kg BW daily of bismuth from bismuth subnitrate, and chickens fed diets containing 1 g/kg bismuth from bismuth trioxide, did not display signs of toxicosis after exposure for 8 or 9 weeks, respectively (Hermayer et al., 1977; Pollet et al., 1979). Bismuth subcitrate is the most bioavailable of the common forms of bismuth and appears to be the most toxic. Rats fed 314 mg/kg BW daily of bismuth as bismuth subcitrate displayed functional and histopathological signs of toxicosis (Leussink et al., 2001).

Factors Influencing Toxicity

Absorption of bismuth in rats is increased by compounds containing sulfhydryl groups, such as cysteine, and by complexing agents, such as EDTA or citrate (Allain et al., 1991; Chaleil et al., 1981). Cysteine increases the acute toxicity of bismuth (Chaleil et al., 1981; Krari et al., 1995).

Selenium affects the metabolic fate of bismuth (Szymanska et al., 1978), and bismuth affects selenium metabolism (Gregus et al., 1998), but the impact on toxicity is not clear. In rats, bismuth diminishes both the biliary excretion of selenium and its accumulation in erythrocytes. Bis-

muth also induces the expression of metallothionein in the liver and especially the kidney (Szymanska et al., 1993). Bismuth also can displace zinc and cadmium from existing metallothionein (Sun et al., 1999) and augments the excretion of zinc and copper (Szymanska et al., 1993). However, the effect of bismuth on the toxicity or the requirement of these metals is not known.

TISSUE LEVELS

Normal bismuth levels in tissues are low, typically below 0.2 mg/kg (Allain et al., 1991). Although the relationship between bismuth intake and bismuth accumulation in tissues of production animals is not known, this metal is not bioaccumulated in rats or dogs (Table 6-2). Even at high daily doses of bismuth, well above therapeutic levels, muscle levels are relatively low (<1 mg/kg). The kidney is the primary organ that accumulates bismuth. Dogs dosed daily with 108 mg/kg BW daily of bismuth ammonium citrate, which is a relatively soluble form, accumulated 58 mg/kg bismuth (wet weight) in their kidneys. Such levels in animal products would not be expected to be of concern for human health.

MAXIMUM TOLERABLE LEVELS

The MTL of bismuth is defined as the dietary level that, when fed for a defined period of time, will not impair accepted indices of animal health or performance.

Acute

Dogs can tolerate a daily dose of 108 mg/kg BW daily of bismuth from bismuth subcarbonate or bismuth ammonium citrate for 2 weeks without clinical signs of toxicosis (Hall and Farber, 1972). Similarly, rats can tolerate 157 mg/kg BW daily of bismuth from bismuth subcitrate for 2 weeks (Leussink et al., 2001).

Chronic

Pigs tolerate 333 mg/kg BW daily of bismuth from bismuth subnitrate (Pollet et al., 1979), and chickens tolerate 1,000 mg/kg diet of bismuth from bismuth trioxide (Hermayer et al., 1977).

Via Water

Most bismuth compounds are not sufficiently soluble in water to cause toxicity.

FUTURE RESEARCH NEEDS

Little is known about the relationship between bismuth intake and the levels in edible products, such as meat, milk, and eggs. Very high levels of bismuth are sometimes administered to farm animals, and research is needed to determine if food residue problems could occur.

SUMMARY

Bismuth is a rare element that normally occurs at low levels in soil and water and is not bioaccumulated by plants or animals. It is not normally added to the feed of animals and occurs at very low levels in feedstuffs and mineral supplements. Bismuth metal and its compounds are poorly absorbed by the gastrointestinal tract and are relatively nontoxic. Toxicosis is likely only if pharmaceutical preparations containing bismuth are chronically administered at high doses.

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TABLE 6-1 Effects of Bismuth Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	9	1 yr	107.6 mg/kg BW/d	Bi ammonium citrute	14 d	Oral	No adverse effects	Hall and Farber, 1972
	3			Bi subcarbonate			No adverse effects	
Rats	20	11–12 wk	157 mg/kg BW/d 314 mg/kg BW/d 627 mg/kg BW/d	Bi subcitrate	14 d	Oral	No adverse effects Kidney necrosis and impaired function Kidney necrosis and impaired function	Leussink et al., 2001
Rats	40	100 d	8 g/kg diet 16 g/kg diet 40 g/kg diet	BiOCI	2 yr	Diet	No adverse effects No adverse effects No adverse effects	Preussmann and Ivankovic, 1975
Rats	10	120–150 g	172 mg/kg BW/d	Tripotassium dicitrbismuthate	14 mo	Oral	No adverse effects	Lee et al., 1980
Chickens, hens	4	22 wk	1 mg/kg diet 10 mg/kg diet 100 mg/kg diet 1,000 mg/kg diet	Bi trioxide	8 wk	Diet	No adverse effects	Hermayer et al., 1977
Duck, mallard	20	8 wk	0.46 g/d	Bi metal	1 dose	Oral	No adverse effects	Ringelman et al., 1993
Pig	4	16–20 kg	333 mg/kg BW/d	Bi subnitrate	9 wk	Diet	No adverse effects	Pollet et al., 1979

[a]Number of animals per treatment group.
[b]Quantity of bismuth dosed. SI conversion: 1 mg bismuth equals 4.78 μmoles bismuth.

TABLE 6-2 Bismuth Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)[a]

Animal	Quantity	Source	Duration	Route	Muscle	Kidney	Liver	Blood Serum	Bone	Reference
Dogs	108 mg/kg BW/d	Bi subcarbonate Bi ammonium citrate	14 d	Gavage	UD[b] UD	8.7 58.1	UD 1.4	UD UD	—	Hall and Farber, 1972
Rabbits	28 mg/kg BW/d	Bi ammonium citrate	28 d	Gavage		1.23				Zommer-Urbanska et al., 1994
Rats	104.5 mg/kg BW/d	Bi subcitrate	14 d	Gavage	—	34.5	4.5	—	<1.5	Slikkerveer et al., 1993
Rats	172 mg/kg BW/d	Tripotassium dicitrbismuthate	14 mo	Gavage	0.13	13.9	0.4	—	—	Lee et al., 1980
Rats	400 mg/kg BW/d	Bi nitrate	30 d	Gavage	0.17	5.6	0.1	0.06	1.3	Allain et al., 1991

[a]Values are on a fresh tissue basis.
[b]UD = undetectable (< 1 mg/kg).

7

Boron

INTRODUCTION

Elemental boron (B) is a relatively inert metalloid that exists as either black monoclinic crystals or yellow-brown amorphous powder when impure at room temperature. However, boron as an element does not occur in nature; it is always found bound to oxygen or in the borate form. The most common commercial compounds of boron are anhydrous, pentahydrate and decahydrate (tincal) forms of disodium tetraborate (borax, $Na_2B_40_7$), colemanite ($2CaO{\cdot}3B_2O_3{\cdot}5H_2O$), ulexite ($Na_2O{\cdot}2CaO{\cdot}5B_2O_3{\cdot}16H_2O$), boric acid ($H_3BO_3$), and monohydrate and tetrahydrate forms of sodium perborate ($NaBO_3$) (Woods, 1994). The borate industry began in 1865 with the mining of borate pandermite (priceite, $4CaO{\cdot}5B_2O_3{\cdot}7H_2O$) in Turkey. Shortly thereafter, several borate deposits were found in California and Nevada, including ulexite and colemanite in Death Valley. Subsequently, tincal, colemanite and kernite ($Na_2O{\cdot}2B_2O_3{\cdot}4H_2O$) were found and mined in the Mojave Desert (Woods, 1994). In addition to Turkey and the United States, other countries producing borates are Peru, Chile, Russia, and China. Sodium perborates are hydrolytically unstable compounds containing boron-oxygen-oxygen bonds; they are used as bleaches in detergents. The end uses of boric acid and borates are diverse and include glass, enamel, and synthetic gems manufacturing; wood and leather preservatives; flame retardants; cosmetics; medical products; detergents; insecticides; fertilizers; and neutron absorbers for the nuclear industry. Boron halides and hydrides are used as catalysts and in jet and rocket fuels. Elemental boron and its carbides and nitrides are used in high-temperature abrasives and in steelmaking (Larsen, 1988).

Boric acid is thought to be the most prevalent form of boron in animals and humans. Boric acid is colorless and odorless with either a white granular powder or transparent crystalline form, which are readily soluble in water. It is a weak Lewis acid, accepting OH^- to form the tetrahedral, tetrahydroxy borate anion at a pK_a of 9.2 (Coughlin, 1996).

ESSENTIALITY

In 1923, signs of boron deficiency for several leguminous plants were reported (Warington, 1923). Shortly thereafter, Sommer and Lipman (1926) reported that boron was essential for the completion of the life cycle of a number of plants. This evidence was enough for wide acceptance of boron essentiality for plants. More than 70 years elapsed before a biochemical function for boron in plants was described. Boron cross-links two dimers of rhamnogalacturonan-II, a small structurally complex pectic polysaccharide found in primary cell walls of plants (Matoh and Kobayashi, 2002). This function is not adequate to explain all boron deficiency signs in plants, which most often occur in dicotyledons raised on light-colored sands and silt loams in humid regions of the world including the eastern United States (Sprague, 1972). Boron stimulates the growth of yeast (*Saccharomyces cerevisiae)* (Bennett et al., 1999) and is an essential component of the sensor protein of autoinducer-2, an extracellular signaling molecule used for cell-to-cell communication by some bacteria (Chen et al., 2002). Boron has been found to be essential for frogs (Fort et al., 1999a,b) and zebrafish (Eckhert and Rowe, 1999) to complete their life cycle. Substantial evidence from experiments comparing very low intakes (<100 µg/kg diet for animals, 0.25 mg/d for humans) with physiological intakes (1–3 mg/kg diet) of boron suggests that boron is needed for optimal bone health, brain function, and immune function in higher animals and humans (Nielsen, 1996, 2002a). However, although the evidence is similar, boron is not consistently accepted as an essential nutrient for higher animals as it is for plants. Apparently, the lack of a clearly defined specific biochemical function is a major obstacle to wide acceptance of boron essentiality by animal and human nutritionists.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Less modern methods involving colorimetric, gravimetric, and titrametric techniques can adequately determine boron in materials that contain high amounts such as fertilizer, soils, and some botanical materials. The spectrophotometric method whereby the colored complex of Azomethine-H and boron is measured is an international standard method and still used (Lopez et al., 1993). Accurate analysis of boron in amounts found in most biological materials requires more modern techniques and instrumentation, which indicate that boron concentrations reported in the older biological and food composition literature often may be too high. Analytical techniques that utilize inductively-coupled plasma, such as inductively-coupled argon plasma spectrometry (ICAP), inductively-coupled plasma mass spectrometry (ICP-MS, probably the most sensitive technique), and inductively-coupled plasma emission spectroscopy (ICP-ES) have become the instruments of choice for boron analysis. However, other methods including neutron-activation-mass spectrometry and prompt gamma-activation analysis have been used to accurately measure boron in biological materials. Most of the difficulties encountered in boron analysis occur during sample preparation (Downing et al., 1998). Because boron is so prevalent in the environment, contamination is a major problem in analysis of boron in low amounts. Sources of this contamination have been listed (Downing et al., 1998). Among those listed are sample contact with borosilicate glass and ashing procedures using the same digestion vessels for both high and low boron-containing samples, which can result in cross contamination because of the difficulty in removing all boron that has bound to or migrated into the vessel surface. Another significant concern in boron analysis is its volatility and mobility. Because some boron in biological materials is water soluble, it is mobile and could be lost if a sample is slowly frozen for storage or freeze-drying; quick freezing in liquid nitrogen will reduce the redistribution or loss of boron. Many boron compounds volatilize at temperatures far below those required for dry ashing (Hunt and Shuler, 1989). Ashing a neutral or acidic sample requires boron to be quickly converted into a nonvolatile compound. This can be accomplished by ashing at low temperatures or by making the sample basic (Downing et al., 1998). Because of the contamination and loss concerns, the validity of any boron analysis can only be assured by the use of quality control procedures such as the concomitant analysis of standard reference materials.

REGULATION AND METABOLISM

Absorption and Metabolism

About 85–90 percent of ingested borax, boric acid, and, apparently, dietary boron is rapidly absorbed and excreted mostly in the urine shortly after ingestion (Jansen et al., 1984; Hunt et al., 1997; Nielsen and Penland, 1999; Sutherland et al., 1999). Because there is no usable radioisotope of boron, the study of its metabolism is difficult. It is likely, however, that most ingested boron is converted into boric acid, the dominant species of boron compounds hydrolyzed at the pH of the gastrointestinal tract, and then absorbed and excreted mainly in that form. Absorption of boron through the intact skin is negligible, and, thus, is not a toxicological concern (Wester et al., 1998). However, toxic amounts can be absorbed through damaged skin (Nielsen, 1970; Draize and Kelley, 1959). Several cases of boron toxicosis, some resulting in death, have been reported in humans who had open skin lesions treated with medications containing high amounts of boric acid (Pfeiffer et al., 1945); such medications are no longer in use. The boron hydrides (boranes), which are highly toxic, can be absorbed by the lungs (NRC, 1980). Inhaled borates also can be absorbed by the lung but are relatively nontoxic.

Metabolic Interactions

The most relevant property of boric acid, the prevalent form of boron in vivo, is its ability to form complexes with substances containing *cis*-hydroxyl groups (Hunt, 2002). During transport in the body, boric acid most likely is weakly attached to organic molecules containing *cis*-hydroxyl groups. The interaction with organic compounds containing hydroxyl groups most likely also affects the response to deficient and toxic intakes of boron. For example, boron toxicity is alleviated by riboflavin (Roe et al., 1972). It is also hypothesized that boron can enhance the function of other nutrients and hormones acting at the cell membrane level (Nielsen, 2002a). Thus, changes in the status of some nutrients and hormones that act at the cell membrane level apparently can affect the response to various intakes of boron. Among the nutrients that affect the response to dietary boron are vitamin D, magnesium, molybdenum, calcium, selenium, protein, and omega-3 fatty acids (Hoffman et al., 1991; Hunt, 1994; Nielsen, 2002b). Estrogen status apparently affects the response to boron deficiency: postmenopausal women on hormone replacement therapy showed changes in plasma copper and serum 17β-estradiol concentrations when deprived of boron, but those not on hormone replacement therapy did not (Nielsen et al., 1992). High intakes of boron have been investigated as antidotes to fluoride toxicity in rabbits (Baer et al., 1977; Elsair et al., 1980), pigs (Seffner and Teubener, 1983), and sheep (Wheeler et al., 1988).

Mechanisms of Toxicity

The mechanism of toxicity for boron has not been firmly established. Ku and Chapin (1994) studied the possible mechanisms through which boric acid causes toxicologic manifestations in testicular function of the rat. Their find-

ings indicated that boron toxicosis did not occur through inducing a riboflavin deficiency or impairing phosphorus, calcium, and zinc metabolism or utilization. They did obtain findings suggesting that boron toxicosis may be the result of an impairment of energy metabolism through the inhibition of some enzymes that produce utilizable energy substrates. Also, they suggested that toxic amounts of boric acid affected the DNA synthetic activity of both mitotic (spermatogonial) and meiotic (post-spermatogonial) germ cells of the rat.

SOURCES AND BIOAVAILABILITY

Sources of exposure to boron have been reviewed (Howe, 1998; IPCS, 1998). Boron enters the environment mainly through the weathering of rocks; volatilization from seawater; burning of oil, coal, and wood; industrial and household use of boron-containing products (especially soaps and detergents); fertilizers; and sewage and sludge disposal. Boron-containing vitreous materials such as fiberglass, borosilicate glass, enamels, frits, and glazes are not significant sources of exposure because the boron is tightly bound in the glassy structure. Significant amounts of boron are not present in the atmosphere (average of 20 ng/m^3 over continents). The Earth's crust contains about 10 mg B/kg, ranging from 5 mg/kg in basalts to 100 mg/kg in shales. Boron occurs in soils at concentrations ranging from 10 to 300 mg/kg (average 30 mg/kg). Concentrations in surface waters range widely from 0.00l to 150 mg/L (Coughlin, 1998). The average boron concentration in the oceans is 4.6 mg/L. Most freshwater concentrations of boron (mostly in the boric acid form) are below 0.4 mg/L and not lowered by treatment for drinking water. Boron accumulates in aquatic and terrestrial plants but does not magnify through the food chain. On a dry weight basis, boron concentrations range between 26 and 382 mg/kg in submerged aquatic freshwater plants, 11 and 57 mg/kg in freshwater emergent vegetation, and 2 and 95 mg/kg in terrestrial plants (IPCS, 1998). Among plants, monocotyledons generally contain less boron than dicotyledons. Boron deficiency occurs in a wide variety of plants when their dry matter concentrations are less than 10 mg/kg (Bell, 1997). A rough method of diagnosing boron toxicity in plants is determining boron in plant components; dry tissue boron concentrations exceeding 250 mg/kg often indicate boron toxicity (Nable et al., 1997). On a wet weight basis, boron concentrations in marine invertebrates and fish are similar to their environment, or between 0.5 and 4 mg/kg (IPCS, 1998). The preceding values indicate that diet and sometimes drinking water are the major sources of exposure to boron. For example, the daily boron intakes for humans were estimated to be 0.44 μg from ambient air, 0.5 μg from soil, 0.1 mg from consumer products, 0.2–0.6 mg from drinking water, and 1.2 mg from the diet (IPCS, 1998).

Boron supplements for the alleviation of arthritis in humans appeared in 1976 (Newnham, 1994). Shortly after boron was indicated as being beneficial to postmenopausal women (Nielsen et al., 1987), the presence of boron in over-the-counter supplements increased markedly. Most supplements provide boron in amounts that would not be toxic for humans, usually 3 mg or less daily. Although boron supplements for animals are not approved in the United States, one report (Newnham, 2002) indicates that they have been used in other parts of the world. Boron supplements reportedly are effective in the treatment of arthritic dogs, horses, and cattle; the dose administered was 3 mg B/day per 25 kg BW for 2 to 4 weeks (Newnham, 2002). It would not be surprising to find that boron supplementation to poultry may be considered because of reports showing that this improved bone strength and growth (Rossi et al., 1993; Wilson and Ruszler, 1997; Kurtoğlu et al., 2001). Boron supplementation of pigs may be considered because it may increase body weight, bone strength, and immune function (Armstrong et al., 2002; Armstrong and Spears, 2003).

As indicated in the absorption discussion above, boron in foods, feeds, water, and supplements are generally highly available.

TOXICOSIS

Single Dose

The oral LD_{50} for boron (provided as boric acid and borax) varies among animal species. Values in mg B/kg BW are in the range of 400–700 for mice and rats (Pfeiffer et al., 1945; Weir and Fisher, 1972); 210 for guinea pigs (Verbitskaya, 1975); and 250–350 for dogs, rabbits, and cats (Pfeiffer et al., 1945; Verbitskaya, 1975). The signs of toxicity in rats, mice, and guinea pigs upon administration of large single doses of borax and boric acid used to determine the LD_{50} were depression, ataxia, occasional convulsions, decreased body temperature, and violet-red color of skin and all mucous membranes (Pfeiffer et al., 1945; Weir and Fisher, 1972). Goats dosed with 2 g soluble borate fertilizer had increased cerebrospinal fluid monoamine metabolites and exhibited seizure-like activity suggesting a central nervous system effect (Sisk et al., 1990). Necropsy signs of boron toxicity in mice, rats, and dogs included glomerular damage (altered permeability of capillaries) and tubular damage (cellular vacuolization and shedding of cells into the tubular lumen) in the kidney, and an increase in small dark cells (probably microglia) in the spinal cord and in the gray matter of the brain cortex (Pfeiffer et al., 1945). The reported single toxic dose of boric acid for humans varies from 20 to 45 g (3.5 to 7.9 g boron) for adults (Potter, 1921) and 1 to 6 g (0.175 to 1.05 g boron) for infants (McNally and Rust, 1928; Young et al., 1949). However, these values are based on old reports and may be questionable. Of the 784 poisoning cases examined by one group (Litovitz et al., 1988), no deaths or severe manifestations of toxicity were found in patients ingesting boric acid in single doses ranging from 10

mg to 88.8 g (1.75 mg to 15.54 g boron); 88 percent did not exhibit any toxicity symptoms in this group whose median age was 2 years. In the patients that showed symptoms, the most common were vomiting, abdominal pain, and diarrhea. Lethargy, headache, lightheadedness, and atypical rash were seen less frequently. Similar findings were obtained from the records of another 300 cases of single, acute ingestions of 10 to 297 g of boric acid (1.75 to 52 g of boron) (Linden, et al., 1986). The initial responses to the lethal doses reported in the older literature included nausea, vomiting (sometimes with blood), abdominal pain, and diarrhea. Shock with low blood pressure, tachycardia, and cyanosis sometimes occurred. Death apparently was the result of central nervous system depression. Autopsy signs of toxicity included a cloudy swelling of the kidneys, centrolobular hepatic necrosis, and hemorrhagic enteritis. It should be noted that no human deaths from large single dose boric acid ingestion were reported between 1928 and 1988 (Litovitz et al., 1988).

Acute

The topic of the first publication in the *American Journal of Physiology* was boron toxicity in dogs (Chittenden and Gies, 1898). This study found that daily doses of 3 g of boric acid or 5 g of borax for 8–10 days had no physiological or pharmacological effect in adult dogs weighing 8 to 12 kg. However, doses of 5 to 10 g B/d resulted in an increase in urinary nitrogen, sulfur and phosphorus excretion, and doses of borax and boric acid equal to 1.5–2.0 percent of daily diet intake induced nausea and vomiting. Rabbits orally administered 800 mg boric acid/kg BW/day for 4 days exhibited anorexia, weight loss, and diarrhea; 850 and 1,000 mg boric acid/kg BW resulted in 100 percent mortality (Draize and Kelley, 1959). Cattle consuming water containing 150 or 300 mg B/L as boric acid for 30 days exhibited lethargy, inflammation, and edema in the legs and around the dewclaws; occasional diarrhea; and decreased food consumption, weight gain, hematocrit, and hemoglobin concentration (Green and Weeth, 1977). Rats fed 262.5 mg B/kg BW/day as boric acid or borax in the diet exhibited reduced weight gain and died within 3 to 6 weeks (Weir and Fisher, 1972). Administration of 250 mg boric acid/kg BW on gestational days 6–19 to rabbits resulted in increased prenatal mortality, decreased litter size, and malformed fetuses (primarily cardiovascular defects) (Price et al., 1996). Fish are not especially sensitive to boron as borate or boric acid. The concentration of borate acutely toxic to freshwater fish (lethal to 50 percent in 1 to 6 days) ranges from 14 to 3,400 mg/L of water (ECETOC, 1996). Rainbow trout and zebra fish are the most sensitive species tested to date. The LC_{50} for most fish ranged between 200 and 1,000 mg/L of water. Eleven infants fed formulae that were accidentally prepared with a 2.5 percent boric acid solution for 1 to 5 days (2 to 14.06 g boric acid, or (0.350 to 2.458 g boron total) exhibited diarrhea, vomiting, erythema, exfoliation, desquamation of the skin, and central nervous system irritation; five of the infants died (Wong et al., 1964).

Chronic

Table 7-1 summarizes the doses and effects of a chronic consumption of high or nonphysiologic amounts of boron by various animals and humans. Table 7-1 indicates that intakes of boron in the form of boric acid and borates that are approximately 100- to 1,000-fold greater than normal are needed to induce reproductive and developmental toxicity in animals. Thus, other than waterfowl in specific habitats, it is unlikely that boron toxicity under normal environmental conditions is a concern for animals. High concentrations of boron have been found in irrigation drainwaters and food consumed by waterfowl in certain areas of the western United States, such as the San Joaquin Valley and Kesterson National Wildlife Refuge in California. Reported waterfowl food boron concentrations (mg/kg dry weight) in these regions include aquatic insects, 150; algae, 400; wetland plants, up to 1,860; and seeds, up to 3,500 (ECETOC, 1996). An excellent summary of the fairly extensive human toxicity reports has been given by Culver and Hubbard (1996). Prolonged consumption of boric acid by humans has been reported to result in mild gastrointestinal irritation, anorexia, disturbed digestion, nausea, vomiting, and an erythematous rash. Seven infants provided soothers dipped in a proprietary borax and honey mixture containing about 3 g B/28 mL (23 mg boron/mL) for 4 to 10 weeks (calculated to be about 30 to 90 mg B/day) suffered from seizures that stopped upon cessation of use of the mixture (O'Sullivan and Taylor, 1983).

Factors Influencing Toxicity

Interaction with polyhydroxyl compounds can affect the response to high intakes of boron. Substances possessing *cis*-hydroxyl groups on adjacent carbons form very stable diesters with boron that are almost undissociable in water. This indicates why supplementing the diet with riboflavin, a dihydroxy compound, protects chickens against boric acid toxicity (Roe et al., 1972). The toxicity of boron to fish is increased with increasing water hardness (Birge and Black, 1977). High dietary selenium (15 and 60 mg/kg diet) and low dietary protein (7 percent compared to 22 percent of the diet) increased the toxicity of boron in mallard ducklings. Feeding 1 g B/kg diet to ducks enhanced the accumulation of toxic amounts of selenium in liver caused by low dietary protein when dietary selenium was high (Hoffman et al., 1991).

TISSUE LEVELS

Boron is distributed throughout the soft tissues and fluids at concentrations mostly between 0.015 and 2.0 mg/kg wet weight. Table 7-2 shows representative values in various

organs and fluids from various animals and humans. The values show that tissues from animals fed nontoxic amounts of boron are not of potential concern for human boron toxicity. As with other mineral elements, overcoming homeostatic mechanisms by high boron intakes will elevate tissue and blood boron concentrations. However, the values in Table 7-2 indicate that no animal tissue or fluid used as a food will accumulate boron to the extent that it would be of potential toxicological concern for humans. The uptake and retention of boron are highest in bone.

MAXIMUM TOLERABLE LEVELS

The most sensitive indicators of boron toxicity in animals have been decreased weight and rib anomalies in the developing rat fetus which occurs with intakes of about 13 mg B/kg BW/day (IPCS, 1998). Thus, 10 mg B/kg BW/day may be a reasonable MTL for animals; this translates into about 135 mg/kg diet. Other effects of boron toxicity and the dose at which they occur in mg/kg BW/day include further rib anomalies in fetuses and testicular pathology in growing rats at about 25; decreased fetal body weight and increased fetal cardiovascular malformations in the rabbit, and severe testicular pathology in the rat at about 40; testicular atrophy and sterility in the rat at about 55; and decreased fetal body weight in the mouse at 80 (IPCS, 1998). Mallard duckling growth was adversely affected with dietary boron concentrations of 30 and 300 mg/kg, and survival was reduced at 1,000 mg/kg (IPCS, 1998). Based on studies with reconstituted water, the NOAEL concentration for early life stages of rainbow trout, considered the most sensitive fish species, has been found to range from 0.009 to 0.103 mg B/L. However, studies using natural water collected from three sources containing a variety of boron concentrations, including 0.75 mg/L in the Firehole River in Yellowstone National Park, showed no adverse effects on embryo-larval stages of rainbow trout (Black et al., 1993). Lowest observed effect (detectable change in embryo-larval morphology or survival) concentrations ranged from 1.10 to l.73 mg B/L for the three sources of natural water. Therefore, under natural conditions, the no-effect-level for fish is apparently around 1 mg B/L (ECETOC, 1996).

HUMAN HEALTH

Based on developmental effects in rats, mice, and rabbits, the acceptable upper limit of daily intake of boron has been estimated to be in the range of 11 to 20 mg/day for adults (Murray, 1996; WHO, 1996; NRC, 2001). However, the NOAEL for humans can be established at 1 g boric acid (175 mg B)/day for adults, or about 2.5 mg B/kg BW/day, and the chronic-adverse-effect level is 5 mg B/kg BW/day (Culver and Hubbard, 1996). These relatively high values indicate why boric acid and borates were used for many years as food preservatives without apparent widespread toxicity. This practice stopped when boric acid and borates became considered as poisons because of numerous reports of toxic effects after accidental and inappropriate domestic and medical misuse of these compounds. Thus, intakes of boron at or below the MTL suggested above for animals would not be expected to result in tissue boron concentrations that would be of concern for human health. The guide level for drinking water in Europe of 1 mg B/L is considered conservative with an ample safety margin (ECETOC, 1994). Respiratory exposure to boron in industry has not caused chronic pulmonary effects, and skin exposure does not cause dermatitis (Culver and Hubbard, 1996).

FUTURE RESEARCH NEEDS

The toxicity of boron for captive animals has been relatively well studied. However, there is a need to more clearly define the mechanisms of boron toxicity. Also, more needs to be known about the effects of boron at low levels of intake, including establishing the basis for its apparent essential or beneficial actions, and the intake below which these actions are compromised. The mechanisms through which boron is absorbed from the gastrointestinal tract need further definition. Further knowledge in these areas should help identify interactions with other nutrients and the environment that could modify the toxic and beneficial actions of boron.

SUMMARY

Boron is always found in nature bound to oxygen or as borates. Boric acid is the prevalent form of boron in animals and humans. It is a weak Lewis acid that forms complexes with biological substances containing *cis*-hydroxyl groups. Boron is accepted as essential for plants because it is required to complete their life cycle. To date, only one essential function has been found for boron in plants; it cross-links two dimers of rhamnogalacturan-II, a small structurally complex pectic polysaccharide found in primary cell walls. Although boron has been found essential to complete the life cycle of some animals (frog, zebrafish), it is not generally accepted as essential, apparently because it lacks a defined biochemical function, for higher animals and humans. Analytical techniques that utilize inductively-coupled plasma have become preferred for the analysis of boron in biological materials. Ashing of samples is a critical step in the accurate analysis of boron in low amounts because of contamination and volatilization concerns. About 85–90 percent of dietary boron is rapidly absorbed and excreted mostly in the urine. Toxic amounts of boron can be absorbed through damaged, but not intact, skin. The diet and sometimes drinking water are the major sources of exposure to boron. Boron is a relatively nontoxic element. Reported oral LD_{50} values for boron in mg/kg BW are in the range of 400–700 for mice and rats, 210 for guinea pigs, and 250–350 for dogs. Cattle con-

suming water containing 150–300 mg B/L exhibited toxicity signs including decreased food consumption and weight. Boron concentrations found lethal to 50 percent of tested freshwater fish ranged from 14 to 3,400 mg/L of water. This large range occurred because rainbow trout and zebrafish were much more sensitive than most other fish tested, and one species was very nonsensitive. The LC_{50} for most fish ranged between 200 and 1,000 mg/L of water. Chronic toxicity studies indicate that intakes of boron in the form of borates and boric acid approximately 100- to 1,000-fold greater than normal are needed to induce reproductive and developmental toxicity in animals. The most sensitive indicators of boron toxicity in animals have been decreased weight and rib anomalies in the developing rat fetus which occurs with intakes of about 13 mg/kg BW/day. Thus, other than for waterfowl and fish in some specific habitats, it is unlikely that boron toxicity under normal environmental conditions is a concern for animals.

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TABLE 7-1 Effects of Boron Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	5	Adult	175 mg/kg 1,750 mg/kg	Borax and Boric acid	90 d	Diet	No adverse effects Decreased hemoglobin, testes and spermatogenic epithelium atrophy, thyroid and adrenal histological changes	Weir and Fisher, 1972
Dogs	8	Adult	117 and 350 mg/kg 1,170 mg/kg	Borax and Boric acid	2 yr 38 wk	Diet	No adverse effects Testicular degeneration and spermatogenesis cessation	Weir and Fisher, 1972
Mice	19-20 pairs	11 wk	175 mg/kg 787.5 mg/kg 1,575 mg/kg	Boric acid	14–27 wk	Diet	No adverse effects Impaired fertility, decreased sperm number and motility, litter size, and weight. Infertile, decreased weight	Fail et al., 1991
Rats	10 10 10 10	81-119 g	175 mg/kg 525 mg/kg 1,750 mg/kg and 5,250 mg/kg	Borax and Boric acid	90 d	Diet	No effect concentration Some testes atrophy Decreased food consumption and growth, atrophy of testes, paw and tail skin desquamation	Weir and Fisher, 1972
Rats	70 and 70 70	81–119 g	117 mg/kg and 350 mg/kg 1,170 mg/kg	Borax and Boric acid	2 yr	Diet	No adverse effect on fertility, lactation, litter size, weight, and appearance Atrophied testes, decreased growth and hemoglobin, skin desquamation, not fertile	Weir and Fisher, 1972
Rats	6	30 d	75 mg/L	Borax	45 d	Water	No adverse effect	Green et al., 1973
Rats	11	30 d	150 mg/L	Borax	45 d	Water	Reduce testis size with irreversible pathology	Green et al., 1973
Rats	11 6	21 d 30 d	300 mg/L	Borax	49 d 45 d	Water	Decreased body size, prepubescent fur, non-pigmented incisors, aspenrnia, impaired ovarian development	Green et al., 1973
Rats	18	200–250 g	500 mg/kg 1,000 mg/kg and 2,000 mg/kg	Borax	30–60 d	Diet	No adverse effect Gonadal tissue changes, reduced fertility (irreversible after 60 d of 2000 mg/kg diet)	Lee et al., 1978; Dixon et al., 1979
Rats	15	Weanling	150 mg/L 300 mg/L	Borax	70 d	Water	Decreased fat in bone and plasma Decreased weight, atrophic scrotal sacs, coarse haircoat, decreased fat and calcium in bone	Seal and Weeth, 1980

Rats	360	60–70 d, 200–220 g	525 and 788 mg/kg 1,050 and 1,575 mg/kg	Boric acid	Killed weekly for 9 wk	Diet	Inhibited spermiation; resolved after 16 weeks fed low-boron diet Decreased weight gain and food consumption at 15,75 mg/kg diet; testicular atrophy; did not resolve after 32 weeks fed low-boron diet	Ku et al., 1993; Chapin and Ku, 1994
Rats	60 60 60	Time-mated females; unborn pups examined	131 mg/kg 175 mg/kg 350 mg/kg	Boric acid	20 d	Diet	No effect Decreased growth, rib anomalies, did not persist with 20 d low-boron diet Decreased growth, rib anomalies that persisted after 20 d low-boron diet	Price et al., 1998
Ducks, mallard	30 30	Adult	300 mg/kg 1,000 mg/kg	Boric acid	~42 d	Diet	No adverse effects Decreased hatching success, weights, and duckling survival	Smith and Anders, 1989
Ducks, mallard	10 10 10	Day-old	100 mg/kg 400 mg/kg 1,600 mg/kg	Boric acid	10 wk	Diet	No adverse effects Less active; decreased brain ATP Decreased growth, some mortality, less active, decreased brain ATP	Hoffman et al., 1990
Cows	2	Lactating adults	145–157 mg/kg	Borax	42 d	Diet	No adverse effect	Owen, 1944
Cows	12	Yearling, 290 kg	150 mg/L	Borax	30 d	Water	Decreased feed consumption, weight loss, edema and inflammation of legs	Green and Weeth, 1977
Fish, large-mouth bass		Ernbryo-Larval	>1.39 mg/L 12.17 mg/L 92 mg/L	Boric acid	11 d	Water, recon-stituted	No effect concentration Some mortality and teratogenesis Lethal to 50% of fish	Black et al., 1993
Fish, channel catfish		Ernbryo-Larval	22–155 mg/L	Boric acid	9 d	Water	Lethal to 50% of fish in hard and soft reconstituted water	Birge and Black, 1977
Fish, channel catfish		Ernbryo-Larval	71–155 mg/L	Borax	9 d	Water	Lethal to 50% of fish in hard and soft reconstituted water	Birge and Black, 1977
Fish, rainbow trout		Ernbryo-Larval	79–100 mg/L	Boric acid	32 d	Water	Lethal to 50% of fish in hard and soft reconstituted water	Birge and Black, 1977
Fish, rainbow trout		Ernbryo-Larval	27–54 mg/L	Borax	32 d	Water	Lethal to 50% of fish in hard and soft reconstituted water	Birge and Black, 1977
Fish, rainbow trout		Ernbryo-Larval	0.1 mg/L 138 mg/L	Boric acid	32 d	Water	Low effect concentration Lethal to 50% of fish in hard reconstituted water	Black et al., 1993
Fish, rainbow trout		Embryo-Larval	0.10 mg/L 1.34 mg/L	Boric acid	36 d	Water	No effect concentration Low effect concentration	Birge et al., 1984

continued

TABLE 7-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Fish, rainbow trout		Embryo-Larval	1.10–1.73 mg/L	Borate	36 d	Water	Lowest effect concentrations in three sources of natural water	Black et al., 1993

[a]Number of animals per treatment.
[b]Mineral concentrations are expressed as mg/kg diet. SI conversion: 1 mg boron equals 92.6 µmoles boron.

TABLE 7-2 Boron Concentrations in Fluids and Tissues of Animals (mg/kg or Mg/L)[a]

Animal	Boron Intake	Egg/ Milk	Plasma/ Serum	Skeletal Muscle	Liver	Heart	Kidney	Bone	References
Humans	Low Normal		0.020–0.067 0.028–0.075		0.08–3.0 0.30–6.52 DW	0.12–4.83 DW	0.1–5.5 0.48–8.4 DW	0.4-–.3	Shuler et al., 1990; Ward, 1993; Nielsen and Penland, 1999
Mice	Low Normal				0.007–0.034 0.007–0.163			0.270–0.522 0.440–0.620	Lanoue et al., 1999
Rats	Low Normal High Toxic		 NQ–1.05 8.30–24.06 16	0.194–0.252 DW 0.188–0.75 DW 14.23	0.37–0.41 DW 0.30–1.13 8.68–27.60 13.13	 0.23–1.15 11.3–20.3	 0.38–3.37 15.80–44.20 19.80	0.21–0.45 DW 1.3–1.6 DW 47.40	Nielsen, 1998; Naghii and Samman, 1996; Bai and Hunt, 1996; Ku et al., 1991
Chickens	Low Normal High Toxic	 0.12	0.051–0.077 0.152–0.177	 0.46–0.69 DW 2.93–13.65 DW 13.78–20.18 DW	 0.49 DW 2.41–8.15 DW 14.08 DW			0.25-0.61 DW 0.40-1.33 DW 3.18–6.70 DW 10.73 DW	Hunt, 1989; Wilson and Ruszler, 1998; Anderson et al., 1994
Ducks	Normal High Toxic	NQ[b] 3–13 DW 49			NQ NQ–3 DW 33–51 DW				Smith and Anders, 1989
Cows	Normal Toxic	0.13-0.23	0.53 11.2–18.9	<0.05	<0.07				Green and Weeth, 1977; Anderson et al., 1994
Fish	Normal			0.24					Anderson et al., 1994

[a]Values are on a wet weight basis, unless indicated as DW (dry weight).
[b]NQ = not quantifiable.

8

Bromine

INTRODUCTION

Bromine (Br) is a halogen and the only nonmetallic element that is a liquid at ambient temperature and pressure. Naturally occurring bromine is found bound to metals in the form of inorganic salts—the bromides. Seawater is the greatest reservoir for bromine on Earth and contains 65 mg/L. The concentration in brine or salt lakes may be enriched by up to 100-fold. Bromine is abundant in saline deposits originating from evaporated lakes. Bromine levels in soils typically range from 1 to 20 mg/kg, although some volcanic soils have considerably higher levels.

Bromine-containing compounds have a variety of agricultural, medicinal, and industrial uses (Lyday, 2002). Methyl bromide is used to fumigate the soil for controlling insects, nematodes, weeds, and pathogens in more than 100 food crops and in forest and ornamental nurseries. This gaseous form of organic bromide is also used for postharvest protection and quarantine treatments of foods, feedstuffs, and wood products in warehouses, ship holds, freight cars, and other areas of storage. Methyl bromide was defined by the Montreal Protocol of 1991 as a chemical that contributes to depletion of the Earth's ozone layer, so most countries have agreed to phase out its use. Potassium bromate is used in the baking industry to help bread rise in the oven and to create a good texture in the finished product. Bromine is also used in the production of brominated vegetable oils that are used as emulsifiers in some soft drinks and fruit drinks.

Many bromide-containing drugs were developed in the early 20th century and used as sedatives and anticonvulsants, but they have mostly been replaced by other drugs. However, bromide salts are still commonly used to treat epilepsy in dogs (March et al., 2002). Bromine is used to estimate the extracellular space in physiology research. Industrial uses of bromine-containing compounds include drilling fluids, fuel additives, photographic chemicals, rubber additives, and polybrominated biphenyls used as flame retardants. These industrial compounds are not expected to be found in feed ingredients or water consumed by animals and are not considered in this analysis.

ESSENTIALITY

Several studies have demonstrated improved growth in chicks and mice fed diets supplemented with bromide, but evidence is conflicting and not robust. There are no known essential biochemical functions of bromine and it is not usually considered as an essential nutrient (NRC, 1980).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Total bromide in foods is usually measured following ashing in alkali (Greve, 1983) and determining the bromide ion content using gas chromatography or high performance liquid chromatography (HPLC). Ashing converts organic forms of bromine into inorganic salts.

Bromine introduced into foodstuffs by fumigation with methyl bromide is unstable and converts to inorganic forms rapidly over time. However, some conditions may result in trace levels of unconverted fumigant. Given the differing toxicological profiles of organic and inorganic forms, their analytical differentiation may be desirable. Procedures for the separation of organic bromides by extraction permit the measurement of both organic and inorganic bromide concentrations (Getzendaner, 1975).

REGULATION AND METABOLISM

Bromide has very similar physiochemical properties as chloride and has a comparable metabolic profile (reviewed by van Leeuwen and Sangster, 1987). Like chloride, bromide is very efficiently absorbed from the gastrointestinal tract and found predominantly in the extracellular fluids, gastric secretions, and saliva. Urine is the primary excretory pathway for bromide (Pavelka et al., 2000). The glomerulus

filters bromide, which competes with chloride for reabsorption in the tubules. Most of the bromide in saliva and gastric juices is reabsorbed in the small intestine, and little bromide is found in the feces.

Steady state pharmokinetics of bromide can be described by a simple one-compartment model with a linear relationship between plasma bromide concentrations and the ingested dose (van Logten et al., 1973; van Logten et al., 1974; Rauws, 1975). The half-life of bromide at normal dietary chloride levels is 10–12 days in humans (van Leeuwen and Sangster, 1987), 15 days in dogs (March et al., 2002), and 3 days in rats (Rauws and Van Logten, 1975). The half-life is strongly dependent upon the dietary chloride level and is increased 10-fold by a chloride deficiency and decreased similarly by dosing chloride. Thus, sodium chloride is used to treat bromide toxicosis.

Bromide replaces chloride in intra- and extracellular fluids, and hypochloremia is diagnostic of bromide toxicity. The hydrated bromide ion is slightly larger than the hydrated chloride ion and has slightly different transport kinetics across the cell membrane. Bromide causes hyperpolarization of nerve cell membranes, which is thought to be the basis of its pharmacological properties on the central nervous system.

The thyroid gland partially discriminates between iodine and bromide, but bromide levels are often higher in this tissue than others, especially during an iodine deficiency. Thyroid peroxidase does not oxidize bromide, and bromine does not substitute for iodine in thyroxine (Hosoya, 1963). However, high levels of bromide decrease iodine uptake by the thyroid and may exacerbate an iodine deficiency (Pavelka et al., 2002). Conversely, dietary bromide reduces the toxicity of iodine in chicks (Baker et al., 2003).

SOURCES AND BIOAVAILABILITY

Bromine is not typically supplemented to the diets of animals. Dietary levels result from background levels in feed ingredients and contaminating levels introduced by the use of bromine-based fumigants and disinfectants.

Natural bromine levels in crops range from approximately 8 to 50 mg/kg dry matter (summarized in van Leeuwen and Sangster, 1987). Marine plants are the exception and some types of algae can concentrate very high levels of bromine. Fishmeal has the highest natural bromide level (12.6 mg/kg) of feedstuffs normally used in animal diets (Greve, 1983).

Fruits, grains, and vegetables grown in soils fumigated with methyl bromide may have higher levels of bromide. Bromide residues are especially high when planting occurs closely after soil fumigation (Roughan and Roughan, 1984) and can reach levels up to 4,037 mg/kg dry weight in foliage vegetables (Maw and Kempton, 1982). However, when good agricultural practices are followed, most foods have levels below 40 mg/kg (Greve, 1983).

Postharvest fumigation of foods and feed ingredients can result in high levels of bromide residues. Fumigation of feed grains and hay, especially during shipment or storage, is currently a common practice. Residue levels in fumigated wheat of 70 mg/kg (Corvi et al., 1977) and in corn fumigated in a ship's hold of 200 mg/kg have been reported (Thompson and Hill, 1969).

Other dietary sources of bromine include salt that has been prepared from brines containing high levels of bromide. Bakery wastes may contain trace levels of potassium bromate added in the baking process. Polybrominated biphenyls and other industrial chemicals have contaminated animal feeds due to mislabeling or mishandling; these isolated events are increasingly rare due to improvements in material handling practices (Reich, 1983).

Bromide salts are presumed to be rapidly and completely absorbed in the gastrointestinal tract using transport systems for the chloride ion. Bioavailability studies have not been formally conducted, but 96 percent of an oral bromide dose is absorbed in humans (Vaiseman et al., 1986).

TOXICOSIS

The toxicological profile of bromine depends on its form. Currently, most toxic studies on bromine concern inhalation toxicity of methyl bromide gas to human applicators. Though this gas is highly toxic, it is not a known contaminant of animal feeds or water and is not currently of concern for animal safety. Inorganic bromide salts that results from the decomposition of methyl bromide following fumigation of hay, feed ingredients, or occasionally complete feeds is the primary source of bromine in animal diets (Knight and Costner, 1977; Knight and Reina-Guerra, 1977). Data on which to base the toxicity of bromide in animal feeds come mostly from studies in humans and laboratory animals, with only a few studies available with production or companion animals (Table 8-1). The historical medical use of bromide salts at relatively high levels compelled extensive research in the first half of the 20th century on bromide intoxication (bromism). Toxicity of bromide depends on its replacement of chloride in body fluids. Because of the large pool size of body chloride, expression of bromide toxicosis typically occurs only after chronic exposure to relatively high levels of bromide (Trump and Hochberg, 1976).

Potassium bromate is more toxic than bromide salts due to its oxidizing properties. It also is a carcinogen (Kurokawa et al., 1986; IARC, 1986; DeAngelo et al., 1998), but it is not a known contaminant of animal feeds. Polybrominated biphenyls are known liver carcinogens in rats at levels of around 10 mg/kg (NTP, 1993).

Single Dose

Bromide salts irritate the gastric mucosa and cause nausea and vomiting (Trump and Hochberg, 1976). The result-

ing feed refusal coupled with the pharmokinetics of bromide in the body make it unlikely that systemic toxicosis would occur when animals are presented with a single meal of a feed containing very high levels of bromine.

Acute

The acute (48-hour) NOAEL for bromide as sodium bromide in water for guppies and medaka is 78 and 250 mg/L (Canton et al., 1983). Lethality (LC_{50}) occurs at 16,000 and 25,000 mg/L, respectively. Orally dosed bromide salts have very low acute toxicity in rodents and an LD_{50} of 3.5 to 7 g Br/kg BW has been reported (Smith and Hambourger, 1925; Voss et al., 1961).

Chronic

In humans, symptoms of chronic bromide intoxication are apathy, headaches, ataxia, memory loss, drowsiness, and loss of emotional control. Dermal symptoms, including acne or a nodular rash, may also occur. Tremors, hallucination, stupor, and coma occur in severe cases (reviewed in van Leeuwen and Sangster, 1987). Shifts in EEG activities, increased circulating thyroxine, and other endocrine changes are the primary physiological disruptions of a chronic toxicosis (Sangster et al., 1983). A summary of studies in humans indicates that the daily NOAEL for bromide in humans is 4 mg/kg BW (van Leeuwen and Sangster, 1987). The Food and Agriculture Organization/World Health Organization (FAO/WHO Pesticide Committees, 1967) applied a margin of safety and set the acceptable daily intake of bromide at 1 mg/kg.

The most sensitive indicators of bromide toxicosis in rodents are changes in behavior and weight gain (Hansen and Hubner, 1983). In mice, the NOAEL is between 400 and 1,200 mg/kg diet (Hansen and Hubner, 1983). In rats, disturbances in thyroid and renal function occur at dietary levels between 1,200 and 19,200 mg/kg diet (Loeber et al., 1983) and a decrease in fertility occurs at 1,200 mg/kg (van Leeuwen et al., 1983). A chronic 2-year study using feed fumigated with methyl bromide found that residual bromide levels of 500 mg/kg diet were not toxic to rats as indicated by normal livability and normal histopathology, but this level did cause a small reduction in body weight; 200 mg/kg had no effect on body weight (Mitsumori et al., 1990).

Potassium bromide is frequently used as therapy for epileptic dogs, and toxicosis occurs occasionally (Yohn et al., 1992; Podell, 1998). Characteristic clinical signs are ataxia and lameness, especially of the pelvic limbs, but the maximum tolerable level has not been determined.

Bromide toxicity is treated by removal of the bromide source and administering sodium chloride. In humans, complete recovery follows normalization of blood bromide levels.

Factors Influencing Toxicity

The primary factor affecting toxicity of bromide is the dietary chloride level because high dietary chloride increases the renal excretion of both chloride and bromide. For example, the daily bromine intake needed to maintain near toxic levels of serum bromide is about twice as high for dogs fed 1.3 percent dietary chloride as those fed 0.4 percent and three times as high as those fed 0.2 percent (Trepanier and Babish, 1995). In rats the biological half-life of bromide increases from 3 days at normal dietary chloride levels to 25 days when sodium chloride is excluded from the diet (Rauws and Van Logten, 1975).

TISSUE LEVELS

In rats, plasma and tissue bromine levels increase linearly with dietary levels. Bromide replaces about half of the chloride in plasma and tissues at a dietary level of 19,200 mg/kg (van Logten et al., 1974). Lynn et al. (1963) found that dairy cows fed 9.5 to 38 mg/kg dietary bromide as NaBr have milk levels ranging from 1 to 12 mg/kg on a wet weight basis (Table 8-2). Bromide in cow's milk was proportional to the level in the blood, which was proportional to the level in the feed. More recently, Vreman et al. (1985) reexamined the relationship between dietary and milk bromide levels. Diets contained 22, 69, or 115 mg/kg inorganic bromide residue that resulted from the decomposition of methyl bromide fumigate and were fed for 5 weeks. Milk bromide increased linearly with dietary bromide, and the average ratio between dietary bromine concentration and milk bromine concentration was 0.27. At the end of the study, tissues were sampled and muscle bromide concentrations increased linearly with dietary bromide, with the 115 mg/kg dietary level giving 20.8 mg/kg fresh muscle.

MAXIMUM TOLERABLE LEVELS

The MTL of bromine is defined as the dietary level that, when fed for a defined period of time, will not impair accepted indices of animal health or performance. Bromide is the mostly likely form of bromine that animals are exposed to through their feed and water. No adverse effects of bromide at levels of 300 mg/kg diet or below have been reported in chronic feeding studies in rodents, and levels of 500 mg/kg slightly reduce growth but do not cause pathology. Hatchling chicks tolerate 5,000 mg/kg for 4 weeks without impaired growth or efficiency of feed conversion. Dogs tolerate 20 mg/kg BW/day with no adverse effects. Maximum tolerable levels have not been determined for other animal species.

HUMAN HEALTH

Tissue levels of bromine in farm animals have not been determined at dietary levels that are toxic. However, extrapolation of muscle bromine concentrations known to occur in animals fed subtoxic levels to animals fed maximum tolerable levels indicates that residue levels would not be of concern for human health. The daily NOAEL for bromide in humans is 4 mg/kg BW (van Leeuwen and Sangster, 1987).

FUTURE RESEARCH NEEDS

Bromine toxicity has not been a problem in animal nutrition so very little research has been done. Accurate estimates of maximum tolerable dietary levels cannot be made for any species except rodents, and more research is needed. Residue levels in meat, milk, and eggs of animals fed maximum tolerable dietary levels of bromide are also needed. Finally, there are insufficient data available to estimate maximum tolerable bromide levels in water of any species.

SUMMARY

Bromine is widely distributed in nature and is found almost exclusively as bromide salts. There are no known essential biochemical functions of bromine, and it is not usually considered as an essential nutrient. Bromine is not typically supplemented to the diets of animals and dietary levels result from background levels in feed ingredients and contaminating levels introduced by the use of bromine-based fumigants and disinfectants. Bromide has very similar physiochemical properties as chloride and has a comparable metabolic profile and tissue distribution. Bromide competes with chloride in metabolism and causes hyperpolarization of nerve cell membranes, which is thought to be the basis of its pharmacological properties on the central nervous system. Because of the large pool size of body chloride, expression of bromide toxicosis typically occurs only after chronic exposure to relatively high levels of bromide. It is rapidly excreted, primarily in the urine, so toxic levels decline quickly after the source has been removed.

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TABLE 8-1 Effects of Bromine Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	6	11.3 kg, adult	20 mg/kg/d	KBr	115 d	Diet	No adverse effects, normal behavior	March et al., 2002
Rats	4 4 4 4	110–130 g	300 1,200 4,800 19,200	NaBr	28 d	Diet	No adverse effects No adverse effects No adverse effects Increased relative kidney weight, behavioral effects	van Logten et al., 1973
Rats	20 20 20 20 20	50–60 g	75 300 1,200 4,800 19,200	NaBr	90 d	Diet	No adverse effects No adverse effects Increased relative thyroid weight Increased relative thyroid weight Increased relative thyroid, spleen, and arenal, weight, behavioral effects	van Logten et al., 1974
Rats, female	3	200 g, 14 d prior to mating	1,000 mg/L	Bromide (salt not specified)	16 d after delivery	Water	No effect on maternal weight or milk production, decreased pup weight, decreased transfer of iodine to pups, decreased serum thyroxine in dams and pups	Pavelka et al., 2002
Rats	120 120 120 120	40 d	80 200 500 500	Inorganic bromide[c] KBr	2 yr	Diet	No adverse effects No adverse effects Decrease body weight of males, but not females No adverse effects	Mitsumori et al., 1990
Chickens	29	1 d	8 15	NaBr	31 d	Diet	No adverse effects	Bosshardt et al., 1956
Chickens	20 20 30 90 20	1 d	2,500 5,000 5,000 (low fat) 10,000 20,000	NaBr	28 d	Diet	No adverse effects No adverse effects Reduced weight gain Reduced weight gain and increased mortality 100% mortality	Doberenz et al., 1965
Swine	8	20 kg	200	Equimolar mix of NaBr, NH_4BR, KBr	To 90 kg	Diet	No adverse effects	Barber et al., 1971
Cattle, lactating, dairy	4	Unspecified	19 43	Inorganic bromide[c] NaBr	72 d	Diet	No adverse effects No adverse effects	Lynn et al., 1963

[a]Number of animals per treatment group.
[b]Quantity in mg Br/kg of feed (dry matter basis) unless otherwise noted. SI conversion: 1 mg bromine equals 12.5 µmoles bromine.
[c]Inorganic salts from the decomposition of methyl bromide used to fumigate the feed.

TABLE 8-2 Bromine Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)[a]

Animal	Quantity[b]	Source	Duration	Route	Plasma/ Serum	Muscle	Liver	Kidney	Milk	Reference
Dogs	20 mg/kg/d	KBr	115 d	Diet	200					
Rats	0	NaBr	28 d	Diet	16		8	56		van Logten et al., 1973
	300				160		96	112		
	1,200				647		248	415		
	4,800				1,998		719	1,278		
	19,200				4,474		1,678	2,716		
Rats	0	NaBr	28 d	Diet	16			8		van Logten et al., 1974
	75				48			24		
	300				168			96		
	1,200				615			336		
	4,800				1,998			1,119		
	19,200				4,075			2,397		
Cattle, lactating, dairy	10	NaBr	22 d	Diet					3	Lynn et al., 1963
	19		19 d						5	
	43		28 d						12	
	10	Inorganic bromide[c]	39 d		15				7	
	19		39 d		33				8	
	43		39 d		68				15	
Cattle, lactating, dairy	22	NaBr	30 d	Diet	21	3	4	14	6	Vreman et al., 1985
	69				58	9	12	31	17	
	115				95	2	27	88	31	

[a]Values are on a wet weight basis.
[b]Quantity in mg Br/kg of feed (dry matter basis) unless otherwise noted.
[c]Inorganic salts from the decomposition of methyl bromide used to fumigate the feed.

9

Cadmium

INTRODUCTION

Cadmium (Cd) is a soft metal with a silver-white color. It has an atomic number of 48, a molecular weight of 112.41, and eight naturally occurring isotopes. It is a divalent 4d transition metal with chemical properties that are similar to zinc. The pure metal crystallizes in distorted but closely packed structures with relatively weak bonding. Consequently, it is soft enough to cut with a knife and for a metal has a low melting point of 321°C. Cadmium has a relatively high vapor pressure, and the vapor released from cadmium metal quickly forms a surface oxide layer when exposed to air. Cadmium is reactive with halides, sulfur, oxygen, and phosphorus, but is unreactive toward carbon, hydrogen, or nitrogen (Butterman and Plachy, 2004).

Cadmium is the 63rd most abundant element in the Earth's crust (0.000016 percent) and is naturally found in association with zinc and, to a lesser extent, with lead and copper. It is extracted mainly as a by-product of zinc mining and processing. In 2000, it was refined in 27 countries, with production dominated by Asia, followed by Europe and then North America. World production in 2000 was 19,700 metric tons, but production is declining due to an increase in the recovery of cadmium by recycling and lower demand because of regulatory pressure to find less toxic substitutes (Plachy, 2002).

Cadmium forms numerous alloys and compounds with other elements and is used in batteries, solders, semiconductors, solar cells, plastic stabilizers, and to plate iron and steel. Cadmium salts have a wide spectrum of vivid colors and are used as pigments in a variety of applications such as coloring plastics and ceramics. However, this use is declining because of replacement by less toxic pigments. End uses for cadmium in 2002 were batteries, 78 percent; pigments, 12 percent; coatings and platings, 8 percent; all other, 2 percent (Plachy, 2002). Cadmium can enter the environment from a variety of anthropogenic sources including by-products from zinc refining, coal combustion, mine wastes, electroplating processes, iron and steel production, pigments, fertilizers, and sewage sludges.

Sulfate, nitrate, chloride, and acetate salts of cadmium are very soluble in water. Cadmium oxide, hydroxide, and sulfide have very low solubilities. Organo-cadmium compounds are not known to occur in nature; however, cadmium readily forms complexes with proteins, organic acids, and other organic compounds.

ESSENTIALITY

Cadmium is not considered an essential nutrient for animals. However, a number of studies with rodents, chickens, and livestock have reported increased weight gain when low levels of cadmium were added to the diets (Bokori and Fekete, 1995). The bases for these effects are unknown and may be the result of antibiotic or pharmacologic actions. Cadmium is an essential nutrient for *Thalassiosira weissflogii*, a common marine diatom. In this organism cadmium is a cofactor for an isoform of carbonic anhydrase that is needed under conditions of low zinc, which are typical of many marine environments (Lane and Morel, 2000). Higher plants have not been shown to need cadmium.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Cadmium levels in feeds and tissues are most commonly measured by atomic absorption spectrophotometry or inductively-coupled atomic emission spectroscopy. Both of these techniques have sufficient sensitivities to monitor cadmium levels of concern in toxicosis (ATSDR, 1999). Atomic absorption spectrometry can detect cadmium in foods and tissues at approximately 10 μg/kg. Samples are usually prepared by digestion with nitric acid and microwave digestion techniques give excellent recoveries. Dry ashing is also commonly used for preparing foods for cadmium analysis and little loss of cadmium occurs at temperatures below 450°C (IPCS,

1992a). Cadmium is a relatively ubiquitous element and careful attention to possible contaminating sources is needed. In particular, colored plastics such as pipet tips can be a source of cadmium contamination. Determining low levels of cadmium in biological specimens requires special attention to background detection (Cerny and Bhattacharyya, 2003).

Cadmium concentrations in tissue of living animals can be measured by neutron activation analysis or x-ray fluorescence. These techniques take advantage of the capture characteristics of the thermal neutrons of the naturally occurring stable isotope ^{113}Cd (IPCS, 1992a).

REGULATION AND METABOLISM

Absorption

Cadmium can be absorbed in toxicologically relevant amounts from oral or inhalation exposure. Absorption by dermal exposure is relatively insignificant unless exposure time is very long (Wester et al., 1992). Intestinal absorption of cadmium is relatively low compared to similar divalent cations such as zinc and iron. Absorption of an experimental dose of cadmium after dosage of soluble salts is about 1–2 percent in mice and rats, 0.5–3.0 percent in monkeys, 2 percent in goats, 5 percent in pigs, 5 percent in lambs, and 16 percent in cattle (ATSDR, 1999). Cadmium is absorbed predominantly in the duodenum and proximal jejunum. Specific cellular importers for cadmium have not been identified, and inadvertent uptake through pathways intended for the essential nutrients iron, zinc, manganese, and cysteine seem to mediate cadmium transport across the brush border (Zalups and Ahmad, 2003). For example, cadmium can be absorbed by the divalent metal transporter-1, which normally mediates dietary iron absorption (Bressler et al., 2004). Cadmium bound to peptides and proteins may also enter the enterocyte via endocytosis.

The proportion of cadmium absorbed by the intestines is dependent on the dose, with higher doses being absorbed more efficiently (Matsuno et al., 1991a). High levels of cadmium disrupt tight junctions between intestinal enterocytes and impair epithelial barrier function (Duizer et al., 1999). Thus, cadmium may increase its own bioavailability by causing disruption of the intestinal paracellular barrier. Absorption decreases markedly with chronic exposure (Muller et al., 1986), apparently due to the induction of protective mechanisms such as intraepithelial metallothionein.

The bioavailability of cadmium appears to be related to its solubility in the digestive tract. Highly soluble salts of cadmium, such as chloride, nitrate, acetate, and sulfate, are absorbed much better than poorly soluble forms, such as cadmium sulfide and cadmium sulfoselenide pigments (ATSDR, 1999). Cadmium bound to metallothionein or the cadmium incorporated into pig liver is absorbed by rats less efficiently than cadmium chloride, indicating that cadmium in foods of animal origin may be less available than soluble salts (Groten et al., 1990, 1994), although at low levels, inorganic and organic forms appear to be absorbed at similar efficiencies.

The efficiency of cadmium absorption increases with iron or calcium deficiency (Brzoska and Moniuszko-Jakoniuk, 1998). High dietary levels of calcium, chromium, magnesium, or zinc decrease cadmium uptake (Foulkes, 1985). Cadmium can also decrease the absorption of calcium and exacerbate a deficiency (Fullmer et al., 1980).

Transport and Distribution

After absorption, cadmium is transported in the blood bound primarily to albumin, with lesser amounts bound to globulins, metallothionein, cysteine, glutathione, or directly to cells (Zalups and Ahmad, 2003). It distributes throughout the body with highest concentrations in the kidney and liver, which account for more than half of the total body burden. Initially, the liver takes up the highest amount of cadmium, but over a few days cadmium in the liver is released and taken up by the kidney. Thus, the kidney usually has the highest concentration of cadmium of any tissue in the body. Blood cadmium levels are indicative of recent exposure to cadmium but are not especially indicative of total body burden. Urine levels are a better indicator of total body burden (ATSDR, 1999).

Cadmium is not transported efficiently into milk or eggs. Little cadmium is incorporated into hair, and levels in hair are not sensitive indicators of exposure (Combs et al., 1983). The transport of cadmium across the placenta is also inefficient and fetal levels are considerably lower than maternal levels (Smith et al., 1991a). Pregnant goats fed diets with high levels of cadmium do not pass on sufficient levels of cadmium to their kids during gestation and lactation to raise their offsprings' tissue cadmium levels above normal (Telford et al., 1984b).

Cadmium (+2) readily complexes to anionic groups, especially sulfhydryl groups, in proteins and other molecules. Metallothionein, a low molecular weight protein that is very high in cysteine, is capable of binding up to seven cadmium atoms per molecule. Cadmium is a strong inducer of metallothionein synthesis and cadmium also decreases the degradation rate of this intracellular chelator (Laurin and Klasing, 1990). Cadmium displaces zinc and copper from previously synthesized metallothionein. Metallothionein is important in modulating the fate of cadmium in tissues by affecting the duration of its retention in tissues and reducing its cellular toxicity (Klaassen and Liu, 1998). The kidney is a major site of metallothionein synthesis and consequently accumulates cadmium.

Excretion

Absorbed cadmium is excreted slowly with daily losses of approximately 0.009 percent of the total burden via the

urine and 0.007 percent via feces. Most of the cadmium loss into the intestines is derived from hepatocellular secretion of cadmium into bile. Circulating cadmium-protein complexes are filtered at the glomerulus and then resorbed from the glomerular filtrate by the cells in the S1 and S2 segments of the proximal convoluted tubules (Dorian et al., 1995). This region of the renal cortex concentrates cadmium and is susceptible to necrosis. The half-life of cadmium in rodents is very long and estimates vary from several months to a year or more (ATSDR, 1999). In beagles, the biological half-life of cadmium fed at either 1, 3, 10, or 50 mg/kg diet is between 1 and 2 years (Matsuno et al., 1991b). Because of this low excretory rate, cadmium accumulates with age, and older animals have significant levels of cadmium in their kidneys even if the levels in their diets and water were consistently low.

Mechanism of Toxicity

The toxic effects of cadmium are thought to be caused by free cadmium ions, and cadmium bound to metallothionein is usually less active (Goyer et al., 1989). Specific mechanisms of toxicity vary according to cadmium concentration and cell type but generally are linked to cadmium's disruption of the cellular redox state and to its structural similarities with zinc, calcium, and other divalent cations (Pinot et al., 2000). The redox activity of cadmium depletes antioxidants and glutathione, causes oxidative stress, enhances lipid peroxidation, and perturbs the lipid composition of membranes (Gill et al., 1989; Xu et al., 2003). Cadmium affects the function of glomerular membranes by depleting polyanions and interfering with membrane charge (Cardenas et al., 1992). Cadmium-induced reactive oxygen intermediates can lead to decreased DNA synthesis and strand breaks.

Cadmium ions displace zinc and other metals from the binding sites of many metalloproteins, perturbing their architecture and functions. In the testes, cadmium interferes with zinc-protein transcription factors leading to apoptosis (Xu et al., 1999). Cadmium binding to metal transcription elements induces expression of a variety of genes including metallothionein, heme oxygenase, and heat shock proteins. Cadmium also has estrogenic effects by binding to the estrogen receptor (Stoica et al., 2000), causing reproductive disruption and accelerated puberty in mammals (Johnson et al., 2003). Cadmium may permeate or block calcium channels and acts to mobilize free-calcium in the cell, causing cell-specific disregulation in function.

Mitochondria are especially susceptible to cadmium toxicity. Cadmium binds to thiol groups of proteins in the membrane of this organelle. Thiol group inactivation leads to oxidative stress and membrane depolarization. Impairment in mitochondrial function results in ATP depletion and necrotic death of the cell. In some cell types, cadmium-induced leakage of mitochondrial enzymes leads to apoptotic death (Pinot et al., 2000). Thus, depending on the cell type, cadmium may cause either necrotic or apoptotic cell death.

SOURCES AND BIOAVAILABILITY

Sources

Cadmium is sparsely distributed in the environment and normally ranges in concentrations between 0.1 and 1 mg/kg in the Earth's crust (Elinder, 1992). Levels 10- to 20-fold higher may accumulate in some types of sedimentary rocks. Levels in the oceans range from 0.001 to 0.1 µg/L, with higher levels found in phytoplankton-rich areas. Levels in natural surface and ground water are usually less than 1 µg/L (ATSDR, 1999; Pinot et al., 2000). However, spillage or leaching of water from mines can contaminate surface or ground waters, resulting in much higher levels. Levels in topsoil are usually higher than in subsoils due to atmospheric fallout and contamination. Unpolluted soils in the United States contain an average of 0.25 mg/kg (EPA, 1985). Higher concentrations of cadmium usually result from human activities such as mining, smelting, fuel combustion, and disposal of batteries and other cadmium-containing products. For example, soil levels within 1 kilometer of a smelter were 72 mg/kg, compared with 1.4 mg/kg between 18 and 60 kilometers away. In 1998, there were 776 hazardous waste sites in the United States with focally high levels of cadmium contamination; animals grazing near these sites may consume high levels of cadmium via food or water.

The most likely source of high cadmium exposure for most animals kept in captivity is the mineral supplement used in the feed. In particular, phosphate and zinc sources can be important contributors (King et al., 1992; Linden et al., 1999; Nicholson et al., 1999; Sapunar-Postruznik et al., 2001; Linden et al., 2003). Natural cadmium levels found in phosphates from sedimentary rocks range from 3 to 100 mg/kg, depending upon their location (Singh, 1994). Phosphates from the southeastern United States have approximately 35 mg/kg (IPCS, 1992a). Analysis of 16 calcium phosphates used in animal feeds in the United States found cadmium levels ranging from <1 to 67.3 mg/kg (Sullivan et al., 1994). Calcium phosphate is often added to animal feeds at levels greater than 1 percent of the diet, which can result in dietary cadmium levels that are high enough to cause unacceptably high levels in livers and kidneys intended for human consumption.

Cadmium accumulates in plants grown in high-cadmium soils. Its mobility and bioavailability to plants is highest in acid soils. Cadmium taken up by plants is concentrated in the leaves with lower levels in seeds and roots (He and Singh, 1994), so grazing animals receive the highest exposure (Hansen and Hinesly, 1979). Application of manure, sewage sludge, or phosphate fertilizers can enrich soils in cadmium. Consequently, limits on the maximum cadmium concentration in fertilizers and biosolids applied to land, the maximal loading rate (kg/ha/y) of cadmium applied to land, and monitoring procedures are set by the EPA and many state agencies (ATSDR, 1999; NRC, 2002).

In aquatic and benthic environments, cadmium concentrations are relatively uniform throughout the food web and cadmium does not appear to biomagnify as it moves up the food chain. In freshwater, the cadmium level in animals is dependent on their ability to absorb it from the water rather than their trophic level (IPCS, 1992b).

Cadmium levels in human foods are highest in shellfish, oysters, salmon, and fungi, and are lowest in fruits, dairy products, legumes, meat, and poultry, with the exception of liver and kidney products. Potatoes and leafy vegetables have higher cadmium levels than most other foods of plant origin (ATSDR, 1999).

The toxicity of cadmium is well recognized, and its concentrations are monitored and regulated by a wide variety of government agencies (ATSDR, 1999). Cadmium is considered a hazardous waste, and the EPA regulates levels in industrial and municipal wastewaters, sewage sludge, and air emissions. State public health departments or departments of natural resources monitor cadmium levels in fish and wildlife that are potentially consumed and issue advisories to limit or prevent consumption of species of concern found in contaminated environments. The World Health Organization has set a 1 mg/kg upper limit for cadmium in complete feeds for animals in order to prevent high levels in food products (IPCS, 1992a).

Bioavailability

The bioavailability of various chemical forms of cadmium is dependent upon its chemical form. Cadmium is likely to be in the free ionic form in fresh water, whereas in foods it generally exists in a complex with a variety of ligands, including proteins such as metallothionein. The toxicological properties of cadmium ions do not seem to be dependent on the anion present. Cadmium chloride is the most common form used in animal experimentation because it is highly soluble and has a high bioavailability. In general, the bioavailability of cadmium chloride supplemented to water is similar to that supplemented to food (Ruoff et al., 1994). The cadmium found in a commercial calcium phosphate was found to be somewhat less bioavailable than cadmium chloride to pigs (King et al., 1992). The bioavailability of cadmium in animal tissues is usually less than that of cadmium chloride. For example, cadmium-metallothionein and cadmium in pig liver have lower bioavailabilities and toxicities than cadmium chloride (Groten et al., 1990, 1994). The bioavailability of cadmium in horse kidney is lower than that of cadmium chloride and is not affected by cooking (Lind et al., 2001). The cadmium in the hepatopancreas of crab has lower bioavailability than cadmium chloride (Lind et al., 1995). The decreased bioavailability of cadmium associated with high protein foods may be due to the ability of undigested oligopeptides to decrease the amount of free cadmium available to be absorbed from the intestine by complexing cadmium (Kojima et al., 1985). The bioavailability of cadmium in foods of plant origin such as mushrooms and lettuce is similar to that in cadmium salts (McKenna et al., 1992; Lind et al., 1995). Phytate, which decreases the bioavailability of many divalent cations, appears to increase cadmium bioavailability to rats (Rimbach et al., 1995).

The bioavailability of cadmium in water to aquatic organisms decreases with increasing organic carbon and oxygenation. Dissolved organic matter and particulates may absorb and render unavailable substantial amounts of cadmium. For these reasons, the effective cadmium content of water is often expressed as the dissolved metal concentration, which is defined as the amount of metal in solution that passes through a 0.45 μm filter (EPA, 2001). Bioavailability of cadmium to aquatic organisms in freshwaters decreases with increasing water hardness and especially calcium content. In saltwaters with salinities above 10 g/kg, insoluble complexes of cadmium chloride predominate and these are of considerably lower availability than ionic cadmium (Kramer et al., 1997). Thus, toxicity thresholds for aquatic animals must be interpreted in the context of the characteristics of the water.

TOXICOSIS

Repeated exposure of humans to excessive levels of cadmium-containing dust or fumes, found usually at cadmium-producing and/or -consuming factories, can have irreversible effects on kidneys and lead to reduced lung capacity and emphysema. High levels of cadmium in dust and soils found in or near toxic waste sites may also pose an inhalation threat, but this route of exposure is not likely to be of concern in animal husbandry and is not considered in this report. Toxicological profiles following exposure by this route have been extensively reviewed (IPCS, 1992a; Jarup et al., 1998; ATSDR, 1999).

Virtually all major organ systems are affected by chronic consumption of foods or water containing high levels of cadmium, with the kidney and the liver as the primary target organs in most species (Swiergosz-Kowalewska, 2001). Usually, nephrotoxicity is the primary pathology leading to initial signs of toxicosis. Cadmium causes damage to proximal tubule cells and interstitial fibrosis in the kidney cortex, resulting in proteinuria, glycosuria, amino aciduria, and polyuria. In dogs, mesangium cells of the glomerulus also become necrotic and the glomerular basement membrane thickens in rats. Cadmium causes renal damage in rats when its concentration in the kidney is 10 mg/kg regardless of the type of cadmium complex that is administered (Min et al., 1986).

Histopathological changes in the liver induced by chronic cadmium exposure include intralobular fibrosis, cirrhosis, focal mononuclear infiltration, and proliferation of the smooth endoplasmic reticulum. A mild osteomalacia has frequently been reported after chronic cadmium exposures. This may be due to direct effects on the turnover of bone matrix by stimulation of bone remodeling pathways (Regunathan et al., 2002), though it can also be secondary to decreased cal-

cium and phosphorus absorption (Sugawara and Sugawara, 1974). The effect on bone is exacerbated by multiparity (Whelton et al., 1994). In multiparous mice, decreased bone calcium is a more sensitive indicator of cadmium toxicosis than changes in renal function, but this is not seen in nonreproducing females (Bhattacharyya et al., 1988a). Anemia is also a commonly reported sign of cadmium toxicosis, though it may be secondary to decreased iron absorption. Cadmium causes decreased hematopoiesis and the resulting anemia is often one of the first detectible changes in toxicosis. Cadmium-induced infertility, pancreatic necrosis, and decreased immunocompetence also contribute to the toxicity profile of cadmium. After withdrawal of cadmium, tissue cadmium levels decay only very slowly, and the signs of toxicosis require a half year or more to resolve (Nomiyama and Nomiyama, 1984).

Because cadmium is poorly excreted and builds up over time, animals with higher rates of food intake appear to be affected at lower levels of dietary cadmium than animals with lower food intakes. Thus, the dietary concentration at which cadmium becomes toxic appears to be higher in small animals, such as rodents and quail, than in larger animals, such as monkeys and pigs, because mass-specific metabolic rate and food intake decrease with body size.

In experimental animals, cadmium is carcinogenic after oral exposure. This is due to cadmium's prooxidant properties, effects on cell proliferation, differentiation, and apoptosis, aberrant activation of protooncogenes, and inhibition of DNA repair and DNA methylation (Waalkes, 2003; Waisberg et al., 2003). In humans, occupational exposure by inhalation of cadmium dusts and fumes has been related to lung cancer; however, carcinogenic activity in human populations exposed to high dietary levels of cadmium is not well documented by epidemiological studies (Pinot et al., 2000; Satoh et al., 2002). In 1993, cadmium was classified as a human carcinogen by the International Agency for Research on Cancer. Cadmium is teratogenic in birds and mammals (ATSDR, 1999; Kertesz and Fancsi, 2003).

The rate of cadmium accumulation in the kidney and liver of rats is very similar for soluble cadmium delivered via the feed versus the water. When cadmium dosage is expressed on a daily intake basis (mg/kg BW/day), toxicity does not differ between food and water exposure (Ruoff et al., 1994). Because the data on the toxicity of cadmium in water are very limited in terrestrial animals other than rodents, it is useful to assume that intakes determined to be toxic when consumed in feeds are appropriate for estimating toxicity of cadmium in water.

Toxicity of cadmium to fish and aquatic invertebrates is variable across species. Among fish, salmonids are particularly sensitive (EPA, 2001). Cadmium interferes with calcium uptake from water and causes hypocalcemia, malformations of the spine, and infertility. The most sensitive life stages are the embryo and early larva. The EPA has conducted an extensive review of the published literature on cadmium toxicity to 125 species of aquatic animals (EPA, 2001). Table 9-1 includes data from some of the studies on economically important species; however, the EPA document should be consulted for additional information. The acute toxicity of cadmium to freshwater fish is highly dependent on the hardness of the water. For example, the LC 50 of cadmium chloride to rainbow trout is 0.83 µg/L of cadmium at 30 mg/L hardness, but 5.23 µg/L at 90 mg/L hardness (Hansen et al., 2002a). Water used in aquaculture normally has a hardness considerably >50 mg/L. Because water hardness has such a marked impact on cadmium toxicity in aquatic environments, EPA toxicity criteria are standardized to a hardness of 50 mg/L and include a regression equation to adjust for other water hardness values.

Studies on cadmium toxicity to animals since 1979 are listed in Table 9-1. Studies before 1980 are summarized in the previous version of this publication (NRC, 1980). Only selected studies in mice and fish are provided in Table 9-1, but thorough reviews of this extensive literature are available elsewhere (IPCS, 1992a,b; ATSDR, 1999; EPA, 2001).

Single Dose

Numerous human and animal studies indicate that a single oral dose of cadmium causes severe irritation to the gastrointestinal epithelium (Andersen et al., 1988). Symptoms include nausea, vomiting, salivation, abdominal pain, and diarrhea (ATSDR, 1999). Desquamation and necrosis of the gastric and intestinal mucosa and dystrophy of the liver, heart, and kidneys are the primary pathological indications (Tarasenko et al., 1974). The single oral dose LD_{50} for cadmium in rodents is dependent on the solubility of the source. Cadmium oxide and the highly soluble sulfate, chloride, nitrate, and iodide salts of cadmium have an LD_{50} of about 50 mg Cd/kg BW, whereas insoluble cadmium metal and cadmium sulfide have an LD_{50} of about 900 mg/kg BW in mice (ATSDR, 1999). A single oral dose of 15.7 mg Cd/kg BW as cadmium chloride in water to mice does not cause histopathology of the stomach or liver (Andersen et al., 1988).

Acute

In rats, an acute dose of cadmium (30 mg/kg BW/day) administered by gavage causes severe necrosis, hemorrhage, and ulcers in the gastrointestinal epithelium (Andersen et al., 1988; Basinger et al., 1988). The pain of the gastroenteritis induced by high cadmium intake usually limits consumption and limits toxicity. In Japanese quail, feeding a diet containing 75 mg/kg cadmium as cadmium sulfate results in decreased egg production within 10 days of exposure (Bokori et al., 1995). In chickens, 50 mg/kg cadmium in the diet as cadmium chloride impairs egg production and egg weight (Hennig et al., 1968).

The EPA (2001) has summarized all studies that examined

the acute toxicity of cadmium for more than 100 species of aquatic animals and then calculated the geometric mean toxicity for individual species (Species Mean Acute Value, or SMAV). These SMAV for cadmium are based on mortality (LC 50) and range from 11,859 µg/L for crayfish to 1.6 µg/L for brown trout. The SMAV for other economically important freshwater animals are tilapia, 10,633; channel catfish, 5,055; common carp, 4,238; coho salmon, 6.2; rainbow trout, 2.1; striped bass, 2.9; and brook trout, 1.8 µg/L. All of these values are adjusted to a water hardness of 50 mg/L. Tolerance to cadmium increases with increasing hardness with a slope of 1.01; consequently fish in water with a hardness of 200 mg/L will be about four times less sensitive (EPA, 2001).

Cadmium is much less toxic in saltwater than freshwater, and coho salmon have a SMAV of 1,500 µg/L when raised in saltwater compared to 6.2 µg/L in freshwater. The SMAV for other saltwater species are sheepshead minnows, 50,000; striped mullet, 7,079; blue crab, 2,594; Pacific oyster, 228; American lobster, 78; and striped bass, 75 µg/L.

Chronic

Ruminants including cattle, sheep, and goats have been fed crops grown in high-cadmium soils or diets supplemented with sewage sludge resulting in dietary cadmium levels of up to 13 mg/kg dry matter (Tables 9-1 and 9-2). In these studies, decreased performance was not reported and in some cases performance increased. Goats fed 28.5 mg/kg cadmium as cadmium chloride did not have significant changes in growth, feed intake, feed efficiency, or organ weights (Combs et al., 1983). A diet containing 10 mg/kg cadmium from cadmium chloride did not influence milk production in Holstein cows, but only four animals were used per treatment level (Sharma et al., 1979). A diet containing 40 mg/kg cadmium from cadmium chloride did not influence feed intake of calves, but 160 mg/kg resulted in decreased feed intake (Powell et al., 1964). Unfortunately, sensitive measures of toxicosis, such as renal pathology or proteinuria, have not been measured in ruminants so a LOAEL cannot be accurately determined, but 10 mg/kg dry matter seems to be well tolerated using growth and general health as criteria. Several experiments have examined the effects of cadmium chloride in the water on sheep. In one experiment, cadmium at 3 mg/kg BW resulted in decreased testes weight, decreased semen volume, and lesions in the Sertoli cells, seminiferous tubules, and primary and secondary spermatocytes (Lymberopoulos et al., 2000). Cadmium at 2.6 mg/kg BW given in water to lactating ewes resulted in pathological changes in the liver, kidney, and mammary gland.

Among nonruminants, pigs fed 10 mg/kg cadmium from cadmium chloride have normal growth rates, but those fed 50 mg/kg develop anemia (Cousins et al., 1973, Sharma et al., 1979). Dogs given 5 mg/L cadmium in water for 4 years develop signs of glomerular and tubular pathology, but those given 2.5 mg/L are normal (Anwar et al., 1961). Functional indications of cadmium toxicosis in rats and mice, such as increased blood pressure, have been observed at levels as low as 1 mg/kg diet, although most studies do not report changes at this level. Indications of pathology in the kidney or other tissues occur at 5 mg/kg diet, and decreased growth rates occur at about 30 mg/kg diet or 30 mg/L water (IPCS, 1992a,b; ATSDR, 1999). In monkeys, a diet containing 3 mg/kg cadmium did not cause any pathological or histopathological effects after 9 years, but 10 mg/kg resulted in decreased body weights (Masaoka et al., 1994). In another study, rhesus monkeys given 5 mg/kg cadmium BW/day orally developed renal failure and increased urinary excretion of total protein if they concurrently suffered from protein calorie malnutrition (Prasad and Nath, 1995). Dogs fed cadmium at 1, 3, 10, and 30 mg/kg from cadmium chloride for 3 months were clinically normal and did not have histopathological evidence of hepatic or renal damage (Loeser and Lorke, 1977). However, dogs fed 50 mg/kg of dietary cadmium from cadmium chloride for 8 years had renal atrophy and renal tubule degeneration (Hamada et al., 1994) and diminished renal function (Kodama et al., 1992). Cadmium at 15 mg/L in the drinking water of ovariectomized beagles induced bone loss over a 7-month period (Bhattacharyya et al., 1992; Sacco-Gibson et al., 1992).

Diets containing cadmium at 10 mg/kg do not decrease gain or cause anemia when fed to chickens, ducks, or quail. A diet containing cadmium at 20 mg/kg resulted in anemia and increased serum glutamic-pyruvate transaminase in mallards (Cain et al., 1983). Laying hens fed 3 mg/kg cadmium as cadmium sulfate had increased egg production, whereas those fed 12 mg/kg had decreased egg production in one experiment and decreased egg shell thickness in a second experiment (Leach et al., 1979). Broiler chickens fed 25 mg/kg cadmium from cadmium sulfate develop focal fatty infiltration of the liver, histiocytic infiltration of the jejunal mucosa, focal lymphohistiocytic interstitial infiltration, and fibrosis of the kidney (Bokori et al., 1996).

In fish, growth rate is not a good indicator of chronic toxicity. For example, female rainbow trout grow at a normal rate when exposed to high levels of cadmium in the water, but their sexual development is markedly impaired and any eggs that are produced are sterile (Brown et al., 1994). Unfortunately, the majority of toxicity studies in fish use growth rate and mortality as the primary endpoints. Based on the available literature, EPA has calculated mean chronic toxicity levels for a variety of aquatic animals (Mean Chronic Value, or MCV). The MCV for freshwater fish are tilapia, >23.6; smallmouth bass, 8.1; Atlantic salmon, 7.9; brook trout, 2.6; coho salmon, 4.2; and rainbow trout, 1.3 µg/L. All of these values are adjusted to a water hardness of 50 mg/L. In these chronic studies, tolerance to cadmium increases with increasing hardness with a slope of 0.74. Con-

sequently, fish in water with a hardness of 200 mg/L will be about three times less sensitive (EPA, 2001).

Factors Influencing Toxicity

The toxicity of cadmium is affected by the nutritional and physiological state of an animal. In Japanese quail, deficiencies of zinc, iron, copper, calcium, or protein increase the tissue accumulation and toxicity of cadmium (Fox et al., 1979, 1984). In rodents, physiological states that induce an increase in iron absorption, such as a fast growth rate or pregnancy, increase cadmium absorption and toxicity (Schafer et al., 1990, Waalkes 1986). In humans, adult females absorb more cadmium than males, and this is at least partially due to higher incidence of anemia in females (Choudhury et al., 2001). Low dietary calcium increases cadmium absorption and accumulation in tissues leading to cadmium toxicosis at lower levels than in well-nourished animals (Brzoska and Moniuszko-Jakoniuk, 1998). Furthermore, cadmium interferes with the metabolic functions of calcium, magnifying the deficiency. A similar situation occurs with zinc (Brzoska and Moniuszko-Jakoniuk, 2001). Age also is an important factor: in rats, cadmium is absorbed at higher rates in immature animals than in adults (Foulkes et al., 1991). Kidney damage from causes unrelated to cadmium toxicity, such as diabetes and old age, are expected to increase the sensitivity to nephrotoxicity caused by cadmium (Buchet et al., 1990).

Cadmium toxicity can be markedly (>80 percent) reduced by high levels of several minerals, especially calcium, phosphate, zinc, or iron (Groten et al., 1991). For example, a high level of dietary calcium decreases absorption and decreases the toxicity of absorbed cadmium (Brzoska and Moniuszko-Jakoniuk, 1998). Zinc protects against cellular toxicity of cadmium by reducing the accumulation of cadmium in cells and also by inducing metallothionein (Kaji et al., 1988). High levels of dietary zinc ameliorate the inhibition in egg production caused by cadmium in the diet of laying hens (Nolan and Brown, 2000). High intakes of vitamin E also reduce the development of toxicosis in growing chickens (Gupta and Kar, 1999), as does cysteine (Czarnecki and Baker, 1982).

For aquatic organisms, the toxicity of cadmium decreases with increasing salinity of the water. The complexing of ionic cadmium with chloride and the high level of calcium in seawater decrease cadmium absorption (Engel and Fowler, 1979). Thus, the accumulation and toxicity of cadmium for a given species can be several orders of magnitude lower in ocean environments than freshwater environments. The protective effect of calcium has been attributed to the lower toxicity of cadmium in hard water than in soft water (Gill and Epple, 1992; Brzoska and Moniuszko-Jakoniuk, 1998). Consequently, animals in saltwater environments receive most of their cadmium from their food, whereas those in freshwater obtain important amounts of cadmium from both food and water (IPCS, 1992b).

TISSUE LEVELS

Cadmium accumulation is greatest in the kidney, followed by liver, testes, pancreas, and spleen. Muscle and bone do not accumulate cadmium at high levels (Table 9-2). There are no major differences in the amount of cadmium that accumulates across different types of muscles. Tissue levels increase with the time of exposure; when dietary levels are high, tissue levels eventually reach a plateau. For example, in ducks fed 20 mg/kg cadmium, muscle cadmium increases from 0.006 to 0.025 mg/kg fresh tissue during the first 30 days and then further increases to 0.077 mg/kg during the next 30 days, but does not increase further thereafter (White et al., 1978). The plateau in muscle usually occurs relatively rapidly (months), but cadmium in kidney and liver accumulates over a much longer period of time. Once elevated levels of cadmium have accumulated, the withdrawal of cadmium from the feed for a month or more does not result in appreciable loss of cadmium from tissues (White and Finley, 1978; Sharma et al., 1979; Baxter et al., 1982).

Phosphate sources used in animal diets are likely sources of cadmium contamination. In one study with pigs, a rock phosphate that contained an exceptionally high level of natural cadmium contamination (100.3 mg/kg) was incorporated into growing and finishing feeds to provide 1.2 mg/kg cadmium final feed. Pigs fed this diet to a 90-kg slaughter weight did not have detectable levels of cadmium in their muscle or fat (<0.3 mg/kg dry weight), but their livers and kidneys contained 0.35 and 1.68 mg/kg, respectively (King et al., 1992).

The relationship between cadmium intake and the concentration in the liver and kidneys of rats is linear over a very wide range (Ruoff et al., 1994). At realistic levels of intake (<10 μg/kg BW/day), the slope of the relationship between intake and tissue cadmium (wet weight) is 0.11 for kidney cortex and 0.04 for liver. Several dietary factors can modify the amount of cadmium that accumulates in tissues. High levels of dietary zinc, calcium, or cysteine decrease accumulation (Czarnecki and Baker, 1982; Lamphere et al., 1984).

In fish, the deposition of cadmium is usually highest in the gills and kidney, with lower levels in the liver. More than 90 percent of the total whole body cadmium in rainbow trout exposed to high levels of cadmium is found in these three tissues (Kay et al., 1986). Experiments examining cadmium accumulation from the water typically use water with sufficient hardness to prevent acute toxicity and mortality in the fish.

Cadmium transfer from the laying hen to the egg is very inefficient. Hens with very high cadmium burdens (100 mg/kg in liver) lay eggs with undetectable levels of cadmium in the albumen and approximately 0.1 mg/kg dry weight in the yolk (Sato et al., 1997; Leach et al., 1979). Cadmium transfer to milk is similarly inefficient (Sharma et al., 1979; Smith et al., 1991b). Feeding heifers a diet containing 5 mg/kg cad-

mium for more than a year before parturition and throughout lactation did not result in significantly elevated milk cadmium levels during any part of lactation. Using radioactive cadmium, it was found that most cadmium in milk is bound to casein, with lesser amounts bound to albumin and lactose, and none detectable in fat (Van Bruwaene et al., 1982).

Regardless of the level of cadmium fed, the concentration of cadmium in meat, milk, and eggs is always lower than the level in the diet that the animal consumed (on a dry matter versus dry matter basis). Thus, foods derived from these products decrease exposure of humans to environmental cadmium. However, levels in kidney and liver are always considerably higher than the levels in the diet and these tissues magnify environmental cadmium (Morcombe et al., 1994).

Kidneys can make their way into a variety of human foods. The kidneys of poultry are located in recesses of the sacrum and ilium and remain with the carcass after the rest of the viscera are removed during processing. After egg production ceases, hens are usually more than 1 year old and have kidney cadmium levels of 0.80 mg/kg (Murphy et al., 1979). In the United States, mechanically deboned meat made from the frames of spent hens was found to contain 0.02 mg/kg (Murphy et al., 1979). However, if only the back, which contains the kidneys, was used to make mechanically deboned poultry meat, the cadmium concentration would be 0.096. Consequently, a regulation was issued by the Food Safety and Inspection Service requiring the removal of kidneys from mature chickens and turkeys at slaughter. However, the value of this requirement has been questioned based on surveys of cadmium levels in products (Vos et al., 1990). Mechanically deboned meat from broilers should not be a problem. Broilers, which are usually marketed at approximately 6–7 weeks of age, have 0.05 mg/kg cadmium in their kidneys and <0.005 mg/kg fresh weight in their muscle (Murphy et al., 1979).

MAXIMUM TOLERABLE LEVELS

The MTL of cadmium is defined as the dietary level that, when fed for a defined period of time, will not impair accepted indices of animal health or performance. Animals are able to tolerate acute exposure to 25 mg/kg cadmium in the diet for a few days. Subtle histological signs of toxicosis in rodents occur after chronic consumption of 1 mg cadmium/kg diet for several months. Dietary levels of 10 mg/kg are tolerated chronically by poultry and livestock species, but these levels result in unacceptable levels of cadmium in kidneys, livers, and, in some cases, muscles of animals. Rhesus monkeys can tolerate 3 mg/kg diet for 9 years without signs of pathology. Dogs can tolerate 10 mg/kg diet for 8 years. The toxicity of cadmium in water appears to be very similar to that in feeds. For this reason, the total daily intake of both feed and water sources should be considered. The toxicity of cadmium to fish is extremely variable with trout being the most susceptible. Cadmium at a level of 1 μg/L is tolerated by all species in all water conditions. With hard water, trout and all other species tested can tolerate 4 μg/L.

HUMAN HEALTH

Animals are able to tolerate levels of cadmium in their diet that result in accumulation of cadmium in their kidneys and livers at concentrations that are of concern for humans consuming foods made from these organs. Consequently, maximum dietary levels in the feed of animals used for human food should be set on the basis of human health and not animal health.

Based on residue levels, the World Health Organization has set a 1 mg/kg upper limit for cadmium in complete feeds for animals (IPCS, 1992a).

FUTURE RESEARCH NEEDS

The level of cadmium in the diet of agriculturally important animals should be based on cadmium content of tissues used as human foods and not on the maximum tolerable level for the animal itself. Few studies have examined the dose–response relationship between dietary cadmium and tissue cadmium in production animals at levels <1 mg/kg diet. Better definition of the dose-response relationship is required to establish the maximal tolerable levels of cadmium in animal feeds based on residue levels. Because cadmium accumulates over many years, examining this relationship in relatively long-lived animals such as dairy cattle and laying hens is especially needed.

Toxicity of cadmium to aquatic species has mainly utilized growth rate and mortality as endpoints. Use of more sensitive criteria such as pathological changes in kidneys, gills, and liver is needed to define more precisely chronic toxicities. Cadmium tolerance and signs of toxicosis in companion animal species have not been investigated. Given the high proportion of kidney and liver tissues in meat meals that are commonly fed to these species, investigation is warranted.

SUMMARY

Cadmium is industrially valuable for batteries, pigments, and coatings. It enters the environment by a wide variety of anthropogenic means and may also be high in phosphate and zinc supplements fed to animals. Cadmium is known to be essential in diatoms, but essentiality has not been established in animals. Cadmium is not absorbed very efficiently, but once it enters the body, it is excreted very slowly and accumulates according to lifetime exposure. The bioavailability of cadmium is mostly similar across feed types and is also similar between feed and water. High intakes of cadmium from the feed or from water can cause decreased growth rates, anemia, kidney damage, osteomalacia, infertility, and hypertension. High dietary cadmium can also interfere with

the absorption and metabolism of other nutrients, especially zinc and calcium. Cadmium accumulates to very high levels in the kidney and liver. This accumulation limits the safe levels of cadmium that can be fed to animals destined for human consumption. Cadmium is not transferred efficiently to milk or eggs, and these foods have much lower levels of cadmium than those found in the diet of the cow or hen. Levels in muscle are also lower than dietary levels. Further research is needed to determine the dose–response relationship between dietary cadmium at low levels of supplementation and the levels in tissues destined for human consumption.

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TABLE 9-1 Effects of Cadmium Exposure in Animals

Animal	N[a]	Age or Weight	Quantity	Source	Duration	Route	Effect(s)	Reference
Monkeys, rhesus	8	3 yr	3 mg/kg 10 mg/kg 30 mg/kg 300 mg/kg	$CdCl_2$	462 wks	Diet	No adverse effects Decreased body weight Decreased weight, proteinuria, glucosuria, renal pathology Decreased weight, proteinuria, glucosuria, renal pathology	Masaoka et al., 1994
Dogs, beagle	2	6–8 mo	1 mg/kg 3 mg/kg 10 mg/kg 50 mg/kg 100 mg/kg	$CdCl_2$	8–9 yr	Diet	No adverse effects No adverse effects No adverse effects Renal pathology, loss of renal function Renal pathology, loss of renal function	Hamada et al., 1994; Kodama et al., 1992
Mice	8–10	67 d females (6 rounds reproduction)	5 mg/kg 50 mg/kg	$CdCl_2$	252 d	Diet	Decreased femur calcium during reproduction Decreased body weight, femur calcium during reproduction	Bhattacharyya et al., 1988b
Mice	68 96 77	67 d females (6 rounds reproduction)	5.0 mg/kg 50 mg/kg	$CdCl_2$	252 d	Diet	No adverse affects No effect on fertility, decreased litter size and pup growth	Whelton et al., 1988
Rats	20	9.5 wk	0.3 mg/kg 3 mg/kg 30 mg/kg 90 mg/kg 0.3 mg/kg 3 mg/kg 30 mg/kg 90 mg/kg	$CdCl_2$ Cd-metallothionein	40 wk	Diet	No adverse effect No adverse effect Basophilic renal tubules, glomerular nephrosis Renal pathology, enzymuna No adverse effect No adverse effect No adverse effect Basophilic renal tubules, glomerular nephrosis	Groten et al., 1994
Chickens, leghorn	10	1 d	10 mg/kg 100 mg/kg	$CdCl_2$	5 wk	Diet	No adverse effects Decreased feed intake, weight gain and cross linking of pulmonary elastin	Lefevre et al., 1982
Chickens, leghorn	6	1 yr	2 mg/kg 10 mg/kg	$CdCl_2$	24 wk	Diet	No adverse effects No adverse effects	Sharma et al., 1979
Chickens, leghorn	15	48 wk	3 mg/kg 12 mg/kg 48 mg/kg	$CdSO_2$	12 wk	Diet	Increased egg production Decreased egg production Decreased egg production	Leach et al., 1979
Chickens, leghorn	100	1 d	0.09 mg/kg 0.57 mg/kg 0.97 mg/kg	Corn and soybeans	80 wk	Diet	No adverse effects No adverse effects No adverse effects	Hinesly et al., 1985

continued

TABLE 9-1 Continued

Animal	N[a]	Age or Weight	Quantity	Source	Duration	Route	Effect(s)	Reference
Chickens, broiler	10	1 d	25 mg/kg 75 mg/kg	$CdSO_4$	36 wk	Diet	Normal growth, renal and hepatic pathology Decreased growth, renal and hepatic pathology	Bokori et al., 1996
Chickens, broiler	10	21 d	2.5 mg/kg/kg BW 10.5 mg/kg/kg BW 30 mg/kg/kg BW	$CdSO_4$	3 wk	Water	Enlarged testicles, depletion of sperm, Decreased BW; testicular and muscular pathology High mortality	Bokori et al., 1995
Quails, Japanese	10	155–200 g	75 mg/kg 150 mg/kg 300 mg/kg	$CdSO_4$	37 d	Diet	Kidney and pancreatic pathology Decreased body weight, egg production, kidney pathology Decreased body weight, egg production, kidney pathology	Bokori and Fekete, 1995
Ducks, mallard	6	1 d	5 mg/kg 10 mg/kg 20 mg/kg	$CdCl_2$	12 wk	Diet	No adverse effects No adverse effects Decreased hematocrit and increased serum glutamic pyruvate trunsaminase	Cain et al., 1983
Ducks, mallard	12	44 wk	50 mg/kg 150 mg/kg 450 mg/kg	$CdCl_2$	42 d	Diet	No adverse effects No adverse effects Decreased body weight, increased plasma uric acid and corticosterone	Di Giulio and Scanlon, 1984
Pigs, Yorkshire	10	9 wk	2 mg/kg 10 mg/kg	$CdCl_2$	6 mo	Diet	No adverse effects No adverse effects	Sharma et al., 1979
Pigs	5	8 kg	0.44 mg/kg[b] 0.89 mg/kg 2.21 mg/kg 4.43 mg/kg 0.60 mg/kg 1.20 mg/kg	$CdCl_2$ Rock phosphate Rock phosphate	Until market weight of 90 kg	Diet	No adverse effects No adverse effects No adverse effects No adverse effects No adverse effects No adverse effects	King et al., 1992
Pigs	12	8 kg	83 mg/kg	CdCl	9 wk	Diet	Decreased growth, anemia	Osuna et al., 1981
Cows, Holstein	4	2 yr	2 mg/kg 10 mg/kg	$CdCl_2$	12 wk	Diet	No adverse effects No adverse effects	Sharma et al., 1979
Goats	2	21.2 kg	10.4 mg/kg 18.0 mg/kg 28.5 mg/kg	$CdCl_2$	125 d	Diet	No adverse effects No adverse effects No adverse effects	Combs et al., 1983

Sheep, Chios	7	12 wk	3 mg/kg BW/d	$CdCl_2$	7 mo	Water	Decreased testes weight, semen volume, sperm concentration, histopathology of seminiferous tubules	Lymberopoulos et al., 2000
Sheep, Sarda	10	Lactating ewes	2.6 mg/kg BW/d	$CdCl_2$	108 d	Water	Histopathology of liver, kidney, mammary gland	Floris et al., 2000
Sheep	5	84 kg rams	7.5 mg/d	$CdSO_4$	7 wk	Water	No change in spermatogenesis, subtle change in grazing behavior	Berry et al., 1999
Fish, African catfish	4 replicates of 24	Eggs, larvae	0.05 mg/L 0. 15 mg/L 0.5 mg/L 1.5 mg/L 5.0 mg/L	$CdCl_2$	5 d	Water	No adverse effects on embryo or larvae No adverse effects on embryo or larvae Malformations, decreased pigmentation 100% lethal to larvae 100% lethal to larvae	Nguyen and Janssen, 2002
Fish, Tilapia	150	Juvenile	1.5 µg/L 6.8 µg/L 14 µg/L 28 µg/L 52 µg/L	$Cd(NO_3)_2$	16 wk	Water	No adverse effects No adverse effects No adverse effects No adverse effects No adverse effects	Papoutsoglou and Abel, 1988
Fish, bull trout	6 tanks	212 mg	0.052 µg/L 0.089 µg/L 0.197 µg/L 0.383 µg/L 0.789 µg/L	$CdCl_2$	55 d	Water	No adverse effects No adverse effects No adverse effects No adverse effects Decreased growth, increased mortality	Hansen et al., 2002b
Fish, rainbow trout	300	2 g	1.47 mg/kg 786 mg/kg 1,395 mg/kg 2,265 mg/kg	$Cd(NO_3)_2$	36 d	Feed	No adverse effects on growth or mortality No adverse effects on growth; slight increase in mortality Increased mortality Increased mortality	Szebedinszky et al., 2001
Fish, rainbow trout	450	5.6 g	0.02 µg/L 0.07 µg/L 0.11 µg/L	$CdCl_2$	30 d	water	No adverse effects No adverse effects No adverse effects	Hollis et al., 2000
Fish, rainbow trout	6 tanks	2.8 g	0.7 µg/L 3.0 µg/L	$Cd(NO_3)_2$	30 d	water	No adverse effects No adverse effects	Hollis et al., 2001
Fish, rainbow trout	10	NA[c]	9 µg/L 18 µg/L	$CdCl_2$	8 wk	Water	Increased mortality Increased mortality	Kay et al., 1986
Fish, rainbow trout	25	270 d	0.47 µg/L 1.77 µg/L 3.39 µg/L 5.48 µg/L	$CdSO_4$	65 wk	Water	No adverse effects No adverse effects, decreased viability of eggs Normal growth, impaired sexual development, nonviable eggs Normal growth, no sexual development	Brown et al., 1994

[a]Number of animals per treatment group.
[b]Quantity of cadmium dosed. SI conversion: 1 mg cadmium equals 8.90 µmoles cadmium.
[c]Not provided.

TABLE 9-2 Cadmium Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)

Animal	Quantity	Source	Duration	Route	Muscle	Kidney	Liver	Spleen	Bone	Egg	Milk	Serum	Reference
Chickens, broiler	0.0 mg/kg[c] 0.5 1.0 5.0 10.0	$CdCl_2$	45 d	Diet		0.06[a] 0.25 0.98 1.64 4.12							Om et al., 2002
Chickens, leghorn	0.2 mg/kg 3 mg/kg 12 mg/kg 48 mg/kg	$CdSO_4$	48 wk	Diet	0.12 [b] 0.57 1.66 6.46	17.1[b] 273.9 708.3 540.7	2.99[b] 33.47 41.82 203.54			0.16[b] 0.13 0.14 0.22			Leach et al., 1979
Chickens, leghorn	0.32 1.88 13.06	$CdCl_2$	24 wk	Diet	0.163[a] 0.140 0.263					— 0.06[a] 0.09	-		Sharma et al., 1979
Chickens, leghorn	0 mg/kg[c] 3 mg/kg 20 mg/kg	$CdCl_2$	8 wk	Diet	0.027[a] 0.162 0.473	0.264[a] 1.54 56.78	0.089[a] 0.77 25.51						Pribilincova et al., 1995
Chickens, leghorn	0.09 mg/kg 0.57 mg/kg 0.97 mg/kg	Corn and soybeans	80 wk	Diet	0.062[b] 0.113 0.131	9.5[b] 45.8 69.5	1.43[b] 4.86 6.66	0.36[b] 1.19 1.49	0.145[b] 0.152 0.221	< 0.06[b] < 0.06 < 0.06			Hinesly et al., 1985
Chickens, Plymouth Rock	0.2 mg/kg 3 mg/kg 12 mg/kg 48 mg/kg	$CdSO_4$	2 wk	Diet	0.07[b] 0.15 0.26 0.75	0.39[b] 9.27 49.69 239.07	0.23[b] 4.75 15.13 87.19						Leach et al., 1979
Quails, Japanese	0.04 mg/kg 0.55 mg/kg	Oats	10 wk	Diet	0.006[b] 0.029	0.853[b] 6.218	0. 180[b] 1.515			0.005[b] 0.009			Bache et al., 1986
Quails, Japanese	0 mg/kg[c] 0.61 mg/kg 1.2 mg/kg	Sunflower meal[c]	15 wk	Diet	0.011[b] 0.026 0.071	0.16[b] 6.52 25.8	0.07[b] 3.09 7.63			0.006[b] 0.010 0.016			Stoewsand et al., 1986
Ducks, mallard	< 0.1 mg/kg 50 mg/kg 150 mg/kg 450 mg/kg	$CdCl_2$	42 d	Diet		2. 9[b] 208.8 335.2 540.7	0.9[b] 54.0 135.4 435.8						Di Giulio and Scanlon, 1984
Pigs	0.08 mg/ke 0.24 mg/kg	Corn[c]	17.6–90 kg	Diet	0.02[b] 0.02	0.64[b] 1.50	0.13[b] 0.21						Lisk et al., 1982
Pigs	<0.10 mg/kg 0.44 mg/kg 0.89 mg/kg 2.21 mg/kg 4.43 mg/kg 0.60 mg/kg 1.20 mg/kg	$CdCl_2$ Rock phosphate Rock phosphate	8–90 kg BW	Diet	<0.3[b] <0.3 <0.3 <0.3 <0.3 <0.3 <0.3 <0.3	0.39[b] 1.23 2.17 5.72 12.25 1.13 1.68	0.30[b] 0.30 0.38 0.94 2.01 0.30 0.35						King et al., 1992

Cattle	3.5 mg/kg[b] 13 mg/kg	Sludge fertilization	8 yr	Diet		8.9[b] 43.8	1.09[b] 7.72		0.064[b] 0.245		0.002[b] 0.003	0.004[a,f] 0.002	Fitzgerald et al., 1985
Cattle, Herefords	0.1 mg/kg[b] 10.7	Sewage sludge	36 wk	Diet	0.14 0.43	3.5[b] 57.0	0.9[b] 19.9						Baxter et al., 1982
Cows, Holstein heifers	0.25 mg/kg[b] 1.0 mg/kg 5.0 mg/kg	$CdCl_2$	394 d	Diet	0. 18[b] 0.39 0.24	2.95[b] 18.75 132.17	0.48[b] 2.03 14.41	0.31[b] 0.70 1.28	0.94[b] 1.27 0.84	0.038	0.032[a] 0.034	0.075 0.077 0.074	Smith et al., 1991a,b
Cows, Holstein heifers	0.18 mg/kg 2.40 mg/kg 11.29 mg/kg	$CdCl_2$	12 wk	Diet	0.054 0.064 0.018	1.35[a] 3.58 8.83	0.64[a] 0.73 3.21				0.019[a] 0.015 0.015		Sharma et al., 1982
Steers, Herefords	0.14 mg/kg[b] 10.6 mg/kg	Sewage sludge	106 d	Diet	<0.01[b] 0.03	1.19[b] 14.55	0.19[b] 4.92	0.02[b] 0.43	0.02[b] < 0.01				Johnson et al., 1981
Goats	0 mg/kg[c] 10.4 mg/kg 18.0 mg/kg 28.5 mg/kg	$CdCl_2$	125 d	Diet	0.08[b] 0.13 0.00 0.01	2.15[b] 27.23 55.75 73.00	1.45[b] 5.62 10.25 15.97						Combs et al., 1983
Goats	<0.2 mg/kg[b] 0.7–1.8 mg/kg 1.3–2.7 mg/kg 1.7–5.3 mg/kg	Corn silage[e]	3 yr	Diet	0.06[b] 0.05 0.07 0.12	3.09[b] 10.9 24.7 22.4	0.26[b] 1.72 2.10 2.94					0.001[a] 0.002 0.001 0.001	Bray et al., 1983; Dowdy et al., 1983
Goats	0. 14 mg/kg[b] 3.81	Grass-legume silage	135 d	Diet	0.04[b] 0.04	1.06[b] 1.65	0.10[b] 0.39					0.013[b] 0.050	Telford et al., 1984b
Sheep	0.3 mg/kg[b] 0.9 mg/kg	Sewage solids	90 d	Diet	<0.07[b] <0.07	<0.07[b] <0.07	<0.07[b] <0.07	<0.07[b] <0.07	2.3[b] 2.1				Sanson et al., 1984a
Sheep	0.2 mg/kg[b] 0.5 mg/kg	Sewage solids	2 yr	Diet	<0.07[b] <0.07	3.1[b] 3.7	0.6[b] 0.8	0.4[b] 0.5	3.2[b] 2.3				Sanson et al., 1984b
Sheep, Dorset	0.05 mg/kg[b] 1.7 mg/kg	Com silage[e]	274 d	Diet	0.005[b] 0.01	5.4[b] 18.5	1.2[b] 5.8	0.04[b] 0.23	0.01[b] 0.02				Heffron et al., 1980
Sheep, Finn-Dorset	0.26 mg/kg[b] 0.41 mg/kg	Sugar beets[e]	12 wk	Diet	0.04[b] 0.03	0.34[b] 0.43	0.07[b] 0.17						Telford et al., 1984a
Sheep, Morlam	0.09 mg/kg[b] 1.14 mg/kg[b]	Forage[e]	152 d	Diet	0.03[b] 0.09	0.55[b] 0.83	0.22[b] 0.40						Hogue et al., 1984
Sheep, Morlam	0.29 mg/kg[b] 3.88 mg/kg	Corn[e]	225 d	Diet	0.02[b] 0.02	2.91[b] 17.84	0.30[b] 3.19						Telford et al., 1982
Sheep, Romney	0.175 mg/kg[b] 0.5 mg/kg	Pasture	1.5 yr	Diet	0.014[a] 0.005	0.33[a] 1.11	0.12[a] 0.41						Lee et al., 1994
Fish, channel catfish	1,120 µg/L 112 µg/L 11. 2 µg/L 1.12 µg/L 0. 112 µg/L <0.03 µg/L	$CdCl_2$	7 d	Water	0.05[a] <0.03 <0.03 <0.03 <0.03 <0.03	23.90[a] 5.20 2.10 1.04 0.77 0.27	9.60[a] 2.00 0.74 0.43 0.25 0.08					0. 18[a,f] 0.06 <0.03 <0.03 <0.03 <0.03	Bentley, 1991

continued

TABLE 9-2 Continued

Animal	Quantity	Source	Duration	Route	Muscle	Kidney	Liver	Spleen	Bone	Egg	Milk	Serum	Reference
Fish, Tilapia	5 mg/L	$CdCl_2$	0 d 15 d 30 d	Water		0.25[a] 5.60 14.4	0.2[a] 3.5 8.6						Rani, 2000
Fish, Tilapia	1.5 µg/L 6.8 µg/L 14 µg/L 28 µg/L 52 µg/L	$(CdNO_3)_2$	16 wk	Water	0.06[a] 0.12 0.23 0.72 0.92								Papoutsoglou and Abel, 1988
Fish, Tilapia	1 µg/L 100 µg/L	$CdCl_2$	140 d	Water	0.015[a] 1.702	0.424[a] 596.3	0.038[a] 140.7	1.546[a] 30.66					Allen, 1995
Fish, bull trout	< 0.013 µg/L 0.052 µg/L 0.089 µg/L 0.197 µg/L 0.383 µg/L 0.789 µg/L	$CdCl_2$	55 d	Water	0.08[b,d] 0.17 0.20 0.38 0.57 0.91								Hansen et al., 2002b
Fish, rainbow trout	1.47 mg/kg 3.9 mg/kg 21.8 mg/kg 117 mg/kg 1,419 mg/kg	$(CdNO_3)_2$	36 d	Feed	0.46[a,g] 0.19 0.28 0.66 1.80	1.5[a] 1.2 2.4 6.9 10.9	0.35[a] 0.29 0.20 0.93 6.47						Szebedinszky et al., 2001
Fish, rainbow trout	0.02 µg/L 0.07 µg/L 0. 11 µg/L	$CdCl_2$	30 d	Water	0.31[b,d] 0.32 0.33								Hollis et al., 2000
Fish, rainbow trout	0.7 µg/L 3.0 µg/L	$Cd(NO_3)_2$	30 d	Water	0.6[a,d] 1.1	0.5 9.4	1.3 3.9						Hollis et al., 2001
Fish, rainbow trout	9 µg/L	$CdCl_2$	0 2 12 22 32 wks	Water		0.02[a] 0.2 0.6 5.2 8.6	0.05[a] 0.2 0.4 0.7 1.1						Kay et al., 1986
Fish, rainbow trout	<0.1 µg/L 3.6 µg/L 6.4 µg/L	$CdCl_2$	178 d	Water	0.03[b] 0.06 0.06	0.3[b] 18 30	0.02[b] 10 18						Giles, 1988
Fish, rainbow trout	0[c] 54 µg/L	$CdCl_2$	8 wk	Water		0.02[a] 10.4	0.05[a] 1.2	0.005[a] 0.7					Thomas et al., 1985

[a]Data are on a fresh tissue basis.
[b]Data are on a dry tissue basis.
[c]Basal level not provided.
[d]Whole body cadmium.
[e]Grown in cadmium-contaminated soil.
[f]Whole blood.
[g]Carcass (muscle, skin, and bone).

10

Calcium

INTRODUCTION

Calcium (Ca) atoms do not exist in their free state—but calcium-containing minerals are common in nature. Fairly pure deposits of calcium carbonate in the form of limestone are common and are a major source of calcium used in animal diet formulation. Nearly pure calcium sulfate is also found in the form of gypsum that is used in Portland cement, drywall, and plaster production as well as animal feeds. Calcium hydroxide (lime) is not commonly used as a dietary supplement but is often used as an astringent to reduce bacterial loads during disinfection of animal pens. Calcium phosphate salts are used for the manufacture of certain types of glass, but they are more commonly used as a source of calcium and phosphate in animal feeds. Calcium chloride is used in animal feeds when acidification of blood and urine is desired. Calcium polysulfide (lime sulfur, "wettable sulfur") is used as a fungicide, especially in orchards and around ornamental shrubbery.

Calcium is a positively charged alkaline Earth metal with an atomic weight of 40.08 and an equivalent weight of 20.04. Calcium is a divalent cation. Chloride and sulfate salts of calcium are water soluble; most other inorganic calcium salts are only slightly soluble in water. Organic salts of calcium, such as calcium propionate, are very soluble and are increasingly being used in diets for man and animals.

ESSENTIALITY

Extracellular calcium is essential for formation of skeletal tissues, transmission of nervous tissue impulses, excitation of skeletal and cardiac muscle contraction, blood clotting, eggshell formation, and as a component of milk. Intracellular calcium, while 1/10,000 the concentration of extracellular calcium, is involved in the activity of a wide array of enzymes and serves as an important "second messenger" conveying information from the surface of the cell to the interior of the cell.

About 98 percent of the calcium in the body is located within the skeleton where calcium, along with phosphate anion, serves to provide structural strength and hardness to bone. The other 2 percent of the calcium in the body is found primarily in the extracellular fluids of the body. Plasma calcium concentration is normally 90–110 mg/L or 2.25–2.75 mM. The ionized calcium concentration of the plasma of mammals and birds must be maintained at a relatively constant value of 1–1.25 mM to ensure normal nerve membrane and muscle end plate electrical potential and conductivity, forcing vertebrates to evolve an elaborate system to maintain calcium homeostasis. This system attempts to maintain extracellular calcium concentration constant by increasing calcium entry into the extracellular fluids whenever there is a loss of calcium from the extracellular compartment. When dietary calcium is inadequate this system may sacrifice bone calcium stores in an attempt to maintain normal blood calcium concentration.

Most farm species will require diets that are between 0.5 and 1 percent calcium. Optimal growth and reproduction, including lactation, will require more calcium than maintenance of the adult animal. Hens laying eggs require about 3.5 percent calcium in the diet to maintain quality of the eggshell.

Cold-blooded species can vary greatly in their requirement for dietary calcium. Softshell turtles raised for meat grew best when their diet was 5.7 percent calcium (and 3 percent phosphorus) (Huang et al., 2003). Some fish species reared in fresh water or seawater can obtain all the calcium they need from the water in which they reside. In freshwater species, the gills can absorb calcium from the water. Brook trout, for example, can obtain as much as 80 percent of their skeletal calcium requirement from water (McCay, 1936). Sea fish drink enough seawater to obtain much if not all of their calcium needs. However some freshwater and saltwater fish also require calcium supplementation for optimal growth (about 0.2 percent calcium added to the diet) as they cannot obtain enough calcium from the water alone (Robinson et

al., 1986; Chavez-Sanchez et al., 2000; Hossain and Furuichi, 2000).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Determination of calcium in feeds and tissues is best accomplished by wet or dry ashing of the sample followed by resuspension of the ash in an acidic solution for analysis by atomic absorption spectrophotometry. Atomic absorption is conducted at a wavelength of 422.7 nm and can detect as little as 0.01 mg Ca/L. Phosphate, sulfate, and aluminum that might be in the sample can interfere with calcium absorption spectra, but their effect is masked by the addition of lanthanum to the standards and samples being analyzed. Since low calcium values result if the pH of the sample is above 7, both standards and samples are prepared in dilute hydrochloric acid solution. Concentrations of magnesium greater than 1,000 mg/L can also cause low calcium values. Concentrations of up to 500 mg/L each of sodium, potassium, and nitrate cause no interference. Anionic chemical interferences can be expected if lanthanum is not used in samples and standards (EPA, 1983). The nitrous oxide-acetylene flame will provide two to five times greater sensitivity than an air-acetylene flame, but is not necessary for routine analysis of feeds or biological samples. Near-infrared spectrophotometry is not a satisfactory method of determining calcium content of feedstuffs and forages, though it is often used for that purpose.

Calcium concentrations can also be measured to ppb (ug/L) levels using inductively coupled plasma optical emission spectrophotometry (ICP-OES). ICP-OES uses radio frequency-generated plasma to excite the electrons of the calcium atoms, which then produce photons unique to calcium. Photomultiplier tubes detect and quantitate the photons emitted by the excited calcium atoms, allowing quantitation of the calcium concentration. An inductively-coupled plasma source atomizes and excites even the most refractory elements with high efficiency, so there is less interference and about 10-fold greater sensitivity using ICP-OES than using atomic absorption spectrometry.

REGULATION AND METABOLISM

When calcium loss exceeds entry, hypocalcemia can occur. This results in loss of nerve and muscle function which can, in some instances, lead to recumbency (milk fever in cows) or tetany (lactating dogs, cows, pigs). During vitamin D intoxication, calcium can enter the extracellular compartment faster than it leaves, resulting in hypercalcemia, which can lead to soft tissue deposition of calcium and then eventually necrosis of the tissues.

Calcium leaves the extracellular fluids during bone formation, in digestive secretions, sweat, and urine. An especially large loss of calcium occurs during lactation or egg-shell formation. Calcium lost via these routes can be replaced from dietary calcium, from resorption of calcium stored in bone, or by resorption of a larger portion of the calcium filtered across the renal glomerulus (i.e., reducing urinary calcium loss). Whenever calcium loss from the extracellular fluids exceeds the amount of calcium entering the extracellular fluids, plasma calcium concentration decreases. The parathyroid glands monitor carotid artery blood calcium concentration and secrete parathyroid hormone when they sense a decrease in blood calcium concentration. Parathyroid hormone immediately increases renal calcium reabsorption mechanisms to reduce urinary calcium loss. This will succeed in returning blood calcium concentration to normal if the loss from the extracellular compartment is small, because normally only small amounts of calcium are excreted in the urine each day. For example, a 600-kg cow typically excretes 0.5–2 g calcium in her urine each day. When calcium losses are larger, parathyroid hormone will stimulate processes to enhance intestinal calcium absorption and resorption of bone calcium stores.

Bone is a living tissue that is constantly undergoing formation and resorption. In young animals the rate of formation exceeds the rate of resorption, resulting in net bone accretion. In mature animals, portions of the skeleton, presumably those traumatized with microfractures during normal wear and tear, are resorbed and reformed constantly. In humans it is estimated that the entire adult skeleton is rebuilt every 7 years (Frost, 1964). Parathyroid hormone can uncouple bone resorption from bone formation, stimulating resorptive mechanisms of bone osteoclasts while inhibiting formation mediated by bone osteoblasts. The net result is an efflux of calcium from bone to extracellular fluids. When excessive calcium enters the blood, another hormone, calcitonin, produced within the thyroid gland, is secreted. Calcitonin inhibits osteoclastic bone resorption and stimulates renal excretion of calcium.

Ultimately dietary calcium must enter the extracellular fluids to permit optimal performance of the animal. Calcium absorption can occur by passive transport between epithelial cells across any portion of the digestive tract whenever ionized calcium concentration in the digestive fluids directly over the mucosa exceeds 6 mM (Bronner, 1987). These concentrations are reached when calves are fed all-milk diets and when cows are given oral calcium drenches for prevention of hypocalcemia (Goff and Horst, 1993). In nonruminant species, studies suggest that as much as 50 percent of dietary calcium absorption can be passive (Nellans, 1988). It is unknown how much passive absorption of calcium occurs from the diets typically fed to ruminants, but the diluting effect of the rumen would likely reduce the degree to which passive calcium absorption would occur. Active transport of calcium appears to be the major route for calcium absorption in mature animals and this process is controlled by 1,25-dihydroxyvitamin D, the hormone derived from vitamin D. Vitamin D, produced within the skin or provided in the diet,

is converted to 25-hydroxyvitamin D in the liver and can be further metabolized to 1,25-dihydroxyvitamin D in the kidneys. Parathyroid hormone indirectly stimulates intestinal calcium absorption because it is the primary regulator of renal production of 1,25-dihydroxyvitamin D. The 1,25-dihydroxyvitamin D is released to the circulation and interacts with nuclear receptors within the intestinal epithelium, primarily in the small intestine, causing transcription and translation of calcium transport proteins. Vitamin D-dependent calcium-binding protein captures calcium at the apical surface of epithelial cells and ferries the calcium to the basolateral side of the cell. There it is pumped into the extracellular space against a concentration gradient by a 1,25-dihydroxyvitamin D-dependent calcium pump (Wasserman, 1981; Bronner, 1987). By carefully regulating the amount of 1,25-dihydroxyvitamin D produced, the amount of dietary calcium absorbed can be adjusted up or down to maintain constant extracellular calcium concentration.

Freshwater fish can obtain calcium from the water across their gills. If the water in which they are reared is high in calcium, they have little need for dietary calcium and little need for vitamin D as intestinal calcium absorption is not critical to meeting their calcium requirement. However, when water calcium content is low the diet must supply calcium, and the fish require vitamin D to use intestinal calcium absorption efficiently (Lovell and Li, 1978). When freshwater fish must rely on their diet to meet calcium needs, they also face another problem that terrestrial animals do not encounter: many do not possess gastric glands and cannot secrete hydrochloric acid into the ingesta. Therefore the inability to solubilize dietary calcium can reduce absorption of calcium incorporated into feed. Saltwater fish drink seawater where the calcium is already in a soluble form, so the calcium is readily available for intestinal absorption.

SOURCES AND BIOAVAILABILITY

Calcium content of grains and high-starch feedstuffs is very low and generally of low importance as a source of calcium that might cause toxicity in an animal. Legumes can be an important source of calcium for herbivores but the bioavailability of calcium in plants is generally lower than that of mineral sources (Martz et al., 1990). Common mineral sources of calcium used to supplement diets of animals include calcium carbonate, calcium sulfate, mono- and dibasic calcium phosphate, and calcium chloride. Bone meal and oyster shells are also good sources of calcium. In ruminants, calcium carbonate may have low bioavailability due to its poor solubility. However, if finely ground, the calcium in calcium carbonate becomes readily solubilized upon contact with the acid of the abomasum and availability can be quite high. In pigs, particle size of calcium carbonate had no effect on calcium bioavailability (Ross et al., 1984). Calcium from these mineral sources can be from 70–90 percent available. During positive calcium balance, intestinal mechanisms for absorption are shut down in most species. Notable exceptions are the horse and rabbit (and possibly other hind gut fermenters). In these species calcium absorption appears to be independent of vitamin D and a large proportion of dietary calcium is always absorbed (Bourdeau et al., 1986; Brommage et al., 1988; Maenpaa et al., 1988).

Water can supply a small portion of the dietary calcium requirement of birds and mammals but does not contain enough calcium to cause toxicosis. Many freshwater fish thrive in the hardest waters (hardness up to 300 mg calcium carbonate/L) as it reduces the difference between the osmolarity of the fish's body and the water and reduces the energy required by the fish to osmoregulate (Boyd, 1979).

TOXICOSIS

Single Dose

If a large bolus dose of calcium is given to an animal orally it is possible for the concentration of calcium directly over the mucosa of the intestinal tract to rise to the level at which passive absorption of calcium occurs by paracellular transport (Bronner, 1987). This pathway is not regulated by the calcium homeostatic mechanisms. As a result, very large amounts of readily soluble calcium administered as a single dose can cause hypercalcemia for several hours (Goff and Horst, 1994) (Table 10-1). Moderate hypercalcemia (plasma calcium between 120 and 150 mg/L or 3–3.75 mM) causes increased urine excretion (diuresis) and depresses feed intake. Severe hypercalcemia (plasma calcium between 150 and 250 mg/L or 3.75–6.25 mM) will begin the process of metastatic calcification of soft tissues, which may or may not be reversible. In some cases blood calcium can increase to the point that the heart stops during systole (plasma calcium above 280 mg/L or 7 mM) (Littledike et al., 1976). These definitions of hypercalcemia do not apply to laying hens—their total plasma calcium content is typically 200–400 mg/L or 5–10 mM. However, their ionized calcium content is 40–50 mg/L or 1–1.25 mM, similar to other animals (Puls, 1994).

In cattle, oral calcium drenches are used to prevent and/or treat the hypocalcemia that is common in dairy animals around the time of calving and the onset of lactation. Calcium chloride and calcium propionate are very soluble calcium salts that have been used widely for this purpose (Jonsson and Pehrson, 1970; Goff et al., 1996). Administering a concentrated solution that supplied 50 g of calcium from calcium chloride into the oral cavity increased plasma calcium by 30–40 mg/L or 0.75–1.0 mM, which was maintained for 4–6 hours. Administering 100 g of calcium from calcium chloride increased plasma calcium by 60–80 mg/L (1.5–2.0 mM), which stayed elevated for 6–8 hours. These doses did not cause the toxic effects associated with severe hypercalcemia (Jonsson and Pehrson, 1970; Goff and Horst, 1994). However, it was noted that repeated doses of the

higher quantities of calcium chloride induced a life-threatening metabolic acidosis, a result of the chloride anion being absorbed along with the calcium (Goff and Horst, 1993). The use of chloride salts of calcium has also been associated with erythema and necrosis of the abomasum and rumen (Wentink and van den Ingh, 1992). Using calcium propionate as the source of calcium has similar effects on plasma calcium concentration but does not cause metabolic acidosis (Goff and Horst, 1994; Pehrson et al., 1998). Placement of the calcium into the back of the pharynx can elicit closure of the esophageal groove in cattle, allowing a proportion of the solution being administered to bypass the rumen. This causes a greater increase in blood calcium than occurs if the calcium is placed into the rumen via use of a tube or hose ending in the rumen (Goff and Horst, 1993). Cows receiving 146 g calcium as a drench delivered into the esophagus (beyond the pharynx) on the day of calving as calcium propionate had rapid increases in plasma calcium, with no deleterious effects noted. Cows receiving 219 g calcium as calcium propionate also had rapid increases in plasma calcium. The hypercalcemia was not considered severe and lasted less than 24 hours. However, it was accompanied by profound hypomagnesemia, which was considered an undesirable effect. Calcium propionate delivered into the esophagus at a quantity of 1.36 kg (supplying 285 g calcium) caused severe hypercalcemia in one of four cows given this dose at calving. The same dose given to two steers was found to be lethal in both (Goff et al., 2002), suggesting the calcium demands of lactation offered some protection from the development of acute severe hypercalcemia following oral administration of calcium.

Administering 50 g calcium from calcium carbonate to dairy cows did not alter blood calcium concentration, suggesting that the more soluble the form of calcium administered in a single oral dose the greater the risk of hypercalcemia developing (Goff and Horst, 1993).

Calcium polysulfide (lime sulfur), used to control fungal diseases in trees and shrubs, has the potential for accidental incorporation into diets as a calcium source. The animals could become sick from the metabolic acidosis induced by the anionic portion of the calcium polysulfide molecule. The calcium itself is not the toxic moiety (Horowitz et al., 1997).

Acute

In animals with intact calcium homeostatic mechanisms, short-term increases in dietary calcium are very well tolerated. The production of 1,25-dihydroxyvitamin D will be downregulated to decrease intestinal calcium absorption of dietary calcium. If kidneys are functioning properly, any excess calcium that is absorbed will be rapidly excreted with the urine. In the horse and rabbit the intestine is not a regulatory point of calcium homeostasis. Intestinal calcium absorption mechanisms are always turned on in these species, and feeding high dietary calcium increases the amount of calcium that enters the blood. Instead, these species use renal calcium excretion to control blood calcium concentration. During renal failure these species are in danger of developing hypercalcemia even when dietary calcium is within normal limits.

Young growing pigs may be more likely than other species to develop hypercalcemia with increasing dietary calcium (Reinhart and Mahan, 1986; Hall et al., 1991). In one case report, a 5.62 percent calcium diet was accidentally fed to piglets after weaning (the requirement was about 0.8 percent diet calcium). Several of the piglets were severely hypercalcemic with blood calcium greater than 200 mg/L (5 mM). All grew poorly and had severely reduced feed intake. They were dehydrated, had markedly dry and hard feces, and were drowsy (Kamphues et al., 1989). In this case, dietary calcium concentration was high enough to permit calcium to be absorbed by the paracellular, vitamin-D independent pathway at a greater rate than the kidneys could excrete the calcium.

Chronic

Feeding excessive dietary calcium long term is generally not associated with any specific calcium-based toxicity. Hypercalcemia generally does not occur if calcium homeostatic mechanisms are intact. The main effect directly attributable to calcium is a reduction in feed intake as more calcium mineral is added to the diet. This may simply be a palatability issue or it could be mediated by calcitonin, a hormone produced in the gut following a meal, and by thyroid C-cells, in response to even slight elevations of calcium concentration. Excessive calcitonin can inhibit feed intake (Freed et al., 1979). A second common effect of calcium in the diet is to reduce the availability of other minerals in the diet, such as phosphorus or zinc, especially if the animal is receiving a diet that is marginally adequate in these other minerals.

Rabbits can be fed as much as 4.5 percent calcium diets with no ill effects (Chapin and Smith, 1967). Horses fed diets that were 2.5 percent calcium for four years exhibited no ill effects provided the diets also had adequate phosphorus (Jordan et al., 1975). Renal excretion of calcium increases with increased dietary calcium concentration in rabbits and horses to prevent development of hypercalcemia and its attendant problems (Schryver et al., 1974).

Feed intake in dogs and cats is largely dictated by energy content of the diet and therefore feeding standards for dogs and cats often express calcium and phosphorus contents of diets in terms of g/1,000 kcal metabolizable energy. For this report, the diet calcium levels will be expressed as g/kg diet DM. If the original literature cited expressed diet calcium only in terms of diet energy, the assumption was made that typical dog and cat diets are 4,000 kcal ME/kg DM. For puppies, the dietary calcium and phosphorus allowances are 12 g Ca and 10 g P/kg DM (NRC, 2006). For kittens, the dietary calcium and phosphorus allowances are 8 g Ca and

7.2 g P/kg DM (NRC, 2006). These allowances decrease with age as growth slows in the animals.

In large-breed dogs, bone growth is especially rapid the first three months of life. Disturbances in endochondral ossification are a frequent cause of clinical problems in the giant breed dogs, particularly in Great Danes. Giant breed dogs fed high calcium diets may be at greater risk of developing osteochondrosis, rickets, and other bone problems than dogs fed lower calcium diets, especially if the diet provides enough energy and protein to support rapid growth, or is marginal in phosphorus. Ad libitum feeding may increase the risk of osteochondrosis over feed restriction, where growth is slowed (Hedhammer et al., 1974). Feeding Great Dane puppies diets that were 2.72 percent calcium and 0.9 percent phosphorus greatly increased the incidence of osteochondrosis and skeletal problems and caused significant hypophosphatemia in some animals (Hazewinkel et al., 1985; Goedegebuure and Hazewinkel, 1986). If dietary phosphorus is increased along with the dietary calcium, the negative effects of dietary calcium are largely, but not entirely, nullified (Weber et al., 2000; Lauten et al., 2002). Schoenmakers et al. (2000) concluded that feeding increased diet calcium alone decreased the amount of phosphorus available for bone formation, which resulted in rachitic lesions, while increasing both diet calcium and phosphorus increased the risk of osteochondrosis lesions in fast-growing Great Danes. When the diet calcium to phosphorus ratio exceeds 2:1 there is risk of increased osteochondrosis in the Great Dane breed (Hazewinkel et al., 1991; Schoenmakers et al., 2000). The Great Dane breed may be rather unique in its sensitivity to dietary calcium concentration, as Giant Schnauzers fed similar diets show no osteochondrosis bone lesions (Weber et al., 2000). Miniature poodles fed diets that were as high as 3.3 percent calcium and just 0.9 percent phosphorus still managed to grow well and had no bone problems, though they did have a temporary decrease in feed intake at 11 weeks of age when compared to poodle pups fed a 1.1 percent calcium, 0.9 percent phosphorus diet (Nap et al., 1993). A summary of trials performed by a commercial pet food manufacturer demonstrated that puppies across a variety of breeds exhibited no skeletal problems when fed up to 2.0 percent calcium on a DM basis—even if diet phosphorus was not increased. When diet calcium exceeded 2.3 percent, feed intake and growth were depressed in many breeds (Laflamme, 2000).

Few studies have examined the effects of elevated diet calcium in cats. Howard et al. (1998) observed reduced feed intake and growth in kittens fed 2.3 percent calcium diets. The kittens also exhibited pronounced hypomagnesemia.

Calves are typically fed milk (whole milk is approximately 0.95 percent calcium on a DM basis) or milk replacer that is 0.9–1.1 percent calcium. At weaning, the diet calcium is generally reduced to 0.6–0.7 percent. Maintenance diets for adult cattle are typically 0.45–0.6 percent calcium. In lactating cows the diet calcium is increased to 0.7–0.9 percent calcium to accommodate lactational demands for calcium. In cattle, dietary calcium concentrations greater than 1 percent have been associated with reduced DM intake and lower performance (Miller, 1983). The amount of mineral added to some of these rations to achieve the higher calcium levels is often unpalatable and also replaces energy or protein that could be used by the animal for growth or other functions. Beede et al. (2001) fed 0.47, 0.98, 1.52, and 1.95 percent calcium diets to cows in late gestation that were receiving a high-chloride diet to prevent milk fever. Cows fed 1.5 percent calcium diets tended to eat less than cows offered 0.98 percent or 0.47 percent calcium diets, while those fed the 1.95 percent calcium diet had significantly lower feed intake. Dietary calcium did not influence the degree of hypocalcemia experienced at calving or milk production in the subsequent lactation.

When diets suitable for lactating cows (0.85 percent calcium) were fed to mature bulls for prolonged periods, the bulls were reported to have developed osteopetrosis of the bone and ultimobranchial tumors of the thyroid gland, presumably from excessive calcitonin production (Krook et al., 1969). Similarly, high calcium diets fed to cows in late gestation were thought to lead to benign hyperplasia of the thyroid gland and excessive secretion of calcitonin. The high-calcium diets were also found to depress secretion of parathyroid hormone prior to calving (Black et al., 1973; Yarrington et al., 1977). Other experiments demonstrated that very low calcium diets fed in late gestation could prevent milk fever, a condition of severe hypocalcemia experienced by some cows at the onset of lactation (Boda and Cole, 1954; Goings et al., 1974; Green et al., 1981). These observations gave rise to the theory that high calcium diets fed to the late gestation dairy cow caused milk fever to develop. Studies now suggest milk fever is instead caused by excessive dietary potassium or sodium, not calcium (Ender et al., 1971; Goff and Horst, 1997). The high cation diets induce a metabolic alkalosis in the cow, which interferes with parathyroid hormone function, predisposing the cow to hypocalcemia (Gaynor et al., 1989; Phillippo et al., 1994).

Calcium, fed in the form of limestone, has some rumen buffering activity in cattle. Wheeler and Noller (1976) fed lactating cows up to 1.48 percent calcium diets. Though there was a small decrease in feed intake with the higher calcium diets, there was no effect on fat-corrected milk production and there was more efficient use of dietary starch. Similar observations were made by Clark et al. (1989). However, in their study, feeding a diet that was 3.5 percent calcium carbonate (estimated to be 1.7 percent calcium) did significantly decrease feed intake, though fat-corrected milk production was not affected.

Very high diet calcium concentration can reduce digestibility of the diet. Ammerman et al. (1963) found that diets that were 4.4 percent calcium reduced protein and energy digestibility of the ration of beef steers. In another study, veal calves aged 8 weeks were fed iso-energetic amounts of

0.71 percent or 1.16 percent calcium milk replacers for a period of 10 weeks. The extra calcium was added in the form of calcium formate. Feces were collected during the final week of the trial. The high calcium diet raised the amount of energy contained in excreted feces by 70 percent. The extra fecal energy output was mostly in the form of crude fat, presumably from formation of insoluble calcium soaps of the fatty acids in the diet. The high versus low calcium intake not only depressed apparent digestibility of total lipids but also that of crude protein, carbohydrates, and ash (lower ash digestibility was largely a result of dietary calcium that was not absorbed). However, the final body weight of the two dietary groups was similar, so it is difficult to use these data to suggest that 1.16 percent calcium (DM) in milk replacer is necessarily excessive (Xu et al., 1999). When weaned calves were fed diets that were 0.34 percent phosphorus and either 0.17, 0.67, 1.31, or 2.35 percent calcium, all grew normally during the four-week trial, suggesting little effect on diet digestibility. However, in those calves fed the 1.31 or 2.35 percent calcium diets, there was a reduction in soft tissue levels of trace minerals such as zinc, manganese, and copper. The 2.35 percent calcium diet also depressed blood phosphorus concentration to levels that might ultimately have led to rickets had the study gone on longer (Alfaro et al., 1988). Fontenot et al. (1964) demonstrated that diets containing 1.2 percent calcium could induce zinc deficiency in lambs receiving no supplemental zinc. However, if zinc were added to the diets, the lambs could be fed 2.4 percent calcium diets with no effects on growth.

In pigs, feed intake and growth are markedly reduced whenever the dietary calcium:phosphorus ratio exceeds 3:1 (Reinhart and Mahan, 1986). Thus, starting pigs requiring 0.60 percent dietary phosphorus would be expected to suffer reduced feed intake and growth when dietary calcium exceeds 1.8 percent, and finishing pigs requiring 0.45 percent dietary phosphorus would suffer reduced growth rate when dietary calcium exceeds 1.35 percent, unless diet phosphorus was also increased. High calcium content is reported to reduce diet digestibility in pigs. Zimmerman et al. (1963) and Combs et al. (1966) found that addition of calcium to the diet of baby pigs reduced growth, feed efficiency, DM digestibility, and bone mineralization, especially when the calcium level exceeded 1.0 percent of the diet. These effects were observed even though the dietary calcium to phosphorus ratios were less than 2:1. The experiments of Zimmerman et al. (1963) and Combs et al. (1966) suggest that the maximal tolerable dietary calcium level is 1.0 percent, although the study by Reinhart and Mahan (1986) demonstrated that increasing dietary phosphorus could increase the tolerable dietary calcium level.

Fangauf et al. (1961) and Urbanyi (1960) in separate experiments fed chicks diets that ranged from 1.2 to 6.5 percent calcium. Levels above 2 percent depressed feed intake and weight gain and increased mortality rate. Shane et al. (1969) report that visceral gout and nephrosis occurred in growing pullets fed a 2.5 percent calcium diet from 8–20 weeks of age. Hurwitz et al. (1995) fed chicks of fast-growing (Cobb) and slow-growing (Leghorn) breeds diets that were 0.4, 0.6, 0.8, 1.0 (considered the required level of dietary calcium), 1.5, and 2.0 percent calcium with 0.7 percent phosphorus (considered the required phosphorus level). Chicks of the slow-growing breed grew equally well with 1.0, 1.5, and 2.0 percent calcium diets. However, chicks of the fast-growing breed fed 2 percent dietary calcium weighed significantly less than fast-growing chicks fed the 1.0 percent calcium diet. When dietary phosphorus was increased to 0.9 percent the negative effect of the 2 percent calcium diet on growth was greatly ameliorated, but not eliminated.

For hens consuming 100 g feed/day the dietary calcium requirement is 3.25 percent (NRC, 1994). The 1980 NRC *Mineral Tolerance of Domestic Animals* set 4 percent diet calcium as a maximum tolerable level in laying hens based largely on the work of Gutowska and Parkhurst (1942), who reported that laying hens fed a 3.95 percent calcium diet suffered reduced egg production and feed efficiency, though eggshell strength was not affected. However, in another study, laying hens fed diets as high as 5 percent calcium showed no ill effects (Harms and Waldroup, 1971). In fact, recent studies with modern high-producing hens suggest the calcium requirement of laying hens may be closer to 5 percent calcium (Bar et al., 2002).

The greater potential risk from high-calcium diets is interference with absorption of other minerals, causing deficiency of these minerals, despite their inclusion in the diet at levels that would ordinarily be considered sufficient. High-calcium diets can interfere with the absorption of phosphorus, which is of particular concern in nonruminant animals. Much of the phosphorus of plant seed origin fed to these animals is bound to an inositol backbone. Up to 6 phosphate molecules can be bound to each inositol molecule, forming inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate, also known as phytate. Nonruminant animals vary in their ability to digest phytate to free up the phosphorus. Poultry can, under some extreme circumstances, use as much as 50 percent of dietary phytate phosphorus. However, in general, swine and poultry hydrolyze only a small portion of the phytate phosphorus they are fed. The endogenous phytase of most nonruminant animals exists primarily in the mucosa of the small intestine and the phytate must be soluble at the pH found in the small intestine for the phytase to effectively hydrolyze the phytate. Phytases originating from feed ingredients or microbial synthesis are also most active when the phytate is soluble. Unfortunately the phytate molecule, with its protruding phosphate, is a highly negatively charged particle. Cations (and in some cases proteins or starches) can then form bonds with the phosphate moieties. The propensity to form bonds with a cation is so great that much of the feedstuff phytate ingested is already chelated to cations, such as potassium, iron, calcium, magnesium, zinc, and copper. Phytate bound to divalent cations, especially calcium, is rela-

tively insoluble at the pH of the small intestine and is therefore resistant to intestinal mucosa phytase digestion. Phytate bound to calcium is soluble at the acidic pH of the stomach and will be digested by phytases of microbial origin that are active at low pH. Calcium, being the most prevalent divalent cation in many diets, is commonly chelated to phytate, but other divalent and trivalent minerals can also become bound to phytate. A single phytate molecule can bind more than one divalent cation. The more cations attached to the phytate the lower the solubility of the complex and the less prone it is to hydrolysis by phytases (Wise, 1983). For this reason, therefore, the greater the calcium content of the diet the less available phytate phosphorus is to the nonruminant animal. In ruminants the activity of microbial phytase, either within the rumen or as the ingesta passes through the abomasum or true stomach, does a thorough job of digesting phytate and freeing the bound minerals. Therefore high calcium diets fed to ruminants do not interfere with phosphorus and trace mineral absorption to the same extent as in nonruminants (Kincaid, 1979).

In nonruminants only small amounts of phytate are digested by the endogenous phytases of the gastrointestinal tract. Dietary calcium reduces the efficacy of intestinal phytase and also the efficacy of phytase of fungal origin added to the diet. In poultry and swine, the ability of calcium to interfere with phytase activity occurs even at dietary calcium concentrations considered essential to meet the calcium needs of the bird (0.9 percent calcium diets) (Atia et al., 2000; Applegate et al., 2003) or pigs (0.8 percent calcium diets) (Sandberg et al., 1993; Lei et al., 1994). However, inhibition of phytate digestion becomes more critical at dietary calcium levels that exceed the requirement of the animal. For example, in fast-growing broilers, increasing dietary calcium from 0.8 to 2.0 percent reduced growth rate and caused pronounced hypophosphatemia. This effect seemed to be due to decreased absorption of phosphate from the diet of the birds (Hurwitz et al., 1995). It was also noted that increasing diet calcium in broilers using calcium carbonate caused hypophosphatemic rickets, while the same level of diet calcium achieved by supplementation with dicalcium phosphate did not. This suggests that the bone problems arose because calcium carbonate interfered with phosphorus metabolism rather than as a direct effect of calcium toxicity (Ogura, 1981).

Feeding excessive calcium could interfere with trace mineral absorption (especially zinc and other trace minerals bound to phytate) and replaces energy or protein the animal might better use for increased production. In swine, excess calcium greatly reduces zinc availability, especially if phytate is high (Morgan et al., 1969). A diet containing 1.71 percent calcium and 68 mg Zn/kg fed to growing boars induced parakeratosis (abnormal keratinization of the skin) due to zinc deficiency. The dietary zinc requirement of boars at this age is 50 mg/kg diet, so this diet would otherwise have been considered more than adequate in zinc (Beilage et al., 1992).

High dietary calcium may also increase the requirement for vitamin K. Pigs fed a 2.7 percent calcium diet based on corn and soybean meal with no supplemental vitamin K developed internal hemorrhaging and died within 28 days. Supplementing the diet with 5 mg menadione prevented further problems (Hall et al., 1991).

In fish, dietary calcium can also interfere with trace mineral absorption and retention (Hardy and Shearer, 1985; Kaushik, 1995). For example, Redlip mullet (*Liza haematocheila*) fed a 2.5 percent calcium diet were reported to have reduced bone zinc, manganese, and iron content when compared to fish fed a 0.2 percent calcium diet (Hossain and Furuichi, 2000). High water calcium concentration also reduces gill uptake of zinc from the water. Although this effect might induce a secondary zinc deficiency, it may be used as a means of protecting fish when water is contaminated with excessive zinc or other heavy metals (Barron and Albeke, 2000).

Female rats given 0.50, 0.75, 1.00, or 1.25 percent dietary calcium as calcium carbonate in AIN-76A diets for 6 weeks before mating, during mating, and for 20 days of gestation had dose-related linear decreases in the iron content of the liver, and in the zinc, iron, and magnesium contents of the kidney. Even though the highest calcium diet represents only a moderate increase in calcium above requirement, the fetuses from these rats had dose-related decreases in their whole-body contents of phosphorus, iron, copper, and magnesium (Shackelford et al., 1994).

Factors Influencing Toxicity

One factor that can upset calcium homeostasis is excessive (toxic) intake of vitamin D, or ingestion of plants containing glycosides of 1,25-dihydroxyvitamin D. In this case the intestinal calcium absorption mechanisms are fully engaged and diets high in calcium are more rapidly able to induce hypercalcemia and metastatic calcification than low calcium diets.

In the horse and the rabbit, compromised renal function will not allow excretion of excess calcium absorbed from the diet and hypercalcemia will develop.

TISSUE LEVELS

Normal total plasma or serum calcium concentrations are between 90 and 110 mg/L or 2.25–2.75 mM for many mammals and growing birds. Horses and rabbits tend to be slightly higher, and up to 130 mg Ca/L plasma or serum may be considered normal (Table 10-2). The rabbit, in particular, will exhibit higher blood calcium concentration as dietary calcium content increases. Laying hens have a special calcium binding protein circulating in their blood, allowing them to rapidly deposit calcium into the oviduct during eggshell formation, and they will normally have total plasma or serum calcium concentrations ranging from 180–360 mg/L

or 4.5–9 mM. Ionized calcium concentration—which is calcium that is not bound to proteins or other organic moieties in the blood—is relatively constant in all species, including poultry that are laying eggs, and is usually 40–60 mg Ca/L (1–1.5 mM) plasma or serum.

Elevations in blood calcium concentration are common in cases of acute calcium toxicity. Plasma total calcium concentrations exceeding 280 mg/L will cause cardiac arrest in many species, since this often means ionized calcium concentration is also greatly increased. Typically these high blood calcium values are only encountered when animals are receiving intravenous calcium treatments at too high a dose or too rapid a rate of administration. Blood calcium concentrations exceeding 150 mg/L (3.75 mM) for more than 12–24 hours will begin to cause metastatic calcification of soft tissues, particularly the kidneys, stomach, and vasculature, which would severely compromise renal function within 1–2 weeks. Chronically elevated blood calcium concentrations above 120 mg/L (3 mM) can also cause metastatic calcification of soft tissues but the process is slower and would occur over weeks to months. Normal liver and kidney calcium content is generally below 200 mg/kg wet weight or about 800 mg/kg on a dry weight basis. Kidney or liver tissue calcium contents greater than 300 mg/kg wet weight would indicate metastatic calcification. Chronically elevated blood calcium concentrations can occur with extremely high calcium diets, but this is rare. More commonly it is a sequelae of vitamin D intoxication, primary hyperparathyroidism, or, in some species such as the horse and rabbit, a consequence of renal failure. Urine calcium concentrations are a poor indicator of dietary calcium excess. Bone ash and bone calcium are sensitive to calcium deficiency states but do not reflect a calcium toxicity situation.

Cow's milk contains approximately 1.2 g Ca/L. Sheep milk contains approximately 2.0 g Ca/L and goat milk about 1.5 g Ca/L. These values change with breed slightly but they are not impacted by dietary calcium. Sow's milk is about 1.3 g Ca/L. Because sow's milk is about 20 percent dry matter, a pig that is consuming only sow's milk is receiving a diet that is approximately 0.65 percent calcium on a dry matter basis.

MAXIMUM TOLERABLE LEVELS

Single Dose

The criteria used to determine the maximum tolerable single oral dose of calcium is that quantity of calcium that is not expected to cause severe hypercalcemia, defined as plasma calcium greater than 150 mg/L (3.75 mM) for more than 12 hours, as this degree of hypercalcemia appears to have severe consequences on feed intake, renal function, and hydration state. The maximum tolerable dose of calcium that can be given once to adult cattle as a drench without causing life-threatening hypercalcemia is 150 g calcium. This would be approximately equivalent to 0.25 g Ca/kg BW per os. Data for making a recommendation for any other species do not exist, and the safety of 0.25 g Ca/kg is likely reduced in nonruminant animals. In adult ruminants, the rumen fluids tend to dilute the calcium administered so that the concentration of calcium overlying intestinal mucosa is lower, rendering passive transport of calcium as less efficient (Goff and Horst, 1994). In nonruminants, the lack of dilution of the administered calcium before it reaches the small intestine would allow a greater portion of the administered calcium available for passive absorption.

In some cases, the anion accompanying the calcium will prove more toxic (due to induction of metabolic acidosis or caustic damage to tissues) than the calcium in the salt, reducing the tolerable amount of that particular calcium salt. For instance, the chloride of calcium chloride can induce a metabolic acidosis at a dose below the level where the calcium causes life-threatening hypercalcemia.

Acute

The criteria used to determine the maximum tolerable acute dietary calcium level is that quantity of calcium that is not expected to cause severe hypercalcemia, defined as plasma calcium greater than 150 mg/L (3.75 mM), for more than 12 hours when animals are introduced to the diet.

Very high concentrations of dietary calcium are tolerated by all species for short periods, provided the animal's kidneys are functioning or there is not excessive stimulation of 1,25-dihydroxyvitamin D receptors in the intestine of the animal (vitamin D intoxication, ingestion of plants containing glycosides of 1,25-dihydroxyvitamin D) causing uncontrolled absorption of calcium across the gut. It is likely that there is a dietary calcium level that will cause acute toxicosis from excessive hypercalcemia in every species if animals could be induced to consume these diets. However, for most species, acute toxicosis due to hypercalcemia has not been reported and a maximal tolerable level based on this criterion cannot be supported by data. This likely reflects the observation that feed intake decreases as the concentration of calcium increases in the diet (as in the studies of Reinhart and Mahan, 1986, and of Beede et al., 2001). Feed intake often declines quickly once diet calcium levels exceed 2–3 percent (though laying hens routinely consume 4.5 percent calcium diets with no ill effects). This is well below the 5 percent calcium diet level associated with acute toxicity when fed to piglets, which is the only documented instance of a high calcium diet causing acute toxic hypercalcemia in any species (Kamphues et al., 1989).

Chronic

If feed intake is significantly reduced by addition of calcium to the diet, animal growth will suffer. In assessing the maximum tolerable level of chronic dietary calcium expo-

sure, a reduction in feed intake accompanied by a reduction in performance (growth, milk or egg production, reproduction) will be considered the criteria comprising evidence of calcium toxicosis. More stringent criteria could have been used based on the evidence that addition of calcium to diets reduces the availability of phosphorus and trace minerals to nonruminants. Unfortunately, this effect occurs even at calcium concentrations in the diet that are suggested to be the required concentrations of calcium in the diet of nonruminants. Under these criteria, any time the calcium content of the diet exceeds the amount required to meet the calcium needs of the body there is a detrimental effect, because the amount of phosphorus and trace mineral required by the animal may be increased. Though these effects are generally of negligible consequence, there is a point at which these effects of calcium excess become intolerable and should be reduced. In nonruminant animals with fast growth rates (broilers, turkey poults, pigs, rats) the maximum tolerable calcium would be considered, by this criteria, to be very close to the calcium requirement of the animal because of the effect diet calcium has on use of phytate-bound minerals. These effects were generally not considered in determining the maximum tolerable levels, since most practical diets provide enough excess phosphorus or trace mineral to allow the animal to tolerate minor calcium effects on their availability. However, when studies demonstrated that the reduction in availability of secondary minerals caused by excess diet calcium resulted in increased clinical disease, such as rickets or parakeratosis, then the induction of secondary mineral deficiency was considered a significant criterion for determining the maximum tolerable dietary calcium level.

In preruminant calves, diet calcium concentration exceeding 1.2 percent DM can interfere with fat use, but does not impair growth or feed intake. The maximum tolerable dietary calcium for suckling calves is at least 1.2 percent and likely higher. In cattle, sheep, goats (and presumably other ruminants) the effects on phytate digestion are inconsequential. Adult cows were fed diets containing up to 1.5 percent calcium (in several studies), with little effect on feed intake and no significant effect on performance. Diets containing 1.9 percent calcium or greater can cause a significant decrease in feed intake in cattle. Although high calcium in the diet causes zinc deficiency in sheep fed diets without added zinc, few data demonstrate a detrimental effect of high calcium diets in sheep or goats. Therefore, for ruminants, the maximum tolerable dietary calcium level is set at 1.5 percent. In the horse and rabbit (and presumably other hind gut fermenters), 2 percent calcium is the maximum tolerable level, provided sufficient available phosphorus is included in the diet.

For growing poultry chicks, the maximum tolerable dietary calcium level is 1.5 percent. Diets containing 2 percent calcium will affect growth and bone ash of fast-growing chicks when diets contain required levels of phosphorus. High-producing laying hens can tolerate 5 percent calcium diets. Growing pigs have the lowest tolerance for dietary calcium, and detrimental effects are possible whenever the dietary calcium to phosphorus ratio exceeds 2:1. The maximum tolerable level of dietary calcium for pigs is 1 percent, though increasing dietary phosphorus can increase the maximum tolerable level to some extent. In fish the data are too limited to allow an estimate of the maximum tolerable dietary calcium concentration. The only data available suggest that diets that contain 2 percent or greater calcium interfere with use of trace minerals and affect growth when compared to diets that contain 0.2 percent calcium. Since fish are often fast growing, especially when young, and most are unable to digest phytate, it would seem reasonable to believe that dietary calcium levels resulting in calcium to phosphorus ratios less than 2:1 would not cause any detrimental problems, similar to other single-stomached species. Because most fish require 0.45 percent phosphorus diets (Lovell, 1991), the maximum tolerable dietary level is presumed to be 0.9 percent.

For most breeds of dog, the maximum tolerable dietary calcium concentration is at least 2 percent, including growing puppies of larger breeds. The tolerable level can be increased somewhat for most dogs provided the diet calcium to phosphorus ratio does not exceed 3:1. Most breeds will suffer a reduction in feed intake and growth if diet calcium exceeds 2.3 percent. For Great Dane and perhaps some other large breed puppies, rickets may occur if the diet calcium to phosphorus ratio exceeds 4:1. The maximum tolerable dietary calcium concentration for these giant breeds is 1.1 percent unless phosphorus is also added to the diet to bring diet calcium to phosphorus ratios between 1.2:1 and 1.4:1. If phosphorus is added to maintain this ratio, even Great Danes can be expected to tolerate up to 2.0 percent calcium diets. Diets containing more than 2.3 percent calcium risk increased occurrence of osteochondrosis in these breeds, even if dietary phosphorus is raised. In cats 1 percent calcium diets are well tolerated and result in optimal bone density (Pastoor et al., 1994). Feeding cats 2.3 percent calcium diets reduced growth rate and depressed feed intake and also caused a negative magnesium balance (Howard et al., 1998). There are no data to allow a more specific recommendation.

FUTURE RESEARCH NEEDS

Only with extremely high dietary calcium is it likely that hypercalcemia and its attendant problems will become manifest. However, it is increasingly clear that feeding animals more calcium than they require to meet their physiological requirements can reduce the efficiency of absorption of several other minerals, which might result in deficiencies of these minerals secondary to excess dietary calcium. Research to define the calcium level at which significant interaction with use of these other nutrients, in particular phytate phosphorus and zinc, could increase awareness of these subtle detrimental dietary calcium effects. Unfortunately, because

the consequences of dietary calcium insufficiency can be severe and because calcium is an inexpensive feed additive, the tendency of nutritionists formulating animal diets is to provide calcium in excess of levels needed to meet the animal's requirement. To keep calcium to phosphorus ratios optimal, excess diet calcium encourages oversupplementation of dietary phosphorus, the most expensive and environmentally damaging of minerals added to animal diets.

SUMMARY

Animals can tolerate relatively high amounts of dietary calcium for relatively long periods. Using hypercalcemia as the primary indicator of acute toxicity resulting from excessive absorption of dietary calcium, the level of dietary calcium that would cause this effect is generally so high that feed intake of the animal would be greatly reduced, limiting the development of hypercalcemia. When animals are fed calcium above the maximum tolerable levels over a longer period of time, the main effects observed would include interference with use of other minerals, especially phosphorus and zinc, and/or a significant reduction in feed intake affecting performance.

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TABLE 10-1 Effects of Calcium Exposure in Animals

Toxicity from a Single Dose of Calcium per Dose (quantity of calcium dosed)

Animal (N)	Age	Quantity	Source	Route	Effect(s)	Reference
Cats	Juvenile	2.5% powder	Calcium polysulfide (lime sulfur)	Topical (SID for 10 days)	Well tolerated, despite ingestion during grooming	Ackerman, 1984
Cattle (8)	Adult nonlactating Jersey	50–75g	Concentrated calcium chloride or calcium propionate solution	Drench	Increased blood calcium by 30–40 mg/L for 4–6 hr	Goff and Horst, 1993, 1994
Cattle (8)	Adult nonlactating Jersey	100 g	Concentrated calcium chloride solution	Drench	Increased blood calcium by 60–80 mg/L for 6–8 hr	Goff and Horst, 1993
Cattle (1)	Periparturient Jersey	100 g given twice	Concentrated calcium chloride solution	Drenches 12 hr apart	Severe metabolic acidosis	Goff and Horst, 1993
Cattle (4)	Periparturient Holstein	146 g	Calcium propionate in 9 L water	Esophageal tube	Moderate (desired) hypercalcemia with no ill effects	Goff et al., 2002
Cattle (4)	Periparturient Holstein	219 g	Calcium propionate in 9 L water	Esophageal tube	Moderate hypercalcemia accompanied by significant secondary hypomagnesemia	Goff et al., 2002
Cattle (4)	Petiparturient Holstein	285 g	Calcium propionate in 9 L water	Esophageal tube	Severe hypercalcemia in one of four, hypomagnesemia in all	Goff et al., 2002
Cattle (2)	Holstein oxen (900 kg)	285 g	Calcium propionate in 9 L water	Esophageal tube	Severe hypercalcemia leading to death	Goff et al., 2002
Cattle (99)	Periparturient Swedish Red and White	40 g (multiple doses)	Calcium propionate (boluses)	Per os	Well tolerated	Pehrson et al., 1998

Acute Calcium Toxicity (from <10 days ingestion of the diet)

Animal (N)	Age	Diet % Ca	Source	Duration	Effect(s)	Reference
Pigs (150)	Weanlings	5.62%	$CaCO_3$	Diet	Moderate to severe hypercalcemia, dehydration, reduced feed intake	Kamphues et al., 1989

Chronic Calcium Toxicity (from >10 days ingestion of the diet)

Animal (N)	Age	Diet % Ca	Source	Duration	Effect(s)	Reference
Cats (30)	10 wk	0.60% Ca 0.66% P	$CaCO_3$	12 wk	Control diet	Howard et al., 1998

continued

TABLE 10-1 Continued

Chronic Calcium Toxicity (from > 10 days ingestion of the diet)

Animal (N)	Age	Diet % Ca	Source	Duration	Effect(s)	Reference
Cats (30)	10 wk	2.3% Ca 0.7% P	$CaCO_3$	12 wk	Decreased feed intake and growth Elevated plasma calcium concentration and decreased plasma magnesium concentrations	Howard et al., 1998
Dogs (3 of each breed)	9 wk Great Dane and Giant Schnauzer	0.8% Ca 0.6% P		24 wk	Control; no skeletal or growth abnormalities	Weber et al., 2000
Dogs (3 of each breed)	9 wk Great Dane and Giant Schnauzer	1.5% Ca 1.23% P	$CaCO_3$	24 wk	No skeletal or growth abnormalities	Weber et al., 2000
Dogs (9)	3 wk Great Dane	1.04% Ca 0.82% P, restricted intake	$CaCO_3$	24 wk	Control diet. Minor osteochondrosis lesions in some dogs	Schoenmakers et al., 2000
Dogs (9)	3 wk Great Dane	3.11% Ca 0.87% P, restricted intake	$CaCO_3$	14 wk, then switch to control diet	Moderate hypercalcemia accompanied by severe hypophosphatemia. Reduced feed intake and growth. Pronounced rickets, which was not corrected when dogs were put back on control diet. Some loss of renal tuction	Schoenmakers et al., 2000
Dogs (6)	3 wk Great Dane	3.10% Ca 2.77% P, restricted intake	$CaCO_3$	14 wk, then switch to control diet	Observed more osteochondrosis lesions than in control diet dogs. Bone lesions resolved when dogs were put on control diet	Schoenmakers et al., 2000
Dogs (6)	2 mo miniature poodles	1.1% Ca 0.9% P fed ad libitum	$CaCO_3$	18 wk	Normal growth	Nap et al., 1993
Dogs (5)	2 mo miniature poodles	3.3% Ca 0.9% P fed ad libitum	$CaCO_3$	18 wk	Reduced feed intake by 11 wks on diet. Slight reduction in total growth Hypophosphatemia, but no clinical bone problems	Nap et al., 1993
Dogs	Weanling, medium-size breeds	1.9%	$CaCO_3$	6 mo	No skeletal or growth abnormalities	Laflamme, 2000
Dogs	Weanling, medium-size breeds	2.3%	$CaCO_3$	6 mo	No skeletal abnormalities Depressed feed intake and growth	Laflamme, 2000

Dogs (12)	Weanling Great Dane	2.05% (ad libitum intake)	$CaCO_3$	9–12 wk	Osteochondrosis Distortion of cartilaginous tissues Inhibition of chondrolysis Excessive thickness of cortical bone	Hedhammar et al., 1974
Dogs (11)	Weanling 4–5 wk Great Dane	0.78% Ca 0.67% P	$CaCO_3$	Up to 12 mo	Control diet with 4,900 kcal/kg feed ad libitum to promote rapid growth	Lauten et al., 2002
Dogs (10)	Weanling 4–5 wk Great Dane	2.67% Ca 2.27% P	$CaCO_3$	Up to 12 mo	No significant difference from controls	Lauten et al., 2002
Dogs (6)	5–10 wk Great Dane	3.3% Ca 0.9% P	$CaCO_3$	26 wk	Hypophosphatemia and temporary reduction in feed intake. No growth or skeletal problems when compared to 1.1% Ca, 0.9% P diet	Hazewinkel et al., 1991
Dogs (6)	5–10 wk Great Dane	3.3% Ca 3.0% P	$CaCO_3$	26 wk	No growth or skeletal problems when compared to 1.1% Ca, 0.9% P diet. No abnormalities in blood minerals	Hazewinkel et al., 1991
Rats, female	6 wk before mating	0.5, 0.75,1.0 and 1.25% Ca	$CaCO_3$	Fed until end of gestation	Dose-related decrease in maternal tissue Fe, Zn, and Mg. Dose-related decrease in fetal whole body Fe, P, Cu and Mg	Shackelford et al., 1994
Chickens (36)	Laying hens 2 strains 400–560 d	4.8% Ca	$CaCO_3$	56–84 d	No ill effects on bird health, slight reduction in egg wt with one strain of bird but increased eggshell wt in both strains	Bar et al., 2002
Chicken (36)	Laying hens	5% Ca	$CaCO_3$	Lay cycle	No ill effects on bird health or productivity	Harmis and Waldroup, 1971
Chickens	Chicks	1.2–6.5%	$CaCO_3$	> 2 wk	Above 2% calcium depressed feed intake and gain and increased mortality	Fangauf et al., 1961
Chickens (50)	8 wk	0.6 vs 3.0%	$CaCO_3$	12 wk	3% Ca caused visceral urate deposits and nephrosis and increased mortality	Shane et al., 1969
Chickens (1,715)	1 d	0.83% and 1.35% Ca (0.52% P)	$CaCO_3$	10 wk	1.35% Ca diet chicks weighed less, and had reduced feed efficiency	Smith and Taylor, 1961
Chickens	Chicks	0.9% Ca (NRC requirement)	$CaCO_3$		Reduced phytate digestion	Applegate et al., 2003
Chickens (30)	7-d broiler vs leghorn	0.8, 1.0, 1.5, and 2.0% Ca (0.7% P)	$CaCO_3$	14 d	2% Ca caused hypophosphatemia and large reduction in growth in fast-growing broiler chicks, but not in slow-growing Leghoms. Insignificant reduction in growth of broilers fed 1.5% Ca diet	Hurwitz et al., 1995

continued

TABLE 10-1 Continued

Chronic Calcium Toxicity (from >10 days ingestion of the diet)

Animal (N)	Age	Diet % Ca	Source	Duration	Effect(s)	Reference
Turkeys	Poults	0.9% Ca (NRC requirement)	$CaCO_3$		Reduced phytate digestion	Atia et al., 2000
Pigs, herd	Growing boars	1.71%	Unknown	Several weeks	Parakeratosis, reduced growth	Beilage et al., 1992
Pigs (30)	2 wk	0.5, 0.7, 0.9, and 1.0% Ca (0.5 or 0.7% P)	$CaCO_3$	5 wk	Reduced growth and bone mineralization at 0.9 and 1.0% Ca	Zimmerman et al., 1963
Pigs (24)	2 wk	0.48, 0.88, and 1.32% Ca (0.44% P)	$CaCO_3$	6 wk	Reduced weight gain and feed efficiency as diet Ca increased. Diet phosphorus digestibility not affected.	Combs et al., 1966
Pigs (8)	18 kg	2.7% Ca (0.9% P, 200 mg/kg Zn)	$CaCO_3$ Dicalcium phosphate	28 d	Increased clotting time and a hemorrhagic condition related to vitamin K deficiency induced by high calcium diet	Hall et al., 1991
Pigs (37)	4 wk	1.95% or 2.60% Ca (0.65% P)	$CaCO_3$ Dicalcium phosphate	28 d	Reduced daily gain and feed efficiency (not observed at 1.30% Ca when diet P was 0.65%). Reduced feed intake when diet Ca:P ratio was 3:1 or greater	Reinhart and Mahan, 1986
Pigs (37)	4 wk	0.94%,1.41, or 2.60% Ca (0.47% P)	$CaCO_3$ Dicalcium phosphate	28 d	Reduced daily gain and feed efficiency (not observed at 0.71% Ca when diet P was 0.47%). Reduced feed intake when diet Ca:P ratio was 3:1 or greater	Reinhart and Mahan, 1986
Horses (15)	4 mo	2.5% Ca	$CaCO_3$	4 yr	No effect on weight gain, mature height, or reproduction. Small decrease in cortical bone area and small increase in medullary area. No lameness or clinical repercussions	Jordan et al., 1975
Cattle	Mature bulls	0.85%	$CaCO_3$ (primarily)	Several years	Osteopetrosis, hypercalcitoninism	Krook et al., 1969
Cattle (84)	Late gestation dairy cows	0.98,1.52% or 1.95% Ca	$CaCO_3$	3 wk	Maximal feed intake at 0.98% Ca; slight reduction in feed intake at 1.52% Ca; marked reduction in feed intake at 1.95% Ca	Beede et al., 2001
Cattle (2)	2 yr	4.4%	$CaCO_3$	4 wk	Reduced digestibility of diet protein and energy	Ammerman et al., 1963

Cattle (4)	261 kg steers	0.90% Ca 0.34% P	Limestone	2 wk	No effect on fat or organic material digestibility compared to 0.45% Ca diet	Zinn and Shen, 1996
Cattle (19)	8 mo	16, 51, 106, or 160 g Ca/d (12 g P/d constant)	$CaCO_3$	140 d	Reduced weight gain as calcium in diet increaded	Dowe et al., 1957
Cattle (4)	Mid-lactation Holsteins	1.7% Ca	$CaCO_3$	14 d	Significant decrease in feed intake, but no effect on fat-corrected milk production and increased starch digestibility, suggesting more efficient use of feed energy	Clark et al., 1989
Cattle (18)	Lactacting Holsteins	0.48, 0.51, 0.87, 1.41, 1.48% Ca diets	Limestone and dicalcium phosphate	21 d	No effect on fat-corrected milk production. Increased starch digestibility. A small decrease in feed intake with 1.41% calcium but not with 1.48% calcium diet	Wheeler and Noller, 1976
Cattle (19)	6–8 wk	0.71% or 1.16%	Ca formate	27 wk	Reduced digestibility of dietary fat, phosphorus, and magnesium calcium soaps formed with diet fat	Xu et al., 1999
Cattle (4)	63 d	0.17, 0.67, 1.31, or 2.35% Ca (0.34%P)	$CaCO_3$	4 wk	Small reduction in tissue Zn, Mn, and Cu with 1.31 or 2.35% Ca also caused hypophosphatemia but no effect on growth rate	Alfaro et al., 1988
Fish (60)	2-wk mullet (seawater)	0.2 vs 2.5%	Tricalcium phosphate	10 wk	Reduced bone Zn, Mn, and Fe	Hossain and Furuichi, 2000

SI conversion: 1 mg calcium equals 25 μmoles calcium.

TABLE 10-2 Calcium Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)[a]

Animal	Serum	Muscle (wet wt basis)	Liver[b] (wet wt basis)	Kidney[b] (wet wt basis)	Bone g Ca/100 g (fat free, dry wt basis)	Milk (g/L) or Egg (g Ca/eggshell)
Chicken, broiler	90–130		75–80	170–600	14–20	
Laying hen	200–400				18–22	2.0–2.1
Pig	90–120		34–65	60–125	32–39	
Cow	90–100	30–300	30–200	45–200	24.5–26.0	1.2
Sheep	90–110	55–96	40–80	60–140		1.8–2.0
Fish	80–100	With bone 1,900–4,100 Without bone <1,000 Whole fish meal[c] 40–50 g/kg dry wt				

NOTE: Dietary calcium excess does not increase the calcium content of animal tissues significantly, unless accompanied by vitamin D intoxication.
[a]Data largely adapted from Puls, 1994.
[b]Vitamin D intoxication can cause metastatic calcification of soft tissues increasing calcium content of these tissues several-fold above normal upper limits.
[c]Menhaden or anchovy fish meal fed to livestock.

11

Chromium

INTRODUCTION

Chromium (Cr) is a metallic element that occurs in each of the oxidation states from –2 to +6. Only the 0, +2, +3, and +6 valence states are commonly found. Elemental chromium (0) does not occur naturally. Chromium (+2) is unstable and is readily oxidized to trivalent chromium (+3). Chromium occurs naturally primarily in the trivalent form, largely as ferrochromite ($FeCr_2O_4$) ores. Trivalent chromium (+3) forms stable complexes with both organic and inorganic ligands. Hexavalent chromium (+6) has strong oxidizing properties and is produced almost entirely by industrial processes (WHO, 1988). Chromium (+6) is spontaneously reduced to chromium (+3), when present in a soluble form.

The principal component of chromium ores is chromite, a black mineral consisting largely of iron and chromium (+3) oxides, with lesser amounts of magnesium and aluminum oxides (Katz and Salem, 1994). Chromium has not been mined in the United States since 1961. Most of the chromium ores processed in the United States are imported from South America, Turkey, and Russia (ATSDR, 2000).

Approximately 60 percent of the chromium processed is used in the production of stainless steel and other alloys. Ferrochromite used in the production of stainless steel and other alloys is prepared by the reduction of chromite with carbon (Katz and Salem, 1994). Chromium (+3) is used in the refractory industry as a component in chrome and chrome-magnesite, magnesite-chrome bricks, and granular chrome-bearing and granular chromite, which are used as linings for high-temperature industrial furnaces (ATSDR, 2000). Both trivalent and hexavalent chromium are produced in the chemical industry and used for chrome plating, leather tanning, and manufacturing of pigments and wood preservatives.

ESSENTIALITY

Early studies by Schwartz and Mertz (1959) showed that chromium was a component of a glucose tolerance factor that corrected impaired glucose metabolism in rats fed certain diets. Later studies demonstrated that chromium potentiates insulin function (Offenbacher et al., 1997). Clinical signs of chromium deficiency, including glucose intolerance, weight loss, and nerve and brain disorders, have been observed in humans receiving long-term parenteral nutrition (Anderson, 1995). Addition of small amounts of chromium (+3) chloride ($CrCl_3$) to the total parenteral nutrition solution corrected deficiency signs in all patients. Recent studies suggest that chromium affects insulin by binding to a low-molecular weight oligopeptide named chromodulin (Vincent, 2001). Chromodulin tightly binds four chromic ions and amplifies insulin receptor tyrosine kinase activity; thus, it enhances insulin action. Without chromium, apochromodulin is largely ineffective in stimulating insulin-dependent tyrosine kinase activity. Activation of apochromodulin is specific for chromium.

Until recently, practical diets fed to domestic animals were assumed to provide sufficient chromium to meet animal requirements. Although responses to chromium supplementation have been variable, a number of studies in the past 10 years have indicated that supplementation of diets with organic forms of chromium may affect animal metabolism and production criteria as well as the composition of animal products produced. Addition of chromium picolinate to diets of calves (Bunting et al., 1994) and growing pigs (Amoikon et al., 1995) increased glucose clearance rate following intravenous glucose administration. In swine, chromium picolinate has increased carcass lean and decreased carcass fat in growing-finishing pigs (Page et al., 1993) and increased litter size in sows (Lindemann et al., 1995; Lindemann, 1999). Chromium supplementation to poultry diets has improved growth rate in broilers (Lien et al., 1999) and reduced serum and egg yolk cholesterol in hens (Lien et al., 1996). A number of studies in cattle indicate that chromium supplementation from various sources may increase immune response and reduce incidence of disease, especially in stressed animals (Spears, 2000). Addition of chromium

methionine to lactating dairy cow diets increased milk production and feed intake (Hayirli et al., 2001).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Contamination of biological samples with chromium during collection, storage, and preparation of samples for analysis can present a major source of error. Prior to 1978, researchers were unaware that chromium concentrations they measured in tissues, blood, urine, and foods far exceeded true concentrations, because of background and environmental contamination (Guthrie et al., 1978). Chromium is generally present in tissues, plasma, and urine in the ng/g or ng/L range. Therefore, it is essential to minimize contamination from dust and stainless steel material (scalpels, knives, trays, needles, and grinding and homogenizing equipment) used in collection and preparation of samples. Stainless steel instruments should be replaced with titanium, glass, quartz, or polyethylene instruments (Katz and Salem, 1994). Needles made from nickel or platinum should be used for collection of blood samples for chromium analysis. Reagents of the highest purity also must be used to avoid contamination. Reagent blanks should be carried through sample preparation and analysis to correct for background contamination. Losses of chromium through volatilization may occur during heating or acid digestion of samples in open systems (WHO, 1988). A reference material (obtained from the National Institute of Standards and Technology) with a certified value for chromium is necessary to validate analytical procedures.

Low concentrations of chromium in biological samples can be measured using graphite furnace atomic absorption spectrometry, neutron activation analysis, or mass spectrometry. Graphite furnace atomic absorption spectrometry is the most commonly used method, and has a detection limit of 0.005 µg/L when an appropriate background correction method is used. Flame atomic absorption spectrometry and inductively-coupled plasma atomic emission spectrometry are not sensitive enough to detect chromium concentrations typically found in biological samples, but can be used to determine potentially toxic concentrations of chromium in feedstuffs.

REGULATION AND METABOLISM

Absorption and Metabolism

Metabolism of chromium is greatly affected by valance state. Hexavalent chromium is absorbed to a greater extent than the trivalent form (Donaldson and Barreras, 1966; Kerger et al., 1996) and readily enters cells through nonspecific anion carriers. In contrast, chromium (+3) is poorly absorbed and enters cells with very low efficiency. Insignificant amounts of hexavalent chromium are likely to be consumed orally because chromium (+6) is reduced in the environment to chromium (+3), the more stable oxidation state. Furthermore, chromium (+6) consumed is totally or at least partly reduced to chromium (+3) in the acid environment of the stomach (Donaldson and Barreras, 1966). Hexavalent chromium also does not generally occur naturally and is produced almost totally from human activities.

Absorption of chromium by humans consuming self-selected diets ranged from 0.5 to 2.0 percent, with higher absorption values observed in individuals consuming a low chromium intake (Anderson and Kozlovsky, 1985). Estimated absorption of chromium from soluble sources, such as $CrCl_3$ and chromium acetate, in humans and rats has ranged from 0.4 to approximately 2.0 percent (Donaldson and Barreras, 1966; Juturu et al., 2003). Absorption of chromium from chromium (+3) oxide was negligible in rats (Juturu et al., 2003). It is this property that allows use of chromic oxide as a nondigestible marker for digestibility studies. Gargus et al. (1994) reported a mean absorption of 2.8 percent in humans administered 400 µg Cr/day as chromium picolinate.

Chromium is transported in the blood primarily bound to transferrin (Offenbacher et al., 1997). Plasma or serum chromium concentrations in humans are generally less than 0.30 µg/L. Blood chromium concentrations are not in equilibrium with tissue chromium concentrations and do not reflect body stores. Tissue chromium concentrations are low (generally less than 100 ng/g DM) with kidney, liver, spleen, and bone containing the highest concentration.

The active form of chromium that potentiates insulin function remains unclear. Early studies suggested that chromium was a component of a glucose tolerance factor (Mertz, 1993). Glucose tolerance factor isolated from brewer's yeast contained chromium (+3), nicotinate, glycine, cysteine, and glutamate (Toepfer et al., 1976). More recent research suggests that chromodulin is the active form of chromium involved in facilitating insulin action (Vincent, 2001). Chromodulin is an oligopeptide comprised primarily of glycine, cysteine, aspartate, and glutamate, and has been isolated from the liver or kidney of a number of animal species (Vincent, 2001). In response to insulin release, chromium from the blood is transported to insulin-sensitive cells where chromium binds to apochromodulin. When insulin concentrations decrease, chromodulin is believed to be released from cells and excreted in the urine. Urine is the major route of excretion for absorbed chromium. Consumption of high carbohydrate diets that increase blood insulin concentrations increases urinary excretion of chromium (Offenbacher et al., 1997).

Metabolic Interactions and Mechanisms of Toxicity

Studies have indicated that chromium (+3) absorption is increased by ascorbic acid, amino acids, and oxalate (Offenbacher et al., 1997). Zinc may reduce chromium absorption; however, increasing dietary zinc did not reduce

toxicosis of chromium (+3) in chicks (Chung et al., 1983). Iron overload in humans increases saturation of transferrin with iron and may impair chromium transport (Offenbacher et al., 1997). High dietary concentrations of chromium (+3) alleviate signs of vanadium toxicosis in chicks (Hill and Matrone, 1970).

Trivalent chromium has a low order of toxicity, because of low intestinal absorption and limited entry into cells. Hexavalent chromium is several-fold more toxic than trivalent chromium (WHO, 1988; Dayan and Paine, 2001). Hexavalent chromium not reduced to chromium (+3) in the gastrointestinal tract or blood can cross cell membranes where it is ultimately reduced to chromium (+3) by reducing agents such as glutathione, cysteine, and ascorbic acid. The mechanism of chromium toxicosis is believed to be due to chromium (+3) and/or chromium (+5) and chromium (+4), intermediates in the reduction of hexavalent to trivalent chromium, binding to intracellular macromolecules, including DNA (ATSDR, 2000). Toxicity of chromium also may be related to chromium (+3, +4, or +5) induced free radical formation (Bagchi et al., 2002).

SOURCES AND BIOAVAILABILITY

Chromium occurs naturally in the Earth's crust and also is released into the environment from combustion processes and ore processing industries. Naturally occurring chromium is almost always in the trivalent state, while chromium (+6) is almost entirely derived from human activities (WHO, 1988). Concentrations of chromium in the ambient air in the United States range from 0.005 to 0.525 μg/m^3. In U.S. river and ocean waters, chromium concentrations average 10.0 and 0.3 μg/L, respectively (ATSDR, 2000). Soil chromium varies greatly with a mean concentration of 37.0 mg/kg (USGS, 1984). Chromium is present in soils primarily in carbonate and oxide forms that are not mobile in soil. Extractable chromium, measured following extraction of soil with acids or chelating agents, provides a much better estimate of chromium available for plant uptake than total soil chromium.

Chromium analysis of animal feedstuffs has received little attention. However, most human food sources are low (<0.10 mg/g DM) in chromium. Feed phosphate sources appear to be a major source of chromium in certain animal diets. Feed grade monocalcium phosphate and defluorinated phosphate sources vary in chromium content but average 83 and 110 mg Cr/kg, respectively (Sullivan et al., 1994). At present, chromium picolinate, chromium propionate, and chromium methionine can be supplemented to swine diets, at levels from 0.2 to 0.4 mg Cr/kg diet, in the United States. Chromium supplementation is currently not permitted in other animal species.

When given orally, hexavalent chromium has a much greater bioavailability than even water-soluble forms of inorganic chromium (+3). However, as discussed earlier, chromium (+6) is not likely to be consumed orally. Chromium (+3) oxide is essentially unavailable when given to rats, compared to the more soluble $CrCl_3$ and chromium (+3) acetate (Juturu et al., 2003). Certain organic forms of chromium (+3) appear to be more bioavailable than inorganic trivalent forms. Rats supplemented with 5 mg Cr/kg from chromium picolinate or a chromium dinicotinic acid-diglycine-cysteine-glutamic acid complex had higher chromium concentrations in kidney and liver than those receiving $CrCl_3$ after three weeks (Anderson et al., 1996). When supplemented at 20 to 100 mg Cr/kg for 20 weeks, rats fed chromium picolinate also had much higher liver and kidney chromium concentrations than rats fed $CrCl_3$ (Anderson et al., 1997a). In contrast to these findings, absorption of ^{51}Cr from $CrCl_3$ and chromium picolinate has been similar in short-term studies with rats (Olin et al., 1994; Anderson et al., 1996).

TOXICOSIS

Chromium toxicity has been extensively reviewed (WHO, 1988; Katz and Salem, 1994; EPA, 1998; Dayan and Paine, 2001). Toxicosis of chromium is a major concern in humans exposed in occupational and industrial settings to chromium via inhalation and dermal contact. The major form of chromium believed to be responsible for toxicosis in humans is chromium (+6). Hexavalent chromium can act as a carcinogen, an allergen, and an acute irritant in humans and experimental animals (Dayan and Paine, 2001). Chromates (+6) are used in oil fields as a corrosion inhibitor and in drilling muds. Toxicosis from chromates (+6) has been suspected in cattle exposed to oil field wastes (Kerr and Edwards, 1981). However, chromium (+6) is generally not consumed orally for reasons described earlier. This discussion will focus on chromium (+3). Trivalent chromium is relatively nontoxic via oral intake.

Single Dose and Acute

Large doses of chromium (+3) are required to produce signs of acute toxicosis. A single oral dose of 650 mg Cr/kg BW produced no overt toxicosis in young rats (NRC, 1980). The LD_{50} in rats for chromium (+3) as $CrCl_3 \cdot 6H_2O$, $Cr(NO_3)_3 \cdot 9H_2O$, and $Cr(CH_3COO)_3 \cdot H_2O$ is approximately 365, 422, and 2,376 mg Cr/kg BW, respectively (Katz and Salem, 1994). Female rats are slightly more sensitive to acute doses of chromium than males (ATSDR, 2000). Signs of acute toxicosis in rats include hypoactivity, mydriasis, lacrimation, and diarrhea. Exposing carp to water containing 20 mg Cr/L as $CrCl_3$ for 48 hours resulted in 100 percent mortality (Table 11-1); however, 10 mg/L had no effect on mortality (Muramoto, 1981). Chromium sulfate caused lower mortality in carp than $CrCl_3$ when supplied at 20 mg Cr/L. The LC_{50} for chromium in prawn larvae was estimated at 12.5 mg/L for $CrCl_3$ (Marino-Balsa et al., 2000).

Chronic

A number of studies evaluating oral ingestion of high concentrations of chromium (+3) are summarized in Table 11-1. Body weight gain in young chicks was reduced by addition of 1,500 mg Cr/kg diet as $Cr_2(SO_4)_3$ (Chung et al., 1983) or 2,000 mg/kg diet as $CrCl_3$ (Hill and Matrone, 1970). In laying hens, 50 mg Cr/kg diet from either $CrCl_3$, chromium yeast, or a chromium aminoniacinate complex did not affect egg production, but reduced activity of liver cytochrome P-450 dependent monooxygenases (Guerra et al., 2002). They suggested that 50 mg Cr/kg may impair cytochrome P-450 catalyzed drug metabolism. Considering the life expectancy of laying hens, the biological significance of this finding is unclear.

Providing chromium ($CrCl_3$) in drinking water at 328 mg/L in rats (Bataineh et al., 1997) or 656 mg/L in mice (Elbetieha and Al-Hamood, 1997) for 12 weeks reduced body weight gain. In male mice, $CrCl_3$ at 1,640 mg Cr/L of water reduced fertility, and 656 mg/L reduced implantation and viable fetuses in pregnant females (Elbetieha and Al-Hamood, 1997). Mice born and raised to mice exposed to 328 mg Cr ($CrCl_3$)/L of water during gestation and lactation (20 d) had reduced body weight at 60 days of age (Al-Hamood et al., 1998). Fertility was impaired in female but not male mice exposed prenatally and postnatally to chromium (Al-Hamood et al., 1998). Reduced sexual behavior (measured by number of mounts and number of males ejaculating) was observed in male rats given 328 mg Cr/L of water for 12 weeks, but fertility was not affected (Bataineh et al., 1997).

Limited research has evaluated chronic toxicity of organic forms of chromium. Rats supplemented for 20 weeks with 100 mg Cr/kg diet from chromium picolinate had chromium concentrations in liver and kidney that were approximately 7-fold higher than observed in rats fed a similar concentration of $CrCl_3$ (Anderson et al., 1997a). However, no signs of toxicosis, including pathological changes in liver or kidney, were seen in rats fed either chromium source. Chromium (+3) oxide is very insoluble and did not produce toxicosis even when provided in the diet of rats at up to 34,215 mg Cr/kg for 2 years (Ivankovic and Preussmann, 1975).

Factors Influencing Toxicity

Soluble forms of chromium (+6) are several-fold more toxic than soluble forms of chromium (+3). Animals are not usually exposed to chromium (+6) orally for reasons discussed previously. Toxicity of chromium (+3) is affected by solubility of the compound. Insoluble Cr_2O_3 has not produced toxicosis in animals (Ivankovic and Preussmann, 1975; NRC, 1980) while soluble forms such as $CrCl_3$ and $Cr_2(SO_4)_3$ can reduce body weight gain and impair reproduction when provided in feed or water at high concentrations (Table 11-1). Organic forms of chromium may be more toxic than $CrCl_3$. Chromium picolinate feeding resulted in higher tissue chromium concentrations than $CrCl_3$ (Anderson et al., 1997a). However, elevated tissue chromium concentrations in rats supplemented with chromium picolinate has not caused toxicosis.

TISSUE LEVELS

Chromium is widely distributed in the body at very low (ng/g DM) concentrations. Representative values in tissues from various animals are shown in Table 11-2. Addition of 100 mg Cr/kg as $CrCl_3$ to turkey diets for 5 weeks greatly increased tissue chromium concentrations; however, even at the high level of supplementation, tissue chromium only exceeded 1 mg/kg DM in kidney (Anderson et al., 1989). In growing pigs, supplementation with 0.2 to 0.3 mg Cr/kg as chromium picolinate increased chromium concentrations in liver, kidney, and heart, but not in muscle (Ward, 1995; Anderson et al., 1997b). Liver and kidney chromium concentrations were much higher in rats fed chromium picolinate than in those fed $CrCl_3$ at high concentrations (Anderson et al., 1997a). Supplementation of cattle diets with low concentrations of chromium did not increase tissue chromium (Spears et al., 2004). Tissue chromium data are not available for animals fed toxic concentrations of chromium. It is unlikely that chromium accumulation in animal products, other than possibly kidney, would cause a toxicological concern for humans.

MAXIMUM TOLERABLE LEVELS

The maximum tolerable level for chromium is defined as the dietary level that, when fed for a defined period of time, will not impair animal health and/or performance. Valance state and chemical form of chromium affect the maximum tolerable level for chromium. Hexavalent chromium (+6) is much more toxic than trivalent chromium (+3). Maximum tolerable levels for chromium (+6) have not been defined for most domestic animals because chromium (+6) is generally not ingested orally.

Chromic oxide, an insoluble form of chromium (+3) is very poorly absorbed, and has been widely used as an indigestible marker in animals for periods of several weeks at concentrations as high as 3,000 mg Cr/kg diet with no evidence of adverse effects (NRC, 1980). Feeding diets containing over 30,000 mg Cr/kg from Cr_2O_3 for two years also did not produce adverse effects in rats (Ivankovic and Preussmann, 1975).

Soluble forms of chromium (+3), such as $CrCl_3$ and $Cr_2(SO_4)_3$, reduce body weight gain in young chicks when fed at 1,500 to 2,000 mg Cr/kg diet. Young chicks can tolerate 500 mg Cr (from soluble forms)/kg diet for 21 to 35 days without adverse effects (Hill and Matrone, 1970; Chung et al., 1983). Exposure of rats and mice to 328 to 656 mg Cr/L in drinking water for 12 weeks reduced body weight and impaired reproduction (Elbetieha and Al-Hamood, 1997; Al-

Hamood et al., 1998). Developmental effects were also observed in mice exposed prenatally and postnatally to 328 mg Cr/L of water (Al-Hamood et al., 1998). Dietary supplementation of rats for 20 weeks with 100 mg Cr/kg from either $CrCl_3$ or chromium picolinate did not produce toxicosis, but increased liver and kidney chromium concentrations (Anderson et al., 1997a). Based on available literature, the maximum tolerable level for chromium (+3) was set at 3,000 mg/kg for Cr_2O_3. For more soluble forms of chromium (+3) the maximum tolerable level was set at 500 mg/kg for poultry and 100 mg/kg for mammalian species. Limited data in fish suggest that soluble forms of chromium (+3) can be tolerated for 48 to 72 hours at 8.0 to 10.0 mg/L of water. In animal species other than poultry and fish, rat and mice data were used to establish maximum tolerable levels. At dietary concentrations of chromium up to the maximum tolerable levels indicated, any increase in chromium concentration in edible tissue, with the possible exception of kidney, should not present a human health concern.

FUTURE RESEARCH NEEDS

Studies are needed to define the maximum tolerable level of chromium for most domestic animals. Research is particularly needed to determine the concentration of chromium from various organic chromium supplements that can be tolerated without adverse effects.

SUMMARY

Chromium occurs in a number of oxidation states, with chromium (+3) being the most stable form. Trivalent chromium potentiates insulin activity and is generally recognized as an essential trace mineral. Chromium is found naturally almost entirely as chromium (+3), but both trivalent and hexavalent chromium are produced and used in the chemical industry. Hexavalent chromium is much more toxic than chromium (+3). However, chromium (+6) released into the environment is largely reduced to chromium (+3). Almost all of the chromium consumed orally is in the trivalent form. Chromium (+3) is relatively nontoxic due to its poor intestinal absorption and limited entry of absorbed chromium (+3) into cells. Chromium oxide even at dietary concentrations over 30,000 mg Cr/kg diet did not produce toxicosis in rats. The level of chromium (+3) in soluble forms, such as $CrCl_3$, needed to cause adverse effects in animals is above 100 mg/kg. Fish can tolerate at least 8 mg Cr/L of water for 48 to 72 hours without adverse effects. The amount of soluble chromium (+3) needed to produce adverse effects is at least 100 times the amount needed to meet animal requirements.

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TABLE 11-1 Effects of Chromium Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Cats	6 7	3.6 kg 7.1 kg	100 µg/d	Cr picolinate	6 wk	Oral, by tablet	No adverse effects No adverse effects	Cohn et al., 1999
Dogs	13	Diabetic	200–800 µg/d	Cr picolinate	3 mo	Oral, by capsule	No adverse effects	Schachter et al., 2001
Mice	10	50 d (males)	656 mg/L	$CrCl_3$	12 wk	Water	Reduced body weight, increased testes, and decreased preputial gland weight as % of BW	Elbetieha and Al-Hamood, 1997
	9		1,640 mg/L				Reduced fertility, reduced body weight, increased testes, and decreased preputial gland weight as % of BW	
	14	50 d (females)	656 mg/L				Reduced number of implantations and viable fetuses in pregnant females	
			1,640 mg/L				Reduced number of implantations and viable fetuses in pregnant females	
Mice	25	Mature	328 mg/L	$CrCl_3$	Day 12 of gestation though d 20 of lactation	Water	Impaired development in offspring	Al-Hamood et al., 1998
	9	Male offspring				Placental and milk	Reduced body weight at 50 d	
	10	Female offspring					Reduced body weight at 50 d, delayed puberty, reduced fertility	
Rats	8	28 d	5–100 mg/kg	$CrCl_3$	20 wk	Diet	Increased liver and kidney chromium concentration	Anderson et al., 1997a
			5–100 mg/kg	Cr picolinate			Increased liver and kidney chromium concentration	
Chickens		1 d	500 mg/kg	$CrCl_3$	21 d	Diet	No adverse effects	Hill and Matrone, 1970
			1,000 mg/kg				No adverse effects	
			2,000 mg/kg				Reduced (23%) BW at 21 d	
Chickens	18	1 d	500 mg/kg	$Cr_2(SO_4)_3$	35 d	Diet	No adverse effects	Chung et al., 1983
			1,500 mg/kg				Reduced (15%) weight gain	
			2,500 mg/kg				Reduced (28%) weight gain	

continued

TABLE 11-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Chickens	6	Laying hens	25 mg/kg	$CrCl_3$	28 d	Diet	No adverse effects	Guerra et al., 2002
			50 mg/kg				Reduced liver cytochrome P-450 dependent monooxygenases	
			25 mg/kg	Cr yeast			No adverse effects	
			50 mg/kg				Reduced liver cytochrome P-450 dependent monooxygenases	
			25 mg/kg	Cr aminioni-acinate			No adverse effects	
			50 mg/kg				Reduced liver cytochrome P-450 dependent monooxygenases	
Turkeys	80	7 d	20–80 mg/kg	$CrCl_3$	14 d	Diet	Increased weight gain	Steele and Rosebrough, 1979
Turkeys	144	1 d	20 mg/kg	$CrCl_3$	21 d	Diet	Increased feed intake and body weight	Rosebrough and Steel, 1981
Swine	7	20 kg	25 mg/kg	$CrCl_3$	35 d	Diet	No adverse effects	Mooney and Cromwell, 1997
Prawns		Larval stage	4 mg/L	$CrCl_3$	72 h	Water	No adverse effects	Marino-Balsa et al., 2000
			8 mg/L				No adverse effects	
			16 mg/L				Lethal to 55% of larvae by 72 h	
			32 mg/L				Lethal to 100% of larvae by 72 h	
			64 mg/L				Lethal to 100% of larvae by 72 h	
Fish, carp		10.5–12.5 g	5 mg/L	$CrCl_3$	48 h	Water	No adverse effects	Muramoto, 1981
			10 mg/L				No adverse effects	
			20 mg/L				Lethal to 100% of fish by 24 h	
			5 mg/L	$Cr_2(SO_4)_3$			No adverse effects	
			10 mg/L				No adverse effects	
			20 mg/L				Lethal to 80% of fish by 48 h	
Fish, channel catfish	25	4.9 g	34–6,409 mg/kg	Cr_2O_3	10 wk	Diet	No adverse effects	Ng and Wilson, 1997
Fish, rainbow trout	12	5.5	1–3 mg/kg	$CrCl_3$	56 d	Diet	No adverse effects	Tacon and Beveridge, 1982
			6 mg/kg				Reduced growth and feed efficiency	

[a]Number of animals per treatment.
[b]Quantity of chromium dosed. SI conversion: 1 mg chromium equals 19.2 μmoles chromium.

TABLE 11-2 Chromium Concentrations in Fluids and Tissues of Animals (mg/kg)[a]

Animal	Quantity	Source	Duration	Route	Muscle	Liver	Kidney	Heart	Egg white	Egg yolk	Reference
Cattle	Control		146 d	Diet		25	13				Spears et al., 2004
	+0.4 mg/kg	$CrCl_3$				21	12				
	+0.8 mg/kg	Cr nicotinate				20	11				
Cattle	Control		125 d	Diet	116	41	108				Spears et al., 2004
	+0.8 mg/kg	Cr methionine			71	38	127				
Pigs	Control		80 d	Diet	2	9	10	8			Ward, 1995
	+0.2 mg/kg	$CrCl_3$			6	17	15	19			
	+0.2 mg/kg	Cr picolinate			3	19	42	12			
Pigs	Control		50 d	Diet	<1.5	21	27	4			Anderson et al., 1997b
	+0.3 mg/kg	Cr picolinate			<1.5	29	57	11			
Rats	Control		20 wk	Diet		6	8				Anderson et al., 1997a
	+100 mg/kg	$CrCl_3$				90	700				
	+100 mg/kg	Cr picolinate				550	2,200				
Turkey hens	Control		35 d	Diet	3	6	14	4	14	29	Anderson et al., 1989
	+25 mg/kg	$CrCl_3$			4	106	367	8	9	27	
	+100 mg/kg	$CrCl_3$			10	494	933	35	15	56	
	+200 mg/kg	$CrCl_3$			13	959	2,254	52			

[a]Values are on a dry matter basis.

12

Cobalt

INTRODUCTION

Cobalt (Co) is a silvery-white, hard metal with an atomic number of 27 and an atomic weight of 58.93. Most common compounds of cobalt have an oxidation state of +2 or +3 (Greenwood and Earnshaw, 1997). The +2 valence is stable in aqueous solution and is the major form of cobalt found in simple salts. In contrast, cobalt (+3) is a strong oxidizing agent and is unstable in aqueous solution. However, cobalt (+3) can form numerous coordination complexes with nitrogen-donor ligands and is the form of cobalt present in vitamin B_{12}. Cobalt is known for its magnetic properties. The element is easily magnetized and retains its magnetism under a wide range of environmental conditions.

Linnaeite (Co_3S_4), safforite ($CoAs_2$), glaucodot (CoAsS), and erythrite ($Co_3(As_2O_4)_2$) are the most important ores of cobalt (ATSDR, 2001). These ores are associated with nickel, copper, and lead, and most of the cobalt produced is recovered as a by-product of ores treated for recovery of nickel or copper. The largest cobalt reserves are in Zaire, Zambia, Morocco, Canada, and Australia. Cobalt deposits in the United States are not concentrated enough to allow economical recovery of the metal. With the exception of a negligible amount of by-product cobalt produced from some mining operations, no cobalt is mined or refined in the United States (ATSDR, 2001). Reported U.S. use of cobalt metal in 2003 was 8,000 metric tons (USGS, 2004).

A major use of metallic cobalt is in the production of superalloys that are used in gas turbine aircraft engines (ATSDR, 2001). It is also used in magnetic alloys and alloys that require hardness, wear resistance, and corrosion resistance. In addition, cobalt compounds are used (1) as pigments (blue) in glass, ceramics, and paint; (2) as catalysts in the petroleum industry; and (3) to hasten the oxidation and, thus, drying of oil-based paints. Gamma rays from radioactive ^{60}Co are widely used to sterilize medical devices, as an external source in radiography and radiotherapy, and for food irradiation.

ESSENTIALITY

The only known function of cobalt is as an essential component of vitamin B_{12} (cobalamin). Vitamin B_{12} contains cobalt (+3) surrounded by six coordinately linked groups to form an octahedron. Vitamin B_{12} is a cofactor for the enzymes methylmalonyl CoA mutase and methionine synthase. Methylmalonyl CoA mutase is responsible for conversions of methylmalonyl CoA to succinyl CoA and is important in propionate metabolism. Methionine synthase is involved in the regeneration of methionine following loss of its methyl group and in the maintenance of biologically active folate concentrations in tissues.

Mammals lack the ability to synthesize vitamin B_{12}, and nonruminant animals require a dietary source of vitamin B_{12}. Vitamin B_{12} can be synthesized from inorganic cobalt by certain bacteria and algae (Smith, 1997). Ruminal bacteria synthesize enough vitamin B_{12} to meet the requirements of ruminants provided that adequate dietary cobalt is supplied. Ruminants require 0.10 to 0.15 mg Co/kg diet to allow sufficient synthesis of vitamin B_{12} by ruminal bacteria to meet the animal's requirement. In certain areas of the world, forage cobalt concentrations are well below requirements of ruminants due to low soil cobalt concentrations. Cobalt deficiency in ruminants, caused by low dietary intake of cobalt, results in loss of appetite, reduced growth rate or even loss in body weight in severe cases, and anemia (Underwood and Suttle, 1999).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Biological samples of plant and animal origin are generally low (<0.5 mg/kg DM) in cobalt. Graphite furnace atomic absorption spectrometry with Zeeman background correction is the method of choice for measuring low concentrations of cobalt (Smith, 1997). Neutron activation analysis and differential pulse anodic stripping voltammetry

also have been used for determining low concentrations of cobalt. Flame atomic absorption spectrometry is not sensitive enough to detect cobalt concentrations normally found in biological samples.

Contamination of samples with cobalt during collection, storage, and preparation of samples for analysis should be avoided. Disposable syringes and technical-grade anticoagulants may contaminate blood samples with cobalt. Wet ashing with acid is the preferred method for preparing solid samples for cobalt analysis. Dry ashing may result in losses of cobalt.

REGULATION AND METABOLISM

Absorption and Metabolism

In weanling rats fed physiological concentrations of cobalt, from $CoCl_2$, apparent and true absorption of cobalt averaged 28 and 29.8 percent, respectively (Kirchgessner et al., 1994). Mice absorbed 26 percent of an oral dose of labeled cobalt (Toskes et al., 1973). Absorption of cobalt was very high in young rats (1 to 20 days of age) and decreased greatly by 30 days of age (Naylor and Harrison, 1995). Cobalt absorption also declined with age in guinea pigs. However, independent of age, cobalt absorption was much lower in guinea pigs than in rats (Naylor and Harrison, 1995). In nonruminants, cobalt and iron appear to share a common intestinal transport system and cobalt absorption is greatly increased in iron deficiency (Thomson et al., 1971). Absorption of cobalt is very low (1 to 2 percent) in ruminants (Looney et al., 1976; Van Bruwaene et al., 1984). Low absorption of cobalt in ruminants may relate to binding of cobalt by ruminal microorganisms. A portion of cobalt ingested by ruminants is used by ruminal bacteria for synthesis of vitamin B_{12}. It has been estimated that the efficiency of cobalt conversion to vitamin B_{12} ranges from 1.7 to 18 percent (Smith and Marston, 1970).

Absorbed cobalt is primarily excreted in urine with small amounts excreted via fecal endogenous routes (Kirchgessner et al., 1994). Cobalt concentrations in tissues are generally low (1 mg/kg DM or less), but liver and kidney cobalt concentrations increase dramatically when animals are fed high dietary cobalt (Henry et al., 1997). Liver has the highest concentration of cobalt followed by the kidney and heart. Because cobalt is required in the mammalian system in the form of vitamin B_{12}, transport and intermediatory metabolism of cobalt per se has received little attention.

Metabolic Interactions and Mechanisms of Toxicity

Sulfur amino acids, especially cysteine, may form stable complexes with cobalt and reduce its absorption. Supplementing cysteine or methionine in excess of dietary requirements reduced liver and kidney cobalt concentrations in chicks fed high dietary cobalt (Southern and Baker, 1981).

Iron and cobalt interact in the intestinal mucosal and are mutually antagonistic (Thomson et al., 1971). Supplementation of cobalt at 100 mg/kg diet or higher reduced serum and liver iron in pigs (Huck and Clawson, 1976). In chicks supplemented with high concentrations (100 to 400 mg/kg diet) of cobalt, liver and kidney cobalt concentrations were greatly elevated when chicks were fed an iron-deficient diet (Blalock, 1985). The addition of 100 or 1,000 mg Fe/kg to the high cobalt diets greatly reduced liver and kidney cobalt concentrations.

Feeding elevated but nontoxic concentrations of cobalt increased zinc absorption in chicks fed diets low in zinc (Chung et al., 1977). Similarly, growth and zinc status of pigs fed a low zinc diet were improved by the addition of 27 or 54 mg Co/kg diet (Chung et al., 1976).

A number of possible mechanisms for cobalt toxicity may exist. Exposure to cobalt may cause oxidative damage to tissues by causing generation of oxygen radicals and free radical-induced DNA damage (Kasprzak et al., 1994). In support of this hypothesis, selenium and vitamin E administration offered protection against cobalt-induced cardiomyopathy in pigs (Van Vleet et al., 1977). Increasing dietary cysteine, a precursor of the antioxidant glutathione, also alleviated growth depression resulting from cobalt toxicosis in chicks (Southern and Baker, 1981). Another possible mechanism of cobalt toxicity relates to the ability of cobalt to block calcium channels and thus, alter cellular calcium influx (Yamatani et al., 1998). Some cobalt toxicosis signs may relate to the effect of cobalt on heme and heme-containing enzymes (ATSDR, 2001). Cobalt is believed to inhibit two different sites in the heme biosynthetic pathway.

SOURCES AND BIOAVAILABILITY

Cobalt occurs naturally in the Earth's crust at an average concentration of 20 to 25 mg/kg (ATSDR, 2001). Soils generally contain from 1 to 40 mg Co/kg and the average cobalt concentration in U.S. soils is 7.2 mg/kg. Cobalt concentrations in surface and groundwater are usually between 1 and 10 µg/L. Elevated concentrations of cobalt in soil and water can result from activities such as mining and processing of cobalt-bearing ores, disposing of cobalt-containing wastes, burning of fossil fuels, copper and nickel smelting and refining, and applying cobalt containing sludge or fertilizers to soil (ATSDR, 2001).

Most animal feedstuffs are low in cobalt (<0.5 µg/kg). Concentration of cobalt in plant material is dependent on soil cobalt, soil pH, and plant species. Soil uptake of cobalt by forages decreases as soil pH increases. Legumes are higher in cobalt than grasses when grown on cobalt adequate soil (Underwood and Suttle, 1999). Soils deficient in cobalt (< than 3.0 mg Co/kg soil) are found in certain areas of the southeastern and northeastern United States. Ruminants grazing or consuming forages grown in these areas require cobalt supplementation to prevent deficiency. Cereal grains

usually contain deficient concentrations (≤0.06 mg/kg) of cobalt. Feeds of animal origin, with the exception of liver products, are also low in cobalt.

Cobalt is generally supplemented to ruminant diets as $CoCO_3$, $CoSO_4$, or various organic forms. Kawashima et al. (1997a) compared bioavailability of different cobalt sources based on tissue cobalt accumulation in lambs fed high (40 mg Co/kg) dietary cobalt. Feed grade sources of $CoCO_3$ and cobalt glucoheptonate were similar in bioavailability to reagent grade $CoSO_4 \cdot 7H_2O$. Cobalt oxide was approximately 20 percent as bioavailable as $CoSO_4 \cdot 7H_2O$ based on accumulation of cobalt in various tissues. In vitro studies indicated that Co_3O_4 was totally unavailable for synthesis of vitamin B_{12} by ruminal microorganisms (Kawashima et al., 1997b). Bioavailability of cobalt from cobalt propionate was similar to $CoCO_3$ in steers based on plasma and liver vitamin B_{12} concentrations (Tiffany et al., 2003). Absorption of cobalt from cobalt naphthenate, an organometallic cobalt-containing compound used as a drier in paint, appears to be similar to $CoCl_2$ in rats (Firriolo et al., 1999).

TOXICOSIS

Cobalt toxicosis in animals is very rare because concentrations of cobalt normally present in animal diets are much lower than those needed to cause toxicosis. Suspected cases of cobalt toxicosis, due to excessive cobalt addition to water, feed, or pastures, have been reported in cattle (Dickson and Bond, 1974). Cobalt toxicosis is not likely to occur in nonruminants unless environmental contamination of feed or water occurs.

Single Dose and Acute

High oral doses of cobalt caused sedation, diarrhea, tremors, convulsions, and death in rats (Speijers et al., 1982). Histological changes were observed in heart, liver and kidney of rats that died. The LD_{50} in rats for $CoCl_2 \cdot 6H_2O$ has ranged from 42.4 to 190 mg Co/kg BW in various studies (ATSDR, 2001; Speijers et al., 1982). Oral administration of 100 mg Co/kg BW, as $CoSO_4$, for 3 days in young pigs resulted in cardiomyopathy, listlessness, diarrhea, and high mortality (Van Vleet et al., 1977). Severity of cardiomyopathy was greater in pigs that were inherently susceptible to stress compared to nonstress-susceptible pigs (Van Vleet et al., 1977).

Chronic

Studies evaluating oral ingestion of high concentrations of cobalt are summarized in Table 12-1. Cobalt concentrations as low as 100 mg Co/kg diet have reduced weight gain in young chicks (Hill, 1974, 1979a; Ling and Leach, 1979). Lower concentrations of cobalt may affect disease susceptibility as chicks fed 50 mg Co/kg diet had increased mortality following experimental infection with *Salmonella gallinarum* (Hill, 1974). Addition of 200 mg Co/kg or higher to chick diets has increased mortality in noninoculated birds (Hill, 1974; Diaz et al., 1994) and resulted in histological lesions in the digestive tract, liver, heart, and skeletal muscle (Diaz et al., 1994).

The amount of cobalt that can be tolerated without adverse effects in rats appears to be similar to chicks. In rats fed an iron-adequate diet, 50 mg Co/kg did not affect weight gain, but addition of 100 mg Co/kg diet or higher reduced weight gain and feed intake and caused some mortality (Huck, 1975). However, as little as 25 mg Co/kg diet reduced weight gain and feed intake when rats were fed an iron-deficient diet (Huck, 1975). Myocardial damage has been seen in adult rats given 12.4 mg Co/kg BW for 21 days (Morvai et al., 1993). Testicular degeneration has been observed in male rats (Corrier et al., 1985; Anderson et al., 1992) and mice (Anderson et al., 1993) given high oral doses of cobalt. Oral administration of cobalt at 5.2 mg Co/kg BW or higher during gestation has impaired fetal development in mice and rats, and increased mortality in pregnant rabbits (Szakmary et al., 2001).

Based on limited research, higher concentrations of cobalt are required in pigs than in chicks and rats to cause toxicosis. Addition of up to 200 mg Co/kg diet did not cause observable adverse effects in young pigs (Huck and Clawson, 1976). Cobalt concentrations of 400 or 600 mg/kg diet caused anorexia, growth depression, reduced blood hemoglobin and hematocrit, incoordination, muscular tremors, stiff legs, and humped back in pigs (Huck and Clawson, 1976).

Older studies indicated that cattle can tolerate up to 0.86 mg Co/kg BW without adverse effects (Ely et al., 1948; Keener et al., 1949). Higher concentrations of cobalt reduced feed intake and caused hyperchromemia (Keener et al., 1949). No recent studies examining cobalt toxicosis in cattle were found in the literature. Becker and Smith (1951) reported that 4.4 mg Co/kg BW or higher, administered via water, decreased feed intake in sheep. A daily dose of 11.0 mg Co/kg BW caused anemia and high mortality in sheep (Becker and Smith, 1951). In a more recent study, adult rams were given 3.0 or 4.5 mg Co/kg BW for 70 days (Corrier et al., 1986). The dose of cobalt was then increased to 10 or 15 mg/kg BW for an additional 39 days. Tissue cobalt concentrations were greatly elevated in cobalt-dosed rams, but no pathological or clinical manifestations of toxicosis were found in this study. The addition of 40 mg Co/kg diet for 60 days also did not affect performance or general health of lambs (Henry et al., 1997).

Factors Influencing Toxicity

Iron status greatly affects the concentration of cobalt required to produce a toxicosis. Cobalt absorption is increased in iron deficiency (Valberg et al., 1969; Blalock, 1985), and

iron deficiency in chicks (Chetty, 1972) and rats (Huck, 1975) lowered the concentration of cobalt required to depress growth by over 50 percent. The addition of 100 to 200 mg Fe/kg to diets adequate in iron partially alleviated cobalt-induced growth depression but did not affect increased susceptibility of chicks to *Salmonella gallinarum* (Hill, 1974).

Increasing dietary concentrations of methionine or cysteine alleviates cobalt toxicosis. Addition of 0.5 or 1.0 percent DL-methionine to a corn-soybean meal-based diet completely alleviated growth depression and reduced serum iron concentrations in pigs fed 600 mg Co/kg diet (Huck and Clawson, 1976). However, serum cobalt concentrations in pigs were not reduced by methionine supplementation. Increasing dietary methionine or cysteine partially alleviated reduced growth and feed efficiency noted in chicks fed 250 or 500 mg Co/kg diet (Southern and Baker, 1981). Cysteine was more efficacious than methionine, when provided on an isosulfurous basis, in alleviating cobalt toxicosis in chicks. Sulfur amino acids may reduce cobalt toxicity by forming stable complexes with cobalt in the digestive tract that are poorly absorbed and/or by enhancing the detoxication of absorbed cobalt (Baker and Czarnecki-Maulden, 1987). Increasing dietary protein from 10 to 20 or 30 percent partially prevented growth depression but not increased susceptibility to *Salmonella gallinarum* observed in chicks fed toxic levels of cobalt (Hill, 1979a).

Selenium and vitamin E status may affect toxicity of cobalt. Intramuscular injection of selenium and vitamin E, one day prior to oral administration of 100 mg Co/kg BW, protected against cobalt-induced myocardial damage (Van Vleet et al., 1977). Ascorbic acid addition to diets partially prevented growth depression observed in chicks fed 200 mg Co/kg (Hill, 1979b). Cobalt toxicosis in chicks was exacerbated by duodenal coccidiosis (*Eimeria acervulina*) in chicks (Southern and Baker, 1982).

Based on studies showing a much higher cobalt absorption in young (1 to 20 days) rats and guinea pigs than in older (30 to 200 days) animals, one would expect that lower concentrations of cobalt may be required to produce toxicosis in young animals. Young calves appeared to be affected more by lower concentrations of cobalt than older calves (Keener et al., 1949).

TISSUE LEVELS

Cobalt is found in tissues at low concentrations (<1.0 mg/kg DM) when animals are fed normal dietary cobalt concentrations. Exposure of animals to high dietary cobalt greatly increases concentrations of cobalt in a number of tissues. Representative values in tissues from various animals fed normal or high concentrations of cobalt are shown in Table 12-2. Cobalt concentrations in liver and kidney increase to the greatest extent with smaller increases in hearts of animals fed high concentrations of cobalt. Increases in muscle cobalt in animals given high concentrations of cobalt are relatively small, and muscle cobalt was only 0.26 mg/kg DM in lambs supplemented with 40 mg Co/kg diet for 60 days (Henry et al., 1997).

MAXIMUM TOLERABLE LEVELS

The maximum tolerable level for cobalt is defined as the dietary level that, when fed for a defined period of time, will not impair animal health and/or performance. A number of factors can affect the maximum tolerable level for cobalt, especially iron status of the animal and dietary concentration of sulfur amino acids. Age and antioxidant status of the animal also appear to affect the level of cobalt that can be tolerated without adverse effects on animal performance or health.

Chicks and rats are able to tolerate 50 mg Co/kg diet without adverse effects on growth (Hill, 1974; Huck, 1975). However, a 50 mg Co/kg diet increased disease susceptibility in chicks experimentally inoculated with *Salmonella gallinarum* (Hill, 1974). In pigs, cobalt concentrations greater than 200 mg/kg diet were required to depress growth and feed efficiency (Huck and Clawson, 1976). Older studies suggested that calves can tolerate up to 0.86 mg Co/kg BW (Ely et al., 1948). This would be 34 mg/kg diet if dry matter intake is assumed to be 2.5 percent of BW. Controlled studies to assess cobalt tolerance have not been reported in older cattle. Yearling sheep tolerated 3.5 mg Co/kg BW (approximately 144 mg Co/kg diet) for 56 days (Becker and Smith, 1951), while adult male sheep tolerated approximately 180 mg Co/kg diet for 109 days without adverse effects (Corrier et al., 1986). Studies have not been conducted to determine if such high concentrations of cobalt can be safely tolerated by younger lambs or during fetal development.

Based on available literature, the maximum tolerable levels for cobalt were set at 25 mg/kg diet for chicks, rats, sheep, and cattle, and 100 mg/kg diet for swine. Insufficient data are available to set a maximum tolerable level for cobalt for dogs and cats. At dietary cobalt concentrations up to the maximum tolerable levels indicated, increases in cobalt in edible tissue are not likely to present a human health concern. However, animals fed diets containing the maximum tolerable level of cobalt may have kidney cobalt concentrations that exceed standards for human health (see Maximum Tolerable Levels chapter).

FUTURE RESEARCH NEEDS

Additional work is needed with swine to confirm earlier research indicating that growing pigs could tolerate considerably higher dietary concentrations of cobalt than chicks or rats. The most pressing need for cobalt toxicosis research is in ruminants, especially cattle. Studies examining cobalt tolerance in growing calves were conducted in the 1940s, and no research has been conducted to define levels of cobalt that can be safely tolerated by older cattle. In contrast, cobalt

studies in sheep have all been conducted with yearling or adult animals, and these studies indicate that sheep may be able to tolerate much higher cobalt concentrations than cattle. It is unclear if the discrepancy in cobalt tolerance reported between sheep and cattle is truly a species difference or an age effect.

SUMMARY

Cobalt functions as an essential component of vitamin B_{12}. Nonruminant animals do not require a dietary source of cobalt because mammalian tissues are unable to synthesize vitamin B_{12} from inorganic cobalt. Ruminal microorganisms synthesize vitamin B_{12} from cobalt, and the vitamin B_{12} produced serves to meet the animal's requirement. Therefore, ruminants have a dietary requirement for cobalt. Cobalt toxicosis is generally not a practical problem because concentrations of cobalt needed to cause toxicosis are much higher than those normally found in animal diets. Errors in formulation of mineral supplements for ruminants could result in cobalt toxicosis. However, toxic levels of cobalt are over 100 times greater than dietary requirements for ruminants.

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TABLE 12-1 Effects of Cobalt Exposure in Animals

Animal	N[a]	Age or weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Mice		Male	400 mg/L	$CoCl_2$	13 wk	Water	Testicular degeneration	Anderson et al., 1993
Mice	19	20–24 g	10.5 mg/kg BW	$CoCl_2 \cdot 7H_2O$	Days 6–15 of gestation	Oral, by gavage	Increased frequency of malformed fetuses	Szakmary et al., 2001
Rabbits	8		4.2–42.0 mg/kg BW	$CoCl_2 \cdot 7H_2O$	Days 6–20 of gestation	Oral, by gavage	Reduced gain or loss of weight in dams, increased mortality of dams	Szakmary et al., 2001
Rats	8	59 g	25–50 mg/kg 100–400 mg/kg	$CoCl_2 \cdot 6H_2O$	35 d	Diet (iron adequate)	No adverse effects Reduced weight gain and feed intake, high (25–100%) mortality	Huck,1975
Rats	8	59 g	25 mg/kg 100–400 mg/kg	$CoCl_2 \cdot 6H_2O$	35 d	Diet (iron deficient)	Reduced weight gain and feed intake Reduced weight gain and feed intake, high (25–100%) mortality	Huck,1975
Rats		100 d (males)	265 mg/kg	$CoCl_2 \cdot 6H_2O$	98 d	Diet	Testicular degeneration and necrosis	Corrier et al., 1985
Rats	6	150–250 g	4.2 mg/kg BW	$CoCl_2 \cdot 7H_2O$	56 d	Diet	Reduced weight gain	Clyne et al., 1988
Rats	20	240–280 g (pregnant females)	6.2 mg/kg BW 12.4 mg/kg BW 24.8 mg/kg BW	$CoCl_2 \cdot 6H_2O$	Day 6–15 of gestation	Oral, by gavage	No adverse effects Reduced weight gain Reduced weight gain and feed intake; elevated hemoglobin and hematocrit	Paternain et al., 1988
Rats		Male	400 mg/L	$CoCl_2$	13 wk	H_2O	Testicular degeneration	Anderson et al., 1992
Rats	8	340–390 g	12.4 mg/kg BW	$CoCl_2$	21 d	Water	Myocardial damage	Morvai et al., 1993
Chickens	60-80	1 d	50 mg/kg 100–400 mg/kg	$CoCl_2 \cdot 6H_2O$	35 d	Diet	Increased mortality after inoculation with *Salmonella gallinaium* Reduced weight gain, increased mortality, especially after inoculation with *Salmonella gallinaium*	Hill, 1974

Chickens	20	1 d	100–300 mg/kg	$CoCl_2 \cdot 6H_2O$	21 d	Diet	Reduced weight gain, increased mortality after inoculation with *Salmonella gallinaium*	Hill, 1979a
Chickens	16	1 d	50 mg/kg 100–200 mg/kg		21 d	Diet	No adverse effects Reduced weight gain	Ling and Leach, 1979
Chickens	15	7 d	250–500 mg/kg	$CoCl_2 \cdot 6H_2O$	15 d	Diet	Reduced weight gain and gain/feed	Southern and Baker, 1981
Chickens	60	1 d	125 mg/kg 250–500 mg/kg	$CoCl_2 \cdot 6H_2O$	14 d	Diet	Reduced weight gain, feed intake, and gain/feed Reduced weight gain, feed intake, and gain/feed, increased mortality, histological lesions in digestive tract, liver, heart and skeletal muscle	Diaz et al., 1994
Swine	16	13.3 kg	100–200 mg/kg 400 mg/kg	$CoCl_2 \cdot 6H_2O$	105 d	Diet	No adverse effects Reduced weight gain and feed intake, decreased hemoglobin and hematocrit, decreased serum iron	Huck and Clawson, 1976
Swine	8	21.7 kg	200 mg/kg 400–600 mg/kg	$CoCl_2 \cdot 6H_2O$	84 d	Diet	No adverse effects Reduced weight gain and feed intake, increased gain/feed, stiff legs, humped back, incoordination, muscular tremors	Huck and Clawson, 1976
Swine	10	18 kg	100 mg/kg BW	$CoSO_4$	3 d	Oral	Cardiomyopathy, listlessness, diarrhea, vomiting, anorexia, 60% mortality	Van Vleet et al., 1977
Cattle		84–148 kg	0.77–0.86 mg/kg BW 0.93–2.38 mg/kg BW	$CoSO_4$	4–21 d	Diet	No adverse effects Loss of appetite	Ely et al., 1948
Cattle	7	1–42 wk	0.22–0.66 mg/kg BW	$CoSO_4$	Up to 28 wk	Water	No adverse effects	Keener et al., 1949
	7	1–42 wk	1.1–11.0 mg/kg BW		Up to 28 wk		Indications of hyperchromemia	
	7	12–14 wk	22.0–44.0 mg/kg BW		4–16 wk		Loss of appetite, incoordination, hyperchromemia	

continued

TABLE 12-1 Continued

Animal	N[a]	Age or weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Sheep	3 5 5	38.1 kg 44.4 kg 43.9 kg	0.22–3.5 mg/kg BW 4.4 mg/kg BW 11.0 mg/kg BW	$CoCl_2 \cdot 6H_2O$	8 wk	Water	No adverse effects Decreased feed intake Decreased feed intake, anemia, 60% mortality	Becker and Smith, 1951
Sheep	4	35–45 kg (male)	3.0–4.5 mg/kg BW 10.0–15.0 mg/kg BW	$CoCl_2 \cdot 6H_2O$	70 d	Gelatin capsule	No adverse effects No adverse effects	Corrier et al., 1986
Sheep	3	60 kg	20–40 mg/kg	$CoSO_4 \cdot 6H_2O$	60 d	Diet	No adverse effects	Henry et al., 1997

[a]Number of animals per treatment.
[b]Quantity of cobalt dosed. SI conversion: 1 mg cobalt equals 17.0 µmoles cobalt.

TABLE 12-2 Cobalt Concentrations in Fluids and Tissues of Animals (mg/kg)

Animals	Quantity	Source	Duration	Route	Serum	Liver	Kidney	Muscle	Heart	Spleen	Reference
Rats	Control	$CoSO_4 \cdot 7H_2O$	56 d	Diet	0.001			0.02[a]	0.05[a]		Clyne et al., 1988
	4.2 mg/kg BW				0.12			0.48	1.62		
Chicks	Control	$CoCl_2 \cdot 6H_2O$		Diet		0.14[a]	0.15[a]				Blalock, 1985
	+100 mg/kg					1.50	2.83				
	+200 mg/kg					1.11	8.25				
	+400 mg/kg					4.53	10.24				
Pigs	Control	$CoCl_2 \cdot 6H_2O$	84 d	Diet		1.16[b]	0.39[b]		ND[c]	0.62[b]	Huck and Clawson, 1976
	+200 mg/kg					8.40	16.72		2.90	4.46	
	+400 mg/kg					10.85	39.73		5.40	5.99	
	+600 mg/kg					12.72	35.89		9.28	6.89	
Sheep	Control	$CoCl_2 \cdot 6H_2O$	109 d	Gavage	0.09	0.09[a]	0.09[a]				Corrier et al., 1986
	3.0–10.0 mg/kg BW				0.98	8.50	2.10				
	4.5–15.0 mg/kg BW				1.33	8.70	1.90				
Sheep	Control	$CoSO_4 \cdot 7H_2O$	60 d	Diet		0.20[b]	0.77[b]	0.10[b]	0.13[b]	0.10[b]	Henry et al., 1997
	+20 mg/kg					3.74	3.27	0.14	0.59	0.39	
	+40 mg/kg					7.33	4.83	0.26	1.26	0.67	

[a]Wet tissue basis.
[b]Dry tissue basis.
[c]Not detectable.

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Copper

INTRODUCTION

Copper (Cu) is one of the oldest metals known to humans. It occurs naturally, to a small extent as the free metal, but most copper occurs as compounds in the +1 (cuprous) or +2 (cupric) oxidation state. Cuprous compounds are generally colorless and are rapidly oxidized to the cupric form in aqueous solution. Cupric compounds are blue or green in color and +2 is the most important oxidation state of copper. Copper has a high electrical and thermal conductivity, and is highly resistant to corrosion (ATSDR, 2002). These unique properties have made copper one of the most important industrial metals.

Copper is found primarily as oxide, sulfide, or carbonate ores with chalcocite (Cu_2S), chalcopyrite ($CuFeS_2$), malachite ($CuCO_3(CuOH_2)$), and cuprite (Cu_2O) ores as the most important sources. Chile is the world's leading copper producer followed by the United States. In the United States, 1.12 million tons of copper were mined and 860,000 tons were imported for processing in 2003 (USGS, 2004). An additional 210,000 tons of copper were reclaimed in the United States by recycling from old scrap in 2003.

Brass, bronze, gun metal, and monel metal are important alloys that contain copper. Copper alloys are used in construction, electrical products, transportation equipment, industrial machinery and equipment, coins, and consumer products (ATSDR, 2002). Copper compounds are also used as fungicides, in fertilizers, in nutritional supplements for humans and animals, and as algicides in reservoirs and streams.

ESSENTIALITY

Copper was reported to be essential for growth and hemoglobin formation in rats in 1928 (Underwood and Suttle, 1999). Subsequent research indicated that copper was an essential component of a number of enzymes including cytochrome oxidase, lysyl oxidase, superoxide dismutase, tyrosinase, ceruloplasmin, and dopamine β-monooxygenase (Linder, 2002). Copper-dependent enzymes function in energy metabolism, maturation and stability of collagen and elastin, pigmentation, the antioxidant defense system, and iron metabolism, as well as other biological processes. Deficiency of copper may result in cardiovascular disorders (cardiac failure or rupture of the aorta), depigmentation and impaired keratinization of hair and wool, anemia, reduced growth, neonatal ataxia, bone abnormalities, and impaired immune responses. Deficiency signs vary depending on animal species and the severity of the deficiency. Copper deficiency is a practical problem in ruminants in many areas of the world. Most copper deficiencies in ruminants are due to the presence of antagonists (molybdenum, sulfur, and iron) in feeds that greatly impair copper metabolism.

Nonruminant animals generally require between 4 and 8 mg Cu/kg diet (Underwood and Suttle, 1999). Copper requirements appear to be higher in horses (Underwood and Suttle, 1999). It is well documented that pharmacological concentrations of copper (125 to 250 mg Cu/kg diet) can stimulate growth and feed efficiency in swine (Cromwell et al., 1989) and poultry (Harms and Buresh, 1987; Pesti and Bakalli, 1996). The mechanism responsible for the growth-promoting action of high dietary copper in nonruminants is unclear. Copper requirements of ruminants vary from approximately 4 to over 20 mg Cu/kg diet, depending on dietary concentrations of copper antagonists (Underwood and Suttle, 1999). Relatively low dietary concentrations of molybdenum and sulfur can increase copper requirements by 2- to 3-fold.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Because of the abundance of copper in the environment, precautions should be taken to avoid contamination when collecting samples for copper analysis (WHO, 1998). Vessels to be used in the collection of samples for copper

determination should be cleaned of dust and washed with dilute acid. Glassware and most plastics are relatively free of copper contamination, but heavily pigmented plastics may contain copper and should be avoided. Deionized water should be used to dilute samples for copper analysis because even distilled water can be contaminated with copper through contact with copper plumbing and brass fixtures. Wet ashing with acid in an open vessel digestion system or closed microwave digestion system is the preferred method for preparing solid samples for copper analysis. Acid(s) used in the digestion process should be extremely low in copper, and a blank should be carried through all procedures to correct for contamination.

Atomic absorption spectrophotometric (AAS) methods (either flame or graphite furnace) are common methods used for determination of copper. Flame AAS has a detection limit of 0.01–0.1 μg/mL, while graphite furnace AAS is much more sensitive with a detection limit of 0.01–1.0 ng/mL (Harris, 1997). Another common method for copper determination is inductively-coupled plasma atomic emission spectroscopy (ICP-AES). Sensitivity of ICP-AES for copper is similar to graphite furnace AAS and this method extends linearity over a wider concentration range than AAS and allows for multielement analysis.

REGULATION AND METABOLISM

Absorption and Metabolism

Major differences in copper metabolism exist between nonruminant and ruminant animals. Studies in humans and rats indicate that copper is fairly well absorbed (~30 to 75 percent) in nonruminants (Linder, 2002). Absorption of copper occurs primarily from the small intestine. Percentage of dietary copper absorbed is affected by dietary copper, being higher when intake of copper is marginal or low relative to requirements. In the intestinal mucosa, a portion of the copper is bound to metallothionein or similar proteins (Linder, 2002). Binding to metallothionein prevents transfer of copper to the intestinal serosa, and metallothionein-bound copper can be lost in the feces during normal turnover of intestinal cells. Metallothionein has a higher affinity for copper than zinc, and high dietary zinc can reduce copper absorption by inducing synthesis of metallothionein (Harris, 1997).

In ruminants, with a functional rumen, copper absorption is low (<1.0 to 10 percent) relative to nonruminants (Underwood and Suttle, 1999). Low absorption of copper in ruminants is largely due to complex interactions that occur among copper, sulfur, and molybdenum in the ruminal environment. Copper absorption is much higher (~75 percent) in young ruminants prior to establishment of a ruminal microflora (Underwood and Suttle, 1999).

Absorbed copper in portal blood binds to albumin and transcuprein for transport primarily to the liver (Linder, 2002). In the liver, copper can be excreted via the bile, stored, or used for synthesis of ceruloplasmin or other copper metalloenzymes. Biliary excretion is the major mechanism responsible for copper homeostasis. Copper excreted in bile is present in forms that are poorly reabsorbed from the small intestine. Liver copper concentrations (15 to 30 mg Cu/kg DM) are well regulated in most nonruminants; however, biliary copper excretion becomes saturated at high dietary copper concentrations, and then liver copper accumulation occurs. Genetic disorders, associated with impaired biliary excretion of copper, occur in humans (Wilson's disease) and certain breeds of dogs (Hyun and Filippich, 2004) that result in elevated liver copper concentrations. Normal liver copper concentrations in ruminants (100 to 400 mg Cu/kg DM) are considerably higher than in pigs and chickens (Underwood, 1977). In fact, concentrations of copper normally found in the liver of most nonruminants would be indicative of copper deficiency in ruminants. Biliary copper excretion is much less effective in regulating liver copper concentrations in ruminants. This is especially true in sheep where increasing dietary copper does not appear to increase biliary copper excretion (Saylor and Leach, 1980).

Liver is the major storage organ for copper, and stored copper is largely bound to metallothionein in most species (Bremner, 1987). When high dietary copper is ingested, binding to metallothionein appears to be an important cellular detoxification mechanism in some animal species (Bremner, 1987). In sheep, a much smaller proportion of liver copper is bound to metallothionein, and sheep have a limited ability to increase metallothionein synthesis in response to increased liver copper (Saylor et al., 1980).

Copper not excreted in bile, stored, or used for copper-dependent enzymes in the liver leaves the liver largely bound to ceruloplasmin, an oxidase and acute phase protein. In most species (except birds), ceruloplasmin is the major form of copper in plasma and is believed to be the major protein that transports copper to extrahepatic tissues. Ceruloplasmin is thought to recognize receptors on the plasma membrane of tissues and to release copper into cells (Harris, 1997). Free copper is highly toxic to cells; cytosolic proteins, referred to as copper chaperones, bind copper ions and deliver them to copper-requiring proteins without releasing free copper ions (Pena et al., 1999). Tissue copper concentrations are highest in the liver followed by the kidney and brain.

Metabolic Interactions and Mechanisms of Toxicity

Interactions that occur among copper, sulfur, and molybdenum are extremely important in ruminant nutrition. Molybdenum in ruminant diets is frequently within the range of 1 to 5 mg Mo/kg DM, while total sulfur usually varies from 0.1 to 0.3 percent. Concentrations of molybdenum and sulfur on the upper end of this range greatly reduce copper bioavailability and increase the risk of copper deficiency. In contrast, low dietary concentrations of molybdenum and sulfur increase the risk of copper toxicosis, especially in sheep.

Sulfide, derived from sulfate reduction and degradation of sulfur amino acids by ruminal microorganisms, is believed to reduce copper absorption via formation of insoluble copper sulfide (Suttle, 1991). Increasing dietary sulfur from 0.1 to 0.4 percent reduced copper bioavailability by 30 to 56 percent in hypocupremic sheep fed low molybdenum diets (Suttle, 1974). Sulfide can also interact with molybdate in the rumen to form various thiomolybdates (Suttle, 1991). Tri-and tetrathiomolybdates reduce copper absorption by forming insoluble complexes with copper that do not release copper even under acidic conditions. When dietary sulfur and molybdenum are high, di- and trithiomolybdates can be absorbed and affect systemic metabolism of copper (Gooneratne et al., 1989). Systemic effects on copper metabolism attributed to thiomolybdates include (1) increased biliary excretion of copper from liver stores; (2) removal of copper from metalloenzymes; and (3) strong binding of copper to plasma albumin, which results in reduced transport of available copper for biochemical processes. Molybdenum and sulfur supplementation have been used to prevent and treat copper toxicosis in sheep (Howell and Gooneratne, 1987). Intravenous administration of ammonium tetrathiomolybdate has effectively been used to treat copper toxicosis in sheep (Howell and Gooneratne, 1987).

In nonruminants, interactions between copper and molybdenum are minimal and only occur at very high concentrations of molybdenum (Mills and Bremner, 1980). Copper does interact with amino acids in nonruminants. Sulfur amino acid requirements for maximal growth are higher in chicks fed pharmacological levels of copper (Robbins and Baker, 1980; Wang et al., 1987). Increasing the concentration of methionine or cysteine above dietary requirements for growth alleviates the growth depression seen in chicks fed excess copper (500 mg Cu/kg) (Jensen and Maurice, 1979; Robbins and Baker, 1980). Cysteine supplementation reduced liver copper concentrations in chicks fed high dietary copper (Persia et al., 2003) and may chelate copper and reduce its absorption (Baker and Czarnecki-Maulden, 1987).

A number of studies indicate that high dietary zinc can reduce copper absorption (WHO, 1998). High doses of zinc are effective in reducing copper absorption in patients with Wilson's disease. High dietary zinc (220 or 420 mg Zn/kg DM) was effective in preventing copper toxicosis by reducing liver copper accumulation in lambs fed high copper diets (Bremner et al., 1976). High dietary iron can reduce copper absorption in nonruminants (Yu et al., 1994) and reduces copper status in cattle and sheep (Spears, 2003).

The mechanism of chronic copper toxicity appears to relate to ionic copper producing oxidative damage to tissues. Copper can shift between an oxidized (Cu^{+2}) and reduced state (Cu^{+1}) in the cell, which allows copper to bind strongly to many types of electron-rich structures (WHO, 1998). Normally, free copper in the cell is maintained at low concentrations by copper-binding compounds such as metallothionein, glutathione, and copper chaperone proteins (Viarengo et al., 2002). However, when the copper concentration in the cell overwhelms metal homeostasis, free copper can alter cellular functions by directly binding to proteins and nucleic acids and by formation of reactive oxygen species. Ionic copper readily participates in the Fenton reaction that results in the production of reactive oxygen species, including the hydroxyl radical (Viarengo et al., 2002). Hydroxyl radicals can cause lipid peroxidation in cell membranes, cleavage of nucleic acids, and oxidation of cellular proteins.

Acute copper toxicosis in several species, including humans, results in nausea and vomiting. It is believed that the mechanism responsible for these signs is due to copper ions stimulating receptors that, in turn, stimulate the vagus nerve, eliciting a reflex response of nausea and vomiting (Araya et al., 2002).

SOURCES AND BIOAVAILABILITY

Copper is found naturally in the Earth's crust at an average concentration of 50 mg/kg (ATSDR, 2002). Seawater contains approximately 0.15 µg Cu/L, and fresh water contains 1.0 to 20 µg Cu/L in uncontaminated areas (WHO, 1998). Natural weathering of soil and discharges from industries and sewage treatment plants can result in copper release into water. Copper compounds are also sometimes applied to water to kill algae. Copper released into water is primarily in particulate form and tends to settle out, precipitate out, or be adsorbed by organic matter, hydrous iron, manganese oxides, or clay in the sediment. This usually results in low concentrations of copper downstream from the source of copper entry. The cuprous (+1) ion is unstable in aqueous solution and tends to disproportionate to the cupric (+2) form and copper metal unless a stabilizing ligand is present (WHO, 1998). The only cuprous compounds stable in water are insoluble ones such as cuprous sulfide. Cupric copper forms coordination compounds or complexes with inorganic and organic ligands. Bioavailability of copper in water is generally low due to adsorption to suspended particles and complexation by dissolved organic matter or inorganic ligands such as carbonate (WHO, 1998). Copper in sediments also appears to be of poor bioavailability because of its ability to react with acid volatile sulfides and form insoluble precipitates (Besser et al., 1996).

Copper concentrations in various soil types in the United States can vary from 1 to 700 mg/kg with an average concentration of 25 mg/kg (WHO, 1998). Soil copper concentrations are highest in areas in close proximity to copper mining and smelting activities. Application of sludge, poultry or swine waste, and copper-containing fungicides can increase soil copper concentrations. The majority of copper deposited in soil will be strongly adsorbed and remain in the upper few centimeters of soil (Georgopoulos et al., 2002). Soil copper can adsorb to organic matter, clay minerals, carbonate minerals, or hydrous iron and manganese oxides. Copper binds to soil more strongly than other divalent

cations (Georgopoulos et al., 2002). Because of the strong adsorption and complexation of soil copper, elevated soil copper often does not greatly increase copper uptake by plants (Suttle and Price, 1976; Payne et al., 1988).

Forages generally contain 3 to 8 mg Cu/kg DM (Minson, 1990). Legumes are usually higher in copper than grasses when grown under temperate conditions. Bioavailability of copper from forages is low in ruminants and is greatly affected by forage concentrations of sulfur, molybdenum, and iron (Underwood and Suttle, 1999). Normal concentrations of copper in cereal grains range from 3 to 8 mg Cu/kg DM, while leguminous and oilseed meals range from 15 to 35 mg/kg DM. Relative to copper sulfate, copper bioavailability from corn gluten meal, soybean meal, and cottonseed meal was 48, 38, and 41 percent, respectively, in chicks (Aoyagi et al., 1995). Feeds of animal origin with the exception of liver products are low in copper. Liver is often used as an ingredient in pet foods. Based on chick studies, using bile copper excretion as a measure of bioavailability, copper in pork liver was essentially unavailable (Aoyagi et al., 1993). However, copper from chicken, turkey, beef, and sheep liver was similar in bioavailability to copper sulfate (Aoyagi et al., 1993).

Use of poultry or swine waste in diets or as pasture fertilizer can result in high intakes of copper by ruminants. Poultry and swine waste are high in copper, especially if waste is obtained from animals fed growth stimulatory concentrations of copper (100 to 250 mg/kg). Copper from swine and poultry waste is equal in bioavailability to copper sulfate when fed to hypocupremic ewes (Suttle and Price, 1976). Application of high copper waste to pasture (Suttle and Price, 1976) or crop land (Payne et al., 1988) did not increase forage or grain copper concentrations greatly. However, application of waste to pastures can result in copper toxicosis due to ingestion of forage contaminated with high copper waste. Copper toxicosis has been reported in ruminants fed poultry waste (Suttle et al., 1978; Tokarnia et al., 2000) or grazing pastures where large amounts of waste have been applied (Sargison and Scott, 1996). However, other studies have shown no ill effects from grazing sheep on pasture fertilized with high-copper pig manure (Prince et al., 1975). In addition, in some studies chicks and sheep have been fed high-copper pig manure directly with no ill effects, apparently because the copper in the manure was poorly bioavailable to the chickens and sheep (Prince et al., 1975; Izquierdo and Baker, 1986).

Another potential source of copper in ruminants is copper used in foot baths. Copper sulfate foot baths are used in ruminants to control foot rot and other lameness-related problems. Ingestion of copper from baths or consumption of pasture where the copper sulfate solution has been disposed of can lead to copper toxicosis. Copper has been used as an anthelmintic to control gastrointestinal parasites in ruminants (Bang et al., 1990). For example, high doses of copper (3 to 4 g) from copper oxide needles have been administrated to sheep (Bang et al., 1990) and goats (Chartier et al., 2000) to control parasite loads.

A major source of copper in animal diets is supplemental copper added to diets or free choice mineral supplements. Errors in copper formulation or excess supplementation with copper can result in toxicosis. Sources of copper supplemented to animal diets include cupric sulfate, tribasic cupric chloride, copper oxide (primarily cupric oxide), cupric carbonate, and various organic copper sources. Copper from copper oxide powder is essentially unavailable when fed to cattle (Kegley and Spears, 1994), hens (Jackson and Stevenson, 1981), chicks (Baker et al., 1991), and swine (Cromwell et al., 1989). Copper oxide needles supply available copper in ruminants because the needles are retained in the digestive tract and release copper over several weeks. Apparently, copper oxide powder passes through the acid environment of the digestive tract before the copper can be solubilized (Spears, 2003). Tribasic cupric chloride is similar in bioavailability to cupric sulfate in chicks (Miles et al., 1998) and swine (Cromwell et al., 1998) and in cattle fed diets low in molybdenum and sulfur (Spears et al., 2004). Relative to cupric sulfate (set at 100 percent), tribasic cupric chloride is more bioavailable (132 to 196 percent) when supplemented to diets high in molybdenum and sulfur (Spears et al., 2004). Feed grade cupric carbonate appears to be somewhat less bioavailable, based on liver copper concentrations, than cupric sulfate (Ledoux et al., 1991; Ward et al., 1996). Results of studies evaluating the bioavailability of copper from different organic sources have been variable, with some studies indicating similar bioavailability and others higher bioavailability relative to cupric sulfate (Spears, 2003).

In field studies with fish, the bioconcentration factor for copper was 10 to 100, indicating a low potential for bioconcentration (ATSDR, 2002). Biomagnification of copper also does not occur in the food chain.

TOXICOSIS

A number of excellent reviews are available on copper toxicosis in mammals and fish (Howell and Gooneratne, 1987; Bremner, 1998; WHO, 1998; ATSDR, 2002; Clearwater et al., 2002). Copper toxicosis can result from a single large dose of copper or from repeated exposure to copper concentrations that exceed animal requirements. A number of studies evaluating oral ingestion of high concentrations of copper are summarized in Table 13-1.

Animal species differ greatly in their ability to tolerate excess copper. Sheep are very sensitive to copper toxicity. Death due to copper toxicosis is a common problem in sheep. The range of dietary copper concentrations required by sheep under some conditions can overlap with dietary concentrations that cause toxicosis under other conditions. For example, 10 mg Cu/kg diet may be required by sheep if dietary sulfur and molybdenum are fairly high. However, if dietary

molybdenum is low, 10 mg Cu/kg diet can cause toxicosis in some breeds of sheep (Hogan et al., 1968). The sensitivity of sheep to copper toxicity appears to relate to their inability to increase biliary copper excretion in response to elevated copper intakes (Bremner, 1998). Cattle and goats are less susceptible to copper toxicity than sheep. Young ruminants are more susceptible than adults because of higher absorption.

In nonruminants, copper homeostatic control mechanisms generally are very efficient in preventing toxicosis. Concentrations of copper needed to cause toxicosis in nonruminants generally exceed requirements by at least 25-fold, and are as high as 50-fold in pigs. Rats can tolerate higher dietary concentrations of copper than swine and poultry, and mice are less sensitive than rats to copper toxicity (Hebert et al., 1993). Most fish can tolerate fairly high concentrations of dietborne copper (Clearwater et al., 2002). However, some fish species are sensitive to relatively low concentrations of copper in water (Taylor et al., 1996).

Liver and kidney are target organs for copper toxicosis in all species. Lesions in the forestomach also occur in rats and mice (Hebert et al., 1993), and proventriculitis (Wideman et al., 1996) and gizzard erosion (Jensen and Maurice, 1978) are seen in poultry exposed to high dietary copper.

Single Dose and Acute

Acute copper toxicosis signs include nausea, vomiting, diarrhea, excessive salivation, abdominal pain, convulsions, paralysis, and sometimes death (NRC, 1980; WHO, 1998). Necropsy of animals with acute copper toxicosis reveals acute gastroenteritis, necrotic hepatitis, and splenic and renal congestion. The acute toxic level of oral copper (as $CuSO_4$) is 9 to 20 mg/kg BW in sheep and approximately 200 mg/kg BW in cattle (NRC, 1980). Administration of copper oxide wire or needles at a rate of 4.1 g Cu/head to sheep (Bang et al., 1990) or 3.4 g Cu/head to goats (Chartier et al., 2000) did not produce toxicosis. Copper oxide needles release copper slowly in the gastrointestinal tract and appear to be relatively safe when administered at the labeled dosage to ruminants with a functional rumen. However, administration of copper oxide needles to young calves has resulted in mortality from copper toxicosis (Hamar et al., 1997; Steffen et al., 1997). In horses, a single oral dose of 125 mg $CuSO_4$ (in solution)/kg BW resulted in gastroenteritis, hemolysis, kidney and liver damage, and death within 2 weeks (Hintz, 1987). Acute toxicosis did not occur when the same amount of copper was added to the feed of horses. The LD_{50} in rats for copper as $CuSO_4$ has ranged from 120 to 244 mg Cu/kg BW in different studies (WHO, 1998).

The 96-hr LC_{50} for Chinook salmon was 54–64 µg Cu/L of water (Hamilton and Buhl, 1990). In channel catfish, the 24-hr LC_{50} was 2,500 to 3,500 µg/L depending on water temperature (Smith and Heath, 1979). In rainbow trout, the 96-hr LC_{50} ranged from 20 µg Cu/L in soft acid water to 520 µg/L in hard alkaline water (Howarth and Sprague, 1978).

Chronic

Chronic copper toxicosis in sheep is characterized by the gradual accumulation of copper in the liver over a period of several weeks or months. The condition is most common in lambs fed large amounts of concentrate feeds, but can occur under grazing conditions if forage molybdenum concentration is low or if sheep are consuming plants that contain hepatotoxic compounds (Underwood and Suttle, 1999). Copper toxicosis in sheep consists of a prehemolytic, and hemolytic phase (Howell and Gooneratne, 1987). Although copper is accumulating in the liver and to a lesser extent in the kidney during the prehemolytic phase, the animal is clinically normal, and depressed animal performance is generally not evident until at least shortly before the hemolytic phase occurs. During the prehemolytic phase, liver necrosis occurs and enzymes (aspartate aminotransferase, glutamate dehydrogenase, and sorbitol dehydrogenase) indicative of liver damage may become elevated in serum, but blood copper is generally normal (Howell and Gooneratne, 1987). Liver concentrations of copper in all cellular fractions are increased, but the proportion of cellular copper in the nuclear fraction is increased during copper loading (Gooneratne et al., 1979).

Sheep show histological and biochemical evidence of liver damage at liver copper concentrations as low as 350 mg Cu/kg DM; however, clinical signs of toxicosis do not usually occur until liver concentrations of 1,000 mg Cu/kg DM or higher are reached (Underwood and Suttle, 1999). Animals that die from copper toxicosis often have liver concentrations that exceed 2,000 mg Cu/kg DM. The hemolytic phase (referred to as hemolytic crisis) is associated with rapid release of copper from the liver into the blood and is characterized by hemolysis, hemoglobinemia, and hemoglobinuria. Clinical signs may include dullness, anorexia, excessive thirst, jaundice of mucous membranes, dark-colored urine, sunken eyes with blood vessels on the surface of the sclera showing a chocolate-brown color, and death (Howell and Gooneratne, 1987). Observed hemolysis is due to entry of excessive copper into erythrocytes. The mechanism whereby copper induces hemolysis is unclear, but may be due to copper inducing production of superoxide radicals that cause erythrocyte membrane damage (Howell and Gooneratne, 1987). Sheep with a mild hemolysis may survive and recover, especially if treated with copper chelating agents such as ammonium tetrathiomolybdate or penicillamine that increase copper excretion.

Sheep that are killed during the hemolytic phase or those that die from toxicosis show major liver and kidney damage (Howell and Gooneratne, 1987). Livers contain areas of necrotic parenchymal cells and swollen copper-containing Kupffer cells high in acid phosphatase. Kidney copper is greatly elevated following the hemolytic phase. The elevated copper causes necrosis in the proximal convoluted tubules and impaired glomerular and tubular function. In addition, kidneys have a characteristic black or dark brown color.

The concentration of dietary copper needed to cause toxicosis in sheep has varied greatly among studies (Table 13-1) and is greatly affected by genetics and dietary factors that will be discussed in the next section. In several studies (Hill and Williams, 1965; Pond, 1989; Zervas et al., 1990) feeding total (diet plus supplemental) dietary copper concentrations of 30 to 40 mg/kg DM resulted in some mortality from toxicosis. Death losses occurred after 3 to 12 weeks of feeding the high copper diets. Elevated plasma aspartate aminotransferase activity was observed in some lamb breeds fed a total dietary copper concentration of 20 mg/kg DM for 9 weeks (Woolliams et al., 1982). Long-term studies have indicated that copper toxicosis can occur in housed sheep fed diets containing approximately 10 mg Cu/kg (Hogan et al., 1968) and in ewes grazing forage containing 7.5 to 15.0 mg Cu/kg DM (MacPherson et al., 1997) if dietary molybdenum is low.

Cattle can tolerate higher dietary concentrations of copper than sheep. However, some breeds of cattle may be more susceptible to copper toxicosis than others. Clinical signs of copper toxicosis in cattle are similar to those described for sheep. The preruminant calf is more susceptible to copper toxicosis than older cattle during relatively short-term copper exposure. Clinical cases of copper toxicosis occurred in 10- to 12-week-old calves fed milk replacer containing 115 mg Cu/kg DM (Shand and Lewis, 1957). In calves (Holstein and Ayrshire-Holstein crossbreds), supplementation of milk replacer with 50 mg Cu/kg DM for 6 weeks reduced intake (Jenkins and Hidiroglou, 1989). Copper addition to milk replacer at 200 mg/kg DM reduced gain and intake while 1,000 mg Cu/kg DM resulted in a 43 percent mortality in calves (Jenkins and Hidiroglou, 1989).

Older cattle (Bonsmara) receiving 10 or 20 mg Cu/kg BW, in an oral drench, for 5 days per week showed clinical signs of copper toxicosis by day 679 after initial dosing (Gummow, 1996). However, liver damage occurred much sooner based on elevated serum asparate aminotransferase activity. A number of factors may influence the ability of older cattle to tolerate copper. In some studies (Chapman et al., 1962; Felsman et al., 1973), cattle have tolerated high doses of copper without showing signs of toxicosis, and under certain conditions (that are not well defined) cattle appear to regulate liver copper concentrations once concentrations reach a certain level. Steers (Hereford x Brahman) dosed daily with 0.13 to 2.0 g Cu/d, via gelatin capsule, for 16 months showed no clinical signs of toxicosis (Chapman et al., 1962). Liver copper concentrations increased during the study, but final liver copper concentrations were approximately 2,000 mg/kg DM regardless of the quantity of copper dosed. Steers (Hereford) administered 3 g Cu/d, via a gelatin capsule, also did not show adverse effects by 64 days; however, this quantity of copper given in water resulted in clinical signs of copper toxicosis, including death, and much higher liver copper concentrations (Chapman et al., 1962). No signs of copper toxicosis were observed in weaned Holstein calves fed a high concentrate diet supplemented with 125 to 900 mg Cu/kg DM for 98 days (Felsman et al., 1973). Liver copper concentrations at the end of the study in copper-supplemented calves were approximately 900 mg/kg DM, regardless of dietary copper (Felsman et al., 1973).

Long-term supplementation of lower dietary copper concentrations than those described above can cause toxicosis in cattle. Bradley (1993) reported the death of 9 of 63 Holstein cows in a herd from copper toxicosis. For over two years, the cows received a diet that analyzed 37.5 mg Cu/kg DM during lactation and 22.6 mg Cu/kg DM during the dry period. Cows exhibited clinical signs of copper toxicosis prior to death and had elevated postmortem liver copper concentrations that ranged from 1,236 to 2,179 mg/kg DM. All cows that died were in late pregnancy. The addition of ammonium molybdate to the diet for 18 days and removal of the copper-containing mineral supplement arrested the outbreak of copper toxicosis (Bradley, 1993). Lactating dairy cows (Holstein) tolerated 40 (Engle et al., 2001) or 80 mg (Du et al., 1996) supplemental Cu/kg DM for 60 days. However, over the 60-day periods, liver copper concentrations increased by over 7 mg Cu/kg DM/day in both studies. If this rate of increase in liver copper had continued, cows may have eventually showed signs of copper toxicosis.

Goats can also tolerate much higher dietary copper concentrations than sheep. Zervas et al. (1990) compared the susceptibility of lambs and goats to chronic copper toxicosis. Lambs supplemented with 30 or 60 mg Cu/kg DM had elevated serum asparate aminotransferase after 50 days, and some lambs died from copper toxicosis beginning as early as 67 days. Goats fed the same diets (30 or 60 mg Cu/kg) had normal serum asparate aminotransferase activity and exhibited no signs of toxicosis during the 137-day study. Lambs stored 6 to 9 times more copper in their livers than goats. Goats fed a total dietary copper of 36 mg/kg DM for 88 days had increased liver copper but no signs of toxicosis or liver damage (Luginbuhl et al., 2000). Goats administered 600 mg Cu/d (gelatin capsule) for 4 weeks showed clinical signs of toxicosis and one of two goats receiving 1,200 mg Cu/d died (Solaiman et al., 2001).

Copper is relatively nontoxic to nonruminant animals. Supplementation of swine diets with 250 mg Cu/kg diet increased liver copper concentration, but generally has produced no negative effects on animal growth or health. In fact, 250 mg/kg of added copper normally stimulates growth rates of weanling pigs (Hill et al., 2000). Addition of 250 mg Cu/kg to sow diets for up to 775 days had no adverse effects on animal health and actually increased piglet birth and weaning weights (Cromwell et al., 1993). In growing pigs, 500 mg Cu/kg diet has reduced growth and hemoglobin concentrations (Bunch et al., 1965; Kline et al., 1971) and resulted in increased mortality in one study (DeGoey et al., 1971).

Poultry are similar to swine in their ability to tolerate excess dietary copper. In long-term studies with laying hens, 400 mg Cu/kg diet or higher reduced feed intake and egg production, and increased gizzard weight (Jackson et al.,

1979; Jackson and Stevenson, 1981). Some studies with broilers have indicated that supplementation with 250 mg Cu/kg diet can cause slight gizzard lining erosion (Robbins and Baker, 1980), proventriculitis (Wideman et al., 1996), and lesions in the oral cavity, tongue, and pharynx (Chiou et al., 1999). In other studies, no adverse effects were noted in broilers supplemented with 375 (Pesti and Bakalli, 1996) or 450 (Ledoux et al., 1991) mg Cu/kg diet. Supplementation with 500 mg Cu/kg diet reduced body weights and feed intake, and caused gizzard erosion in turkey poults (Christmas and Harms, 1979). In older turkeys, addition of up to 500 mg Cu/kg diet did not affect performance (Leeson et al., 1997). Ducklings accumulated more copper in their livers than chicks when fed high dietary copper concentrations (Wood and Worden, 1973) and thus, may be more sensitive to copper toxicosis. Addition of 500 mg Cu/kg to duckling diets caused myopathy characteristic of selenium-vitamin E deficiency (Van Vleet, 1982). High mortality and severe necrosis in the gizzard, skeletal muscle, and intestine were observed in ducklings fed 1,000 mg Cu/kg diet.

Horses and rabbits appear to be more resistant to copper toxicosis than swine or poultry. Ponies fed diets containing up to 791 mg Cu/kg for 6 months showed no signs of toxicosis, including liver damage despite final liver copper concentrations over 3,000 mg/kg DM (Smith et al., 1975). Some of the ponies were pregnant during the study and foaled normally 3 to 4 months after the study ended. Rabbits can tolerate 500 mg Cu/kg diet for up to 32 days without adverse effects (Patton et al., 1982; Grobner et al., 1986). In some studies, high dietary copper has increased gain and reduced mortality from enteritis in rabbits (Patton et al., 1982). Addition of 1,000 mg Cu/kg diet reduced gain, feed intake, and feed efficiency in rabbits (Grobner et al., 1986).

Rats are very tolerant to dietary copper. Diets supplemented with 1,000 mg Cu/kg produced no adverse effects in rats (Hebert et al., 1993; Aburto et al., 2001a). Aburto et al. (2001a) reported reduced body weight and slight liver damage in rats fed 1,250 mg Cu/kg diet for 3 months, and more extensive liver damage in animals fed 1,500 mg Cu/kg. Rats fed 2,000 to 8,000 mg Cu/kg diet for 13 weeks exhibited hyperplasia and hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach, inflammation in the liver, with indications of cellular damage, and an increase in the size and number or cytoplasmic protein droplets present in the epithelium of the proximal convoluted tubules of kidney (Hebert et al., 1993). Severity of stomach, liver, and kidney lesions increased with increasing dietary copper. Some research indicates that rats are able to adapt to prolonged exposure to high copper, with elevated liver and kidney concentrations eventually decreasing, with subsequent recovery from copper-induced liver and kidney damage (Haywood, 1985; Fuentealba et al., 1993). Mice are even less sensitive to copper than rats (Hebert et al., 1993). Chronic copper toxicosis did not affect reproduction in either rats or mice (Hebert et al., 1993).

Copper toxicosis resulting from a genetic disorder in copper metabolism is a problem in certain breeds of dogs, especially in Bedlington terriers (Hyun and Filippich, 2004). Dogs with this disorder absorb normal amounts of copper, but biliary excretion of copper is reduced. Progressive accumulation of copper within hepatic lysosomes results in liver damage that can be fatal. Occurrence of a hemolytic crisis is uncommon in dogs with inherited copper toxicosis, but can be seen in the terminal stages. Once diagnosed, inherited copper toxicosis is treated by restricting copper intake, adding an antagonist, such as zinc, to the diet to reduce copper absorption and/or use of chelators that increase urinary copper excretion (Hyun and Filippich, 2004). Limited research has examined the level of copper needed to cause toxicosis in normal dogs. During a 12-month study, 2 of 12 Beagle dogs fed 8.4 mg Cu/kg BW developed elevated serum alanine aminotransferase (WHO, 1998).

The amount of dietborne copper needed to cause toxicosis differs among fish species (Clearwater et al., 2002). Channel catfish may be most sensitive to dietary copper. Murai et al. (1981) found that only 8 mg Cu/kg diet reduced gain:feed, and that 16 to 32 mg Cu/kg diet reduced gain and gain:feed during a 16-week study with catfish. In contrast, Gatlin and Wilson (1986) reported that copper concentrations up to 40 mg/kg diet caused no adverse effects in channel catfish during a 13-week study. In Atlantic salmon fry, copper concentrations of 500 to 1,750 mg/kg diet reduced weight gain (Berntssen et al., 1999). Atlantic salmon parr are more sensitive to lower dietary concentrations of copper than fry (Clearwater et al., 2002). Lanno et al. (1985) concluded that 665 mg Cu/kg diet was the maximum tolerable level in rainbow trout fry, when copper was supplied from $CuSO_4$. Reduced gain and increased feed:gain were observed in trout fed diets containing 730 mg/kg and increased mortality occurred when diets supplied 1,585 to 3,088 mg Cu/kg (Lanno et al., 1985). Dietary copper concentrations as low as 660 mg/kg increased mortality in rainbow trout fry when supplemental copper was provided from shrimp, enriched with copper (Mount et al., 1994). Waterborne copper released from shrimp may have contributed to the higher toxicosis of copper in this study (Mount et al., 1994). Rainbow trout exposed to water containing 144 μg Cu/L for 12 weeks had reduced growth and increased mortality when fed high carbohydrate diets and maintained at 10°C (Dixon and Hilton, 1985). This concentration of copper in water had no effect when fish were fed low carbohydrate diets and/or maintained at a water temperature of 15°C.

Factors Influencing Toxicity

A number of dietary, physiological, and genetic factors affect the occurrence of copper toxicosis, especially in ruminants. Length of exposure will affect copper toxicity because copper generally accumulates in the liver over time when animals are fed high dietary copper. In ruminants, young animals are more susceptible to copper toxicosis than older

animals during relatively short-term exposure periods (Todd, 1969).

Molybdenum and sulfur are potent copper antagonists that greatly affect the level of copper needed to cause toxicosis in ruminants. Addition of 0.2 percent sulfur and 2 to 16 mg Mo/kg diet reduced liver copper and liver damage in sheep fed a high copper diet (45 mg Cu/kg) for 18 weeks (Suttle, 1977). In sheep previously exposed to high dietary copper, supplementation with 0.3 percent sulfur and 14 or 30 mg Mo/day reduced liver copper concentrations by 34 to 47 percent over a 79-day period (Van Ryssen, 1994). Deaths from outbreaks of copper toxicosis can also be alleviated by supplementation of molybdenum and sulfur (Hidiroglou et al., 1984). However, sheep consuming diets low in molybdenum and sulfur are susceptible to toxicosis at relatively low (10 to 14 mg/kg) dietary copper concentrations. High dietary sulfur intake via feed or water will reduce copper toxicity even if dietary molybdenum is low. Consumption of high sulfate (1,500 mg SO_4/L) water versus normal water greatly reduced liver copper accumulation in cattle fed 100 mg Cu/kg diet (Wright et al., 2000).

Dietary iron and zinc can also influence copper toxicity. High dietary zinc can reduce liver copper and clinical signs of copper toxicosis. In lambs fed diets containing 29 mg Cu/kg, increasing dietary zinc from 43 to 220 or 420 mg/kg reduced liver copper concentrations and liver damage, and prevented onset of a hemolytic crisis (Bremner et al., 1976). Providing high dietary zinc to sheep that had previously been loaded with copper was ineffective in reducing liver copper concentrations over an 88-day period (Van Ryssen, 1994). However, administration of 200 mg Zn/day for 2 years to dogs (Bedlington terriers and West Highland White terriers) with inherited copper toxicosis reduced liver copper concentrations and liver damage (Brewer et al., 1992).

Factors that reduce or eliminate ruminal protozoa increase copper bioavailability and, thus, increase the susceptibility of ruminants to chronic copper toxicosis (Ivan et al., 1986). Liver copper concentrations increased to a much greater extent in fauna-free than in faunated sheep when diets containing 13 mg Cu/kg were fed (Ivan et al., 1986). Lower copper bioavailability in sheep with ruminal protozoa may relate to the role of protozoa in degradation of dietary protein. Increased degradation of dietary protein by ruminal microorganisms increases ruminal sulfide, which subsequently can reduce copper absorption. Feeding monensin, a carboxylic ionophore, increased liver copper in sheep (Van Ryssen and Barrowman, 1987; Ivan et al., 1992). Higher liver copper in sheep fed monensin may relate to the ability of the ionophore to reduce ruminal protozoa numbers.

Plants containing toxins that compromise liver function decrease tolerance for copper in ruminants. Plants belonging to the *Heliotropium echium* and *Senecio* genera contain pyrrolizidine alkaloids that cause liver damage. Concurrent exposure to high copper and *Heliotropium europaeum* in sheep enhanced the toxicity of both substances and caused greater accumulation of copper in liver than high copper alone (Howell et al. 1991). Mycotoxins that cause liver damage can also increase liver copper concentrations and may lower the level of copper needed to produce toxicosis (White et al., 1994).

High dietary copper may increase the requirement for certain antioxidants because of its ability to cause oxidative damage. Addition of 2 mg Se/kg or 200 IU vitamin E/kg to diets adequate in these nutrients prevented mortality and muscle necrosis in ducklings fed 1,500 mg Cu/kg diet (Van Vleet et al., 1981). Dietary selenium did not affect hepatic damage in rats fed 2,000 mg Cu/kg diet (Aburto et al., 2001b). Ascorbic acid supplementation reduced liver copper accumulation, but did not alleviate the growth depression observed in chicks fed 1,000 mg Cu/kg diet (Persia et al., 2003).

Copper toxicosis in poultry is affected by dietary sulfur amino acid level. Addition of 0.4 percent methionine or 0.33 percent L-cysteine to diets adequate in sulfur amino acids prevented growth depression and reduced liver copper in chicks fed 500 mg Cu/kg diet (Jensen and Maurice, 1979). Cysteine supplementation at 0.5 percent also alleviated growth depression and reduced elevated liver copper concentrations in chicks receiving 1,000 mg Cu/kg diet (Persia et al., 2003). In turkey poults, addition of 0.4 percent methionine partially prevented growth depression and gizzard erosion seen in birds fed 500 or 750 mg Cu/kg diet (Christmas and Harms, 1979).

Susceptibility to copper toxicosis, especially in sheep and dogs, is greatly affected by genetics. Breeds of sheep differ in their ability to accumulate copper in their livers when fed low (Littledike and Young, 1993; Suttle et al., 2002) or moderate to high (Woolliams et al., 1982) copper levels. When fed moderately high dietary copper, Texel-sired lambs were most susceptible to copper toxicosis followed by Suffolk lambs (Woolliams et al., 1982). Finnish landrace were intermediate and Scottish Blackface appeared to be least susceptible to copper toxicosis. In crossbred lambs fed diets low in copper (4 to 5 mg/kg), Texel-sired lambs had the highest liver copper followed by Dorset- and Montadale-sired lambs with lambs from Finnsheep or Romanov rams being lowest (Littledike and Young, 1993).

Genetic differences among breeds in copper metabolism also occur in cattle. Simmental and Charolais cows and their calves had lower plasma copper concentrations than Angus when fed diets low in copper (Ward et al., 1995). Simmental cattle also have lower liver copper concentrations than Angus (Mullis et al., 2003). Biliary copper excretion is higher in Simmental compared to Angus (Gooneratne et al., 1994). Copper accumulated more rapidly in the liver of Jerseys than in Holsteins when copper was supplemented at 80 mg/kg diet (Du et al., 1996). During an outbreak of copper toxicosis in nursing beef calves, 32 percent of Angus calves were affected, but only 5.5 percent of Charolais calves (Sargison and Scott, 1996).

Liver copper concentrations vary greatly among and within breeds in dogs (Thornburg et al., 1990). Inherited copper toxicosis affects approximately 70 percent of Bedlington terriers in the United States (Hyun and Filippich, 2004). The condition is caused by an autosomal recessive trait that reduces biliary excretion of copper. Inherited copper toxicosis has also been seen in Doberman pinschers, West Highland White terriers, Skye terriers, Dalmatians, and in some mixed breeds (Hyun and Filippich, 2004).

Acute toxicity of waterborne copper in fish is affected by water hardness, pH, and temperature (WHO, 1998). The 96-hr LC_{50} for rainbow trout increased with increasing water hardness and was higher at alkaline pH (Howarth and Sprague, 1978). The effect of water temperature varies depending on fish species (Smith and Heath, 1979).

TISSUE LEVELS

Representative copper concentrations in tissues from various animals fed normal or high levels of copper are shown in Table 13-2. Copper concentrations are highest in the liver, and liver copper increases in animals fed high dietary copper. Normal liver copper concentrations are higher in ruminants than in pigs and chickens, and relatively small amounts of dietary copper greatly increase liver copper concentrations in ruminants. In nonruminants and fish, liver copper increases to a much smaller extent with increasing dietary copper unless high levels of copper (>100 mg Cu/kg) are fed. Kidney copper concentrations also can increase in animals fed high dietary copper, but to a lesser extent than liver. Muscle contains less than 4 mg Cu/kg DM in most animals and does not increase in animals fed high copper diets. Copper concentration in eggs is also not affected by level of copper in diets of laying hens. The copper content of milk is low and varies with species and stage of lactation (Underwood, 1977). Copper addition to diets already adequate in copper has little effect on milk copper concentration in cows (Underwood, 1977).

MAXIMUM TOLERABLE LEVELS

The maximum tolerable level for copper is defined as the dietary level that, when fed for a defined period of time, will not impair animal health and/or performance. Nonruminant animals are tolerant to high dietary copper concentrations relative to copper requirements. Based on published literature in nonruminants, maximum tolerable levels for copper in mg Cu/kg diet were set by the NRC committee at chicken and turkey, 250; duck, 100; swine, 250; horse, 250; rabbit, 500; rat, 1,000; and mouse, 2,000. Insufficient data are available to set a maximum tolerable level for copper for dogs and cats.

In broilers, addition of 250 mg Cu/kg to diets may increase the incidence of proventriculitis (Wideman et al., 1996) and lesions in the oral cavity, tongue, and pharynx (Chiou et al., 1999), but negative effects on performance are generally not observed. Ponies tolerated up to 791 mg Cu/kg diet for 6 months without showing clinical signs of toxicosis (Smith et al., 1975). However, liver copper concentrations were elevated to concentrations that could cause toxicosis problems in stressful situations that affect liver function. Because of the limited research data, and possible genetic differences in susceptibility to copper toxicity, a maximum tolerable level of 250 mg Cu/kg is recommended in horses.

The maximum tolerable level of dietborne copper for fish is affected by species and fish size or life stage (Clearwater et al., 2002). The upper limit for copper intake from diets in Atlantic salmon and rainbow trout is approximately 100 and 500 mg/kg diet, respectively. The maximum tolerable copper level may be lower for channel catfish, but sufficient data are not available to arrive at a reliable upper limit. Waterborne copper is generally more toxic to fish than dietborne copper. However, it is impossible to set a maximum tolerable water copper concentration for fish because of the major impact of water hardness and pH on copper toxicity from water.

In sheep, the maximum tolerable level was set at 15 mg Cu/kg diet DM. Some sheep breeds may be susceptible to copper toxicosis at lower dietary concentrations if dietary molybdenum and/or sulfur are below normal levels (1 to 2 mg Mo/kg diet; 0.15 to 0.25 percent S) (Hogan et al., 1968; MacPherson et al., 1997). This upper limit for copper is similar to the current European Commission (EC, 2003) limit of 17 mg Cu/kg DM in sheep diets. A recent study (Suttle et al., 2002) suggested that the EC limit of 17 mg Cu/kg diet was too high for some sheep breeds when fed high concentrate diets (contained 0.27 percent sulfur and unspecified molybdenum concentration).

The level of dietary copper that causes toxicosis in ruminants is greatly affected by dietary sulfur and molybdenum, and genetics. Other factors that may affect the maximum level of copper that ruminants can tolerate include: (1) length of exposure, (2) age, (3) initial liver copper concentration at the onset of high copper exposure, (4) dietary toxins that impair liver function and trigger a hemolytic crisis, and (5) dietary concentrations of zinc and iron. Maximum tolerable copper levels for ruminants were set based on available literature, and assuming normal dietary sulfur (0.15 to 0.25 percent DM) and molybdenum (1 to 2 mg/kg DM).

The maximum tolerable level for copper in cattle was set at 40 mg Cu/kg diet DM. Higher concentrations of copper can often be tolerated by cattle for several weeks or even months. However, long-term feeding of diets slightly less than 40 mg Cu/kg can result in copper toxicosis (Bradley, 1993). Feeding high dietary copper will greatly increase liver copper concentrations. Factors that regulate liver copper in cattle are poorly understood, and the minimum liver copper concentration that may result in clinical signs of toxicosis is not known. However, cattle not showing clinical signs of copper toxicosis, but with high liver copper concentrations, would be expected to be more susceptible to a hemolytic crisis when exposed to

toxins that cause liver damage or other stressors. Increased incidence of copper toxicosis in cattle has been reported recently by the Veterinary Laboratories Agency (VLA) in England (Bidewell et al., 2000; VLA, 2001). A few years ago a considerable amount of the supplemental copper provided in cattle supplements was in the form of copper oxide (primarily cupric oxide) powder. Based on research indicating that bioavailability of copper from copper oxide was extremely low, more bioavailable sources of copper have now replaced copper oxide in most ruminant supplements. Supplementation of more bioavailable copper sources to cattle diets may have contributed to increased incidence of copper toxicosis. Recent studies with goats suggest that their tolerance to copper is similar to cattle.

HUMAN HEALTH

Copper in edible tissue is not likely to present a human health concern, when copper is provided in animal diets at the maximum tolerable levels indicated. Muscle copper is low and increasing dietary copper does not increase muscle copper concentration. Copper intake from animal products could possibly be of concern in humans that consume large quantities of liver. Livers from animals fed dietary copper concentrations within the maximum tolerable levels suggested can contain from approximately 20 to 900 mg Cu/kg DM (Table 13-2).

FUTURE RESEARCH NEEDS

Maximum tolerable levels of copper are fairly well defined for poultry and swine, and copper toxicosis is not a common problem in these species. Copper toxicosis in ruminants is primarily due to poor copper homeostasis, resulting in liver copper accumulation. Research is needed to better understand biliary copper excretion in ruminants and factors that affect it. Under certain conditions, which are not well defined, cattle appear to regulate liver copper concentrations once concentrations reach a certain level in spite of high copper intakes. However, in other situations, liver copper continues to increase until ruminants succumb to copper toxicosis. Additional research is also needed to identify factors (dietary toxins, stress, etc.) that may trigger the occurrence of hemolytic crisis, and thus clinical signs of copper toxicosis in ruminants with elevated liver copper concentrations.

SUMMARY

Copper occurs primarily in the cuprous (+1) and cupric (+2) oxidation states. Because of its high electrical and thermal conductivity, and resistance to corrosion, copper is a widely used industrial metal. Copper compounds are also used in nutritional supplements for animals, fungicides, and as algicides in streams and reservoirs. Copper is an essential trace mineral, and functions as a component of a number of enzymes that are involved in maturation and stability of collagen and elastin, energy metabolism, the antioxidant defense system, and pigmentation, as well as other processes. Homeostatic control mechanisms for copper are usually very efficient in preventing copper toxicosis in nonruminants. Concentrations of copper needed to cause toxicosis in nonruminants exceed requirements by at least 25-fold. Ruminants, particularly sheep, are very susceptible to copper toxicity. Copper requirements of sheep generally range from 3 to 8 mg Cu/kg diet DM, while 15 mg Cu/kg diet or even less can cause toxicosis under certain conditions. Cattle and goats can generally tolerate 40 mg Cu/kg diet DM for several weeks or months. Maximum tolerable concentrations of copper for ruminants are greatly affected by dietary concentrations of sulfur and molybdenum as well as dietary toxins that compromise liver function. Susceptibility of sheep and dogs to copper toxicity is greatly affected by genetics. Certain fish species are also quite susceptible to toxicosis from waterborne copper. In all species, liver and kidney are the major organs affected by copper toxicosis.

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TABLE 13-1 Effects of Copper Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effects(s)	Reference
Dogs	12		0.42–2.1 mg/kg BW 8.4 mg/kg BW	Cu gluconate	12 mo	Diet	No adverse effect Elevated serum GPT in 2 of 12 dogs	WHO, 1998
Mice	10	6 wk	300–1,000 mg/L 3,000 mg/L 10,000 mg/L	$CuSO_4$	15 d	Water	No adverse effect Reduced gain, emaciation, abnormal posturing, hypoactivity, dyspnea, tremors, postration 100% mortality	Hebert et al., 1993
Mice	20	6 wk	500–2,000 mg/kg 4,000–16,000 mg/kg	$CuSO_4$	92 d	Diet	No adverse effect Reduced final body weight, stomach lesions	Hebert et al., 1993
Rabbits	31		200 mg/kg	$CuSO_4$	8 wk	Diet	Increased weight gain	King, 1975b
Rabbits	24	28 d	385 mg/kg 495 mg/kg	$CuSO_4$	32 d	Diet	Increased gain and feed efficiency, reduced mortality from enteritis Reduced mortality from enteritis	Patton et al., 1982
Rabbits	20	28 d	50–500 mg/kg 1,000 mg/kg	$CuSO_4$	28 d	Diet	No adverse effect Reduced gain, feed intake and feed efficiency	Grobner et al., 1986
Rats	10	6 wk	300–1,000 mg/L 3,000 mg/L 10,000 mg/L	$CuSO_4$	15 d	Water	No adverse effect Reduced gain, emaciation, abnormal posturing, hypoactivity, dyspnea, tremors, prostration 100% mortality	Hebert et al., 1993
Rats	20	6 wk	500–1,000 mg/kg 2,000 mg/kg 4,000–8,000 mg/kg	$CuSO_4$	92 d	Diet	No adverse effect Stomach lesions, slight liver and kidney damage, increased serum ALT and SDH Reduced final body weight, liver and kidney damage, stomach lesions, decreased hemacrit and hemoglobin	Hebert et al., 1993
Rats	5	10 wk	750–1,000 mg/kg 1,250 mg/kg 1,500–2,000 mg/kg	$CuSO_4$	3 mo	Diet	No adverse effect Reduced body weight, slight liver damage Reduced body weight, liver necrosis	Aburto et al., 2001a
Chickens	33	24 wk Laying hen	100–300 mg/kg 400 mg/kg	$CuSO_4$	336 d	Diet	No adverse effects Reduced feed intake and egg production	Jackson et al., 1979

continued

TABLE 13-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effects(s)	Reference
Chickens	24	26 wk	150 mg/kg	$CuSO_4$	336 d	Diet	No adverse effects	Jackson and Stevenson, 1981
		Laying hen	300 mg/kg				Increased liver Cu, increased gizzard weight	
			450–750 mg/kg				Reduced feed intake, reduced egg number and egg weight, increased liver Cu, increased gizzard weight	
Chickens	15	28 wk Laying hen	200 mg/kg	$CuSO_4$	28 d	Diet	No adverse effects	Chiou et al., 1997
			400 mg/kg				Reduced feed intake and egg production	
			600 mg/kg				Reduced feed intake and egg production, increased serum aspartate aminotransferase, lactate dehydrogenase and creatine kinase	
			800 mg/kg				Reduced feed intake and egg production, increased serum enzymes, liver damage	
Chickens	15	8 d	250 mg/kg	$CuSO_4$	14 d	Diet	Slight gizzard lining erosion	Robbins and Baker, 1980
			500 mg/kg				Reduced gain and gain:feed, gizzard lining erosion	
Chickens	16	1 d	150–450 mg/kg	$CuSO_4$	21 d	Diet	No adverse effects	Ledoux et al., 1991
Chickens	60	1 d	125–375 mg/kg	$CuSO_4$	42 d	Diet	No adverse effects	Pesti and Bakalli, 1996
Chickens	100		250–500 mg/kg	$CuSO_4$	28 d	Diet	Proventriculitis	Wideman et al., 1996
Chickens	20	21 d	250 mg/kg	$CuSO_4$	21 d	Diet	Lesions in oral cavity, tongue and pharynx, increased hemoglobin	Chiou et al., 1999
			500 mg/kg				Severe oral lesions and gizzard erosion, reduced gain and feed intake	
Turkeys	64	1 d	500 mg/kg	$CuSO_4$	21 d	Diet	Reduced body weight and feed intake, gizzard erosion	Christmas and Harms, 1979
Turkeys	96	1 d	500 mg/kg	$CuSO_4$	21 d	Diet	No adverse effect	Harms and Buresh, 1987
			750 mg/kg				Reduced body weight and feed intake	
Turkeys	25	6 wk	250–500 mg/kg	$CuSO_4$	28 d	Diet	No adverse effect	Leeson et al., 1997
Ducks	39	8 d	100 mg/kg	$CuSO_4$	55 d	Diet	Increased weight gain, smaller caeca	King, 1975a

Ducks	15	2 d	500 mg/kg 1,000 mg/kg 1,500 mg/kg	$CuSO_4$	28 d	Diet	Myopathy characteristic of selenium-vitamin E deficiency 80% mortality, myopathy in gizzard, skeletal muscle and intestine 100% mortality, myopathy	Van Vleet, 1982
Swine	16	15 d	250 mg/kg 500 mg/kg	$CuSO_4$	42 d	Diet	Increased liver Cu Reduced gain, reduced hemoglobin and liver Fe	Bunch et al., 1965
Swine	15	23.3 kg	500 mg/kg	$CuSO_4$	96 d	Diet	Reduced gain, reduced hemoglobin and hematocrit, mortality	DeGoey et al., 1971
Swine	24	23.6 kg	250 mg/kg 500 mg/kg	$CuSO_4$	54 d	Diet	Increased liver Cu Reduced gain, decreased hemoglobin and hematocrit	Kline et al., 1971
Swine	18	6.9 kg	400 mg/kg	$CuSO_4$	35 d	Diet	Reduced hemoglobin, elevated liver Cu	Kornegay et al., 1989
Swine	16	28 d	250 mg/kg 650 mg/kg	$CuSO_4$	28 d	Diet	Increased liver Cu Reduced gain and feed efficiency	Edmonds and Baker, 1986
Swine	28	152 kg	250 mg/kg	$CuSO_4$	Up to 775 d	Diet	Increased liver Cu, increased piglet birth and weaning weights	Cromwell et al., 1993
Ponies	5	Yearling	262–791 mg/kg	$CuCO_3$	183 d	Diet	Increased liver Cu, no adverse effects	Smith et al., 1975
Cattle	4	180 kg	0.13–2.0 g/d	$CuSO_4$	16 mo	Gelatin capsule	No adverse effects	Chapman et al., 1962
	2	144 kg	3.0 g/d	$CuSO_4$	64 d	Gelatin capsule	No adverse effects	
	3	144 kg	3.0 g/d	$CuSO_4$	64 d	Water	66% mortality, loss of weight, weakness, hemoglobinuria, incoordination, dullness	
Cattle	7	1 wk	115 mg/kg	$CuSO_4$	13 wk	Diet	Hemolysis, icterus, hepatic necrosis	Shand and Lewis, 1957
Cattle	8	6 wk	125–900 mg/kg	$CuSO_4$	98 d	Diet	No adverse effects	Felsman et al., 1973
Cattle	7	3 d	50 mg/kg 200 mg/kg 500 mg/kg 1,000 mg/kg	$CuSO_4$	6 wk	Milk	Reduced intake Reduced gain and intake Reduced gain and intake, increased plasma GOT 43% mortality, increased plasma GOT, apathy, anorexia, jaundice in mucous membranes of eyes, brownish-red urine	Jenkins and Hidiroglou, 1989
Cattle	63	>2 yr	23–38 mg/kg		2.5 yr	Diet	14% mortality, anorexia, weakness, mental dullness, jaundice, hepatic and renal cortical necrosis, elevated liver Cu	Bradley, 1993

continued

TABLE 13-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effects(s)	Reference
Cattle	8	17–26 mo	80 mg/kg	$CuSO_4$	60 d	Diet	Increased liver Cu	Du et al., 1996
	8		80 mg/kg	Cu Proteinate	60 d	Diet	Increased liver Cu	
Cattle	4	240 kg	0.6 mg/kg BW	$CuSO_4$	745 d	Oral by drench 5d/wk	No adverse effects	Gummow, 1996
			10.0 mg/kg BW				25% mortality, increased serum gamma glutamyltransferase	
			20.0 mg/kg BW				75% mortality, depression, diarrhea, congested mucous membranes, increased serum gamma glutamyltransferase and aspartate aminotransferase	
Cattle			20 or 40 mg/kg	$CuSO_4$		Diet	Reduced gain, feed intake and gain:feed, increased liver Cu	Engle and Spears, 2000
Cattle	16	329 kg	40 mg/kg	$Cu_2(OH)_3 \cdot Cl$	154 d	Diet	Increased gain and feed intake, increased liver Cu	Engle et al., 2000a
Cattle	8	>2 yr	40 mg/kg	$CuSO_4$	61 d	Diet	Increased serum cholesterol, increased liver Cu	Engle et al., 2001
Goats	3	1 yr	5 mg/kg BW	$CuSO_4$	56–113 d	Oral by aqueous solution	Death in 1 or 3 goats	Soli and Nafstad, 1978
Goats	6	12 wk	30–60 mg/kg	$CuSO_4$	137 d	Diet	Increased liver Cu	Zervas et al., 1990
Goats	14	10 mo	10–30 mg/kg	$CuSO_4$	88 d	Diet	Increased liver Cu, no liver damage	Luginbuhl et al., 2000
Goats	2	7–8 mo	50–100 mg/d	$CuSO_4$	9 wk	Gelatin capsule	No adverse effect	Solaiman et al., 2001
			150–300 mg/d		13 wk		No adverse effect	
			600 mg/d		4 wk		Increased plasma aspartate aminotransferase and ϒ-glutamyl transferase	
			1,200 mg/d				Cu toxicosis (anorexia, dark urine, hemolytic crisis) in 1 of 3 goats	
Sheep	18	20 kg	26.6 mg/kg	$CuSO_4$	7 wk	Diet	Elevated liver Cu	Hill and Williams, 1965
			40.7 mg/kg				Reduced gain, mortality, elevated liver Cu	

Sheep	16	6 mo	20 mg/kg BW	$CuSO_4$	10 wk	Oral by drench	Hemolytic crisis, anorexia, icterus, hemoglobinuria	Gopinath et al., 1974
	8	6 mo	30 mg/kg BW	$CuSO_4$	83 d	Oral by drench	Hemolytic crisis, death, elevated serum enzymes and urea	
Sheep	16	10 wk	18.0 to 25.0 mg/kg	$CuSO_4$	10 wk	Diet	No adverse effect except increased liver Cu	Buckley and Tait, 1981
	7	11 wk	37.3 mg/kg	$CuSO_4$	11 wk	Diet	Increased plasma aspartate aminotransferase	
Sheep	39	9 wk	20 mg/kg	$CuSO_4$	13 wk	Diet	Increased plasma aspartate aminotransferase in Texel and Suffolk-sired lambs	Woolliams et al., 1982
Sheep	32	12 wk	30 mg/kg	$CuSO_4$	12 wk	Diet	Increased mortality (41%), reduced gain and feed intake	Pond, 1989
Sheep	6	12 wk	33.5 mg/kg	$CuSO_4$	67 to 91 d	Diet	Mortality (16.6%), increased serum GOT and creatine kinase, increased Cu in liver and other tissues	Zervas et al., 1990
			67.5 mg/kg				Mortality (33%), increased serum GOT and creatine kinase, increased Cu in liver and other tissues	
Sheep	26	8 mo	40.5 mg/kg	Fertilization of pasture	176 d	Diet–pasture	Liver Cu concentrations of approximately 800 mg/kg DM, no clinical signs of toxicosis	Grace et al., 1998
			140.0 mg/kg				Liver Cu concentrations of approximately 2,000 mg/kg DM, no clinical signs of toxicity	
Fish, Atlantic salmon	3,000	Fry (0.9 g)	35 mg/kg	$CuSO_4$	12 wk	Diet	No adverse effect	Berntssen et al., 1999
			500–1,750 mg/kg				Reduced weight gain	
Fish, Chinook salmon		Fry		$CuSO_4$	96 h	Water	LC_{50} was 54–64 mg/L	Hamilton and Buhl, 1990
Fish, channel catfish		8.8 g		$CuSO_4$	24 h	Water	LC_{50} was 2,500 to 3,500 mg/L depending on temperature	Smith and Heath, 1979
Fish, channel catfish	45	14.5 g	2–4 mg/kg	$CuSO_4$	16 wk	Diet	No adverse effect	Murai et al., 1981
			8 mg/kg				Reduced gain:feed	
			16–32 mg/kg				Reduced gain and gain:feed	
Fish, channel catfish	45	83 g	2–40 mg/kg	$CuSO_4$	13 wk	Diet	No adverse effect	Gatlin and Wilson, 1986

continued

TABLE 13-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effects(s)	Reference
Fish, mullet	55	Juvenile	2,400 mg/kg	$CuSO_4$	70 d	Diet	Reduced growth and feed intake, hepatic lipid peroxidation	Baker et al., 1998
Fish, rainbow trout				$CuSO_4$	96 h	Water	LC_{50} ranged from 20 mg Cu/L in soft acid water (30 mg $CaCO_3$/L, pH 5) to 520 mg/L in hard alkaline water (360 mg $CaCO_3$/L, pH 8)	Howarth and Sprague, 1978
Fish, rainbow trout	360	2.5 g	144 mg/L	$CuSO_4$	12 wk	Water	Increased mortality and growth in fish fed high carbohydrate diets at 10°C, but not at 15°C	Dixon and Hilton, 1985
Fish, rainbow trout	40	Fry (8.0 g)	500 mg/kg	$CuSO_4$	20 wk	Diet	No adverse effect	Knox et al., 1984
Fish, rainbow trout	110	Fry (4.0 g)	9.287 mg/kg 730 mg/kg 1,585–3,088 mg/kg	$CuSO_4$	8 wk	Diet	No adverse effect Reduced gain, increased feed/gain Reduced gain, increased feed/gain, increased mortality	Lanno et al., 1985
Fish, rainbow trout	60	Fry	350 mg/kg 660–800 mg/kg	Shrimp (enriched with Cu)	60 d	Diet	No adverse effect Increased mortality	Mount et al., 1994

[a]Number of animals per treatment.
[b]Quantity of copper dosed. SI conversion: 1 mg copper equals 15.7 μmoles copper.

TABLE 13-2 Copper Concentrations in Fluids and Tissues of Animals (mg/kg)

Animal	Quantity	Source	Duration	Route	Liver	Kidney	Muscle	Heart	Egg	Reference
Rabbits	Control + 250 mg/kg + 500 mg/kg	$CuSO_4$	28 d	Diet	12[a] 195 890	10 11 15	2.6 3.6 3.4			Grobner et al., 1986
Chicks	Control + 250 mg/kg	$CuSO_4$	42 d	Diet	3.1[b] 5		0.4[b] 0.4	2.8[b] 3.0		Pesti and Bakalli, 1996
Laying hens	Control + 150 mg/kg + 300 mg/kg + 450 mg/kg	$CuSO_4$	48 wk	Diet	12[a] 16 32 61				3.4[a] 3.4 3.3 3.0	Jackson and Stevenson, 1981
Pigs	Control + 120 mg/kg + 240 mg/kg	$CuSO_4$	132 d	Diet	20[a] 122 439	29[a] 40 44	1.8[a] 1.7 1.7			Bradley et al., 1983
Cattle	Control + 20 mg/kg + 40 mg/kg	$CuSO_4$	155 d	Diet	63[a] 290 380		3.5[a] 4.2 3.0			Engle et al., 2000b
Sheep	Control + 30 mg/kg	$CuSO_4$	91 d	Diet	350[a] 2,400	14[a] 50	5.0[a] 8.0	7.0[a] 26.0		Zervas et al., 1990
Fish, rainbow trout	Control + 300 mg/kg + 1,000 mg/kg	$CuSO_4$	28 d	Diet	38[b] 45 100	1.7[b] 1.7 10.0	0.3[b] 0.3 0.3			Kamunde et al., 2001

[a]Dry tissue basis.
[b]Wet tissue basis.

14

Fluorine

INTRODUCTION

Fluorine (F) is the most electronegative and reactive of all elements and can form compounds with all elements except helium, neon, and argon. It has an atomic number of 9 and an atomic weight of 18.9984. Fluorine is a pale greenish-yellow, highly toxic gas with a characteristic pungent odor. In nature, it does not occur in the elemental state but forms organic or inorganic compounds as fluorides. Fluorine occurs mainly as salts containing the halide anion, fluoride. It is found in both igneous and sedimentary rock and constitutes about 0.06–0.09 percent of the upper layers of the Earth's crust. It ranks 13th in abundance among elements in the Earth's crust. The most important inorganic fluorine-bearing minerals are fluorspar (CaF_2), fluorapatite ($Ca_5(PO_4)_3F$), and cryolite (Na_3AlF_6). The largest known deposits of fluoride are located in the United States, United Kingdom. and Germany.

Inorganic fluorides are used in steel, glass, brick, ceramic, and adhesive manufacture. Hydrogen fluoride (HF), a major industrial compound, is widely used in the production of cryolite, aluminium fluoride (AlF_3), gasoline alkylates, and chlorofluorocarbons. It is also used for cleaning or etching compounds for semiconductors, glass, aluminium, and tanning leather, and it is also used in rust removers. HF is highly soluble in water in which it forms hydrofluoric acid. Calcium fluoride (CaF_2), with a fluorine content of 48.7 percent, is relatively insoluble in water and dilute acids and bases. It is the principal fluoride-containing mineral produced for industrial use as a flux in steel, glass, and enamel production, and as the raw material for production of hydrofluoric acid and anhydrous HF. Sodium fluoride (NaF), fluorosilic acid (H_2SiF_6), and sodium hexafluorosilicate (Na_2SiF_6) are used for controlled fluoridation of drinking water.

Fluorides are ubiquitous in the environment and are released naturally through the weathering and dissolution of minerals, as emissions from volcanoes, and as marine aerosols (Symonds et al., 1988; ATSDR, 2003). Inorganic fluorides are released in the environment from phosphate fertilizer production and use; aluminium smelting; steel production; glass, enamel, brick, and ceramic manufacturing; glue and adhesive production; pesticides; and drinking water fluoridation (Burns and Allcroft, 1964; Neumüller, 1981; Fuge, 1988; Fuge and Andrews, 1988). Transformation and transport of inorganic fluorides in the aquatic environment is influenced by pH, hardness, and the concentration of ion-exchange materials (e.g., bentonite clay and humic acid) in water. In the environment, vaporization, aerosol, and hydrolysis are the main factors that determine the fate of fluorides. Generally, the formation of aluminium and calcium complexes and pH affect the fate of fluorides in soils. In areas of extreme acidity and alkalinity, inorganic fluorides leach into surface or ground water, and their solubilization in water may be further enhanced by the presence of ion-exchange materials (Pickering et al., 1988). During weathering under acidic conditions, certain fluorine-bearing minerals (e.g., cryolite) dissolve rapidly; however, the solubility of fluorapatite and calcium fluoride is relatively slow (Fuge and Andrews, 1988). Soils rich in calcium carbonate or amorphous aluminium hydroxides may bind inorganic fluoride by forming insoluble calcium fluoride or aluminium-fluoro-hydroxide complexes, thus limiting leaching from the soil and uptake by plants (Flühler et al., 1982). Other cations (e.g., iron) also contribute to the fixation of fluoride (Murray, 1983, 1984); however, soil phosphate may contribute to the mobility of inorganic fluoride (Kabata-Pendias and Pendias, 1984). The fate of inorganic fluorides released to soil also depends on their chemical form, rate of deposition, soil chemistry, and climate (Davison, 1983).

ESSENTIALITY

Fluorine is considered an essential element, although specific deficiency signs have not been observed in experimental animals. No one has yet produced an environment suffi-

ciently devoid of this element that survival of an animal was virtually threatened. Fluorine was identified as a constituent of bones and teeth as early as 1905. Attempts to demonstrate the essential role of fluorine in rat experiments with other than dental criteria have not been successful. McClendon and Gershon-Cohen (1953) fed weaning rats for 66 days upon materials grown hydroponically in water considered to be "fluorine free." The rats weighed 51 g and had 10 carious molars per animal compared to fluoride-supplemented rats that weighed 128 g and had 0.5 carious molars per animal. Maurer and Day (1957) purified dietary ingredients and produced a diet that contained about 0.007 μg/g fluoride on which four generations of rats were raised without evidence of impaired general health, dental health, or weight gain as compared to rats raised on the same diet plus 2 μg/mL of fluoride in their drinking water. In the early 1970s, it was reported that mice fed low fluoride (0.005 μg/g diet) exhibited anemia and infertility compared to mice supplemented with fluoride (50 μg/mL drinking water) (Messer et al., 1972, 1973). Subsequently, it was determined that the diets of these mice were low in iron and that high dietary fluoride, similar to that fed in supplemented controls, improved iron absorption or use (Tao and Suttie, 1976). Mice fed low fluoride diets containing sufficient iron neither exhibited anemia nor infertility. Relatively high fluoride supplementation (2.5–7.5 μg/g diet) to rats fed low fluoride (0.04 μg/g diet) slightly improved the growth of suboptimally growing rats. Weber (1966) reported that in six generations of study on mice, no definite differences in reproduction were observed between the low (0.25 μg/g) and high (65 μg/g) fluoride groups. Schwarz and Milne (1972), working in a filtered-air environment, reported a favorable growth response when small increments (1–2.5 μg/g) of fluoride were added to a low fluoride diet for rats. Studies on high fluoride supplements indicate that these growth-promoting effects were probably pharmacological. High or pharmacological amounts of fluoride have been also found to depress lipid absorption and to alleviate nephrocalcinosis induced by feeding phosphorus and to alter soft tissue calcification by magnesium deprivation.

Several decades of research on humans have strengthened the view that fluorine is essential to reduce the prevalence of dental caries, and it should be considered as an essential element on this basis (NRC, 1993). A fluoride supplement of 80 μg/g diet enhanced the growth of broiler chickens (Gutierrez et al., 1993). To date, sufficient evidence of the essentiality of fluorine in farms animals has been provided only by Anke et al. (1997). Female goats developed skeletal abnormalities and poor growth in their offspring for 10 generations on a diet containing < 0.3 mg F/kg DM. These findings need to be confirmed in other animal species before being accepted as evidence for essentiality in higher animals. Fluoride may be needed for proper functioning of some enzymes, given that in vitro its activities, including histidine methyl transferase, stabilize the interaction between guanosine triphosphatase (GTPase) and GTPase-activitating proteins, and affect the posttranslational assembly of glycosaminoglycan chains in mineralizing bone cells (Kirk, 1991).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Fluorine is too reactive to be analyzed directly in biological samples and environmental media, and its measurement is restricted to the detection of the free anion (F^-). The most widely used analytical method is a potentiometric technique that measures free anion using the fluoride ion-selective electrode (F-ISE) (Neumüller, 1981; Harzdorf et al., 1986; ATSDR, 2003). This method has been used for the quantification of fluoride in biological tissues and fluids (e.g., urine, serum, plasma, organs, bone, and teeth), foodstuffs, and environmental media (e.g., air, water, and soil). The variations in the sample preparation procedures and the recovery of this element may affect the detection limits using F-ISE. The values reported by this method range from 0.1 to 300 ng/m^3 in air, from 1 to 1,000 μg/L in water, and from 0.05 to 20 mg/kg in nonskeletal tissues (Harzdorf et al., 1986; ATSDR, 2003).

Fluoride ions form a stable, colorless complex such as $(AlF_6)^{3-}$, $(FeF_6)^{3-}$, and $(ZrF_6)^{3-}$ with certain trivalent ions. Colorimetric methods are based on the bleaching of these metal complexes with organic dyes in the presence of fluoride (WHO, 1984). Other methods for the quantification of fluorine include gas chromatography (GC), ion chromatography, capillary electrophoresis, atomic absorption, and photon activation (Neumüller, 1981; Harzdorf et al., 1986; Wen et al., 1996; ATSDR, 2003). GC methods have been used to measure fluoride concentrations in urine and plasma but require extraction and derivatization using trimethylchlorosilane in toluene to produce trimethylfluorosilane (Ikenishi et al., 1988). Analytical methods based on neutron or proton activation of fluorine-19 have been also developed that measure emitted gamma rays or x-rays using lithium-drifted germanium detectors (Shroy et al., 1982; Knight et al., 1988). These techniques do not depend on the specific sample matrix or chemical form.

Appropriate sample preparation is the critical step in the accurate quantification of fluoride, especially where only the free fluoride ion is measured. For analysis involving biological materials, the most accurate method is microdiffusion techniques such as the acid-hexamethyldisioxane (HMDS) diffusion method described by Taves (1968). Methods involving acid or alkali digestion may not convert all complex organic fluorine into an ionic form that can be conveniently measured (Venkateswarlu, 1983). Open-ashing methods may result in the loss of volatile fluoride compounds or of fluoride itself at temperatures in excess of 550°C, or they may result in contamination with extraneous fluoride (Venkateswarlu, 1983; Campbell, 1987).

REGULATION AND METABOLISM

Approximately 75 to 90 percent of ingested fluoride is absorbed in the stomach and small intestine as well as rumen of animals (Messer, 1984; WHO, 1994; Cerklewski, 1997). The efficiency of fluoride absorption depends on the solubility of the fluoride compound and the presence of other dietary components. In the stomach, low pH conditions favor the formation of highly diffusible hydrogen fluoride (pK_a = 3.4). Therefore, the conditions that promote gastric acidity increase the rate of fluoride absorption, and it is impaired by alkalinity. The absorption from the small intestine occurs as the fluoride ion by non-pH dependent diffusion (Nopakun and Messer, 1990). In the fasted state, fluoride absorption from either fluoridated water or sodium fluoride is almost 100 percent (Rao, 1984; Trautner and Seibert, 1986). Soluble fluorides, such as sodium fluoride, are almost completely absorbed. Less soluble sources, such as bone meal, are relatively poorly absorbed (<50 percent). Calcium, magnesium, aluminum, sodium chloride, and high lipid levels are the main dietary factors that depress fluoride absorption (NRC, 1980; Cerklewski, 1997).

The respiratory tract is the major route of absorption of both gas and particulate fluoride from industrial emissions. Depending on their aerodynamic characteristics and solubility, fluoride-containing particles are deposited in the bronchioles and nasopharynx and absorbed either immediately or gradually released from lungs (McIvor, 1990). Dermal absorption of fluoride has been reported only in the case of burns resulting from exposure to hydrofluoric acid (Burke et al., 1973).

Removal of fluoride from circulation occurs principally through two mechanisms: renal excretion and calcified tissue deposition. Following absorption, the concentration of ionic fluoride (F^-) increases in plasma where it reacts with calcium to form calcium fluoride. Some ionic fluoride may become non-ionic by coordinating with macromolecules such as proteins (Singer and Armstrong, 1964; Kirk, 1991). About 75 percent of fluoride in the blood is in the plasma (Carlson et al., 1960), with 15 to 70 percent (0.01–0.04 mg/L) in the ionic form (Singer and Armstrong, 1964). Nearly 5 percent of plasma fluoride is bound to protein, but most of the bound fluoride is associated with compounds having molecular weights less than albumin. However, HF, not ionic fluoride, apparently is the form that is in diffusion equilibrium across cell membranes. It is removed from circulation by mineralized tissues in exchange for other anions such as hydroxyl ion, citrate, and carbonate and subsequently enters bone crystal lattice. Soft tissue fluoride levels are maintained within narrow limits and are marginally affected by long-term fluoride intake or by short-term fluctuations in plasma levels. However, soft tissue fluoride is readily exchangeable with extracellular fluid fluoride, as demonstrated by the rapid distribution of ^{18}F following intravenous injection (Wallace, 1953; Carlson et al., 1960). The rate of uptake of fluoride into bones and teeth is most efficient during early development stages. Approximately 50 percent of fluoride absorbed each day is deposited in the calcified tissues (bone and developing teeth), which results in approximately 99 percent of body burden of fluoride being associated with these tissues (NRC, 1980). Excretion of fluoride occurs mainly via the urine, which accounts for approximately 90 percent of total excretion. Urinary excretion of fluoride is directly related to urinary pH; thus factors that affect urinary pH, such as diet, drugs, metabolic and respiratory disorders, and altitude residence, can affect how much absorbed fluoride is excreted. Generally, > 20 percent of ingested fluoride is excreted in feces.

Milk and saliva have fluoride concentrations similar to plasma ionic fluoride levels, and variations in plasma fluoride levels are reflected in these secretions. Milk fluoride concentrations are affected only minimally by dietary fluoride (Greenwood et al., 1964). Fluoride crosses the placental barrier of cows, and fluoride levels in the bones of the offspring are correlated with those of maternal blood (NRC, 1974). However, bone fluoride concentration of calves born to cows consuming as much as 108 mg F/kg diet (from sodium fluoride) were low (Hobbs and Merriman, 1962), and it appeared that neither placental fluoride transfer nor milk fluoride concentration were sufficient to adversely affect the health of these calves.

Homeostatic regulation of plasma fluoride concentration involves the kidneys and skeleton (Smith et al., 1950; Singer and Armstrong, 1964). Bone has a great affinity to incorporate fluoride into hydroxyapatitite to form fluorapatite. This results in larger, less soluble, more stable apatite crystals (Zipkin et al., 1964). The fluoride cannot be removed without resorption of this mineral unit. Even low levels of fluoride intake will result in appreciable accumulation of fluoride in the skeleton and teeth. These accumulations can increase, within limits, over a period of time without morphological evidence of pathology. However, in some cases of high-fluoride intake, structural bone changes develop (Shupe et al., 1963b). Most soft tissues do not accumulate much fluoride, even during high intakes, although tendon (Armstrong and Singer, 1970), aorta (Ericsson and Ullberg, 1958), and placenta (Gardner et al., 1952) have a higher fluoride concentration than other soft tissues, possibly associated with their relatively high levels of calcium and magnesium. Kidney will usually exhibit a high fluoride concentration during high fluoride ingestion due to urine retained in the tubules and collecting ducts.

In laboratory animals and humans, approximately 99 percent of fluoride is retained in bones and teeth (Kaminsky et al., 1990; Hamilton, 1992), with the remainder distributed in vascularized soft tissues and the blood (McIvor, 1990). In calcified tissues, the highest concentration is found in bone, dentine, and enamel, and the concentration varies with age, sex, and in different parts of the bone. During the rapid growth phase at the early stages of development, a high por-

tion of fluorine is deposited in skeletal tissues. Retention of fluorine in skeletal tissues is related to the turnover of minerals and previous exposure to this element (Carracio et al., 1983). The selective affinity of fluoride for mineralized tissues is, in the short term, due to uptake on the surface of bone crystallites by the processes of isoionic and heteroionic exchange. In the long run, fluoride is incorporated into the crystal lattice structure of teeth and skeletal tissues by replacing some hydroxyl ions within the unit cells of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), producing partially fluoridated hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_{2-2X}F_{2X}$).

Metabolic Interactions and Mechanism of Toxicity

The mechanism of fluorine toxicity is partially understood, and the clinical manifestations of the toxicosis depend on the age and species of animal, dosage, form and duration of exposure to fluorine, and nutritional status. The biochemical and clinical basis of acute fluorine toxicity has been classified into four major categories: (1) enzyme inhibition, (2) calcium complex formation, (3) shock, and (4) specific organ injury (Hodge and Smith, 1981). Fluoride inhibits a large number of metalloenzymes containing copper, manganese, zinc, nickel, and iron (heme and non-heme) by binding to the metal on the active site (reviewed by Messer, 1984; Kirk, 1991). Enzymes requiring divalent cations (e.g., magnesium) are also inhibited, and in some instances inhibition of fluoride ion is enhanced by inorganic phosphate (e.g., enolase, succinic dehydogenase). Although the inhibition of enzyme activities by fluoride toxicosis interrupts metabolic processes such as glycolysis and synthesis of proteins (Kessabi, 1984), the molecular mechanisms involved in these biochemical processes are mainly speculative. Fluoride concentrations of blood and tissues increase rapidly, and there is a severe hypocalcemia probably from inhibition of lactate required for extrusion of Ca^{2+} from bone (Hodge and Smith, 1981). The mechanism of sudden death during acute toxicity may also involve hyperkalemia or diminished Na+/K+-ATPase activity affecting ATP production and causing glycolysis inhibition (Messer, 1984).

Many of the biochemical actions of fluoride toxicity have been explained in terms of its substitution for hydroxyl ions, which the fluoride ions closely resemble in their physical properties (hydration number, ion radius, and charge), particularly in mineralized tissues where the substitution results in dissolution of minerals in bones. For enolase and pyrophosphatase, which have been examined in considerable detail, the substitution of the fluoride ion for the hydroxyl ion (that normally participates in this enzymatic reaction) results in the formation of stable, less reactive complexes. Chronic toxicosis occurs in extracellular fluid when the fluoride concentration is in the low micromolar range and includes both inhibitory and stimulatory effects on cells that cannot readily be explained in terms of biochemical effects (Messer, 1984; Kirk, 1991).

SOURCES AND BIOAVAILABILITY

Fluoride is present in varying amounts in air, water, soil, and in plant and animal tissues. Active volcanoes and fumaroles, as well as certain industrial processes, may contribute significantly to local concentrations of fluoride. Fluoride levels in surface water vary according to local geology and proximity to emission sources. Fluoride concentrations in surface freshwater range from 0.01 to 0.03 mg F^-/L and the range in seawater is 1.2 to 1.5 mg F^-/L (EPA 1980; IPCS, 2002). Higher concentrations are found in areas where there is geothermic and volcanic activity. In rivers, fluoride concentrations range from < 0.001 to 6.5 mg F^-/L; the average fluoride concentration is approximately 0.2 mg F^-/L (Fleischer et al., 1974). Fluoride levels may be higher in lakes, especially in saline lakes and lakes in closed basins in areas of high evaporation. The Great Salt Lake in Utah has a fluoride content of 14 mg F^-/L (Fleischer et al., 1974). The fluoride content of ground water generally ranges from 0.02 to 1.5 mg/L (Fleischer, 1962; EPA, 1980). Highest fluoride levels in U.S. ground water are generally found in the Southwest, and maximum ground water levels in Nevada, southern California, Utah, New Mexico, and western Texas exceed 1.5 mg/L. In endemic fluorosis areas, deep-well water may percolate through fluorapatite and frequently contains 3 to 5 mg F^-/L, and sometimes 10 to 15 mg F^-/L (Harvey, 1952; Cholak, 1959). The amount of fluoride in water is influenced by pH, water hardness, and the presence of clay that has ion-exchange properties. In the Rift Valley of Kenya and Tanzania, high fluoride levels in water and a high incidence of fluorosis have been correlated with low levels of calcium and magnesium in the water (Gaciri and Davies, 1993).

Industrial pollution furnishes airborne fluorine in one of three principal forms: hydrofluoric acid, silicon tetrafluoride, or fluoride-containing particulate matter. The average fluorine concentrations in air are generally less than 0.1 $\mu g/m^3$ (IPCS, 2002) with higher concentrations in urban than rural areas. Generally, the airborne fluorine contamination in areas located in the vicinity of industrial emissions does not exceed 2 to 3 $\mu g/m^3$. Direct inhalation of fluoride does not contribute significantly to fluoride accumulation in animals; however, these emissions contaminate plants, soil, and water. Gaseous fluorine may be absorbed and incorporated in plant tissues. Particulate fluorides accumulate on plant surfaces and may be ingested by animals as the plant is eaten. Rain may wash off some of these inert particles, but their toxicity is related largely to their solubility in water.

Although soil is undoubtedly the principal source of fluoride in plants, there is no consistent relationship between total fluoride in soil and plants (NRC, 1974). Fluorine content of pastures and forages are particularly low unless they have been contaminated by a deposition of dust and fumes from volcanic and industrial origin or by fluoride-rich geothermic and well water used for irrigation (Shupe, 1980). Animals ingest soil during grazing, which may contribute to

more than 50 percent of the dietary fluorine in grazing sheep and cattle, and this amount may increase to > 80 percent in winter months (Cronin et al., 2000). There is some indication that acid soils promote fluoride uptake in plants, and liming of these soils may reduce it. Fluoride content of soils ranges from 20 to 1,000 mg total F^-/kg in areas without natural phosphate and fluoride deposits. Some soils unusually high in fluoride have been found in Idaho (3,870 mg/kg) and in Tennessee (8,300 mg/kg). The natural fluoride content of the soil increases with increasing depth. Only 5 to 10 percent of the total fluoride content in soil is soluble (Noemmik, 1953). The fluoride content increase with depth is in the usual range in the United States, as 20 to 500 mg/kg (average, 190 mg/kg) from 0 to 8 cm deep and 20 to 1,620 mg/kg (average, 292 mg/kg) from 0 to 30 cm deep (Robinson and Edgington, 1946). The extent of fluoride retention depends upon the amount of clay and pH, and the distribution at various depths follows the clay pattern of soil. Several anthropogenic sources of fluoride can enrich soil, especially phosphate fertilizers, insecticides containing fluorides, and emissions from industry. Soils may contain fluoride in several different minerals.

Natural forage normally contains 2 to 20 mg F/kg dry weight (NRC, 1974). Cereal grains and cereal by-products usually contain 1 to 3 mg/kg (Underwood and Suttle, 1999), which mainly accumulates in the outer layer and embryo (Kumpulainen and Koivistoinen, 1977). The fluorine content of both leaf and root vegetables do not differ appreciably from those of cereals, with the exception of spinach, which is rich in this element. The tea plant and camellia are exceptions, and fluoride concentrations of 100 mg/kg or more have been reported (Underwood and Suttle, 1999). Fluorine is a normal component of calcified animal and fish tissues. Animal by-products containing bone may contribute significant quantities of fluoride to animal diets, depending upon the amount of by-product used (and bone contained) and the dietary history of the animals from which the by-products were derived. Bone ash normally contains less than 1,500 mg F/kg and would contribute only minor amounts. However, cattle grazing fluoride-contaminated pastures can have bone ash containing over 10,000 mg/kg F. Aquatic organisms absorb fluoride to a limited extent from food sources and directly from water where that uptake depends on fluoride concentration, exposure time, and temperature (Hemens and Warwick, 1972; Milhaud et al., 1981; Nell and Livanos, 1988). In natural environments, the proximity of anthropogenic sources, geology, physiochemical conditions, and food source also influence fluoride content in fish, invertebrates, and aquatic plants.

The primary sources of dietary fluorides for farmed animals are phosphorus supplements and feed ingredients of animal origin. Phosphorus supplements greatly vary in their fluoride content, depending on origin and manufacturing processes. The majority of U.S. feed phosphate originates from rock phosphate deposits, which contain 2 to 5 percent (average, 3.5 percent) fluorine (vanWazer, 1961). When processed sufficiently to qualify as defluorinated, feed-grade phosphates must contain no more than 1 part of fluoride to 100 parts phosphorus (AAFCO, 2004). Processed low-fluoride, feed phosphates include mono-, di-, and tricalcium phosphates, mono- and diammonium phosphates, mono- and disodium phosphates, ammonium and sodium polyphosphates, feed-grade phosphoric acid, and defluorinated phosphate. Unprocessed feed phosphates, supplying substantial amounts of fluoride, include soft rock phosphate, ground rock phosphate, and ground low-fluoride rock phosphate. More readily absorbed sources of fluoride, when incorporated in animal diets, are undefluorinated, fertilizer-grade phosphates.

Fluoride levels in blood, urine, and feces are commonly used to assess bioavailability and toxic levels of fluorine for humans and animals from dietary sources; however, skeletal tissue uptake has been used for experimental animal investigations (Rao, 1984). Fluoride is readily absorbed from water, but the absorption from diet may depend on the following factors: concentration, source, chemical form (inorganic or organic), other elements (Al, Ca, Mg, P, Cl, SO_4^{2-}), fluorine exposure from other sources (water, air, etc.), and physiological status (age, acid-base balance, disease, etc.). Soluble forms of fluorine, such as sodium fluoride, are readily absorbed compared with bone meal, rock phosphate, and defluorinated rock phosphate. Fluorine from dicalcium phosphate and raw rock phosphate was 50 percent as available as fluorine from NaF (Clay and Suttie, 1985). The fluorine from hay is available as NaF (Shupe et al., 1962).

Some reduction in fluorine absorption from diet may be associated with insoluble complex formation between the fluoride anion and multivalent cations in the alkaline environment of the small intestine. Calcium and magnesium form insoluble complexes with fluoride, which significantly decreases fluoride absorption (Cerklewski, 1997). Aluminium also forms an insoluble complex with fluoride. Among the anions, only chloride significantly influences fluoride bioavailability. Diets low in chloride reduce fluorine excretion and increase uptake in bone and teeth (Cerklewski, 1997). Fluoride absorption in laboratory animals increased when either fat or protein were increased (McGown et al., 1976; Boyde and Cerklewski, 1987).

TOXICOSIS

The toxicity of fluorine in animals via excessive fluoride ingestion from water or from industrial exposure is referred to as fluorosis, chronic fluorine toxicity, or fluorine toxicosis. The toxicosis is initially manifested as dental fluorosis (mottled enamel) and, at higher levels of intake, skeletal fluorosis. Several reviews have been published on fluoride toxicity in animals and humans (Krishnamachari, 1987; Whitford, 1996; Kirk, 1991; Underwood and Suttle, 1999; Cronin et al., 2000; ATSDR, 2003; Camargo, 2003; IPCS,

2002; McDowell, 2003). Table 14-1 summarizes some studies on the effects of fluorine exposure in animals.

Single Dose

The oral LD_{50} for fluorine varies among animal species and also by gender. In rats, the following LD_{50} values or their range (mg F^-/kg body weight) have been reported for three common fluoride supplements (DeLopez et al., 1976; Lim et al., 1978; Whitford, 1990; ATSDR, 2003): sodium fluoride, 31 to 101; sodium monofluorophosphate, 75 to 102; and stannous fluoride, 45.7. For mice, LD_{50} values reported were 44.3; 58, 54, and 94; and 25.5 and 31.2 for sodium fluoride, sodium monofluorophosphate, and stannous fluoride, respectively (Lim et al., 1978; ATSDR, 2003). The predominant signs of toxicosis in these animals upon administration of a single large oral dose of fluoride were nausea, salivation, lacrimation, vomiting, diarrhea, respiratory arrest, tetany, and coma. Intraperitoneal injection (13.6 and 21.8 mg F^-/kg BW) of sodium fluoride into female rats caused proximal tubular necrosis and marked changes in kidney weight, urine osmolarity and pH, and chloride excretion; however, these effects were less severe in the younger animals (Daston et al., 1985). When sodium fluoride solutions (1 to 50 mmol F^-/L dissolved in 0.1 N HCl) were introduced in the stomach of rats, several histopathological changes in the gastrointestinal tract (hemorrhage, disruption of epithelial integrity and glandular structure, lysis, and loss of epithelial cells) were observed (Easmann et al., 1984, 1985).

The inhalation exposure of rats, mice, and guinea pigs to fluorine gas (F_2) and HF causes severe ocular and nasal irritation, pulmonary congestion, edema, respiratory distress, and erythraemia of exposed skin (ATSDR, 2003). Fluorine is more toxic than HF (Stokinger, 1949; Keplinger and Suissa, 1968). For rats, LC_{50} values (mg F^-/m^3) ranging from 4,065 to 14,400 have been reported for 5-minute exposure (WHO, 1984; ATSDR, 2003) and 1,084 for 60-minute exposure to HF (Rosenholtz et al., 1963). The LC_{50} for mice were approximately 5,000 and 280 when exposed to HF for 5 and 60 minutes, respectively (WHO, 1984; ATSDR, 2003). Direct exposure of the skin to hydrofluoric acid produces severe burns and tissue damage depending upon the concentration and the length of the exposure (Derelanko et al., 1985). In rats, direct application of aqueous solution of sodium fluoride (0.5 and 1.0 percent) to the abraded skin caused necrosis, edema, and inflammation (Essman et al., 1981).

Acute

Acute fluoride toxicosis is rare. It usually results from the accidental ingestion of compounds such as sodium fluorosilicate (Na_2SiF_6), used as a rodenticide, or sodium fluoride, used as an ascaricide in swine. The rapidity with which toxic signs appear depends on the amount of fluoride ingested (Cass, 1961). Toxic signs include high fluoride content of blood and urine, restlessness, stiffness, anorexia, reduced milk production, excessive salivation, nausea, vomiting, urinary and fecal incontinence, chronic convulsions, necrosis of gastrointestinal mucosa, weakness, severe depression, and cardiac failure (Shupe et al., 1963a,b). Death occurred within 12 to 14 hours in dairy cows after ingesting sodium fluorosilicate (Krug, 1927). Acute fluoride toxicosis also occurred in cattle after exposure to wood treatment compounds used on utility poles (Bischoff et al., 1999). The predominant systematic effects of acute oral exposure to sodium fluoride in humans and laboratory animals include hypocalcemia, hyperkalemia, and gastrointestinal pain (ATSDR, 2003).

Chronic

Chronic skeletal fluorosis is prevalent in cattle, sheep, goats, horses, and humans in parts of India, Argentina, Australia, Turkey, Africa, the United States, and other regions of the world (Underwood, 1981; WHO, 1994), often due to consumption of water high in fluoride. The degree to which inorganic fluoride induces skeletal changes varies considerably in farm animals. Cattle are most sensitive to fluorosis, followed by sheep, horses, pigs, rabbits, rats, guinea pigs, and poultry (Franke, 1989). Young animals are most affected by fluorosis. The dietary concentration at which fluoride ingestion becomes harmful in farm animals is not clearly defined. Diagnosis of fluoride toxicosis at low levels of ingestion is difficult because there is an extended interval of time between ingestion of elevated levels and the appearance of toxic signs (Shupe, 1970). Low-level toxicosis also depends upon solubility of the fluoride source, general nutritional status, species of animal, age when ingested, and dietary components that modify toxicity.

Both domestic and wild grazing animals also develop chronic fluorosis due to contamination with volcanic gas and ash (Roholm, 1937; Araya et al., 1990; Shanks, 1997), ingestion of commercial phosphorus supplements (Shupe et al., 1992; Jubb et al., 1993; Singh and Swarup, 1995), phosphate fertilizer residues (Clark and Stewart, 1983), forage or water polluted with industrial emissions (Kay et al., 1975; Singh and Swarup, 1995; Kierdorf et al., 1996), and drinking water from ground and geothermal sources that are rich in fluoride (Harvey, 1952; Shupe et al., 1984; Botha et al., 1993).

The effect of fluorine toxicosis generally takes weeks or months to become manifest and some of the excess fluorine is excreted in urine (Underwood and Suttle, 1999). Plasma fluoride concentrations reflect short-term changes in fluorine uptake with levels of $< 0.1 \mu g\ g^{-1}$ in normal animals and $1 \mu g\ g^{-1}$ indicating a high fluorine uptake (Suttie et al., 1972). The skeleton of animals normally contains the greatest proportion of fluorine within the animal and the normal whole bone fluorine concentration ranges between 300 and 600 $\mu g\ g^{-1}$ (dry fat-free basis), the highest concentration being

within the cancellous bones such as rib and vertebrae (Underwood and Suttle, 1999). In ruminants, teeth contain approximately half the fluorine concentration of bone (Underwood and Suttle, 1999). The period during which developing teeth in cattle are sensitive to excess fluoride is from approximately 6 months to 4 years of age. Teeth that have erupted are not influenced adversely by subsequent fluoride ingestion (Garlick, 1955), and cattle that are more than 3 years old will not develop typical dental lesions. Dental fluorosis is generally diagnosed by examining the incisors. Gross fluoride lesions of the incisor enamel begin with inside mottling (white, chalky patches or striations) and progresses to defined mottling, hyperplasia, and hypocalcification. A scoring system for classification of dental fluorosis has been proposed (NRC, 1974).

The amount of fluoride stored in bone may increase over time with no apparent change in bone structure or function. However, if excess fluoride ingestion is sufficiently high, and occurs over a sufficiently long period of time, morphological abnormalities will develop. In livestock, clinically palpable (bilateral) lesions usually develop first on the medical surface of the proximal third of the metatarsals. Subsequent lesions are seen on the mandible, metacarpals, and ribs. Osteofluorotic lesions tend to be more severe in those bones, and parts of the bones, that are subject to greatest physical stress. Radiographic evidence of osteoporosis, osteosclerosis, osteomalacia, hyperostosis, and osteophytosis or any combination of these lesions has been described (Johnson, 1965; Shupe and Alther, 1966; Shupe, 1969). Grossly, severely affected bones appear chalky white, are larger in diameter, and heavier than normal, and they have a roughened, irregular periosteal surface. In cattle poisoned by industrial fluoride emissions, Krook and Maylin (1979) suggested that the primary target of fluoride was the resorbing osteocyte. Morphological signs of osteolysis were absent, and the failure of resorption caused osteopetrosis with retention of lamellar bone in cortices.

Animal movement may be impaired by intermittent periods of stiffness and lameness, associated with advanced areas with calcification of particular structures and tendon insertions. In animals with marked periosteal hyperostosis, spurring and bridging of the joints may lead to rigidity of the spine and limbs. Anorexia, unthriftiness, dry hair, and thick, nonpliable skin have been noted in fluorotic animals (Roholm, 1937; Shupe et al., 1963b).

Primary adverse effects on reproduction and lactogenesis have not been demonstrated although milk production may decrease with high fluoride intake, secondary to dental and skeletal damage and consequent reductions in feed and water intake (Stoddard et al., 1963). Suttie et al. (1957b) have demonstrated that cows first exposed to fluoride at 4 months of age can consume 40 to 50 mg/kg of fluorine as sodium fluoride in their diet for two or three lactations without a measurable effect on milk production. Milk production was reduced in the fourth and subsequent lactations. Higher dietary fluoride levels (93 mg/kg) affected the milk production in the second lactation slightly and definitely reduced milk yield in a subsequent lactation (Stoddard et al., 1963). Irrespective of level or duration of fluoride intake, clinical signs of toxicosis will normally precede impaired milk production. No characteristic, unequivocal histological or functional changes in blood or soft tissues have been correlated with fluoride intakes sufficient to induce chronic fluorosis of bones and teeth.

Natural surface waters are often contaminated with fluoride from discharges of fluoridated municipal waters, fertilizers, and other industrial pollutants that cause ecological risk to aquatic organisms and plants. In certain areas, high concentrations (10 mg F^{-1}/L) have been measured in rivers (CEPA, 1993; Camargo, 1996). Aquatic organisms accumulate soluble inorganic fluorides. When sodium fluoride was released into an experimental pond, within 24 hours the concentration of fluoride in aquatic vascular plants increased 35-fold and a significant increase in its uptake was observed in algae (14-fold), molluscs (12-fold), and fish (7-fold) (Kudo and Garrec, 1983). Limited data on the toxicity of fluorine in freshwater to algae, aquatic plants, invertebrates, and fish are available (reviewed by Camargo, 2003). Aquatic plants can effectively remove fluoride from contaminated water, and the concentration increases with exposure time. The toxicity is linked to fluoride ions that affect nucleotide and nucleic acid metabolism that influences algal cell division (Antia and Klut, 1981). Some algae can tolerate inorganic fluoride levels as high as 200 mg/L (Antia and Klut, 1981; Camargo, 2003). Fluoride toxicity in aquatic invertebrates and fish increases with increasing fluoride concentrations, exposure time, and water temperature. Marine invertebrates appear to be more tolerant to toxicity than freshwater species (reviewed by Camargo, 2003). Fluorine accumulates in the exoskeleton of invertebrates and bones of fish. The LC_{50} values (mg F^{-}/L) of several fish at different exposure times range from 51 to 460 (Camargo, 2003). In soft water with low ionic content, a fluoride concentration as low as 0.5 mg F^{-}/L produces toxicosis in invertebrates and fish. Among fishes, rainbow trout have been used widely to study fluoride toxicity. In addition to water temperature, calcium and chloride content of water and body size also affect the fluorine toxicity.

In humans, the major toxic effects range from dental fluorosis (Fejerskov et al., 1977; DenBesten and Thariani, 1992), reversible gastric disturbances (Jowsey et al., 1979), and lower urinary concentrating ability (Goldemberg, 1931; Whitford and Taves, 1973) to skeletal fluorosis (Singh and Jolly, 1970) and death (Hodge and Smith, 1965; Church, 1976; Dukes, 1980; Eichler et al., 1982; Gessner et al., 1994).

Reproduction

The adverse effects of high levels of fluoride intakes on reproduction of several laboratory animal species have been reported (IPCS, 2002). Reproductive function was severely affected in female mice orally administered ≥5.2 mg F/kg

BW/day after mating (Pillai et al., 1989), and in male rabbits orally administered 9.1 mg F/kg BW/day for 30 days (Chinoy et al., 1991). Histopathological changes have also been observed in testes of male rabbits fed 4.5 mg F/kg BW/day for 18 to 29 months (Susheela and Kumar, 1991) and male mice fed 4.5 mg F/kg BW/day for 30 days (Chinoy and Sequeira, 1989a,b) and in ovaries of female rabbits injected subcutaneously with 10 mg F/kg BW for 100 days (Shashi, 1990). Exposure of dams to 150 mg/L fluorine in drinking water prior to breeding and during pregnancy and lactation had no adverse effects on the bones of developing rats (Ream et al., 1983a), although effects on the bones of the dams were observed (Ream et al., 1983b). The lowest-observable-effect-level (LOEL) of dams was considered to be 21.4 mg/kg BW/day. In most studies, the fluoride concentrations associated with adverse effects were much higher than those measured in drinking water or animal feeds. The apparent threshold concentration for inducing reproductive effects in animals in drinking water has been listed by NRC (1993): 100 mg/L for mice, rat, foxes, and cattle; 100–200 mg/L for mink, owls, and kestrels; and >500 mg/L for hens.

Genotoxicity and Carcinogenicity

The genotoxicity of fluoride has been examined in a large number of in vitro and in vivo assays (WHO, 1984; Li et al., 1987; Tong et al., 1988; Zeigler et al., 1993; IPCS, 2002). Generally, fluoride is not mutagenic in microbial cells, but increases the frequency of gene-locus mutations in cultured mammalian cells and induces the morphogenic transformation of Syrian hamster embryo cells at cytotoxic concentration in vitro. Although the results of some studies have indicated that sodium fluoride increases unscheduled DNA synthesis in mammalian cells, these results were not confirmed when steps were taken to eliminate potential artifacts (i.e., formation of precipitable complexes of magnesium fluoride and [^{3}H] thymidine). Sodium fluoride has been considered capable of inducing chromosomal aberrations, micronuclei, and sister-chromatid exchanges in vitro in mammalian cells; however, the results from these studies were not consistent. Chromosal aberrations have not been detected in cells exposed in vitro to levels of fluoride less than 10 µg/mL, which is considered as a threshold for the clastogenic activity of fluoride.

The results of early carcinogenicity bioassays conducted with NaF administered in water were largely negative. However, the documentation and protocols of these studies were inadequate according to current bioassay techniques and thus of limited value. Although several carcinogenicity bioassays have been conducted in recent years, the results of two studies were selected to be reliable by NRC (1993). The National Toxicological Program (NTP, 1990) administered 175 mg F/L in drinking water to male and female mice. Although results were negative, there was some evidence of a dose-related increase in the incidence of osteosarcomas in male rats. These results were not confirmed by another study conducted by Procter and Gamble (Maurer et al., 1990) in which fluorine in the diets was at doses higher than those in the NTP study. The later study did produce significant dose-related incidence of osteomas (benign bone tumors) in male and female mice. The available laboratory animal experimental data are insufficient to demonstrate a carcinogenic effect of fluorine (NRC, 1993).

Factors Influencing Toxicity

Toxicity of fluoride, at a given level of exposure, depends on animal species, source, concentration, chemical form (inorganic or organic), other elements, and physiological status (age, acid-base balance, disease, etc.) of the animal. Fluoride is readily absorbed from water; therefore, the toxicity of soluble fluoride compounds will depend on their water solubility. Based on the skeletal accumulation of fluoride by rats, Hobbs et al. (1954) ranked the toxicity of fluoride compounds in the order from highest to lowest as follows: rock phosphate, natural and synthetic cryolite, calcium and magnesium fluosilicates, and calcium fluoride. Fluoride in rock phosphate was considerably less toxic to beef heifers than that in sodium fluoride (Hobbs and Merriman, 1962). Naturally fluorinated water produced toxicity comparable to equal amounts of fluoride from sodium fluoride added to water (Wagner and Muhler, 1957) or to a dry diet (Harvey, 1952; Wuthier and Phillips, 1959). Variations in fluoride intake, with alternate periods of high and low exposure, were more damaging to young cattle than were constant intakes of the same amount (Suttie et al., 1972).

Some dietary components that have been shown to reduce fluoride toxicosis include aluminum, calcium, magnesium, phosphorus, sodium chloride, boron, and sulfates (NRC, 1980; Krishnamachari, 1987). Calcium given orally or intravenously counteracts the effects of fluoride, particularly toxicity of an acute nature (Krishnamachari, 1987). Feeding of calcium carbonate, aluminum oxide, or aluminum sulfate reduces absorption of fluoride and may control chronic fluorosis under some conditions. However, the consumption of a combination of aluminum sulfate with calcium carbonate in a mineral mixture was not effective in reducing bone fluoride accumulation in cattle grazing in a fluoride-contaminated pasture (Allcroft and Burns, 1968). Aluminum compounds may also adversely affect dietary phosphorus retention (Street, 1942; Hobbs et al., 1954; Alsmeyer et al., 1963; Storer and Nelson, 1968); if they are used to alleviate fluoride toxicosis, increased levels of phosphorus must be fed. Aluminum chloride and aluminum acetate also appear to be effective in reducing fluorosis in cattle, but aluminum oxide produces only slight alleviation (Hobbs et al., 1954). For chickens, free-choice access to dietary aluminum sulfate at 800 mg Al/kg completely prevented toxicity of 1,000 mg F/kg; the oxide form was not

effective (Cakir et al., 1978). Aluminum seems to reduce the gastrointestinal absorption of fluoride.

High intakes of boron have been shown to be an effective antidote to fluoride toxicosis in rabbits (Baer et al., 1977; Elsair et al., 1980), pigs (Seffner and Teubener, 1983), and sheep (Wheeler et al., 1988). Boron may induce formation of a complex, BF_4, which is excreted in the urine. Magnesium metasilicate has been tested in chronic fluorosis patients, who responded with partial clinical amelioration of their signs (Rao et al., 1975). Toxicity of fluorine is also affected by dietary fat level, probably due to its effects on absorption of this element. Increase in the level of dietary fat from 5 to 15 or 20 percent enhanced the growth-retardation effect of high fluorine intake in rats (Miller and Phillips, 1953; Buttner and Muhler, 1958) and chickens (Bixler and Muhler, 1960). Fluorine, in the presence of fat, causes delayed gastric emptying, which may account for the increased toxicity of fluorine in rats fed high-fat diets (McGown et al., 1976).

TISSUE LEVELS

The concentrations of fluorine in bone, tissues, blood, eggs, and milk vary with fluorine intakes from water and diet (NRC, 1980). Mineralized tissues contain approximately 99 percent of total body fluorine, with a major proportion concentrated in bone. The skeleton of animals normally contains the highest proportion of fluorine, with normal bone having ranges from 0.3 to 0.6 mg F/g of dry tissue on a fat-free basis (Underwood and Suttle, 1999). The fluorine concentration in the soft tissues is relatively low and changes little with age. Kidneys contain higher levels of fluorine than other tissues partly due to urine retained in this organ. Normal fluorine levels (μg/g, dry matter basis) of cow and sheep in the following soft tissues have been reported (Harvey, 1952; Suttie et al., 1958): liver, 2.3 and 3.5; kidney, 3.5 and 4.2; thyroid, 2.1 and 3.0; and heart, 2.3 and 3.0. Dairy cows fed a ration supplemented with 50 μg F/g as NaF for 5.5 years only increased the levels in heart, liver, thyroid, and pancreas 2- to 3-fold above 3 μg/g found in tissues of normal cows (Suttie et al., 1958). In normal cow's milk, fluorine levels range from 0.01 to 0.4 μg/g (Suttie et al., 1957b; Bergmann, 1995), whereas fluorosis can occur in cattle when levels reach 0.64 μg/g (Suttie et al., 1957b).

Plasma fluoride concentrations are maintained within narrow limits by regulatory mechanisms involving skeletal tissues. In cattle, the plasma fluorine concentration is less than 0.1 μg/g in normal animals and 1 μg/g indicates a high fluorine uptake from diet (Suttie et al., 1972). The fluorine concentration in plasma of healthy humans ranges from 7.6 to 28.5 μg/L (Guy, 1979) and values as high as 106 ± 76 mg/L in adults exposed to 5.03 mg F/L in drinking water have been reported (Li et al., 1995). Elevated intakes of fluoride also result in an increased concentration of fluoride in urine and bone. In several long-term experiments with beef and dairy cattle, the skeletal concentration of fluorine was approximately proportional to the concentration of NaF (NRC, 1974). Urine fluoride levels are approximately correlated with dietary intake, although the duration of fluoride ingestion, sampling time, and total urinary output will introduce variation. With excess ingestion of fluorine, urinary levels increased from 15 to 30 mg/L to an upper limit of 70 to 80 mg/L (Suttie and Kolstad, 1977). The following urine concentrations indicate fluorine status of cattle: normal, <5 mg F/L; borderline toxicity, 20 to 30 mg F/L; systematic toxicity, >35 mg F/L (McDowell, 2003).

Birds fed high-fluoride diets accumulate fluorine in their eggs, particularly in yolk. The fluorine content of normal chicken eggs increased from 0.8 to 0.9 μg/g to as high as 3 μg/g with an increase in dietary rock phosphate concentration (Phillips et al., 1935). Generally the fluorine in deboned poultry meat is low (0.2 mg/kg), but higher values in the range of 0.3 to 2.7 mg/kg in chicken and turkey meat have been reported (Jedra et al., 2001). Fluoride is mainly concentrated in skeletal tissues of fish and exoskeletons of invertebrates. In freshwater and marine fish muscle, it ranges from 0.6 to 26 mg/kg wet weight (Lall, 1994; Camargo, 2003) and in whole fish, from 10 to 60 mg/kg (Soevik and Braekkan, 1981). The average amount in the edible part of muscle is 0.8 mg F/kg, but small bones retained in fish muscle can affect the fluorine content (Lall, 1994). Fluorine in the muscle of crab, shrimps, and prawn from the North Sea varies from 1 to 4 mg/kg (Soevik and Braekkan, 1981). Antarctic krill contains exceptionally large amounts of fluorine (mg/kg dry weight): whole animal, 1,000; muscle, 70; and exoskeleton, 2,000. The concentrations of fluoride in the body of wild invertebrates consumed by fish have ranged from 7 to 3,500 mg/kg (Camargo, 2003). The consumption of these organisms could have a significant effect on the fluorine content of fish tissues.

HUMAN HEALTH

Fluorine is considered a hazardous substance with ingestion of large doses of this element in either food or water or both. Fluoride consumed from water and beverages accounts for more than 70 percent of total fluoride intake, because most animal meat, milk, and eggs contain less than 30 μg F/100 g (Table 14-2). Regular consumption of marine fish and crustaceans can significantly increase fluoride intake. The drinking water in many parts of the world is naturally rich in fluoride, which causes local inhabitants to develop endemic fluorosis. Several reviews related to the risk of chronic fluoride toxicity to humans have been published (Kaminsky et al., 1990; USPHS Ad Hoc Subcommittee on Fluoride, 1991; NRC, 1993; Whitford, 1996; IPCS, 2002; ATSDR, 2003).

MAXIMUM TOLERABLE LEVELS

The tolerance levels for domestic animals are based on clinical signs of fluoride toxicosis, and most of the values

were obtained before 1980. While small intakes of fluoride may be beneficial or even essential, prolonged intakes of oral fluoride concentrations above these maximum tolerable levels may result in reduced performance. The tolerable levels are based on tolerances to sodium fluoride or other fluorides of similar toxicity (fluoride in certain phosphorus sources appears to be less toxic) and assume that the diet is essentially the sole source of fluoride. When water also contains appreciable fluoride (> 3 mg/L), these dietary levels should be reduced. The maximum fluoride levels for young beef or dairy calves and heifers are 35 and 40 mg/kg of diet, respectively; for animals kept for breeding, 30 to 40 mg/kg of diet for heifers; and for mature animals, 40 to 50 mg/kg of diet. Long-term studies (approximately 7 years) conducted with young calves showed that 30 mg F/kg diet caused excessive staining of teeth (Hobbs and Merriman, 1959; Shupe et al., 1963b). Minor morphological lesions may be seen in cattle teeth when dietary fluoride during tooth development exceeds 20 mg/kg, but a relationship between these lesions and animal performance has not been established.

Mature dairy cattle consume more feed in relation to body weight than mature beef cattle, so maximum recommended concentrations of dietary fluoride are 40 mg/kg for dairy cattle and 50 mg/kg for beef cattle. Lifetime fluoride exposure for finishing cattle is less than for breeding cattle, so the maximum tolerable level for this productive class is estimated at 100 mg/kg. Excessive exposure during tooth development in cattle may result in exaggerated tooth wear, impaired mastication, and sensitivity to cold drinking water. Maximum tolerable levels for other species are based on relatively limited published data in some cases, and the level of 40 mg F/kg for horses and rabbits represents an estimate. Levels for lifetime exposure for sheep, swine, and poultry are less than that for cattle and horses. Poultry are relatively less sensitive to fluoride. The maximum tolerable levels are 150, 150, and 200 mg F/kg for swine, turkeys, and chicken, respectively. Sheep raised for lamb or wool can tolerate 60 mg F/kg diet, and finishing lamb can tolerate up to 150 mg F/kg diet (NRC, 1980).

Studies on laboratory species under a wide range of experimental design have been summarized by CEPA (1993) and IPCS (2002), and the NOAEL and the LOAEL are as follows (mg of F/kg of BW/day): rats, 1.8–12.7, 3.2–12.8; and mice, 2.7–9.1, 4.5–5.7. The estimated average safe concentrations (infinite hour $LC_{0.01}$ values) of fluoride for fry (8–10 cm) of rainbow trout and brown trout in soft water (21.2–22.4 mg $CaCO_3$/L) were 5.14 (range 3.10–7.53) and 7.49 (range 4.42–10.96) mg F^-/L, respectively (EPA, 1986; Lee et al., 1995). A fluoride concentration of 0.5 mg F^-/L is considered safe for freshwater upstream migrating salmon inhabiting soft water of low ionic content and 1–1.5 mg F^-/L in hard water with high ionic content (Camargo and La Point, 1995; Camargo, 1996).

The LC_{50} values of fluoride for freshwater, brackish, and marine invertebrates at different stages of development, exposure time, and temperature have ranged from 10.5 to 308 F^-/L. However, the safe concentration (infinite hour $LC_{0.01}$ values) of fluoride for many species in soft freshwater is in the range of 0.5 mg F^-/L (Camargo, 2003).

The lethal dose (LD_{100}) of sodium fluoride in an average human adult has been estimated between 5 and 10 g (32 to 64 mg of F/kg BW) (WHO, 1984; Whitford, 1990). The tolerable upper intake level of fluorine ranges from 0.7 to 2.2 mg/day for children to 10 mg/day for adult men and women (NRC, 1993), approximately 2- to 3-fold higher than the recommended dietary allowance. The EPA derived an oral reference dose (RFD) of 0.06 mg/kg/day for fluorine (ATSDR, 2003) that was based on a NOAEL of 0.06 mg of F/kg of BW/day and the LOAEL of 0.12 mg of F/kg of BW/day from data on fluorosis in children. The maximum amount of fluoride allowed by EPA in drinking water is 4 mg/L.

FUTURE RESEARCH NEEDS

The beneficial effects of fluoride on dental health of farmed and laboratory animals and humans have been known for more than 60 years; however, the exact biochemical mechanisms to qualify fluorine as an essential trace element remain to be established. Numerous publications describe the effects of fluorine supplementation (in some cases, pharmacological dose) that include prevention of dental caries or osteoporosis. As well, the amelioration of anemia and fertility in experimental animals has been reported; however, the biochemical mechanisms remain unclear. The potential hazard of fluoride on farm animals by ingestion of phosphate fertilizers, volcanic ash, and industrial wastes is likely to continue and it will affect terrestrial and aquatic animal production. Research is needed to establish the risk of fluorine toxicity in pastoral systems and aquaculture, as well as to develop sustainable strategies for the future. The impact of human activities—such as aluminum smelters, discharges of municipal waters, and industries manufacturing brick, ceramics, glass, and chemicals containing fluorides—are causing significant increases in the fluoride concentration of surface waters. Despite determining the safe levels of fluoride for certain aquatic organisms, there are limited data on bioaccumulation in the edible portion of fish and shellfish.

SUMMARY

Fluorine is present as fluoride in igneous and sedimentary rock. Most plants have a limited capacity to absorb fluoride from the soil; however, animals may consume significant quantities from contaminated surface waters, deep-well water percolating through fluorapatite; forages contaminated by fluoride-bearing dusts, fumes, or water; animal by-products containing bone high in fluoride; and a variety of inorganic phosphate supplements. Animals normally ingest low levels without harm, and small amounts of fluoride may be beneficial and perhaps even essential. Ingestion of excessive

fluoride chronically induces characteristic lesions of the skeleton and teeth, resulting in intermittent lameness, excessive tooth wear, reduced feed and water intake, and decreased weight gain and milk production. Developing teeth and bone are particularly sensitive, and excessive exposure in early postnatal life is especially damaging. Other disorders caused by acute fluorine toxicosis in animals and/or humans include gastrointestinal irritation, severe cardiac effects (e.g., tetany, cardiovascular collapse, ventricular fibrillation), and parenchymal liver degeneration. In general, those fluoride compounds that are most soluble are most toxic. Aquatic organisms absorb fluorine from their diet and surrounding water and accumulate it in skeletal tissues. Toxicity data are available for terrestrial and aquatic animals; however, oral maximum tolerable levels for fish are not established.

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TABLE 14-1 Effects of Fluorine Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	4	Weanlings	200 mg/kg	NaF	7 wk	Diet (30 mg/kg Mg)	Slight reduction in feed intake and gain; prevented aortic calcification of Mg deficiency	Bunce et al., 1962
Dogs	5					Diet (180 mg/kg Mg)	Slight reduction in feed intake and gain	
Dogs	4–6		250 mg/kg	NaF	3+ wk	Diet (low Mg)	Weight gain reduced 50-75% at 3 wk as compared to low Mg basal; no gain after 3 wk; muscular weakness, convulsions, low serum Mg (due to low Mg?) but no aortic lesions	
Dogs	36	Puppies	5 mg/kg	Bonemeal	1 yr	Diet	No adverse effect	Greenwood et al., 1946
Dogs	22			Defluorinated rock phosphate			No adverse effect	
Dogs	20			NaF			Dental fluorosis	
Rats	5	24 d, 55 g	44 mg/kg	NaF	19 wk	Diet	No adverse effect	Kick et al., 1935
		24 d, 56 g	46 mg/kg	CaF_2			No adverse effect	
		24 d, 55 g	88 mg/kg	NaF			None on gain; excess wear of mandibular incisors	
		24 d, 54 g	92 mg/kg	CaF_2			No adverse effect	
		24 d	100 mg/kg	Rock phosphate	Tbrough 3 litters and 4 generations		No adverse effect	
		24 d, 51 g	220 mg/kg	NaF	19 wk		Decreased feed intake and gain; excess wear of mandibular incisors	
		24 d, 61 g	230 mg/kg	CaF_2			None on gain; excess wear of mandibular incisors	
		24 d	240 mg/kg	Phosphatic limestone	Through 3 litters and 4 generations		Decreased gain; dental fluorosis	
			240 mg/kg	CaF2			No adverse effect	
			250 mg/kg	Rock phosphate			No adverse effect	
Rats	5	24 d	480 mg/kg	Phosphatic limestone	Through 3 litters and 4 generations	Diet	No adverse effect	Kick et al., 1935
			480 mg/kg	CaF_2			No adverse effect	
			510 mg/kg	Rock phosphate			None on gain; failure to reproduce in second generation	
			710 mg/kg				No adverse effect	
			720 mg/kg				Decreased gain; reproductive failure in third generation	
			750 mg/kg	CaF_2			No adverse effect	

continued

TABLE 14-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Rats	8	Males, weanlings	200 mg/kg	NaF	6 wk	Diet (30 mg/kg Mg)	No decreased gain as compared to low Mg basal; calcinosis of low Mg not prevented	Bunce et al., 1962
						Diet (180 mg/kg Mg)	No adverse effect	
Rats		Females, weanlings	450 mg/kg	NaF	30–35 d	Diet	Reduced gain; altered pattern of food consumption (more "nibbling") and reduced glycogen metabolism	Zebrowski and Suttie, 1966
Chickens	50	Females, 1 d	423 mg/kg	Natural diet incl. superphosphate	8 wk	Diet	Slightly lower weight at 6 wk; no effect by 8 wk	Gerry et al., 1947
			462 mg/kg	Natural diet incl. colloidal phosphate			Slightly lower weight at 6 and 8 wk	
Chickens	100	Females	530–546 mg/kg	Natural diet incl. raw rock phosphate	38 wk		Lower weight at 6 and 8 wk; no weight difference at 12, 16, or 38 wk; egg production, hatchability, and growth of chicks to 8 wk unaffected	
			546 mg/kg		8 wk		Lower weight at 6 and 8 wk	
Chickens	50	Females, 1 d	681 mg/kg				Lower weight at 6 and 8 wk	
			721 mg/kg				Lower weight at 6 and 8 wk	
Chickens	100	Females, 1 d 16 wk	40 mg/kg	Natural diet (plus raw rock phosphate)	24 wk		No adverse effect	
			1,017 mg/kg					
Chickens	100	Females, 1 d	1,017 mg/kg	Natural diet incl. raw rock phosphate			Lower weight at 6, 8, 16, and 24 wk	
			1,019 mg/kg		8 wk		Lower weight at 6 and 8 wk	
Chickens	135	Females, 20 wk	27 mg/kg	Natural diet incl. mix of defluor. and nondefluor. dical. phoshate	51 wk	Diet	No adverse effect	Smith et al., 1970
			53 mg/kg	Natural diet incl. mix of defluor. and nondefluor. dical. phoshate	51 wk		No adverse effect	
			87 mg/kg	Natural diet incl. mix of defluor. and dical. phoshate			No effect on egg production; reduced tibia bone ash phosphorous concentration	
Chickens	50	Females, 1 d	37 mg/kg	Natural diet	8 wk	Diet	No adverse effect	Gerry et al., 1947
			40 mg/kg	Natural diet	24 wk		No adverse effect	
			43 mg/kg	Natural diet	8 wk		No adverse effect	

Chickens	100	Females, 1 d	43 mg/kg	Natural diet	38 wk		No adverse effect	
Chickens	50	Females, 1 d	99 mg/kg	Natural diet incl. melted rock phosphate	8 wk		No adverse effect	
		3	18 mg/kg	Natural diet incl. partially defluor. rock phosphate	8 wk		No adverse effect	
Chickens	20	1 d	250 mg/kg	Rock phosphate	8 wk	Diet	No adverse effect	Kick et al., 1933
Chickens	40		360 mg/kg	Rock phosphate NaF			No adverse effect Decreased blood clotting time	
Chickens	20		360 mg/kg	Rock phosphate			No adverse effect	
				NaF			No adverse effect	
			450 mg/kg	NaF			No adverse effect	
Chickens	60		710 mg/kg	Rock phosphate			Reduced feed consumption, gain, and blood clotting time	
			720 mg/kg	NaF			Reduced feed consumption, gain, and blood clotting time	
Chickens	20		890 mg/kg				Reduced feed consumption and gain	
			950 mg/kg	Rock phosphate			Reduced feed consumption and gain	
Chickens	80		1,070 mg/kg	Rock phosphate			Reduced feed consumption, gain, and blood clotting time	
Chickens	60		1,080 mg/kg	NaF			Reduced feed consumption, gain, and blood clotting time	
Chickens	20		1,080 mg/kg	CaF_2			No adverse effect	
			1,580 mg/kg	Rock pho§phate			Reduced feed consumption and gain	
Chickens	20	1 d	1,780 mg/kg	Rock phosphate	8 wk	Diet	Reduced feed consumption and gain	Kick et al., 1933
			1,810 mg/kg				Reduced feed consumption and gain	
			1,820 mg/kg	CaF_2			No adverse effect	
			2,210 mg/kg	NaF	5 wk		Reduced feed consumption and gain; death by 5 wk	
			2,710 mg/kg	CaF_2	8 wk		No adverse effect	
			4,420 mg/kg	NaF	3 wk		Reduced feed consumption and gain; death by 3 wk	
Chickens	20	1 d	250 mg/kg	Rock phosphate	8 wk	Diet	Decreased gain (?)	Kick et al., 1935
Chickens	60		360 mg/kg	Rock phosphate			No adverse effect	
			360 mg/kg	NaF			No adverse effect	
Chickens	20		450 mg/kg	NaF			No adverse effect	
Chickens	60		710 mg/kg	Rock phosphate			Decreased feed intake and gain	
			720 mg/kg	NaF			Decreased feed intake and gain	

continued

TABLE 14-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Chickens	20		890 mg/kg 950 mg/kg 1,070 mg/kg	NaF Rock phosphate Rock phosphate			Decreased feed intake and gain Decreased feed intake and gain Decreased feed intake and gain	Kick et al., 1935
Chickens	60		1,080 mg/kg	NaF			Decreased feed intake and gain	
Chickens	20		1,080 mg/kg 1,080 mg/kg 1,580 mg/kg 1,780 mg/kg 1,810 mg/kg 1,820 mg/kg 2,210 mg/kg 2,710 mg/kg 4,420 mg/kg	CaF_2 Rock phosphate CaF NaF CaF NaF	 5 wk 8 wk 3 wk		No adverse effect Decreased gain Decreased feed intake Decreased feed intake Decreased feed intake No adverse effect Decreased feed intake and gain; death in 5 wk No adverse effect Decreased feed intake and gain; death in 3 wk	
Chickens	18	Pullets, 0.66 kg	444 mg/kg	Rock phosphate	2 yr	Diet	No adverse effect	Snook,1958
Chickens	48	Males and females, 1 d	500 mg/kg 1,000 mg/kg	NaF	4 wk	Diet	Reduction in body wt, 9% Reduction in body wt, 21%; increased plasma alk. phosphatase activity	Weber et al., 1969
Chickens	44	Male, 1 d	800 mg/kg	NaF	6 wk	Diet	Decreased body wt; increased proventriculus wt.	Gardiner et al., 1959
Chickens	25	Males	1,000 mg/kg				Decreased body wt; increased proventriculus wt.	
Chickens	30	Male, 5 wk	800 mg/kg	NaF	4 wk	Diet	Decreased gain, feed consumption, and gain/feed	Gardiner et al., 1968
Chickens	10	1 d	900 mg/kg	NaF	49 d	Diet	Reduced feed intake and gain	Phillips et al., 1935
Chickens	6		1,000 mg/kg		30–35 d		Reduced feed intake and gain	
Chickens	10		34–40 mg/kg BW		30–35 d	IP inj.	Depressed gain	

Chickens	25	4 wk	0 mg/kg		4 wk	Diet	Normal	Abdelhamid and Dorra, 1993
			25 mg/kg	NaF			Cramped chicks, tumbled feathers, greenish feces, pathological changes	
			125 mg/kg				Inactive-cramped chicks, tumbled feathers, curved toes, greenish feces, pathological changes	
			625 mg/kg				Obtused legs, soft greenish feces, pathological changes	
			3,125 mg/kg				Growth stop, inactive-cramped chicks, watery-bloody stinking feces, pathological changes	
Turkeys	28	Males and females, 10–12 wk	43 mg/kg	Natural diet	16 wk	Diet	No adverse effect	Anderson et al., 1955
			100 mg/kg	Natural diet + NaF			No adverse effect	
			200 mg/kg				Decreased gain in males	
			400 mg/kg				Decreased gain in males	
			800 mg/kg				Decreased gain in males and decreased gain/feed	
			1,600 mg/kg				Decreased gain in both sexes, feed consumption and gain/feed	
Pigs	8	Weanlings, 17 kg	100 mg/kg	NaF	160 d	Diet	None on gain; increased femur density	Kick et al., 1935
		Weanlings, 18 kg	100 mg/kg	Rock phosphate	140 d		No adverse effect	
		Weanlings, 18 kg	150 mg/kg	Superphosphate	140 d		Slight decrease in feed intake and gain	
		Weanlings, 18 kg	160 mg/kg	Rock phosphate	160 d		No adverse effect	
		Weanlings, 19 kg	240 mg/kg	Phosphatic limestone	148 d		No adverse effect	
		Weanlings, 19 kg	250 mg/kg	Rock phosphate	148 d		No adverse effect	
		Weanlings, 24 kg	290 mg/kg	NaF	144 d		None on gain; increased femur breaking strength	
		Weanlings, 18 kg	290 mg/kg	NaF	160 d		Decreased feed intake, gain, and femur breaking strength	
Pigs	2	Gilts	290 mg/kg	NaF	2 yr		Femur hyperplasia; decreased feed intake and poor lactation	
Pigs	8	Weanlings, 18 kg	320 mg/kg	Rock phosphate	160 d		Decreased feed intake, gain, and femur breaking strength	
		Weanlings, 24 kg	330 mg/kg	Rock phosphate	144 d		None on gain; increased femur breaking strength	
Pigs	2	Gilts	330 mg/kg		2 yr		None on gestation; decreased feed intake and poor lactation	

continued

TABLE 14-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Pigs	8	Weanlings, 19 kg	360 mg/kg		148 d		No adverse effect	
		Weanlings, 18 kg	580 mg/kg	NaF	160 d		Decreased feed intake, gain and femur breaking strength	
		Weanlings, 24 kg	580 mg/kg		144 d		Decreased feed intake and gain	
Pigs	2	Gilts	580 mg/kg		2 yr		Femur hyperplasia; decreased feed intake and poor lactation	
Pigs	8	Weanlings, 24 kg	650 mg/kg	Rock phosphate	144 d		Decreased feed intake, gain and femur breaking strength	
Pigs	5	Gilts	650 mg/kg		2 yr		Femur hyperplasia; decreased feed consumption and poor lactation	
Pigs	8	Weanlings, 23 kg	700 mg/kg		144 d		Decreased feed intake, gain and femur breaking strength	
		Weanlings, 18 kg	710 mg/kg		140 d		Decreased feed intake, gain and femur breaking strength	
		Weanlings, 19 kg	710 mg/kg		148 d		Decreased feed intake and gain; femoral exostosis	
Pigs	8	Weanlings, 18 kg	910 mg/kg	CaF_2	140 d	Diet	No adverse effect	Kick et al., 1935
		Weanlings, 24 kg	970 mg/kg	NaF	144 d		Decreased feed intake, gain and femur breaking strength	
Pigs	2	Barrows, 120–150 d 27–36 kg	0.4 g/d	NaF	40 d	Diet	Reduced gain, gain/feed, and bone growth	Comar et al., 1953
			0.4 g/d, 200 mg/kg		75 d		No adverse effect	
			1.4 g/d, 1,000 mg/kg				Reduced gain, gain/feed, and bone growth	
			1.6 g/d		40 d		Reduced gain, gain/feed, and bone growth	
			1.6 g/d		75 d		Reduced gain, gain/feed, and bone growth	
Pigs	6	Female	1 mg/kg BW	NaF	3 lactations	Diet	Slight mottling and staining of incisors	Spencer et al., 1971
Pigs		Market pigs	11–14 mg/kg BW	Rock phosphate	3 generations, 5 yr	Diet	Dental fluorosis; slight decrease in feed intake	Fargo et al., 1938
			19–26 mg/kg BW				Dental fluorosis; reluctance to masticate whole corn or drink cold water; decrease in feed intake; no effect on reproduction	

Cows, dairy	4	4–6 yr	3–5 mg/kg 33–35 mg/kg 53–55 mg/kg	Forage, conc. Forage, conc., NaF (30 mg/kg) Forage, conc., NaF (50 mg/kg)	3 yr	Diet	No adverse effect Slight to moderate metatarsal exostosis Slight to extensive metatarsal exostosis	Suttie and Phillips, 1959
Cows, dairy	40	4 yr or older	0.5–1.7 mg/kg BW	NaF	6 yr raised on diets with similar F	Diet	Dental mottling, staining, wear	Neeley and Harbaugh, 1954
Cattle	2	9–11 wk	3–5 mg/kg	Forage, conc.	6 yr	Diet	No adverse effect	Suttie et al., 1972
Cattle	4	2 yr	3–5 mg/kg 23–25 mg/kg 33–35 mg/kg 43–45 mg/kg 53–55 mg/kg	Forage, conc. Forage, conc., NaF (20 mg/kg) Forage, conc., NaF (30 mg/kg) Forage, conc., NaF (40 mg/kg) Forage, conc., NaF (50 mg/kg)	5-1/2 yrs	Diet	No adverse effect Slight to medium dental mottling, staining Slight to heavy dental mottling, staining Slight to heavy dental mottling, staining; slight to medium hypoplasia; slight metatarsal exostosis Slight to heavy dental mottling, staining; slight to medium hypoplasia; moderate to severe exostosis; intermittent lameness after 5 yr; refusal of F-containing feeds	Suttie et al., 1957a,b Ramberg et al., 1970 Suttie et al., 1957a,b; 1958
Cattle	3	Yearlings	8 mg/kg	Hay, pasture, conc.	8 yr 10 yr	Diet	No adverse effect No adverse effect	Hobbs and Merriman, 1962
Cattle	6	Yearlings	10 mg/kg	Pasture, hay, conc.	8 yr		No adverse effect	
Cattle	10				10 yr		No adverse effect	
Cattle	3		18 mg/kg 27 mg/kg 28 mg/kg	NaF (10 mg/kg) Hay, pasture, conc. NaF (20 mg/kg)	 8 yr 10 yr		Slight to medium dental mottling, staining Slight to heavy dental mottling, staining Slight to medium dental mottling, staining Slight to medium dental mottling, staining	Hobbs and Merriman, 1962
Cattle	12	 16–20 mo	31 mg/kg 6–22 mg/kg 2–10 mg/kg	Pasture, hay, conc. Pasture Hay	 7 yr		Slight to heavy dental mottling, staining No adverse effect	
Cattle	3	Yearlings	3–8 mg/kg 44 mg/kg	NaF (30 mg/kg) Hay, pasture, conc.	8 yr 10 yr		Slight to medium dental mottling, staining Slight to heavy dental mottling, staining, negligible to medium wear Slight to heavy dental mottling, staining; trace to slight bone hypertrophy	

continued

TABLE 14-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Cattle	12						Slight to heavy dental mottling, staining, negligible to medium wear; trace to slight bone hypertrophy	
Cattle	9		45 mg/kg		8 yr		Slight to heavy dental mottling, staining, caries	
Cattle	3		48 mg/kg	NaF (40 mg/kg)			Slight to heavy dental mottling, staining	
					10 yr		Slight to heavy dental mottling, staining, negligible to medium bone hypertrophy; decreased feed consumption	
			58 mg/kg	NaF (50 mg/kg)	8 yr		Slight to heavy dental mottling, staining, caries; decreased feed consumption after 3 yr	
					10 yr		Dental mottling, staining, hypoplasia, slight to excessive wear; trace to medium bone hypertrophy; slight exostosis; decreased feed consumption	
Cattle	3	Yearlings	78 mg/kg	NaF (70 mg/kg)	10 yr	Diet	Dental mottling, staining, hypoplasia, slight to excessive wear; general bone hypertrophy; slight exostosis; decreased feed consumption	Hobbs and Merriman, 1962
			108 mg/kg	NaF (100 mg/kg)			Dental mottling, staining, hypoplasia, slight to excessive wear; general bone hypertrophy; decreased feed consumption	
			110 mg/kg				Dental mottling, staining, hypoplasia, slight to excessive wear; general bone hypertrophy, exostosis; decreased feed consumption	
Cattle	12	16–20 mo	162–474 mg/kg 17 mg/kg	Pasture Hay	409 d (then on 2–22 mg/kg F to 7 yr)		Slight to heavy dental mottling, staining; slight bone hypertrophy in two	
					639 d (then on 2–22 mg/kg F to 7 yr)		Slight to heavy dental mottling, staining; slight bone hypertrophy	
					837 d (then on 2–22 mg/kg F to 7 yr)		Slight to heavy dental mottling, staining; slight bone hypertrophy	
Cattle	6		265–474 mg/kg 17 mg/kg	Pasture Hay	209 d (then on 2–22 mg/kg F to 7 yr)		Slight to heavy dental mottling, staining; slight to medium bone hypertrophy	
			410–474 mg/kg 17 mg/kg	Pasture Hay	102 d (then on 2–22 mg/kg F to 7 yr)		Slight to medium dental mottling, staining; slight bone hypertrophy	

Cattle	4	7–8 mo	10 mg/kg 62 mg/kg 68 mg/kg 69 mg/kg	Hay Hay (near steel plant) Hay, NaF (60 mg/kg) Hay, CaF (60 mg/kg)	588 d	Diet	No adverse effect Slight to definite dental mottling, staining, hypoplasia, wear; metatarsal hyperostosis Slight to definite dental mottling, staining, hypoplasia, wear; metatarsal hyperostosis Slight to definite dental mottling, staining; slight metatarsal hyperostosis	Shupe et al., 1962
Cattle	8	3–4 mo, 105 kg	12 mg/kg (dry basis) 27 mg/kg (dry basis) 49 mg/kg (dry basis) 93 mg/kg (dry basis)	Hay, conc. Hay, conc., NaF (15 mg/kg) Hay, conc., NaF (37 mg/kg) Hay, conc., NaF (81 mg/kg)	7 yr	Diet	No adverse effect Slight dental mottling; slight histological bone changes Moderate to marked dental mottling, staining, hypoplasia, slight to definite wear; intermittent lameness after 4 yr; moderate bone changes; slightly decreased feed intake after 4 yr Marked dental mottling, staining, hypoplasia, definite wear; marked bone changes; intermittent lameness after 2.5 yr; decreased feed consumption after 4 yr; decline in milk production (not statistically significant); slight elevation in % eosinophils	Greenwood et al., 1964; Harris et al., 1964; Hoogstratten et al., 1965
Cattle	10	14 wk	40 mg/kg	NaF	32 mo	Diet	Mild hyperostosis of mandibles, metacarpals, and metatarsals; low-TDN delayed incisor eruption and increased fluorosis of incisor 4	Suttie and Faltin, 1973
Cattle	5		43 mg/kg	Forage, conc., NaF (40 mg/kg)	58 mo		General dental mottling; mild hyperostosis of mandibles, metacarpals, and metatarsals	
Cattle	3	6 wk	100 mg/kg	Pelleted forage and conc., NaF (100 mg/kg)	11 mo	Water	Decreased feed intake, weight gain, calcium absorption, and calcium balance	Ramberg et al., 1970
Cattle	20	Yearlings	11–122 mg/kg 12–220 mg/kg Trace 11–122 mg/kg 12–220 mg/kg 0.7–2.6 mg/kg 27–735 mg/kg 50–1,812 mg/kg Trace 27–735 mg/kg 50–1,812 mg/kg 1–1.5 mg/kg	Pasture Hay Water Pasture Hay Water Pasture Hay Water Pasture Hay Water	5.5 yr	Diet Diet and water Diet Diet and water	No adverse effect No adverse effect Slight to medium dental mottling, staining Slight to medium dental mottling, staining	Merriman and Hobbs, 1962

continued

TABLE 14-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Cattle	4	2 yr	0.15–0.3 mg/kg BW	Forage, conc.	6 yr	Diet	No adverse effect	Newell and Schmidt, 1958
			1.0 mg/kg BW	NaF			No to slight dental mottling	
			1.5 mg/kg BW				No to marked dental mottling, staining, wear	
			2.0 mg/kg BW				No to marked dental mottling, staining, wear; occasional unthriftiness	
			2.5 mg/kg BW				No to excessive dental mottling, staining; metatarsal exostosis; unthriftiness	
Cattle	3	6–25 wk	1.0 mg/kg BW	NaF	2 lactations	Diet	Slight dental mottling	Suttie et al., 1961
Cattle	6	6–24 wk	1.2 mg/kg BW				Slight dental mottling	
		8–22 wk					Extensive dental mottling, mild enamel hypoplasia; intermittent lameness in second lactation	
Cattle	4	6–21 wk	1.6 mg/kg BW				Extensive dental mottling, hypoplastic enamel, excessive wear; intermittent lameness in second lactation	
Cattle	3	6–27 wk	2.0 mg/kg BW				Extensive dental mottling, hypoplastic enamel, excessive wear; intermittent lameness in first lactation	
Cattle	2	9–11 wk	1.5 mg/kg BW	NaF	6 yr	Diet	Moderate to general dental mottling, enamel hypoplasia; mild to severe metacarpal and metatarsal hyperostosis and exostosis	Suttie et al., 1972
Cattle	3		1.5 mg/kg BW (6 mo) No suppl. (6 mo)				Slight to general dental mottling, enamel hypoplasia; no to moderate metacarpal and metatarsal hyperostosis	
Cattle	4	19–22 wk	1.5 mg/kg BW				Moderate to general dental mottling, enamel hypoplasia; mild to severe metacarpal and metatarsal hyperostosis and exostosis	
Cattle	3		3.0 mg/kg BW (4 mo) 0.75 mg/kg BW (8 mo)				Moderate to general dental mottling, enamel hypoplasia; severe molar wear; mild to severe metacarpal and metatarsal hyperostosis and exostosis; intermittent lameness	

Cattle	3	13 mo	2.5 mg/kg BW (3 mo) 0 (32 mo)	NaF	35 mo	Diet	Rapid increase in plasma F with rapid decrease postexposure; fluorotic damage to incisors 1 and 2	Suttie and Faltin, 1971
		16 mo	2.5 mg/kg BW (3 mo) 0 (29 mo)		32 mo		Rapid increase in plasma F with rapid decrease postexposure; fluorotic damage to incisors 2 and 3	
Sheep	18	Weanlings, wethers	14 mg/kg 32 kg 18 mg/kg 32 mg/kg 58 mg/kg 112 mg/kg	NaF (50%) F on hay (50%)	14 wk	Diet	No adverse effect No adverse effect No adverse effect No adverse effect No adverse effect	Harris et al., 1958
Sheep	18	Weanlings, wethers	17 mg/kg 50 mg/kg 100 mg/kg 200 mg/kg	Natural diet NaF	84 d	Diet	No adverse effect No adverse effect No adverse effect Decreased feed intake, gain, and carcass grade	Harris et al., 1963
Sheep	6	10–11 mo	2.5 mg/kg 5–7 mg/d 5 mg/kg 9–13 mg/d 10 mg/kg 18–27 mg/d 20 mg/kg 37–53 mg/d	NaF	3.5 yr	Water	No adverse effect Slight dental mottling Dental mottling Marked dental lesions	Peirce, 1952
Sheep	16	2.5–3.5 yr	10 mg/kg 18 mg/d 0.24 mg/kg 20 mg/kg 33 mg/d 0.44 mg/kg	NaF	26 mo	Water	No adverse effect No adverse effect	Peirce, 1954
Sheep	17	Conception	10 mg/kg	NaF	7 yr	Water	Dental lesions and excessive wear; decreased wool production	Peirce, 1959
Sheep	20		20 mg/kg				Dental lesions and excessive wear; decreased wool production; no effect on reproduction; slight increase in mortality	
Fish, brown trout		Fry, freshwater		15.1–15.5°C, hardness 20.6–24.2 mg/L, pH 7.5–7.8	96 h	Water	Acute toxicity, LC_{50} – 164.5 mg F^{-1}/L	Camargo and Tarazona, 1991
Fish, crescent perch		Adult		20–21°C, salinity 10–28	96 h	Water	Acute toxicity, LC_{50} – >100 mg F^{-1}/L	Hemens and Warwick, 1972

continued

TABLE 14-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Fish, fathead minnow		<1 g, freshwater		15–19°C, hardness 10–44 0mg/L, pH 7.5–8.0	96 h	Water	Acute toxicity, LC_{50} – 315 mg F^{-1}/L	Smith et al., 1985
Fish, mullet		Juvenile		20–21°C, salinity 10–28	96 h	Water	Acute toxicity, LC_{50} – > 100 mg F^{-1}/L	Hemens and Warwick, 1972
Fish, rainbow trout		<3 g, freshwater		15°C, hardness 23–62 mg/L, pH 7.4–8.0	96 h	Water	Acute toxicity, LC_{50} – 200 mg F^{-1}/L	Smith et al., 1985
		1.89, freshwater		12°C, hardness 17 mg/L, pH 7.2	96 h	Water	Acute toxicity, LC_{50} – 51 mg F^{-1}/L	Pimental and Bulkley, 1983
		1.89, freshwater		12°C, hardness 49 mg/L, pH 8.3	96 h	Water	Acute toxicity, LC_{50} – 128 mg F^{-1}/L	
		1.89, freshwater		12°C, hardness 182 mg/L, pH 8.3	96 h	Water	Acute toxicity, LC_{50} – 140 mg F^{-1}/L	
		1.89, freshwater		12°C, hardness 385 mg/L, pH 8.7	96 h	Water	Acute toxicity, LC_{50} – 193 mg F^{-1}/L	

[a]Number of animals per treatment.
[b]Quantity expressed as mg/kg or as mg/kg of body weight (BW). SI conversion: 1 mg fluorine equals 52.6 μmoles fluorine.

TABLE 14-2 Fluorine Concentrations in Fluids and Tissues of Animals (mg/kg or µg/L)

Animal	Level and Source of F	Plasma	Bone	Liver or Soft Tissues	Muscle	Milk	Egg Yolk	Reference
Humans	Diet	0.003–0.080	1.5–2.2	19.6–159				Guy et al., 1976; Zipkin et al., 1960; Taves et al., 1983
Chickens, laying hens	16 100 1,300	0.001 0.003 0.010	538 2,247 2,600	5.2 5.5 19.2	4.0 4.0 6.7		4.3 3.3 18.4	Hahn and Guenter, 1986
Meat and poultry	17 varieties of muscle and poultry in Canada			0.04–1.2				Dabeka and McKenzie, 1995
Meat and poultry	7 varieties of meat and poultry in Hungary			0.01–1.7				Schamschula et al., 1988
Cattle, fluorosis			885–6,918			0.072–0.64		Hillman et al., 1979
Cows	12 varieties of diary products in Canada					0.01–0.8		Dabeka and McKenzie, 1995
Cows	13 varieties of dairy products in Hungary					0.045–0.51		Schamschula et al., 1988
Cows	Milk and milk products sampled between 1981–1989 in Gennany					0.019–0.16		Bergmann, 1995
Cows	50 µg/g fluorine in feed ration for 5.5 yr			Liver: 3.6 Kidney: 19.3 Heart: 4.6 Thyroid: 7.3				Suttie et al., 1958
Sheep	10 mg/g liter fluorine in water for 2 yr			Liver: 2.2 Kidney: 16.8 Thyroid: 7.2 Heart: 2.2				Harvey, 1952

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Iodine

INTRODUCTION

The name iodine (I) is derived from the Greek word *iodes*, meaning violet. Iodine (atomic weight 126.9045), a nonmetallic element of the halogen group, is volatile at ambient temperature and pressure. It can exist in various oxidation states with valences of $^{-}1$, $^{+}1$, $^{+}3$, $^{+}5$, or $^{+}7$, but the main oxidation state is $^{-}1$ as iodide. The term iodide refers to its ionic form I^{-}. Iodate refers to IO^{3-}. Elemental iodine occurs as a purple-black solid with rhombic crystalline structure that sublimes into the gaseous form (I_2), giving off an intense violet vapor and a characteristic odor. It is also a component of fallout produced by nuclear explosions. Iodine is less reactive than other halogens (fluorine [F], chlorine [Cl], and bromine [Br]) but readily forms compounds with other elements. It dissolves in alcohol, carbon disulfide, carbon tetrachloride, and chloroform, but it is only slightly soluble in water. In water, hydrogen and iodine form hydrogen iodide. Iodine forms compounds with certain nonmetals (e.g., carbon, nitrogen, phosphorus, and oxygen), and it can be displaced by the other halogens. Among 36 isotopes of iodine with masses between 108–143 (Chu et al., 1999), 14 yield significant radiation. The naturally occurring isotopes of iodine, ^{127}I and ^{129}I, are stable and radioactive. The other isotopes of iodine are instable isotopes. Iodine^{-131} is a radioactive isotope with a half-life of 8 days. It is widely used to diagnose abnormalities of the thyroid gland in humans and experimental animals.

Iodine, the 64th most abundant element (10^{-5} percent of Earth's crust), is widely distributed in nature and present in both organic and inorganic substances in very small amounts. The ocean is the primary source of iodine, containing between 50–60 µg/L. Only a few substances, such as the saltpetre deposits of Chile and some marine products, have concentrations of up to 1,000–2,000 mg/kg. Iodine is present in soil, air, and water and becomes a constituent of plants and animals used for food. The average iodine concentration in air, soil, freshwater, and animal body are 0.7 µg/m^3, 300 µg/kg, 5 µg/l, and 0.4 mg/kg (WHO, 1998; ATSDR, 2004). The iodine content of water reflects the iodine content of the rocks and soils of the region. Thus, crops grown in certain areas, such as the Ganges plains of India, are iodine deficient, and animals and humans in this area suffer from a deficiency of this element. An important source of iodine is caliche, a nitrate rock that contains up to 0.2 percent iodine in the form of iodate salts. Iodine is also found as an iodide in certain seaweeds.

Iodine was first isolated in 1811 by a French chemist, Bernard Courtois, from seaweed ash during the making of gunpowder. In 1895, Baumann discovered iodine in the human thyroid gland and the relationship of iodine deficiency to enlargement of the thyroid gland was first clinically shown by Marine and Kimball in 1922 (Hetzel and Dunn, 1989). A comprehensive review of the historical aspects of goiter has been published (Matovinovic, 1983). Iodine and its compounds are used as supplements in feeds and foods (e.g., iodized salt), agricultural chemicals (e.g., herbicides and fungicides), animal drugs (e.g., ethylenediamine dihydriodide [EDDI]) and sanitizers (e.g., iodophors). EDDI is used at relatively high levels to prevent or treat foot rot and soft tissue lumpy jaw in cattle (Miller and Tillapaugh, 1966). Iodophors are widely used in the dairy industry as teat dips and udder washes. Iodophor solutions are also used as sanitizing agents for cleansing equipment. In industry iodine is used mainly as organoiodine compounds in pharmaceuticals, photography, pigments, sterilization, dyestuffs, and rubber manufacture.

ESSENTIALITY

Iodine is an essential element for animal species, including humans, mainly because it is an integral component of the thyroid hormones, 3,3′,5-triiodothyronine (T_3) and 3,3′,5,5′-tetraiodothyronine (thyroxine, T_4). Thyroid hormones regulate cell activity and growth in virtually all tissues and therefore are essential in intermediary metabolism,

reproduction, growth and development, hematopoiesis, circulation, neuromuscular functioning, and thermoregulation. The concentration of iodine in the environment, as well as in plant material and water, is extremely variable throughout the world and iodine deficiency disorders (IDD) can be a major problem in both domestic animals and humans (Hetzel and Dunn, 1989; Delange, 1994; Underwood and Suttle, 1999).

In all farm animals, deficiency is accompanied by thyroid hyperplasia or "goiter" and a decrease in thyroglobulin concentration within the follicles of the thyroid gland. The goitrous condition is a hyperplastic response of the thyroid glands to an increased stimulation of thyroid growth by thyroid-stimulating hormones produced in the pituitary gland. With mild iodine deficiency, the hyperplastic thyroid gland can compensate for the reduced absorption of iodine (Hetzel and Welby, 1997). Deficiency signs vary depending upon the animal species and the severity of the deficiency. Calves may be born hairless, weak, or dead. Fetal death can occur at any stage of gestation, but cows often appear normal (Hemken, 1960). Reproductive failures have been reported in both male and female cattle, sheep, and pigs suffering from goiter (Underwood and Suttle, 1999). Chickens may show thyroid hyperplasia without a significant effect on growth and reproductive performance. Laying hens fed diets containing 0.01 to 0.02 mg I/kg, produced eggs of low hatchability and poor embryonic development. While 0.35 mg I/kg was adequate to maintain good hatchability, 0.75 mg I/kg was not sufficient to prevent thyroid hyperplasia in embryos (Rogler et al., 1961).

Goitrogenic substances in feed may increase iodine requirement substantially (2- to 4-fold) depending on the amount and type of this natural toxicant (Bell, 1984). Cyanogenetic goitrogens include thiocyanate derived from cyanide in white clover and the glucosinolates found in *Brassica* seeds and forages (e.g., kale, turnips, and rapeseeds). These thiocyanate compounds impair iodine uptake by the thyroid, but their effect can be overcome by increasing dietary iodine intake. Several cases of fetal death, abortion, or the birth of weak lambs or calves associated with hypothyroidism have been reported where plants containing goitrogens constituted an appreciable proportion of the diet during pregnancy (Sinclair and Andrews, 1958). Goitrogens, particularly the thiooxazolidone type, readily pass from the bloodstream to the milk of lactating animals and consequently the milk possesses goitrogenic potency (Arstila et al., 1969; Laurberg et al., 2002).

Recent estimates of iodine requirements (mg/kg diet) published in NRC publications where diet does not contain goitrogens are as follows: chicken and cats, 0.35; turkey, 0.40; beef cattle, 0.50; dairy cattle, 0.25 (growing) and 0.5 (lactating); sheep, 0.1–0.8; horses, 0.10; swine, 0.14; rats and mice, 0.15; nonhuman primates, 2 (reviewed by McDowell, 2003). Lactating animals require more dietary iodine because approximately 10 percent or more of the iodine intake may be excreted in milk, depending upon the rate of milk production (Miller et al., 1975). Iodine requirements may also be influenced by genetic differences, climate, and environment. The thyroid hormone secretion in certain animals has been shown to be inversely related to environmental temperature. Cattle, sheep, and goats show a significant decrease in thyroid hormone production during the summer (ARC, 1980).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Iodine is found in the organic forms in plants and animals and in the inorganic form in natural water samples. Methods for the identification and quantitative determination of iodine depend upon the type of compound and the matrices in which it occurs. Iodine normally exists in nature as iodide (I^-); however, other common forms include iodate (IO^3) and molecular iodine (I_2). For analysis of iodine in most biological samples, a dissolution step (wet or dry ashing) is required prior to analysis in order to convert and isolate it to a chemical or physical form suitable for detection. Spectrophotometry was initially used for the determination of iodine based on the iodized catalyzed reaction of cerium (IV) by arsenic (III) in food and feeds.

Several analytical methods—including chromatography (ion and gas), spectrometry, and neutron activation analysis—are used to determine iodine in feeds and foods. The ionic forms of iodine, iodide or iodate, can be determined using ion chromatography where detection limits with conductivity detection generally range between 0.1–1mg/L. Other analytical techniques, particularly those based on radioiodine measurements, require highly specialized equipment and multi-step sample preparation techniques, which are prone to iodine loss or contamination. Due to the high selectivity and sensitivity obtained by ICP-MS, it is commonly used for the quantitative determination of iodine in biological tissues (Larsen and Ludwigsen, 1997; Julshamn et al., 2001).

Some iodine species, such as hydrogen iodide, are volatile at room temperature and therefore must be stabilized in sample solutions at alkaline pH prior to ICP-MS determination. Volatilization of iodine may lead to memory and adhesion effects in ICP instruments, which may cause problems in sample introduction systems. Several methods such as digestion using perchloric acid in combination with nitric acid have been used successfully to digest samples in polytetrafluoroethylene-lined steel bombs. The acid mixture oxidizes potentially volatile iodine species to nonvolatile species such as iodate. Other analytical approaches using ICP-MS are based on alkali extraction combining potassium hydroxide and tetramethylammonium hydroxide (TMAH) to determine iodine in milk or serum by flow injection ICP-MS or use TMAH as a strong alkali to measure iodine in diets supplemented with the element. The microwave digestion system using 0.5 percent

ammonium solution has also been successfully used for rapid and sensitive determination of iodine (Baumann, 1990; Vanhoe et al., 1993). Volatile and semi-volatile forms of organic iodine may be determined by gas chromatography and gas chromatography-mass spectrometry.

REGULATION AND METABOLISM

Absorption and Metabolism

Ingested inorganic iodine and iodate are reduced to iodide and absorbed almost completely from the gastrointestinal tract (Hetzel and Maberly, 1986). In the ruminant, the rumen is the major site of absorption of iodine and the abomasum is the major site of endogenous secretion (Barua et al., 1964). After absorption, the iodide is rapidly distributed throughout the body. The major sites of iodine concentration are the thyroid and the kidney. In addition, iodine is concentrated by the salivary glands, mammary glands, gastric mucosa, placenta, ovary, skin, and hair (Gross, 1962). In thyroid follicular cells, iodine is transformed through a series of metabolic steps into the thyroid hormones, T_4 and T_3. These two hormones express almost all of the biological effects of iodide. Other forms of iodine in the thyroid include inorganic iodine, 3-monoiodotyrosine (MIT), 3, 5 -diiodotyrosine (DIT), polypeptides containing thyroxine, and thyroglobulin.

Iodide trapped by the thyroid is oxidized by thyroid peroxidase to active iodine, which iodinates the tyrosyl residues in thyroglobulin. This large glycoprotein (MW 660,000), the storage form of thyroid hormones, serves as the substrate for iodination and coupling of peptide-linked iodotyrosines to T_4 and T_3. This coupling reaction is also catalyzed by thyroid peroxidase and results in conversion of an alanine side chain to dehydroalanine. Thyroglobulin consists of two polypeptide subunits that undergo post-translational glycosylation and iodination prior to secretion into the lumen of the thyroid follicle. The iodoprotein is reabsorbed into the thyroid cells and hydrolyzed to release free thyroid hormones for secretion into the circulation sytem and distribution to the peripheral tissues. This process also occurs in lactating mammary glands and, to a limited extent, in the ovum within the ovary. In other sites, the element remains in the form of iodide.

Thyroglobulin in the colloid contains the greatest part of iodine in a normal thyroid. Approximately 70 percent of the dry weight of a thyroid gland is thyroglobulin. One mole of thyroglobulin containing 1 percent iodine consists of 10–12 residues each of MIT and DIT, 3–4 residues of T_4, and less than one residue of T_3. The percent iodine varies and is a function of iodine in the diet. The colloid can store a sufficient amount of thyroid hormones to supply the body needs of humans for several months (Taurog, 2000); however, the storage time may vary among different animals. The iodide pool is replenished continuously, exogenously from the diet and endogenously from the saliva, gastric juice, and breakdown of thyroid hormones and iodothyronines by deiodination. The pool is in a dynamic equilibrium with the thyroid gland and kidneys. Approximately 80–90 percent of iodine intake is excreted via the kidneys in the urine (Vought and London, 1967; Nath et al., 1992); other routes include saliva, bile, sweat, and feces. In lactating animals, milk is also a major route of iodine excretion. Undigested organic iodine is excreted in feces.

Thyroid hormones are primarily transported in blood mainly (>99.7 percent) loosely bound to plasma proteins (thyroxine binding protein, transthyretin, albumin, and lipoproteins). The regulation of thyroid metabolism is a complex process, which involves the thyroid, anterior pituitary, hypothalmus, and peripheral tissues (Hetzel and Welby, 1997). Thyroid-stimulating hormones (TSH) from the pituitary gland stimulate thyroid metabolism via cyclic AMP and the synthesis of thyroid hormones. The TSH released from the pituitary is inhibited through a feedback control by the thyroid hormones, and its release is stimulated by thyrotropin-releasing hormones (TRH), a tripeptide (pyroglutamyl histidyl-prolinamide). Synthesis of TRH occurs in the hypothalamus, and they are transported to the pituitary through blood vessels. The secretion of TRH from the hypothalamus is in turn inhibited by T_3. These interacting factors of the thyroid-pituitary–hypothalamus axis maintain homeostasis with regard to thyroid hormones. Under normal conditions most of the thyroid hormones released from the thyroid gland are in the form of T_4 and only a small amount as T_3. Three selenium-dependent enzymes, deiodinase (Type I, II, and III) convert T_4 into T_3, the biological active form of thyroid hormone (Arthur, 1999). Thiouracil only inhibits Type I deiodinase (ID1). A major difference exists between ruminants and nonruminants with respect to ID1; this enzyme is particularly important in brown tissue metabolism of newborn ruminants and serves as source of T_3 for other tissues (Nicol et al., 1994). Free MIT and DIT are deiodinated by tissue deiodinases. Normal concentrations of thyroid hormones are necessary to maintain a wide range of essential physiological processes including metabolic rate, protein and enzyme synthesis, thermoregulation, and growth and development of most vital organs.

Fish and other aquatic organisms obtain iodine primarily from gills and, to a lesser extent, from food sources. Rainbow trout derive 80 percent of their iodine from water, 19 percent from diet, and less than 1 percent from recycling iodine originating from thyroid hormone degradation (Hunt and Eales, 1979). When dietary uptake is low or absent, fish are able to maintain their plasma iodine levels by uptake of iodide from water and mobilization of this element from tissue stores. Major differences exist between fish and mammals in the handling of iodine and extrathyroidal metabolism of T_4 and T_3 (Higgs et al., 1982).

Metabolic Interactions and Mechanism of Toxicity

The cellular functions of thyroid hormone are mediated through triiodothyronine in a complex molecular mechanism involving steroid and thyroid hormones and nuclear receptors (Hetzel and Welby, 1997). Retinoic acid (vitamin A)

stimulates, synergistically with triiodothyronine, the production of growth hormones in cultured cells. Goitrogenic substances in a wide range of vegetables and plant products are capable of producing thyroid hyperplasia by interfering with thyroid hormone synthesis. The pituitary responds by increasing TSH output, thus causing thyroid gland hypertrophy in an effort to increase thyroid hormone production. Inorganic elements in diets that interfere with iodine metabolism include: (1) high intake of fluorine, arsenic, and calcium, (2) cobalt deficiency or excess, and (3) low manganese intake (Underwood and Suttle, 1999). Higher amounts of dietary potassium increase urinary loss of iodine in cattle (Dennis and Hemken, 1978). Synthetic flavonoids have been shown to interfere with iodine metabolism in a rat fetus by crossing the placenta and reducing the availability of T_4.

Iodine toxicosis in animals and humans markedly reduces thyroidal iodine uptake, and the response is dependent on dose and duration of iodine intake (Hetzel and Welby, 1997). Excess iodine may result in either hypothyroidism, with or without goiter, or hyperthyroidism (thyrotoxicosis), but the mechanisms involved in these responses are not completely understood. Numerous studies conducted on the mechanisms of iodine toxicity show the following direct effects on thyroid glands: (1) inhibition of iodide transport and uptake by the thyroid; (2) accumulation of iodotyrosines; (3) inflammation and degeneration of follicular cells; and (4) damage to follicular cells DNA. Indirect effects of iodine toxicosis include: (1) changes in thyroid hormones transport in plasma; (2) interference in TSH or transthyrectin metabolism; (3) higher hepatic microsomal enzyme activities causing an increase in iodotyrosine excretion; and (4) poor absorption of thyroid hormones and their excretion in feces.

The adverse effects of iodine toxicosis may not be related exclusively to the source of the iodine. Large doses of iodine temporarily inhibit either organic iodine synthesis (Wolff-Chaikoff effect), possibly by binding the active form of iodine as I_3^-, or inhibit thyroglobulin proteolysis with reduction in hormone secretion. Both effects may contribute to the stimulation of release of TSH from the pituitary gland and to an increase in serum concentration of TSH and hypertrophy of the thyroid gland. However, the thyroid gland adapts to excess iodine by decreasing its transport into the cell. The mechanisms for the Wolff-Chaikoff effect involve both iodide transport and iodination reactions, possibly through an inhibition of the expression of sodium-iodine symport (NIS) and thyroid peroxidase. This reaction is mediated by iodide or an iodinated metabolic intermediate (Eng et al., 1999). Iodine may escape when iodide transport into the thyroid gland is either depressed to release it from inhibition of thyroid peroxidase or by other biochemical processes in the production of iodothyronines (Saller et al., 1998). Several chemical inhibitors of iodine thyroid metabolism have been identified (ATSDR, 2004).

The mechanism by which the iodide suppresses iodination and thyroid hormone release appears to involve inhibition of adenyl cylase. The stimulatory actions of TSH on the thyroid gland, which include increased iodide transport and increased iodination of thyroglobulin production and release of T_4 and T_3, occur in response to an increase in intracellular cAMP levels. Iodide inhibits adenylate cyclase in thyroid follicle cells and decreases the TSH-induced rise in intracellular cAMP. However, inhibitors of the iodination reaction, such as prophylthiouracil, can prevent the effects of iodide on adenylate cyclase. This indicates that the active inhibitor may be an endogenous iodinated species, which is produced in the reaction involving thyroid peroxidase.

Excess iodide intake may also be a contributing factor in the development of lymphocytic thyroiditis in rats and mice and autoimmune thyroiditis in humans. Hyperthyroidism has been observed after iodine supplementation of iodine-deficient populations. Chronic iodine deficiency results in thyroid gland proliferation and the development of autonomous nodules that do not respond to serum TSH levels. Under these conditions, excess amounts of iodine cause increased levels and nonregulated thyroid hormone production (Corvilain et al., 1998; Roti and Uberti, 2001).

SOURCES AND BIOAVAILABILITY

Iodine is distributed widely in nature in both organic and inorganic forms in very small amounts. Iodine is present in soil, air, and water and is a constituent of plants and animals used for food. It exists in several chemical forms such as molecular iodine, iodide, iodate, and periodate. It is also subject to oxidation-reduction reactions to yield other forms, as well as microbial alkyation (e.g., methyl iodide). The highest concentration of iodine (0.5 to 2 g/kg) is found in nitrate deposits (saltpetre) of Chile, where it was brought from Antarctic anticyclonic airflows from the Pacific Ocean. Several marine algae (brown, red, and green) cultured in Southeast Asia contain high concentrations of iodine (up to 3 g I/kg). The iodine content of water reflects the iodine content of the rocks and soils of the region. Iodine in water is in the form of iodide and iodate. The iodine content in seawater averages 40– 60 µg/L and unpolluted surface water contains 2– 4 µg/L (NRC, 1974). In certain areas, where water is polluted with municipal wastewater or urban run-off, the concentration may be as high as 8.7 µg I/L (FDA, 1974; WHO, 1988). In Denmark, iodine concentrations ranging from 2 to 139 µg/L have been reported in drinking water (Pedersen et al., 1999; Rasmussen et al., 2000). In surface water, the proportion of iodide to iodate depends on the microbial activity and release of iodine from terrestrial sources (De Luca Rebello et al., 1990). The iodine content of river water may range from 0.1 to 18 µg/L (NRC, 1979), with a relative proportion of iodide to iodate of about 55:45. The average atmospheric iodine concentration ranges between 2–52 ng/m^3 (NRC, 1979; WHO, 1988), with gaseous iodine exceeding particulate iodine by a factor of 2–6 (Whitehead, 1984). The source of atmospheric iodine is mainly from the vaporization of seawater; in this process, iodide is

oxidized by solar light. The concentration of atmospheric iodine is much higher over the ocean. Air pollution, particularly the combustion of gasoline and oil, increases the iodine concentration in air.

The concentration of iodine in common feedstuffs is highly variable. Protein sources of animal origin other than fishery by-products contain relatively high amounts of this element. Oilseed proteins or their concentrates contain 100 to 300 µg/kg and common cereal grains 40 to 100 µg/kg. Concentrations of iodine in forage depend on the iodine content of soil. Many soils of the world are low in iodine and goiter regions have been identified in many areas of all continents. During the glaciation period, iodine was swept away and replaced by crystalline soil that lacks humus to retain sufficient amounts of iodine. Heavy rain and flooding may also leach iodine in certain areas. Soils near the oceans provide iodine to plants. Iodine concentrations in forages from the Great Lakes region and the Northwest United States are generally low enough to cause iodine deficiency in cattle unless iodine is supplemented to diets.

Iodine sources permitted as feed additives include calcium iodate, sodium iodate, potassium iodate, potassium iodide, sodium iodide, EDDI, calcium iodobehenate, cuprous iodide, 3,5-diiodosalicylic acid, pentacalcium orthoperiodate, and thymol iodide (AAFCO, 2004). Iodine occurs in foods largely as inorganic iodide. In this form, it is almost completely absorbed from the gastrointestinal tract (Underwood, 1977). The reported bioavailability of potassium iodide, sodium iodide, EDDI, and pentacalcium orthoperiodate for poultry, cattle, and rats is about 100 percent, and for calcium iodide between 90–95 percent (Ammerman and Miller, 1972; Miller and Ammerman, 1990). Diiodosalicylic acid is a stable compound and well absorbed (80 percent) by rats, but it is not well utilized (~15 percent) by ruminants. Pentacalcium orthoperiodate and EDDI are also more stable and less soluble and are added to mineral blocks for licking by cattle to supply dietary iodine. Limited information is available on the bioavailability of iodine from plant material and animal by-products (Miller and Ammerman, 1990).

Potassium iodate used in salt iodization and as a maturing agent and dough conditioner in bread may appear in animal and human food sources. Some non-food sources also supply iodine (e.g., iodine-containing drugs, antiseptics and disinfectants, and iodized oil used for intramuscular injections). Iodophors used as antiseptic agents for udder washes and milking machines—as well as disinfection of tanks used for handling, storing, and transport of milk—can increase the iodine content of dairy products.

TOXICOSIS

Iodine toxicosis can result from a single large dose of iodine or from repeated exposure to iodine concentrations that exceed animal requirements. Several studies evaluating oral ingestion of high concentrations of iodine are summarized in Table 15-1. Several reviews are available on iodine toxicity in mammals (NRC, 1980; SCF, 2002; McDowell, 2003; ATSDR, 2004); however, reports on iodine toxicity to fish from diet and the aquatic environment are sparse. More than a century ago, in practically all of the areas of Europe affected by goiter, there was a wave of enthusiasm for the use of some form of inorganic iodine to prevent and treat goiter. It appears that indiscriminate use of various iodine preparations was practiced with many cases of toxicosis. The adverse effects of iodine in humans and animals have resulted from the consumption of food supplements (e.g., iodized salt, bread or water), use of iodine-containing pharmaceuticals (e.g., oral, intramuscular injection, applications to skin and mucus membrane), iodine in water supplies and consumption of seaweed or animal meat containing thyroid tissues. Iodine toxicity has been studied in many laboratory animals, dogs, poultry, pigs, cattle, and goats. Significant species differences exist in the tolerance to high levels of iodine because of the differences in basal metabolic rate and iodine metabolism. All species appear to have a wide margin of safety for this element.

Single Dose

The reported oral LD_{50} in rats fed NaI was 3,320 mg I^-/kg BW and the oral LD_{100} for mice fed KI was 1,425 mg I/kg BW (Clayton and Clayton, 1981). The oral LD_{50} of iodate and iodide in mice were 483–698 and 1,550–1,580 mg/kg BW, respectively (Webster et al., 1957). Webster et al. (1966) determined the minimum lethal dose and the maximum allowable dose of potassium iodate for dogs. Oral administration of three single doses of potassium iodate (100, 200, or 250 mg/I kg BW) to dogs caused anorexia and occasional vomiting at the low level; however, the higher levels (200 and 250 mg/kg) caused death preceded by anorexia, prostration, and coma (Webster et al., 1966). Severe retinal degenerative changes have been reported in laboratory animals intravenously administered sodium iodate above 10 mg/kg (Bürgi et al., 2001), but no such retinal changes were observed in guinea pigs given potassium iodate in the drinking water (Highman et al., 1955).

Acute and Chronic

The prolonged administration of large doses of iodine markedly reduces iodine uptake by the thyroid, thus causing antithyroidal or goitrogenic effects in many domestic and experimental animals (Radostits et al., 2000). High levels of iodide inhibit organic-iodine formation and saturate the active-transport mechanism of this ion causing iodide goiter. Significant species differences exist in tolerance to high dietary iodine intakes. In comparison to other trace element toxicities, the dietary level of iodine necessary to cause toxicosis is high relative to the minimal requirement. For ex-

ample, the margin of safety is 1,000 times the minimal requirement of chickens (NRC, 1994) and pigs (Newton and Clawson, 1974; NRC, 1998). A significant amount of data is available on iodine toxicity in humans (Hetzel and Welby, 1997; ATSDR, 2004); however, limited research has been conducted on acute and chronic toxicosis in domestic and laboratory animals since the NRC last report (NRC, 1980). Iodine toxicity data on laboratory animals is considered of limited use for humans (ATSDR, 2004) because of the higher sensitivity of this element to animals and some differences in their dietary requirements and energy metabolism.

Although early work on the minimum toxic iodine intake (as calcium iodate) for calves (80–112 kg) was close to 50 mg/kg diet, some animals showed adverse effects at lower levels (Newton and Clawson, 1974; Newton et al., 1974). Elevated levels of dietary iodine depressed growth rate and feed intake, with the depression being significant for diets containing 50, 100, or 200 mg/kg added iodine. The feeding of either 100 or 200 mg I/kg, and in some cases lower levels, produced toxic signs that included coughing and nasal discharge. All levels of added iodine increased serum iodine, and calves fed 200 mg/kg had significantly lower hemoglobin and serum calcium levels. Calves fed diets with added iodine had heavier adrenal glands, but there was no consistent effect on the weight of the thyroid glands. Based on trends in growth rate and adrenal weights, Newton et al. (1974) concluded that 50 mg/kg diet appeared to be the minimum toxic level for calves. Another study showed that calves (100 kg) tolerated 20 and 40 mg I/kg from EDDI, but daily gains were slightly depressed at 86 and 174 mg/kg diet (Fish and Swanson, 1977). Iodine levels of 71, 140, and 283 mg/kg had no effect, but a level of 435 mg/kg diet depressed daily gains in yearling (320 kg) heifers (Fish and Swanson, 1977). Mild and severe signs of iodine toxicosis were observed in cattle fed 2.2 and 20 mg I/animal for 6 months (Mangkoewidjojo et al., 1980).

Iodine toxicosis has been reported in adult dairy cows with dietary intakes of 50 mg/day (NRC, 1980, 2001). High concentrations of iodine in the diet increase iodine concentration in milk. Humans are more susceptible to iodine thyrotoxicity than cows; therefore, the excess iodine in the dairy cow must be limited due to public health concerns (Hetzel and Welby, 1997). Feeding 170 mg I/day to high-yielding cows for 30 days has resulted in abortions within a 68-day period following treatment (Morrow and Edwards, 1981). Other clinical signs of iodine toxicosis in dairy cows and cattle include excessive nasal and ocular discharge, coughing, nervousness, tachycardia, decrease in appetite, dermatitis and alopecia, exopthalmos, weight loss, decreased milk production, susceptibility to infectious and respiratory diseases, and increased mortality of dams (Olson et al., 1984; Radostits et al., 2000).

Sheep were able to tolerate daily diets of 102 mg I/kg (as KI) and 214 mg I/kg (as EDDI) for 22 days without any adverse effects on growth and feed intake (McCauley et al., 1973). Body weight gains were depressed by daily intakes of 393 mg potassium iodide (300 mg iodine) or 562 mg EDDI (450 mg iodine) per lamb per day. Other signs of iodine toxicosis in sheep include cough, anorexia, hyperthermia, respiratory diseases, and sometimes death (McCauley et al., 1973; Paulikova et al., 2002).

A high incidence of goiter and leg weakness was reported in foals born to mares fed 48–432 mg I/day (Baker and Lindsey, 1968; Drew et al., 1975), although an upper tolerable limit for horses has not been established. Excess iodine caused osteoporosis and increase in serum phosphate and alkaline phophatase levels in thoroughbred horses (Silva et al., 1987).

Pigs and chickens are more tolerant of excess iodine than are cattle. Newton and Clawson (1974) fed levels of iodine ranging from 10 to 1,600 mg/kg diet to growing-finishing pigs and found that the minimum toxic level was between 400 and 800 mg/kg. Growth rate, feed intake, and hemoglobin levels were depressed at 800 and 1,600 mg I/kg, and liver iron levels were significantly depressed at 400 mg/kg diet. A decrease in serum T_3 concentrations was observed in finishing pigs fed 10 mg I/kg diet (Schöne and Leiterer, 1999). During lactation and the last 30 days of gestation, neither 1,500 nor 2,500 mg I/kg diet affected reproduction in sows (Arrington et al., 1965).

Early work on iodine toxicity in chickens showed that their performance was not affected by feeding 500 mg I/kg in the form of potassium iodide for up to 6 weeks (Wilgus et al., 1953). More recent studies on oral iodine toxicity of potassium iodide in chickens fed >900 mg I/kg showed depressed growth and neurological clinical signs (Baker et al., 2003); however, severe growth depression in chickens fed 1,000–1,500 mg I/kg was totally reversed by dietary supplementation of 50–100 mg Br/kg as sodium bromide. In chicken and turkey laying hens, typical iodine toxicosis signs observed with high intake of dietary iodine include decreased egg production, egg size, and hatchability; low fertility; enlarged thyroids in hatching chicks; and wiry down (Perdomo et al., 1966; Arrington et al., 1967; Marcilese et al., 1968; Christensen et al., 1991; Christensen and Ort, 1991). Turkey breeder hens are more sensitive to high dietary iodine than chickens (Christensen and Ort, 1991). When laying chickens were fed 625 to 5,000 mg I/kg, egg production varied inversely with the iodine level and ceased with dietary levels of 5,000 mg/kg (Arrington et al., 1967). Early embryonic death, reduced hatchability, and delayed hatching were also observed; however, egg production commenced within 1 week after cessation of feeding iodine. Diets containing 5,000 mg I/kg caused an increase in serum calcium level, as well as a marked reduction in egg production and in the size of ovaries and oviducts of chickens (Roland et al., 1977).

Marked differences exist between rabbits, hamsters, and rats in their tolerance to high intakes of iodine. Mortality was high in the offspring of rabbits fed 250 mg I/kg in late gestation. However, the feeding of diets containing

2,500 mg/kg iodine to hamsters during gestation did not affect death loss in the offspring (Arrington et al., 1965). The survival of the offspring of rats was not affected by feeding gestating female rats 500 mg I/kg, but high mortality of the young was found when the gestation diets contained 1,000 mg I/kg (Ammerman et al., 1964). No gross lesions or abnormalities in mice or guinea pigs that received 5,000 mg/L of potassium iodate in their drinking water for several weeks were observed (Webster et al., 1959). However, mice showed hemosiderin deposits in the renal convoluted tubules.

High concentrations of dietary iodine (up to 14 mg/kg of diet) in commercial and experimental feed have been shown to cause a transient decrease in serum thyroid hormone levels in cats (Tarttelin et al., 1992; Kyle et al., 1994; Tarttelin and Ford, 1994). Excessive dietary iodine intake may be linked to feline hyperthyroidism; however, epidemiologic studies have not identified a clear relationship between dietary iodine and adenomas or adenocarcinomas of the thyroid gland in cat (Scarlett, 1994). The minimum lethal dose for potassium iodate administered orally to dogs was estimated to be 200 to 250 mg/kg (Webster et al., 1966). In puppies, high levels of iodine (5.6 mg/kg of diet) caused a decrease in uptake of radioiodine by the thyroid gland (Castillo et al., 2001a), and an excessive amount of iodine present in some commercial feed caused impairment of thyroid function and hypothyroidism (Castillo et al., 2001b).

In humans, an iodine intake of 2 mg/day is considered harmful (Hetzel and Welby, 1997). Oral iodine toxicosis has occurred in certain areas of China and Japan in populations exposed to chronic high intakes (50–80 mg/day) of iodine (Wolff, 1969; NRC, 2001). Inhabitants of coastal regions that consume large amounts of seaweed developed goiter from high iodine intake (Hetzel and Welby, 1997). The release of at least 15 different radioactive isotopes of iodine occurs as gases when uranium or plutonium atoms are split resulting in typical signs of iodine toxicosis in animals and humans (ATSDR, 2004). In 1986, the Chernobyl nuclear accident in the Soviet Union caused excess iodine levels in milk from dairy cows grazing on contaminated pastureland. Children drinking contaminated milk showed iodine toxicity (Kazakov et al., 1992; Nauman and Wolff, 1993).

Oral toxicity of iodine in fish has not been studied, but high amount of iodine in water is toxic. An exposure of 8 mg I/L was lethal to mullet (Gozlan, 1968). Acute toxicity of iodine to channel fish varied with the exposure period (LeValley, 1982). Concentrations as low as 0.72 mg I/L resulted in 100 percent mortality during a 24-hour exposure period and 7.2 mg I/L within an hour. Mortality was caused due to gill damage and asphyxiation.

Factors Influencing Toxicity

Natural or synthetic chemicals distributed in plants and plant products used for animal feeds or for forage possess goitrogenic properties and they affect iodine bioavailability and metabolism (Talbot et al., 1976). Although the effects of goitrogens on iodine toxicity are not clearly defined, changes in thyroid metabolism associated with high intake of these compounds may influence iodine toxicity as well as concentration of iodine in tissue and animal products. There are two types of goitrogens: thiocyanates and goitrin (L-5-vinyl-2-thiooxazolidone). Among the naturally occurring goitrogens, the best characterized are the glucosinolate derivatives isolated from the *Brassica* species. Rapeseed, kale, cabbage, and turnip contain high concentrations of goitrogens. Thiocyanates inhibit the uptake of iodine in the thyroid, but their action is reversible by iodine supplementation. However, goitrin inhibits thyroid hormone synthesis, probably through inhibition of thyroid peroxidase, and this effect is irreversible by iodine supplements (Talbot et al., 1976). Iodine supplementation alleviates the hypothyroidism and the adverse effects on performance caused by feeding rapeseed meal to swine, and the efficacy of iodine can be improved by dietary copper supplementation (Lüdke and Schöne, 1988; Schöne et al., 1988). Glucosinolates are inactivated by treatment with copper sulphate solution. Moreover, extrusion of rapeseed with barley eliminates its hypothyroidism effects (Maskell et al., 1988). Other plant products such as soybean, cottonseed, linseed meals, lentils, and peanuts also contain goitrogenic substances (Matovinovic, 1983; Fenwick et al., 2000).

Thiocyanates, perchlorates, and rubidium salts are known to interfere with iodine uptake by the thyroid, and high levels of arsenic can induce goiter in rats (Underwood, 1977). Bromide, fluoride, cobalt, manganese, and nitrate may also inhibit normal iodine uptake (Talbot et al., 1976). Excess calcium intake has been shown to have an antithyroid effect (Taylor, 1954). Adequate amounts of both iodine and selenium are necessary for optimum thyroid metabolism (Hotz et al., 1997). Selenium deficiency can influence iodine bioavailability. High iodine intake when selenium is deficient in diets of rats causes thyroid tissue damage as a result of low thyroidal GSH-Px activity during thyroid stimulation (Hotz et al., 1997).

However, a moderately low selenium intake normalized the circulating T_4 concentration in the presence of iodine deficiency. Protein-calorie malnutrition may reduce iodine absorption and thyroid iodine clearance and radiodide uptake (Ingenbleek and Beckers, 1973, 1978). Excess dietary iodine intake has been also shown to inhibit thyroid activity, presumably by blocking the uptake of iodine by the thyroid (Baker and Lindsey, 1968; Nagasaki, 1974; Newton and Clawson, 1974; Fish and Swanson, 1983; Kaneko, 1989). Certain food coloring agents (e.g., erythrosine), pharmaceutical products, water purification tablets, and disinfectants contain high amounts of iodine and are likely to increase total intake of this element by the animal.

Few studies have been conducted to compare the relative toxicity of various iodine compounds. No differences in toxicity of sodium and potassium iodide to rats have been reported

(Arrington et al., 1965). Iodate salts were more toxic than iodide salts in mice when administered orally, intraperitoneally, or intravenously (Webster et al., 1957). Although absorption of EDDI and sodium or potassium iodide does not differ widely in dairy cows, EDDI was retained in most tissues longer than the iodide salts (Miller and Swanson, 1973). The presence of food in the stomach greatly decreased the acute toxic effects of orally administered iodine to mice and guinea pigs, and small or intermittent doses of potassium iodate in drinking water were better tolerated by these animals than a single dose given by stomach tube (Webster et al., 1959).

TISSUE LEVELS

Iodine concentration in animal products, particularly milk and eggs, is directly influenced by iodine intake from feed and water. In mammals, approximately 80 percent of total iodine is distributed in the thyroid gland, and the remaining amount is found in other soft tissues including muscle, liver, kidney, and heart (Downer et al., 1981; Kaufmann and Rambeck, 1998). The highest iodine concentrations are in fish, shellfish, marine algae, and seaweed. Representative iodine concentrations in tissues from various animals fed normal or high levels of iodine are shown in Table 15-2.

Although iodine is a natural constituent of milk, the concentration is influenced by iodine intake, stage of lactation, season, level of milk production, and the use of iodine-containing disinfectants to maintain udder and dairy equipment. Average iodine concentrations in milk of cows fed 0, 40, 81, 162, 405, and 810 mg I/kg diet as EDDI was 8, 362, 895, 1,559, 2,036, and 2,383 μg/L (Miller et al., 1975). The iodine content of milk varies seasonally, with higher concentrations present in winter (80– 930 μg/L) than in summer (40–200 μg/L) (Varo et al., 1982; Dellavalle and Barbano, 1984; Pennington, 1990; Lee et al., 1994; Larsen et al., 1999; MAFF, 2000). The colostrum of dairy cows is much higher in iodine than milk, and in late lactation there is a decrease in concentration. A mean value of 264 ± 100 μg/L for colostrom compared to 98 ± 82 μg/L for milk was reported (Kirchgessner, 1959). Iodine content of milk is reduced below normal in goiter regions (Feng et al., 1999). Dipping of teats in iodophor after milking increased the iodine levels from 89 and 94 μg/L to 127 and 152 μg/L (Funke et al., 1975). Average iodine concentration reported in sheep and goat milk were 105.5 and 63.0 μg/L, respectively; however, the concentrations were much higher when these animals had access to mineral licks containing the element (Trávniček and Kursa, 2001).

The early work of Fisher and Carr (1974) showed that the iodine content of beef, pork, and mutton was low (27 to 45 μg/kg). A wide range of iodine concentration ranging from 2 to 580 μg/kg has been reported in animal meat products from Europe and the United States (Hemken et al., 1972; Hemken, 1979; Varo et al., 1982; Wenlock et al., 1982; Dunn, 1993; Lee et al., 1994; Jahreis et al., 2001). Generally, hen eggs contain 4–10 μg I/kg, which is located mainly in the yolk. Feeding large amounts of iodine supplements and seaweed to laying hens increases the amount in eggs by 100-fold or more (Underwood and Suttle, 1999). When high concentrations of iodine (100–150 μg/day) were fed to laying hens, the iodine concentration reached 50–120 μg/kg (Marcilese et al., 1968). Iodine concentrations in fish muscle vary widely (30 to 3,500 μg/kg) between species as well as within species, and the concentration is generally higher in marine fish (Varo et al., 1982; Lall, 1994; Julshamn et al., 2001; Karl et al., 2001).

MAXIMUM TOLERABLE LEVELS

The tolerance levels of domestic animals are based on clinical signs of iodine toxicosis, and most animals tolerate high dietary iodine concentrations relative to iodine requirements. Iodine toxicity in domestic animals has not been properly quantified. Based on available literature, maximum tolerable levels (mg I/kg diet) suggested for iodine are cattle, 50; sheep, 50; swine, 400; chicken and turkey, 300. These values are unchanged from those reported by NRC (1980) since recent data do not support a change in these recommendations. Although cattle can tolerate 50 mg/kg iodine, this level in the diet may result in undesirably high levels of iodine in the milk. Among the species studied, horses are most susceptible to iodine toxicity. High levels of iodine, for example of kelp consumption by horses, cause goiter in the offspring of mares. Assuming that mares consume 10 kg air dry diet daily, the maximum tolerable level for iodine in horse diets is 5 mg/kg.

Laboratory animal models have been useful to study the mechanism of iodine toxicity. However, the major toxicokinetics data on oral exposure have been obtained in experimental and clinical studies of humans using biomarkers (e.g., urinary excretion, serum TSH, thyroid scintillation scan, and antibodies) (ATSDR, 2004). Thus, unlike other minerals, most toxicological studies conducted with laboratory animals lack toxicokinetic data, and results do not distinguish specific effects of an overdose of iodine or iodate supplements (Bürgi et al., 2001). Mice showed marked toxic effects of iodate administered in water for 4 weeks such as hemolysis and renal damages, which occurred from 300 mg I/kg BW upward with a no-observable-effect level (NOEL) at approximately 120 mg/kg BW. However, guinea pigs exposed via the same route tolerated 300 mg/kg BW without apparent effect. When given intravenously to rats, doses above 10 mg I/kg BW were highly toxic to retina (Bürgi et al., 2001).

HUMAN HEALTH

Iodine toxicity in humans is well documented and the subject of several comprehensive reviews by national, regional, and international organizations. Estimates of dietary intake of iodine have ranged from <50 μg/day in

iodine-deficient regions to >10 mg/day in populations that regularly consume seaweeds high in iodine. The NRC's Recommended Dietary Allowance (RDA) for iodine is 150 µg/day (2.1 µg/kg/day for a 70-kg adult), with additional allowances for 70 and 140 µg/day during pregnancy and lactation, respectively (NRC, 2001). An upper tolerable level of iodine intake for an adult human is 1,100 µg/day (NRC, 2001). A provisional maximum tolerable daily intake of 1 mg/day (equivalent to 17 µg/kg BW) from all sources has been recommended by WHO (1988). In countries with long-standing IDD, the intake should not exceed 500 µg I/day to prevent hyperthyroidism (AFSSA, 2001). A minimal risk level (MRL) of 0.01 mg/kg/day has been derived for acute duration of oral exposure (1–14 days) to iodine, which is based on a NOAEL of 0.024 mg/kg/day in healthy adult males (ATSDR, 2004). This NOAEL value derived from human adults may also be applicable to children and the elderly. Genotoxicity and carcinogenicity data for iodine are limited and are nonexistent for iodate (Bürgi et al., 2001).

FUTURE RESEARCH NEEDS

The toxicity of iodine in most domestic and aquatic animals should be better defined. There is a need to determine the mechanisms involved in iodine metabolism, particularly the interaction of iodine with other nutrients such as selenium, bromine, and iron. Although the toxicity of iodine for humans has been extensively studied, there is a need for more research to better establish the basis for beneficial or essential action of iodine, and the intake below which this action is compromised. This would be helpful in setting the lower limits of toxicity standards for iodine and would allow better use of animal by-products in human nutrition.

SUMMARY

Iodine is an essential element for all animals. Its only known function in the body is in the synthesis of the thyroid hormones. Sodium iodide, potassium iodide, and ethylenediamine dihydroiodide are well utilized as a source of iodine. Limited information is available on the bioavailability of this element from plant and animal by-products. Species differ widely in their susceptibility to iodine toxicity, but all animals can tolerate iodine levels far in excess of their requirements for this element. Feeding excessive levels of iodine has resulted in decreased egg production in hens, inhibition of lactation in rats, decreased hemoglobin levels in pigs, and goiter and reduced thyroid hormone synthesis in several species. Increasing the iodine intake of lactating cows and laying hens increases the levels of iodine in milk and eggs.

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TABLE 15-1 Effects of Iodine Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Cats	18	2.2 kg	5.6 mg/kg	KI	45 d	Diet	Hypothyroidism, histological changes in bone metabolism	Castillo et al., 2001b
Dogs	4	8–16 kg	59 mg/kg BW	KIO_3	Several months	Oral, milk	Some dogs vomited	Webster et al., 1966
	3	10–15 kg	59 mg/kg BW 118 mg/kg BW 148 mg/kg BW	KIO_3	Single dose	Capsule	No effect 1 of 3 died within 1 day All died within 1 wk	
Mice	80–140	15–20 g	698 mg/kg BW	KIO_3		6% KIO_3 soln (not fasted)		Webster et al., 1957
			483 mg/kg BW	KIO_3		6% KIO_3 soln (fasted)	LD_{50}	
			1,580 mg/kg BW	KI		6% KI soln (not fasted)	LD_{50}	
	130	15–20 g	1,550 mg/kg BW	KI		6% KI soln (fasted)	LD_{50}	
Rabbits	9	Mature	250 mg/kg	NaI or KI	2 d before parturition	Diet	Only 30% survival in young to 3 d	Arrington et al., 1965
	19	Mature	500 mg/kg	NaI or KI	5 d before parturition		Only 3% survival in young to 3 d	
Rats	9	Mature	500 mg/kg	KI	12 wk before mating	Diet	Slight increase in mortality of young	Ammerman et al., 1964
			1,000 mg/kg 1,500 mg/kg 2,000 mg/kg				66% litter survival to 5 d 36% litter survival to 5 d 16% litter survival to 5 d	
Guinea pigs	12	250 g		KIO_3	28 d KIO_3 soln	0.05%	No adverse effect	Webster et al., 1959
					28 d KIO_3 soln	0.5%	No adverse effect	
Hamsters	31	Mature	250 mg/kg	KI	12 d	Diet	Slight reduction in feed intake and decreased weaning weight of offspring	Arrington et al., 1965
Chickens	10	Young	500 mg/kg	Iodinated casein or KI	6 wk	Diet	No adverse effect on growth	Wilgus et al., 1953

Chickens	5	27 wk	625 mg/kg	KI	6 wk	Diet	Reduced egg production and hatchability	Arrington et al., 1967
			2,500 mg/kg				Increased thyroid weights in chicks	
		Mature	625–2,500 mg/kg				Similar to pullets but less pronounced	
Chickens	15	27 wk	5,000 mg/kg	KI	10 d	Diet	Reduced egg production and serum calcium	Roland et al., 1977
Chickens	16	Young	600 mg/kg	EDDI and KI	13 d	Diet	Reduced feed intake	Baker et al., 2003
	16		1,000–1,500 mg/kg	EDDI and KI	13 d	Diet	Reduced growth and feed intake Neurological clinical signs	
Turkeys	8	Brooder hens	35 and 350 mg/kg	KI	20 wk	Diet	Reduced egg production, egg size, hatchability	Christensen and Ort, 1991
Cattle	8	83 kg	10 mg/kg	$Ca(IO_3)_2$	144 d	Diet	No adverse effect	Newton et al., 1974
			100 mg/kg				Reduced gains, feed intake, and hemoglobin	
			200 mg/kg				Reduced gains and feed intake; coughing and nasal discharge	
	8	112 kg		25 mg/kg	112 d	Diet	Reduced gains and feed intake; enlarged adrenals	
			50 mg/kg				Coughing and nasal discharge	
			100 mg/kg				Coughing and nasal discharge	
Cattle	6	100 kg	20 mg/kg	EDDI	12 wk	Diet	No adverse effect	Fish and Swanson, 1977
			42 mg/kg				No adverse effect	
			86 mg/kg				No adverse effect on growth; reduced feed intake	
			174 mg/kg				Slightly reduced growth and feed intake	
Cattle	6	Calf	250–1,250 mg/d	EDDI	6 mo	Diet	Mucopurulent nasal discharge, seromucous ocular discharge, hypersalivation, thick scaly skin, rough hair, alopecia, pneumorda with the 70 apparently dose-related	Mangkoewidjojo et al., 1980
Cattle	4		1,250 mg/d	EDDI	6 mo	Diet	Decrease in persistence of antibody titers to Brucella and Leptospira organisms after vaccination, decreased lymphocyte mitotic activity, phagocytosis by WBC, and WBC counts	Haggard et al., 1980
Cattle	6		0.625–5.0 mg/kg BW	EDDI		Diet	Nasal discharge, occasional lacrimation, moderate coughing	Fish and Swanson, 1982
Cows, dairy	2	Mature	40 mg/d	EDDI	7 wk	Diet	No adverse effect	Miller and Swanson, 1973
			80 mg/d	KI			No adverse effect; milk contained 0.38 mg/kg I	
			80 mg/d	EDDI			No adverse effect; milk contained 0.36 mg/kg I	
			160 mg/d				No adverse effect; milk contained 1.6 mg/kg I	
			400 mg/d				No adverse effect; milk contained 2 mg/kg I	
			800 mg/d				No toxic signs; milk contained 2.4 mg/kg I	

continued

TABLE 15-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Cows, dairy	5	600 kg	47 mg/kg	EDDI	12 wk	Diet	No adverse effect; milk contained 2.4 mg/kg I	Fish and Swanson, 1977
			93 mg/kg				No adverse effect; milk contained 2.8 mg/kg I	
			198 mg/kg				No adverse effect; milk contained 5.0 mg/kg I	
			314 mg/kg				No adverse effect; milk contained 6.4 mg/kg I	
Cows, dairy	45		164 mg	EDDI	3 wk	Diet	Lacrimation, coryza, conjunctivitis, coughing, hair loss, scaly dermatitis, exophthalmos	Hillman and Curtis, 1980
Cows, dairy	20		440 mg	EDDI	1 mo		Milk production reduced by 15%, coughing	Olson et al., 1984
	22		300–600 mg	EDDI	4 yr		Coughing, naso-ocular discharge, salivation, dry coat	
	2		600 mg	EDDI	7 yr		Pneumonia in calves with 50% mortality, decreased milk production	
	13		300–600 mg	EDDI	2 yr		Pneumonia in calves, decreased milk production, naso-ocular discharge	
	7		>68 mg	EDDI	1 yr		Cows with injured hocks not responding to therapy	
	7		250–785 mg	EDDI	3 yr		Naso-ocular discharge, increased calving interval	
Pigs	8	17 kg	400 mg/kg	$Ca(IO_3)_2$	97 d	Diet	No adverse effect on performance; increased thyroid weight and reduced liver iron	Newton and Clawson, 1974
			800 mg/kg				Decreased gains and feed conversion	
Pigs	2	Mature	1,500 mg/kg	KI	30 d	Diet	No adverse effect on reproduction	Arrington et al., 1965
	3		2,500 mg/kg		30 d		No adverse effect on reproduction	
Horses	165	Mature	48 to 55 mg I/mare	Iodized salt		Diet	3% incidence of goiter in foals	Baker and Lindsey, 1968
	60		56 to 69 mg I/mare	Kelp	Several months		10% incidence of goiter in foals	
	6		288 to 432 mg I/mare	Kelp	Several months		50% incidence of goiter in foals	
Sheep	4	30 kg	75 mg/d	EDDI	22 d	Capsule	No adverse effect	McCauley et al., 1973
			150 mg/d				No adverse effect	
			300 mg/d				Slightly reduced daily gains	
			450 mg/d				Anorexia and reduced gains	
			150 mg/d	KI			Slightly reduced gains	
			300 mg/d				Reduced gains and feed intake	
			450 mg/d				Reduced gains and feed intake	

Lambs	4	30 kg	94–785 mg	EDDI and KI	3 wk	Diet	Lethargy, decreased food intake, retarded growth, hyperthermia, coughing, at larger doses death due to bronchopneumonia	McCauley et al., 1973
Fish, catfish	60	.02–7.3g	0.72–7.22 mg/L	KI	24 h	Water	100% mortality	LeValley, 1982

[a]Number of animals per treatment group.
[b]Quantity expressed as mg/kg or as mg per day in diet, or as mg/kg of body weight (BW). SI conversion: 1 mg iodine equals 7.88 μmoles iodine.

TABLE 15-2 Iodine Concentrations in Fluids and Tissues of Animals

Animal	Quantity	Source	Duration	Route	Milk (μg/100 ml)	Egg Yolk (μg/g)	Reference
Chickens	Control (0.5 mg I/kg)		45 d	Diet		0.18	Ryś et al., 1997
	+ 2.0 mg I/kg	Kelp				0.87	
	+ 4.4 mg I/kg	Kelp				2.00	
	+ 2.7 mg I/kg	CaI_2				1.36	
	+ 7.2 mg I/kg	CaI_2				0.93	
	Control		5 d after	Diet		0.13	
	+ 10 mg I/kg live wt	KI	single dose	Diet		12.86	
	+ 10 mg I/kg live wt	Iodized oil		Diet		14.04	
	+ 10 mg I/kg live wt	Iodized oil		Intramuscular injection		0.44	
	Control		21 d after	Diet		0.05	
	+ 10 mg I/kg live wt	KI	single dose	Diet		0.09	
	+ 10 mg I/kg live wt	Iodized oil		Diet		0.01	
	+ 10 mg I/kg live wt	Iodized oil		Intramuscular injection		0.99	
Cows, dairy	Control	KI	5 wk	Diet	1.8		Feng et al., 1999
	+ 10 mg I/kg				15.7		
	+ 20 mg I/kg				23.6		
	+ 30 mg I/kg				30.1		
	+ 40 mg I/kg				36.2		
Cows, dairy Spring	Control (5.8 mg I/cow/d)	EDDI	42 d	Diet	20.6		Brzóska et al., 2000
	+ 28.3 mg I/cow/d						
	+ 43.3 mg I/cow/d				91.4		
	+ 58.3 mg I/cow/d				151.1		
					217.0		
Cows, dairy Summer	Control (5.8 mg I/cow/d)						
	+ 28.3 mg I/cow/d	EDDI	47 d	Diet	15.3		
	+ 43.3 mg I/cow/d						
	+ 58.3 mg I/cow/d				18.6		
					26.8		
Cows, dairy Autumn	Control (5.8 mg I/cow/d)				14.6		
	+ 28.3 mg I/cow/d						
	+ 43.3 mg I/cow/d	EDDI	32 d	Diet	83.1		
	+ 58.3 mg I/cow/d						
					112.9		
					206.3		
					239.2		
Cows, dairy	Control		5 d	Diet			Herzig et al., 2001
	+ 3.8 mg I/kg	KI			20 ± 5.2		
	+ 3.8 mg I/kg	EDDI			50 ± 15.2		
	+ 7.6 mg I/kg	KI			80 ± 3.0		
	+ 7.6 mg I/kg	EDDI			64 ± 13.9		
	+ 11.5 mg I/kg	KI			173 ± 39.3		
	+ 11.5 mg I/kg	EDDI			121 ± 52.8		
Goats	20.5–162.4 μg/kg DM	Hay		Diet	19.3		Trávniček and Kursa, 2001
	+ 3.05 μg I/kg	Hay + iodized salt			142.2		
Sheep	20.5–162.4 μg/kg DM	Hay		Diet	105.5		
	+ 3.05 μg/kg	Hay + mineral lick			243.3		

16

Iron

INTRODUCTION

The atomic symbol for iron is Fe, which is derived from the Latin *ferrum*. Iron (atomic number, 26; atomic weight, 55.847) is a silvery, lustrous metal. There are 14 known isotopes of iron. Common iron consists of a mixture of four isotopes.

The melting point of iron is 1,535°C, boiling point is 2,750°C, specific gravity is 7.874 (20°C), with a valence of 2, 3, 4, or 6. Pure iron is chemically reactive and corrodes rapidly, especially in moist air or at elevated temperatures. Four allotropic forms, or ferrites, are known: α, β, γ, and δ, with transition points at 770, 928, and 1,530°C. The α form is magnetic, but when iron is transformed into the β form, the magnetism disappears, although the lattice remains unchanged.

Iron is relatively abundant in the universe. It is found in the sun and many types of stars in considerable quantity. The core of the earth is thought to be largely composed of iron with about 10 percent occluded hydrogen. On a weight basis, the metal makes up 5 percent of the Earth's crust and is the fourth most abundant element. Pure iron is rarely encountered in commerce; it is usually alloyed with carbon or other metals. Pig iron is an alloy containing about 3 percent carbon with various amounts of sulfur, silicon, manganese, and phosphorus. Iron is hard, brittle, and fairly fusible and is used to produce other alloys, including steel. Wrought iron, which contains only a few tenths of a percentage of carbon, is tough, malleable, and less fusible. The pure metal is very reactive chemically and rapidly corrodes, especially in moist air or at elevated temperatures.

Iron is one of the most useful metals in both technology and biology. Iron compounds are involved in numerous oxidation-reduction reactions, beginning with the reduction of hydrogen and its incorporation into carbohydrates during photosynthesis in the presence of ferredoxins (Fairbanks, 1994). Iron is a vital constituent of plant and animal life and works as an oxygen carrier in hemoglobin.

The ancient Greeks, Egyptians, and Hindus prescribed iron as a treatment for general weakness, diarrhea, and constipation. The role of iron in blood formation became apparent in the 17th century when it was shown that iron salts were of value in treating chlorosis, now known as iron-deficiency anemia, in young women. The first clinical description of iron overload was reported in 1871 (Fairbanks, 1994).

ESSENTIALITY

Iron has been recognized as an essential nutrient for more than 100 years when it was found to be present in all body cells. The largest portion is found as a necessary component of the protein molecules hemoglobin and myoglobin. The major role of iron in both hemoglobin and myoglobin are similar. Hemoglobin is found in red blood cells (erythrocytes) and transports oxygen from the lungs to the tissues, whereas myoglobin binds oxygen for immediate use by muscle cells (Morris, 1987).

Iron is also found in plasma (transferrin), milk (lactoferrin), placenta (uteroferrin), and liver (ferritin and hemosiderin) (Underwood, 1977; Underwood and Suttle, 1999). Species differences in total body iron concentrations occur in the newborn, but become much less pronounced in the adult. For example, the pig has relatively little iron in its body at birth (29 mg/kg) because it is born with low liver iron stores and has no polycythemia (venous hematocrit of greater than 65 percent) of the newborn as does the human infant. The newborn rabbit, by contrast, has an exceptionally high total body iron concentration (135 mg/kg) because of its high liver stores (Underwood, 1977). The iron concentration of skeletal muscle (on a fresh tissue basis) in adult dogs, rabbits, hens, pigs, and cattle ranges from 12 to 16 mg/kg (Georgievskii et al., 1979).

Iron plays an important role not only in oxygen delivery to the tissues, but also as a cofactor with several enzymes involved in energy metabolism and thermoregulation. Mitochondrial iron enzymes are essential for oxidative production of cellular energy. Aerobic metabolism depends on iron because of its role in the functional groups of most of the enzymes of the Krebs cycle, as an electron carrier in cytochromes, and as a means of oxygen and carbon dioxide transport in hemoglobin (Fairbanks, 1994).

Iron deficiency is the most commonly known potential mineral deficiency in humans (Baynes, 1994). It is estimated that about half of the world's population suffers from iron deficiency. Iron deficiency is of limited practical significance in most livestock, but examples of situations in which animals are vulnerable to iron deficiency are newborn pigs, calves raised for veal, copper-supplemented pigs, and animals with parasitic infestations (Underwood and Suttle, 1999). Iron is required in growing, laying, and lactating animals at between 50 and 100 mg/kg, depending on age and species.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

The most common methods currently used for the analysis of iron in biological samples are flame AAS, graphite furnace AAS, ICP-AES, ICP-MS, and x-ray fluorescence spectroscopy. The method of choice for the analysis of iron at very low concentrations is graphite furnace AAS, but AAS and ICP-AES are acceptable for measuring usual or toxic levels. Limits of detection for iron using AAS are on the order of µg/mL (mg/kg) and for graphite furnace AAS are generally in the low ng/mL (ppb) range. Sample preparation usually consists of wet ashing the specimen with strong acid and heat, and redissolving the residue in dilute acid prior to analysis so that all iron species are converted quantitatively to the same iron compound.

To ensure adequate quality control, a reference material should be used that closely matches the matrix type and concentration of the experimental samples to be analyzed. Iron contamination during sample collecting and processing must be avoided. All glass equipment involved in blood collection and storage should be made of iron-free silicate glass and acid washed. Because of the high levels of iron in the atmosphere, samples can become contaminated during the analytical process. It is essential, therefore, to complete all analyses keeping the sample covered or in a hood. Blanks should be included in all analyses.

REGULATION AND METABOLISM

Absorption

Gastrointestinal absorption of iron occurs primarily in the duodenum, and the proportion of the dose that is absorbed decreases as the dose increases. It is likely that iron absorption occurs by the divalent metal transporter-1, which primarily transports non-heme iron (Bressler et al., 2004). The rate-limiting processes in iron absorption are associated with transfer of iron from enterocytes to the blood rather than transport across the apical membrane of the enterocytes. Because of the limited capacity of the body to excrete iron, iron homeostasis is maintained primarily by adjusting iron absorption to bodily needs. The absorption of iron is affected by (1) the age, iron status, and state of health of the animal; (2) the conditions within the gastrointestinal tract; (3) the amount and chemical form of the iron ingested; and (4) the amounts and proportions of various other components of the diet, both organic and inorganic (Underwood, 1977).

Soluble forms of iron are absorbed better than insoluble forms (Skikne and Baynes, 1994). The proportion of a dose of highly soluble iron absorbed by adults may vary from 2 to 20 percent when ingested (Layrisse and Martinez-Torres, 1971). Phytate, milk proteins, and soy proteins reduce iron absorption. Young animals absorb iron more efficiently than do older animals. Pregnancy, lactation, or a deficiency of iron also increases the efficiency of absorption.

Metabolic Interactions

Once absorbed, iron enters the blood where it is taken up by transferrin (Baker and Morgan, 1994). Iron in circulation is primarily bound to transferrin. Transferrin distributes iron throughout the body to wherever it is needed, mostly to erythrocyte precursors in the bone marrow for new hemoglobin synthesis. Cell membranes contain a protein called transferrin receptor. On the cell membrane, diferric transferrin binds to transferrin receptors, and then the iron-transferrin-transferrin receptor complex is internalized by endocytosis. The cellular regulation of iron is dependent on the effect of iron, or iron lack, in stimulating the synthesis of ferritin or of transferrin receptors, respectively (Fairbanks, 1994). This regulatory mechanism involves the effect of iron on iron-responsive elements (IRE) in the untranslated regions of transferrin receptor mRNA and ferritin mRNA. An iron-responsive element binding protein (IRE-BP) appears to be involved in the activation or repression of ferritin and also of the transferrin receptor gene.

Much of the absorbed iron is transferred to the bone marrow where the iron-transferrin receptor complex enters erythroblasts by endocytosis. Iron is released into the cytosol and apotransferrin is returned to circulation. Within the cytosol, iron either is transported to mitochondria to be incorporated into heme or taken up by ferritin within siderosomes (Fairbanks, 1994). Within the mitochondria, iron is inserted into protoporphyrin, forming heme, a reaction catalyzed by heme synthetase (ferrochelatase). Heme inhibits the release of iron from transferrin, an important feedback mechanism for adjusting the iron supply to the rate of hemoglobin synthesis. Approximately 20 to 25 mg of iron is used daily for hemoglobin synthesis in humans.

In addition to the synthesis of heme, iron is also required for the synthesis of iron-containing enzymes (Fairbanks, 1994). Examples of iron-containing enzymes are cytochrome *c*, aconitase, cytochrome oxidase, phenylalanine hydroxylase, and α-glycerophosphate dehydrogenase. These enzymes are sensitive to iron depletion; the degree of loss varies from enzyme to enzyme and from tissue to tissue. Cytochrome *c* and aconitase are readily depleted during iron deficiency, as are the iron-sulfur enzymes in muscle mitochondria.

Iron in excess of need is stored intracellularly as ferritin and hemosiderin, principally in the macrophage system of the liver, spleen, and bone marrow (Fairbanks, 1994). Ferritin is the basic storage form; hemosiderin appears to be aggregated ferritin stripped of its protein component. Mobilization of iron from iron stores requires the presence of the Cu-containing enzyme of the plasma ceruloplasmin (ferroxidase I) (Chung et al., 2004).

Daily excretion of iron is limited, and losses from the body are relatively small except during hemorrhage or menstruation. Although iron is released from breakdown of erythrocytes and is secreted in bile, most of this iron is reabsorbed and used to form new hemoglobin. The primary routes of iron excretion are via feces and urine, and there are additional losses in sweat, hair, and nails (Georgievskii et al., 1979; McDowell, 2003).

Mechanism of Toxicity

Organ damage arising from iron overload is remarkable for the range of tissues affected and for the slow and insidious onset of organ dysfunction with chronic toxicity (Eaton and Qian, 2002). The organs affected most by iron overload are the liver, heart, and pancreatic beta cells; all are tissues with highly active mitochondria, which generate activated oxygen species capable of causing synergistic toxicity with intracellular iron.

One hypothesis concerning the etiology of cell and organ damage arising from iron overload is that excess iron selectively targets mitochondria and, perhaps, the mitochondrial genome (Eaton and Qian, 2002). The leak of electrons from mitochondria accounts for about 90 percent of the activated oxygen generated by most cells. Experimental acute iron loading of cultured (beating) rat myocardial cells caused a decrease in membrane polyunsaturated fatty acid (PUFA) content, loss of thiol-dependent enzyme activities, decreased ATP, and increased lysosomal fragility (Link et al., 1989). Similar changes in mitochondrial energy production with iron loading have been observed in the livers of rats with chronic iron overload (Bacon et al., 1993). It is also known that abnormal accumulation of iron within mitochondria is associated with extensive and irreversible damage. Affected mitochondria show not only iron accumulation but also inactivation of iron-sulfur enzymes—complexes I, II, and III and aconitase (Rotig et al., 1997). One of the pathological consequences of iron overload might be damage to mitochondrial DNA (Eaton and Qian, 2002). There are several reasons for the apparent fragility of mitochondrial DNA (mtDNA): (1) mitochondria generate reactive oxygen species; (2) mitochondria are intrinsically rich in iron; (3) MtDNA is deficient in histones that may normally provide partial protection against oxidant damage; and (4) repair of damage to mtDNA is slow and less effective.

An alternative, but not mutually exclusive, mechanism for the toxic effects of iron on cells and organs involves the proposition that an accumulation of iron within the cellular lysosomal compartment sensitizes the lysosomes to damage and rupture, releasing damaging lysosomal digestive enzymes into the cytoplasm of the cell (Eaton and Qian, 2002). Minimal release of lysosomal enzymes may induce transient reparative autophagocytosis, while moderate lysosomal rupture is followed by apoptosis. Severe oxidative stress, causing massive lysosomal breakdown, causes necrosis.

In summary, iron overload or toxicosis causes a number of serious and life-threatening pathologies. The molecular bases of these changes are not known, but pathologies involving an interaction between iron and mitochondrial respiration seem to be at the core.

SOURCES AND BIOAVAILABILITY

Sources

The iron content of animal feeds is highly variable. The level of iron in herbage plants is determined by the species and type of soil in which the plants grow. Values as high as 700 to 800 mg/kg iron have been recorded for uncontaminated alfalfa and as low as 40 mg/kg for some grasses grown on sandy soils (Underwood, 1977). Most cereal grains contain 30 to 60 mg/kg, and species differences are small. The leguminous and oil seeds may contain 100 to 200 mg/kg iron.

Feeds containing animal products are rich sources of iron. Meat meals and fish meals contain 400 to 600 mg/kg, and blood meals contain more than 3,000 mg/kg iron. Ground limestone, oyster shell, and many forms of calcium phosphate used as mineral supplements contain 2,000 to 5,000 mg/kg iron (Underwood, 1977). Iron is commonly added to animal diets in the form of iron sulfate, iron chloride, iron proteinates, and blood meal. Red iron oxide is added to some pet foods and trace mineral supplements as a coloring agent, but the iron in this source is essentially unavailable to animals.

Water

The EPA has established National Primary Drinking Water Regulations that set mandatory water quality standards for drinking water contaminants (EPA, 1992). These are enforceable standards called "maximum contaminant

levels" (MCL), which are established to protect the public against consumption of drinking water contaminants that present a risk to human health. An MCL is the maximum allowable amount of a contaminant in drinking water that is delivered to the consumer. The MCL set for iron is 0.3 mg/L. Excessive amounts of iron in water cause a rusty color, sediment, metallic taste, and reddish or orange staining of containers and cooking utensils. Iron is not generally listed in tables with safe upper limits of concentrations of minerals in water for livestock and poultry (NRC, 1974). Iron content of surface waters in the United States ranges from 0.10 to 4,600 µg/L with a mean of 43.9 µg/L (NRC, 1974). Well water with high iron content may cause bacteria to proliferate, forming a red, slimy mass.

Bioavailability

Henry and Miller (1995) published a comprehensive report on the bioavailability of iron in a wide variety of feedstuffs and feed supplements. The proportion of dietary iron absorbed is largely determined by the iron requirement of the individual or animal. Absorption is regulated by the body iron stores; the percentage absorbed is inversely proportional to serum ferritin concentrations (Bothwell et al., 1979). Iron bioavailability is also influenced by the composition of the diet.

A variety of factors in feeds can have an enhancing or inhibiting effect on iron bioavailability. Ascorbic acid strongly enhances the absorption of non-heme iron. In the presence of ascorbic acid, dietary ferric iron is reduced to ferrous iron, which forms a soluble iron-ascorbic acid complex in the stomach. Animal tissues enhance the uptake of non-heme iron. It is thought that low molecular weight peptides released during digestion bind iron and improve its bioavailability.

Inhibitors of non-heme iron absorption include phytate, polyphenols, and calcium. Phytic acid (inositol hexaphosphate) is present in legumes, rice, and grains. There appears to be a dose–response relationship between the level of phytate in a food and iron absorption (Hallberg and Hulthén, 2000). Because phytate and iron are concentrated in the aleurone layer and germ of grains, milling to white flour and white rice reduces the content of phytate and iron, thereby increasing the bioavailability of the remaining iron (Larsson et al., 1996). Polyphenols markedly inhibit the absorption of non-heme iron. Iron binds to tannic acid in the intestinal lumen forming an insoluble complex that reduces absorption. The inhibitory effects of tannic acid are dose dependent and reduced by the addition of ascorbic acid (IOM, 2000). Calcium appears to inhibit the absorption of both heme and non-heme iron (Gleerup et al., 1995). The mechanism is not fully understood, but it may involve interference with the degradation of phytic acid and/or inhibition of iron absorption during the transfer through the mucosal cell.

Trace mineral interactions may also alter iron bioavailability. An excess of one trace mineral may impair the absorption or transportation of another mineral with similar divalent form, possibly by competing for intestinal binding sites on the mucosa; conversely, a deficiency of one mineral may be associated with an enhanced absorption of another (Hill and Matrone, 1970). Interactions affecting iron transport that have been described in animals include zinc-copper-iron, calcium-phosphorus-iron, manganese-iron, cobalt-iron, nickel-iron, and iron-cadmium. For example, Baker and Halpin (1991) reported that when young chickens are fed excessive dietary concentrations of manganese, the blood hemoglobin concentrations decrease.

Additional examples of interactions that affect bioavailability of iron for animals, along with a discussion of the effects of feed processing, are provided in the review by Henry and Miller (1995).

TOXICOSIS

Iron toxicosis is not a common problem in most domestic animals, probably because of the limited absorption and uptake of iron when intakes are high. Nevertheless, if intakes are sufficiently high, signs of iron toxicosis occur. When animals consume large amounts of iron over sustained periods, tissue overload occurs, iron binding capacity is exceeded, and reactive (free) iron levels become sufficient to cause peroxidative damage, especially in liver. According to Underwood and Suttle (1999), the underlying pathogenic mechanism is peroxidative damage of lipid membranes and therefore the extent of the damage will depend to some extent on the antioxidant status of the animal. Rats, and probably other animals, become more susceptible to iron overload with age (Wu et al., 1990).

Single Dose

Very large doses of soluble iron sources can be fatal. Accidental fatal toxicoses have occurred in children, and there have been fatalities in adults due to suicides (Abdel-Mageed and Oehme, 1990). The lethal dose in adults is 200 to 250 mg Fe/kg BW. Three to 10 grams of ferrous sulfate are usually fatal in young children (Mullaney and Brown, 1988). Autopsy findings include periportal hepatic necrosis and congestion along with necrosis of gastric and duodenal mucosa. Iron toxicosis in most animals (dogs may be an exception) requires much higher iron levels along with high intakes of cobalt, zinc, manganese, or copper (Abdel-Mageed and Oehme, 1990). A few studies have examined the effect of single doses of iron in animals (Table 16-1). In piglets given 200 mg iron from ferric ammonium citrate within six hours of birth, only 33 percent survived for 21 days. Lesions in piglets dosed with ferrous sulfate include hydropericardium, hydrothorax, and coagulative necrosis of skeletal muscle. Ferrous sulfate given at 200 mg/kg BW to

rabbits caused death within a few hours of administration (NRC, 1980). A lethal dose of iron (200 mg/kg as ferrous sulfate) was administered into the duodenum of seven rabbits as heart rate, right atrial pressure, arterial pressure, cardiac output, left ventricular pressure, right ventricular developed force, and arterial pH were monitored (Artman et al., 1982). The acute lethal dose increased systemic vascular resistance and depressed stroke volume, cardiac output, and ventricular force. In these rabbits, acute iron toxicosis significantly depressed myocardial contractility.

Single dose iron toxicity has also been studied in swine. Eight pigs (mean weight 25 kg) were given 60 mg of iron as either solid iron tablets or chewable multivitamins with iron per kg body weight (Nordt et al., 1999). After 10 hours, gross examination of the esophagus, liver, small and large intestines, and stomach of all the animals was performed by a surgical pathologist. All of the animals survived for the 10-hour study. The group receiving the solid iron tablets had severe esophageal inflammation and focal erosion, but no significant changes were identified in the liver, small intestine, or large intestine of either group.

A newly born pig has very low iron reserves, rapid growth, limited iron intake from the sow's milk, and is generally raised in an environment (away from soil) where exogenous iron is not available; therefore, pigs are routinely supplied with an oral or injectable dose of iron (usually dextrin, dextran, or gleptoferrin). In some cases, after a single dose injection of iron in a newborn pig, toxicosis has occurred. Death may result as quickly as 30 minutes to 6 hours postinjection or even be delayed for 2 to 4 days. A toxic oral single dose of iron from ferrous sulfate is approximately 600 mg/kg BW for 3- to 10-day-old pigs (Campbell, 1961). A single injection of 200 mg/kg BW of iron dextran to newborn pigs from sows fed adequate vitamin E will result in normal hemoglobin concentrations and growth performance (Hill et al., 1999). However, toxicosis signs were observed when 100 mg/kg BW of iron dextran was injected into newborn pigs from vitamin E deficient sows (Lannek et al., 1962).

The severity of iron toxicosis in young pigs from oral doses of 200 mg iron administered within 6 hours of birth depended on the iron source given. Dosing with ferric ammonium citrate resulted in 66 percent mortality by 21 days of age (Cornelius and Harmon, 1976).

Within 8 hours of birth, five foals were given 16 mg/kg BW of ferrous fumarate orally (Mullaney and Brown, 1988). Two of the five foals died 50 hours after dosing, one died at 7 days, and the other two survived. Necropsies showed that the foals had liver disease. The dose of iron causing acute toxicosis in foals (about 16.5 mg/kg BW) is strikingly low when compared with the dose rates of other species. This may be related to the high serum iron and percentage saturation of transferrin levels that foals have at birth. Also, since iron absorption is elevated at birth, a small amount of orally administered iron could be well absorbed and exceed the iron binding capacity of serum and result in free or unbound iron reaching tissues, especially the liver.

Iron toxicity is sometimes encountered in dogs and cats, usually as the result of consumption of large amounts of readily ionizable iron such as multivitamins, dietary mineral supplements, or human pregnancy supplements. Acute doses from 200 to 600 mg of iron per kilogram of body weight are fatal in dogs (Reissman and Coleman, 1955; Bronson and Sisson, 1960; D'Arcy and Howard, 1962). For cats, the LD_{50} (median lethal dose) for an acute dose of ferrous sulfate has been estimated to be 500 mg/kg BW (Hoppe et al., 1955).

A study using rat hepatocytes showed that glutathione and vitamin E can be protective against the lipid peroxidation associated with iron toxicity (Milchak and Bricker, 2002). In piglets, iron toxicosis is often associated with vitamin E deficiency (Lannek et al., 1962).

Acute

Rats injected intraperitoneally with iron dextran at doses of 250, 500, or 1,000 mg/kg BW developed oxidative stress and impaired spermatogenesis in the testes that was dependent on the amount of iron given and its accumulation in the tissue (Lucesoli et al., 1999), suggesting that the toxic effects of iron are dose dependent. Chicks (2-days old) force-fed by oral gavage with either 1, 10, or 100 mg ferrous sulfate (0.2, 2, or 20 mg of iron) survived and showed no gross lesions from the treatment (Wallner-Pendleton et al., 1986). However, administration of 180, 240, or 300 mg ferrous sulfate (36, 48, or 60 mg of iron) by gavage caused 6.6, 16.1, and 26.5 percent of 3-day-old chicks to die within 24 hours, respectively, and 85 percent developed ulcerative hemorrhagic ventriculitis and friable livers (Pescatore and Harter-Dennis, 1989). An LD_{50} of 357 mg ferrous sulfate for chicks has been reported. In laboratory animals, the oral LD_{50} is reported to be 300, 600, and 900 mg/kg BW in guinea pigs, rabbits, and mice, respectively (Somers, 1947).

Brown trout were exposed for 96 hours to lethal concentrations of iron sulfate as commercial grade liquor and analytical grade iron sulfate to determine if gill tissues reflect systemic toxicosis (Dalzell and Macfarlane, 1999). The 96-hour LC_{50} on brown trout of a commercial iron (III) sulfate liquor was 28 mg total iron/L (0.05 mg soluble iron/L); the 96-hour LC_{50} for analytical grade iron (III) sulfate was 47 mg total iron/L (0.24 mg soluble iron/L). Lethal and sublethal exposure to both grades of iron caused accumulation on the gill, which appears to be the main target for iron toxicity. Physical clogging of gills and gill damage was seen. Gill tissue showed no evidence of iron uptake, and iron did not accumulate in plasma of fish exposed to iron compared to controls. Respiratory disruption due to physical clogging of the gills is suggested as a possible mechanism for iron toxicosis in fish.

In humans, acute toxicity resulting from overdose of medicinal iron, especially in children, has been reported (IOM, 2000). Gastrointestinal irritation occurs with doses between 20 and 60 mg/kg BW.

Chronic

Characteristic signs of chronic iron toxicosis include reduced feed intake, growth rate, and efficiency of feed conversion. Cao et al. (1996) fed day-old chicks diets supplemented with 400, 600, or 800 mg/kg added iron as either ferrous sulfate or iron methionine. The basal diet contained 188 mg/kg of iron. Feed intake and weight gain were reduced at all supplemental levels, but the effects were most evident at the 800 mg/kg level.

A moderately high iron intake coupled with removing phytate from a corn-soy diet increased the susceptibility to oxidative stress in weanling pigs (Porres et al., 1999). Two levels of dietary iron were studied: 80 or 750 mg/kg diet as ferrous sulfate. Liver, colon, and colon mucosal scrapings were collected after feeding the diets for four months. Oxidative stress, as measured by thiobarbituric acid reacting substances (TBARS), was increased in the high iron group as well as by adding phytase to the lower iron diet. The data show that intrinsic phytate in corn and soy is protective against lipid peroxidation in the colon in the presence of moderately high levels of dietary iron. Signs of a phosphorus deficiency were noted in pigs fed 5,102, or 7,102 mg/kg iron when phosphorus was present at 0.92 percent of the diet (Furugouri, 1972).

Dose–response studies done in steers show that chronic intakes of high amounts of iron (i.e., 400 or 1,600 mg/kg) as ferrous sulfate reduced daily feed intake and average daily gain; 1,600 mg/kg reduced plasma copper and increased plasma inorganic phosphorus (Standish et al., 1969). Koong et al. (1970) fed six levels of iron between 100 and 4,000 mg/kg as iron citrate to calves weighing about 125 kg. Poor gains and diarrhea occurred in the animals fed 4,000 mg/kg, and they were changed to 2,000 mg/kg after 6 weeks. The body weights and feed consumption data for calves receiving 1,000 mg/kg were not significantly different from those receiving 100 mg/kg. There was a trend toward poorer performance at all dietary iron levels of 500 mg/kg or more.

Milk replacers containing 100, 500, 1,000, 2,000, or 5,000 mg/kg iron were fed to 3-day-old calves for six weeks to estimate the lowest amount of dietary iron (added as ferrous sulfate) that would reduce calf performance (Jenkins and Hidiroglou, 1987). Calves tolerated all iron treatments except 5,000 mg/kg. At 5,000 mg/kg, calves showed reduced weight gains, feed efficiency, and digestibility of DM and protein. There were no other signs of iron toxicosis and no gross abnormalities on postmortem examination. Marked increases in spleen and liver iron occurred with 2,000 and 5,000 mg/kg treatments.

Lambs with an initial weight of 31 kg were fed diets supplemented with iron (as ferric citrate) at either 0 or 760 mg/kg for 76 days (Rosa et al., 1982). The experiment also included supplemental levels of phosphorus and aluminum. The supplemental iron reduced feed intake and weight gain. An increased intake of phosphorus partially alleviated the adverse effects of the excess iron. In a subsequent experiment (Rosa et al., 1986), mature wethers (70.5 kg) were supplemented with ferric citrate to provide either 0 or 1,000 mg of iron per kilogram of diet. The high iron intake reduced weight gain, but this was partially counteracted by supplemental zinc. The effect of supplemental iron on growth and mineral utilization was studied in 24 lambs weighing an average of 29 kg (Prabowo et al., 1988). Treatments consisted of supplemental iron at 0, 300, 600, or 1,200 mg/kg diet as ferrous carbonate. Lambs were slaughtered after ad libitum access to the diet for 98 to 121 days. None of the levels of dietary iron affected lamb gain or feed intake. However, most forms of ferrous carbonate are low in iron bioavailability (Henry and Miller, 1995), and this probably explains the high iron tolerance in this experiment. Supplemental iron did increase iron concentrations in liver, spleen, and bone. Serum and liver copper, as well as copper transport proteins, were decreased by supplemental iron; zinc and manganese status were not affected; and only subtle changes were seen in plasma and tissue phosphorus. Studies of iron toxicosis in adult sheep show that the chronic dose is between 40 and 80 mg/kg BW/day when iron is administered in the soluble form of ferrous chloride (Rallis et al., 1989).

To determine the effect of supplemental iron on liver function in adult ponies, four ponies were given 50 mg Fe/kg BW/day for 8 weeks (Pearson and Andreasen, 2001). Hepatic and serum iron concentrations, percentage saturation of transferrin, and serum ferritin concentrations were increased compared with baseline values and controls, but there were no adverse clinical signs or histological lesions in the liver; liver iron levels returned to normal by 28 weeks.

The effect of increased iron intake on growth and lipid peroxidation in juvenile catfish weighing 32 g was studied in a group of fish fed a fishmeal-based diet containing either 663 or 6,354 mg/kg dry diet as ferrous sulfate for 5 weeks (Baker et al., 1997). The higher iron diet suppressed growth, although tissue iron levels were not altered and hematocrit levels did not change. Lipid peroxidation of the liver and heart increased with the higher dose, and hepatic levels of vitamin E were depleted. These data show that vitamin E is important in preventing damage caused by excessive dietary iron in fish as well as other species.

Factors Influencing Toxicity

The dietary level at which iron becomes toxic is affected by other dietary constituents and by the physiological state of the animal (Underwood, 1977; Abdel-Mageed

and Oehme, 1990). Animals can tolerate considerably higher daily exposure levels of iron when it is consumed in the diet than when it is delivered in the water or the fasting state. Among dietary factors, calcium is important in modulating iron toxicity (IOM, 2000); high calcium intakes reduce iron toxicity. Dietary phytate can also modulate iron toxicity. Pigs fed diets high in iron and low in phytate were more susceptible to oxidative stress than pigs fed high iron–high phytate diets (Porres et al., 1999). High dietary intakes of cobalt, copper, zinc, and manganese, and deficient intakes of nickel will depress iron absorption. Levels of iron intake that would produce signs of toxicosis in the animal would clearly need to be much higher under conditions of abnormally high intakes of one or more of these interacting elements than when such intakes are low or normal.

Several antioxidant enzymes may play vital roles in the impact of overload iron. Cytosolic superoxide dismutase prevents the toxic effects of oxygen (Wisnicka et al., 1998) and chronic iron toxicosis (Zhao et al., 1995). Oral vitamin E can prevent a toxic reaction to oral iron (ferrous sulfate or fumarate) or intramuscular injection of iron either as dextrin or dextran (Tollerz, 1973). In addition, Dougherty et al. (1981) reported that supplemental dietary vitamin E was essential in preventing mortality in rats given intraperitoneal injections of iron. Vitamin E and glutathione status of the animal also influence susceptibility to iron toxicity (Lannek et al., 1962; Milchak and Bricker, 2002).

Once iron has been absorbed, methods to minimize its toxic effects are based on chelation to reduce the body burden. The chelating agents are desferrioxamine, $CaNa_2$-EDTA and DTPA (diethylene triamine penta-acetic acid).

TISSUE LEVELS

Accumulation of iron in tissues is dependent upon the dose, the length of exposure, mode of administration, composition of the diet, and physiology of the animal (Table 16-2). When animals are exposed to excessive amounts of iron, it is preferentially deposited in the liver, spleen, and bone marrow (Underwood, 1977). With very high doses, iron may be deposited in the heart and kidneys. The iron content of milk varies with the species and stage of lactation and is highly resistant to changes in the level of dietary iron (Underwood, 1977). The average iron concentration in human, cow, and goat milk is very similar. A high proportion of values fall between 0.3 and 0.6 μg/mL, with a mean of about 0.5 μg/mL. An average hen's egg contains close to 1 mg of iron, or approximately 20 mg/kg of the edible portion (Underwood, 1977). A high proportion of this iron is present in the yolk. The effect of changes in the chicken's diet on egg iron is unknown. Increases in liver, kidney, spleen, and bone iron of chicks were reported when diets contained from 400 to 800 mg/kg of supplemental iron (Cao et al., 1996). A modest 25 percent increase in muscle iron occurred when steers were fed 1,000 mg/kg iron compared to 100 mg/kg (Standish et al., 1969). In sheep, a supplement of 760 mg/kg of iron as ferric citrate increased iron concentrations in liver, kidney, spleen, and muscle (Rosa et al., 1982). Similar effects were observed in a subsequent experiment. Muscle iron levels did not change in sheep fed 40 or 80 mg Fe/kg BW (Rallis et al., 1989).

MAXIMUM TOLERABLE LEVELS

The maximum tolerable level of iron is defined as the dietary level that, when fed for a defined period of time, will not impair accepted indices of animal health or performance. Dietary iron from natural feed sources is more tolerable at higher concentrations than iron from soluble compounds. Maximum tolerable concentrations of dietary iron have been set at 500 mg/kg for cattle, 500 mg/kg for sheep, 500 mg/kg for poultry, and 3,000 mg/kg for swine. Aquatic animals and fish appear to have damage to gills when iron concentrations are above 0.1 mg/L. These tolerable concentrations were set based on the animal having normal iron status, and fed a source of iron that is highly digestible. Therefore, higher dietary concentrations can probably be fed when iron is supplied from sources with low bioavailability or if the animal is in a deficient state.

HUMAN HEALTH

The Food and Nutrition Board of the Institute of Medicine recently established an upper level for dietary intake of iron by humans. The estimate was based on the gastrointestinal effects of supplemental intakes of iron salts (IOM, 2000). The LOAEL was estimated to be 70 mg/day and the uncertainty factor was 1.5 to permit extrapolation to a NOAEL. The LOAEL of 70 mg/day divided by the 1.5 uncertainty factor gives an upper level of 45 mg Fe/day.

FUTURE RESEARCH NEEDS

There are inadequate data to define accurately maximum tolerable levels for iron from dietary or water sources for most non-laboratory animals. Few studies have included incremental dose levels adequate for determining thresholds for toxicity. Although swine seem to be able to tolerate higher iron concentrations than most other species, long-term studies are needed to confirm this tolerance.

SUMMARY

Iron toxicosis occurs in animals given excessive supplements to prevent deficiencies. Since iron absorption is tightly regulated, most animals do not take up large amounts of iron from their diet. Thus, the primary effect of high iron intakes is gastrointestinal distress. Young animals absorb iron more

efficiently than older animals and have a lower tolerance. Most research on iron toxicity has utilized iron sulfate, which is highly available. Low levels of iron exposure cause subtle changes in growth and feed efficiency. Higher levels of exposure cause accumulation in the liver, spleen, and bone marrow, the major sites for iron storage. Muscle, milk, and eggs are not a major site of iron accumulation.

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TABLE 16-1 Effects of Iron Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Chickens	72	1 d	400 mg/kg 600 800	$FeSO_4 \cdot 7H_2O$	1, 2, or 3 wk	Diet	Basal diet had 188 mg/kg of iron. All supplemental levels reduced feed intake and weight gain with no significant differences among levels	Cao et al., 1996
Chickens	54	1 d	800 mg/kg	$FeSO_4 \cdot 7H_2O$	3 wk	Diet	Reduced feed intake and weight gain	
Chickens	24–30	I d	400 mg/kg 600 800	$FeSO_4 \cdot 7H_2O$ or Fe methionine	2 wk	Diet	Basal diet had 123 mg/kg of iron. Reduced feed intake and weight gain, especially at the highest level	
Swine	13	10 kg	60 mg/kg BW	$FeSO_4$	1 dose	Oral	Lethargy, diarrhea	Dean et al., 1996
Swine	8	28 d	750 mg/kg	$FeSO_4$	15 wk	Diet	Decreased alkaline phosphatase, increased lipid peroxidation and superoxide dismutase in colon	Porres et al., 1999
Ponies	3	1 d	16 mg/kg BW	Fe fumarate	1 dose	Oral	Hepatic pathology, death	Mullaney and Brown, 1988
Ponies	4	79–179 kg	50 mg/kg BW	$FeSO_4$	8 wk	Oral	No adverse effects on liver histology or enzyme activities	Pearson and Andreasen, 2001
Calves	8	3 d	100 mg/kg 500 1,000 2,000 5,000	$FeSO_4 \cdot 7H_2O$	6 wk	Diet	No adverse effect No adverse effect No adverse effect No adverse effect Decreased gain, efficiency of feed conversion	Jenkins and Hidiroglou, 1987
Cows, Holstein	8	Lactating	500 mg/kg	$FeSO_4 \cdot 7H_2O$	4 wk	Diet	Slight decrease in intake, milk yield, and respiration rate when iron added to soy-based diet but not a cotton seed-based diet	Barraza et al., 1991
Cows, Holstein	24	Lactating	500 mg/kg	$FeSO_4$	83 d	Diet	No adverse effect on milk, protein or fat; decreased liver copper stores if dietary copper was low	Chase et al., 2000
Sheep	12	31 kg	760 mg/kg	Ferric citrate	76 d	Diet	Decreased feed intake and weight gain Increased inorganic phosphorus in serum	Rosa et al., 1982
Sheep	12	Wethers 70.5 kg	1,000 mg/kg	Ferric citrate	56 d	Diet	Decreased weight gain	Rosa et al., 1986
Sheep	6	29 kg	300 600 1,200	$FeCO_3$	98–12 1 d	Diet	None of the treatments had an adverse effect on gain, intake or efficiency, but all decreased copper stores	Prabowo et al., 1988
Sheep	5	35–40 kg	40 mg/kg BW[c] 80	$FeCl_3 \cdot 6H_2O$	22 wk	Oral	Hepatocellular necrosis, 1 of 5 sheep died Anorexia, diarrhea, dark green feces, death within 7 wk	Rallis et al., 1989
Fish, catfish	20	32 g	6,354[c] mg/kg	$FeSO_4 \cdot 7H_2O$	5 wk	Diet	Decreased growth and liver vitamin E, increased lipid peroxidation in liver and heart	Baker et al., 1997

[a]Number of animals or pools of animals per treatment group.
[b]Quantity of iron dosed. Unless otherwise indicated, the quantity dosed is additional to the background levels found in the diet. SI unit conversion: 1 mg iron equals 17.9 µmoles iron.
[c]Total dietary iron (basal plus supplemental).

TABLE 16-2 Iron Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)

Animal	Quantity	Source	Duration	Route	Muscle	Kidney	Liver	Heart	Spleen	Bone[e]	Blood	Reference
Chickens	400 mg/kg 600 800	$FeSO_4 \cdot 7H_2O$	1, 2, or 3 wk	Diet		308[b] 337 349	434[b] 756 643		393[b] 405 385	346 385 384		Cao et al., 1996
Chickens	400 mg/kg 600 800	$FeSO_4 \cdot 7H_2O$ or Fe methionine	2 wk	Diet		255[b] 281 288	360[b] 557 709					
Calves	100 mg/kg 500 1,000 2,000 5,000	$FeSO_4 \cdot 7H_2O$	6 wk	Diet	33.3[b] 35.8 35.9 41.0 47.3	171[b] 222 243 358 390	121[b] 181 273 1,295 4,350		473[b] 921 1,068 1,551 2,142		2.41[b,c] 3.27 3.83 4.42 5.53	Jenkins and Hidiroglou, 1987
Cows, Holsteins	0 500	$FeSO_4$	83 d	Diet			208[b] 277[b]					Chase et al., 2000
Sheep	0 mg/kg 760	Ferric citrate	76 d	Diet	65[b] 81	349[b] 678	175[b] 1,777	180[b] 196	981[b] 7,510			Rosa et al., 1982
Sheep	0 mg/kg 1,000	Ferric citrate	56 d	Diet		178[b] 728	212[b] 788		804[b] 5,493			Rosa et al., 1986
Sheep	0 mg/kg 300 600 1,200	$FeCO_3$	98–121 d	Diet	57[b] 58 58 58	230[b] 231 238 236	235[b] 241 283 306		957[b] 1,028 1,311 1,611	30[b] 32 33 35		Prabowo et al., 1988
Sheep	0 mg/kg BW 40 80	$FeCl_3 \cdot 6H_2O$	22 wk	Oral		184[b] 583 589	258[b] 1,170 3,082		779[b] 5,433 6,686		2.32[b,d] 3.09 4.20	Rallis et al., 1989
Sheep	400 mg/kg 800 1,200	$FeSO_4 \cdot 7H_2O$	30 d	Diet	124[b] 123 128	222[b] 285 294	252[b] 523 801	224[b] 222 205	1,1115[b] 2,054 2,687	17 18 23		van Ravenswaay et al., 2001
Sheep	600 mg/kg	$FeCO_3$	30 d	Diet		217[b]	163[b]		513[b]			
Fish, Catfish	664 6,354 mg/kg	$FeSO_4 \cdot 7H_2O$	5 wk	Diet	251[b] 238		523[b] 745				0.060[a,c] 0.062	Baker et al., 1997

[a]Data are on a fresh tissue basis.
[b]Data are on a dry tissue basis.
[c]Plasma.
[d]Serum.
[e]Ash weight basis.

17

Lead

INTRODUCTION

The atomic symbol for lead is Pb, which is derived from the Latin *plumbum*. Lead (atomic number, 82; atomic weight, 207.19; specific gravity, 11.34) is a bluish to silvery-gray metal, although the pure metal is easily tarnished by an oxide film to dull gray. Lead metal is soft, pliable, and has no characteristic taste or smell. It has four naturally occurring isotopes (208, 206, 207, and 204 in order of abundance), but the isotopic ratios for various mineral sources are sometimes substantially different. This property has been used to carry out nonradioactive-tracer environmental and metabolic studies.

Lead has four electrons in its valence shell, but only two ionize readily. The usual oxidation state of lead in inorganic compounds is therefore +2 rather than +4. Lead sulfide, lead oxides, and most other inorganic salts of lead are poorly soluble in water. Exceptions are salts with nitrate, chlorate, and chloride. Organic salts have variable solubility, with lead oxalate being insoluble and acetate being highly soluble.

Lead in the Earth's crust is usually found in the sulfide (PbS) form as galena ores, with smaller amounts in cerrusite ($PbCO_3$) and anglesite ($PbSO_4$). Galena occurs mostly in deposits associated with other minerals, particularly zinc. Mixed lead and zinc ores account for about 70 percent of total lead supplies from mining. In 2002, Australia was the largest producer of lead, with 23 percent of the world's total, followed by China, 21 percent; the United States, 15 percent; Peru, 10 percent; and Mexico, 5 percent. About 97 percent of the lead in the United States is produced in Alaska and Missouri (Smith, 2002).

Production of lead-acid batteries is the dominant use of lead, accounting for about 83 percent of reported lead consumption in 2002 (Smith, 2002). Use of lead in ammunition and as pigments for glass and ceramics continues to account for much of the rest of consumption, although these uses are declining as less toxic substitutes are being developed. Other current commercial uses of lead metal include production of brass and bronze alloys, solders, greases, caulkings, and bearings. Lead sheets are used in building construction, storage tanks, and medical radiation shielding. Organolead compounds were historically used as fuel additives (tetraethyllead and tetramethyllead), but they are not currently an important industrial product.

Lead is considered one of the most significant environmental pollutants, and its use is highly regulated because of its high level of toxicity (IPCS, 1989; ATSDR, 1999). Because of this, lead metabolism and toxicity have been intensely studied in humans and these data are often useful in prediction of toxicity to domestic animals. Lead has been incriminated as one of the most common causes of accidental poisonings in agricultural, companion, and wild animals, as well as humans; however, incidence of toxicosis has been diminishing due to decreased use of lead-containing products (Prescott, 1983; Morgan, 1994; Burger, 1995; Needleman, 2004). Lead poisoning is sometimes referred to as plumbism.

ESSENTIALITY

Lead is not known to be an essential nutrient for animals and does not participate in any known beneficial biochemical functions. However, in several studies, the addition of lead to the diet of rats and pigs improved growth rates and lipid metabolism (Reichlmayr-Lais and Kirchgessner, 1981; Kirchgessner et al., 1991; Manser, 1991) and improved egg production in chickens (Mazliah et al., 1989).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

The most common methods currently used for the analysis of lead in biological samples are flame atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry (GFAAS), anode stripping voltametry (ASV), inductively coupled plasma-atomic emission spectroscopy

(ICP/AES), inductively coupled plasma mass spectrometry, and x-ray fluorescence spectroscopy. GFAAS and ASV are the methods of choice for the analysis of lead at very low concentrations, but AAS and ICP/AES are adequate when levels are in the toxic range. Limits of detection for lead using AAS are on the order of μg/mL (ppm) and for GFAAS are generally in the low ng/mL (ppb) range (Flegal and Smith, 1995). Sample preparation usually consists of wet ashing the specimen with strong acid and heat, and redissolving the residue in dilute acid prior to analysis so that all lead species are converted quantitatively to the same lead compound. Closed vessel microwave digestion gives similar results as wet ashing (McCarthy and Ellis, 1991).

The reference method for the determination of the absolute amounts of lead is by isotope dilution mass spectrometry (Grandjean and Olsen, 1984), but due to equipment costs and required expertise, it is not widely used. Spectrophotometric methods, using diphenylthiocarbazone as the colorimetric reagent, were widely used in the past. They are less sensitive but still useful for toxicity studies.

To ensure adequate quality control, a reference material should be used that closely matches the matrix type and concentration of the experimental samples to be analyzed. Lead contamination during sample collecting and processing must be avoided. All glass equipment involved in blood collection and storage should be made of lead-free silicate glass and acid washed.

REGULATION AND METABOLISM

Absorption and Metabolism

Gastrointestinal absorption of lead occurs primarily in the duodenum, and the percentage of the dose that is absorbed decreases as the dose increases. It is likely that lead absorption occurs by inadvertent uptake through pathways of essential nutrients such as the divalent metal transporter-1, which transports non-heme iron (Bressler et al., 2004). The rate-limiting processes in lead absorption are associated with transfer of lead from enterocytes to the blood rather than transport across the apical membrane of the enterocytes. The efficiency of lead absorption is markedly influenced by the chemical form of the lead, the level of other dietary constituents, and the age and physiological state of the animal. Soluble forms of lead are absorbed better than insoluble forms. The proportion of a dose of highly soluble lead absorbed by adults may vary from less than 10 percent when ingested with a meal to 60–80 percent when ingested after a fast. Calcium and phosphate are particularly effective in reducing lead absorption (Fullmer, 1991; Varnai et al., 2001). Young animals absorb lead considerably more efficiently than older animals. Pregnancy, lactation, or a deficiency of iron or calcium also increases the efficiency of lead absorption. The apparent absorption of lead in adult sheep is 15 percent when included in the diet at 1,000 mg/kg (Pearl et al., 1983).

Absorption of lead from inhalation of particulates and dust is efficient, but dermal absorption of inorganic lead through unabraded skin is considered to be minimal (<0.1 percent) (IPCS, 1995).

Once absorbed, lead enters the blood where >90 percent is taken up by red blood cells (Cake et al., 1996). Most of the lead in red blood cells is bound to hemoglobin within the cell rather than the erythrocyte membrane. Lead in plasma binds to albumin and γ-globulins and complexes with low molecular weight sulfhydryl compounds. After entering peripheral tissues, lead is predominantly bound to protein. High affinity cytosolic lead binding proteins (PbBP) rich in aspartic and glutamic acids have been identified in the cytosol of rat kidney and brain (Fowler, 1998). These proteins possess dissociation constants for lead of 10^{-8} M. Lead also binds to metallothionein. Binding of lead to these proteins attenuates lead-induced inhibition of several enzymes.

Over time lead redistributes from soft tissues to bone where it forms highly stable complexes with phosphate, replacing calcium in hydroxyapatite. As a result, lead is incorporated into bone during the normal mineralization processes that occur during bone growth and remodeling and is also released to the blood during the process of bone resorption (Silbergeld et al., 1993; O'Flaherty, 1998). Physiological states (e.g., pregnancy, parturition, osteoporosis, infection, or prolonged immobilization) that are associated with increased bone resorption promote the release of lead and entry into blood and milk (ATSDR, 1999).

A mother with a high body lead burden can transfer lead transplacentally to her developing fetus. In rats and humans, there is no functional placental-fetal barrier to lead transport. Maternal and fetal blood lead levels are nearly identical, so lead passes through the placenta unencumbered (Goyer, 1990). In cynomolgus monkeys (*Macaca fascicularis*), up to 40 percent of the maternal lead burden that is transferred to the fetus is mobilized from maternal skeleton (Franklin et al., 1997). The transport of lead through the more complex epitheliochorial placenta of pigs is delayed and there appears to be protection against lead accumulation in the fetal brain (Lu et al., 1997). Lead transferred from the hen to the egg is found in the shell and the yolk, but not the white (Mazliah et al., 1989). Lead can also be transferred to milk, and 90 percent is associated with casein (Beach and Henning, 1988).

Lead that is incorporated into hair is a useful indicator of toxic levels of exposure, though levels are affected by hair color, texture, location on the body, and growth phase. Also, external contamination is problematic because cleaning methods that are sufficiently vigorous to remove superficial lead also remove lead from the hair shaft (IPCS, 1995).

Metabolism of inorganic lead consists primarily of reversible ligand reactions, including the formation of complexes and thiols with free amino acids and proteins. Organolead compounds are actively metabolized in the liver by oxidative dealkylation catalyzed by cytochrome P-450.

Tetraethyl and tetramethyl lead are oxidized to triethyl and trimethyl metabolites, respectively, and to inorganic lead. Further biotransformation of these intermediate metabolites is highly species-specific.

The half-life for lead in blood and other soft tissues of adult humans is about 1 month, but it is much longer in the various bone compartments. The lead content of blood steadily declines after exposure is discontinued. Lead is excreted in the urine following glomerular filtration in the kidneys and by the intestines, either by transmucosal losses or through biliary clearance in the form of organolead conjugates. There is considerable species variability in the relative importance of urinary versus intestinal excretion routes. Intestinal excretion appears to dominate in rats and sheep. In dogs, renal excretion dominates at low levels of lead exposure but the proportion of the lead excreted into the gut via bile increases with increasing body burden (Klaassen and Shoeman, 1974).

Mechanism of Toxicity

Proposed toxic mechanisms for lead include its ability to interact with proteins and change their functions, inhibit or mimic the action of calcium, replace zinc as a cofactor in enzymes, and cause oxidative stress (Bressler and Goldstein, 1991; Goering, 1993; Goldstein, 1993; Hsu and Guo, 2002). Lead modifies the folding, binding characteristics or enzymatic activity of proteins by binding to sulfhydryl, amine, phosphate, and carboxyl groups. Lead can replace calcium in some reactions, such as formation of hydroxyapatite. Lead can replace zinc in the catalytically active site of enzymes, such as 5-aminolaevulinic acid dehydratase (Warren et al., 1998). It mimics calcium in the activation of calmodulin and protein kinase C by binding to amino acid carboxyl groups and sulfhydryl groups, respectively. Lead-induced oxidative stress also contributes to the pathogenesis of lead poisoning. The relative importance of these diverse disruptive actions depends upon the cell type and organ system (Goyer, 1993).

SOURCES AND BIOAVAILABILITY

The primary sources of lead exposure to animals are contaminated soils; lead paints that remain on older structures; water from plumbing systems that contain lead; and lead-based products, especially batteries, used crankcase oil, and linoleum (Waldner et al., 2002). Most plants do not take up large amounts of lead from the soil and plant-based feed ingredients are low in lead unless they are contaminated from airborne sources or postharvest (Burrows, 1982). Meat and fishmeals are low in lead unless they become contaminated by exogenous sources. However, some mineral sources have high levels of lead. For instance, samples of feed-grade copper sulfate and complete mineral mixes had 640 and 460 mg/kg, respectively (Bakalli et al., 1995a; Marcal et al., 2001).

The contribution of natural sources to lead concentrations in the biosphere is small and anthropogenic sources dominate. Releases to soil from nonferrous smelters, battery plants, and chemical plants are currently the major anthropomorphic sources of lead. In 1984, combustion of leaded gasoline was responsible for approximately 90 percent of all anthropogenic lead emissions. Use of lead additives in motor fuels was totally banned after 1995, and use in paints and many other applications has also been prohibited. Yet lead is extremely persistent in the environment and historical sources are still a major contaminant. The chemical remnants from leaded fuels now exist in soils primarily as inorganic lead oxides, carbonates, oxycarbonates, sulfates, and oxysulfates. Lead has been mined and purified throughout recorded history, and the legacy of this activity is reflected in the high content of lead in soils at many locations (Eckel et al., 2002). Agricultural use of municipal sludge and other biosolids as fertilizers also contributes lead to soils. Dusting and flaking of paint from older structures is a source of lead contamination in surface dust and soils. Many metropolitan areas have areas of very high lead concentrations due to historical use of lead paints and leaded fuels. Companion animals accumulate lead from soils and dust to a much greater extent than humans living in the same environment, and are particularly at risk to lead exposure from the sanding and scraping of lead-based paints during remodeling of older homes (Berny et al., 1994; Knight and Kumar, 2003). Though most automobile batteries are recycled, toxicosis occasionally occurs from animals ingesting those left in pastures (Oskarsson et al., 1992). Lead used for balancing wheels has also been implicated in toxicosis. A major source of lead to waterfowl and other wildlife is spent lead shot, bullets, cartridges, and the lead sinkers used in sport fishing (Burger and Gochfeld, 2000; De Francisco et al., 2003). Birds retain grit in their gizzard as a digestive aid and lead particles can be retained in the acid environment of the gizzard for long periods of time, increasing its toxicity. Bullets and other ammunition contaminating target ranges have caused lead toxicosis in cattle, and shooting ranges should not be used for pasture.

Lead occurs naturally in the Earth's crust at a concentration of about 13 mg/kg, but there are some areas with much higher concentrations, including the lead ore deposits scattered throughout the world. The lead concentrations in igneous, metamorphic, and sedimentary rocks are in the range of 10–20 mg/kg. The lead content of sandstones and carbonaceous shales from the United States and Europe ranges from 10 mg/kg to 70 mg/kg. Lead in phosphate rocks may exceed 100 mg/kg (IPCS, 1989; ATSDR, 1999). The concentration of lead in the top layers of soil may be due to deposition and accumulation of atmospheric particulates from anthropogenic sources. For example, soils directly beside major roadways where leaded gasoline was used for decades are typically 30–2,000 mg/kg higher than natural levels, but drop exponentially up to 25 meters from the roadway (IPCS, 1989; ATSDR, 1999).

The concentration of lead in surface water is highly variable depending upon sources of pollution; lead content of sediments; and the pH, salinity, and organic matter content of the water. Surface waters in the United States average 3.9 μg/L and are higher in urban areas than in rural areas (ATSDR, 1999). Lead is estimated to be present in seawater at approximately 0.005 μg/L. In seawater and most surface and ground waters, the concentration of dissolved lead is kept low because lead forms carbonates, sulfates, and phosphates that have low water solubilities and precipitate out of the water column. Much of the remaining ionic lead absorbs to organic matter and is removed by appropriate water purification.

Biomagnification of lead in the environment does not typically occur. In general, the highest lead concentrations are found in aquatic and terrestrial organisms that live near lead-contaminated sites. In aquatic organisms, lead concentrations are usually highest in benthic organisms and algae, and lowest in upper trophic level predators such as carnivorous fish. Older organisms tend to contain the greatest body burdens of lead.

The bioavailability of lead in soil to plants is highest in acidic soils that have low organic matter content. Translocation of lead ions in plants is limited, and most lead is found in the cell walls of root cells. The total concentration of lead in soil does not correlate well with the concentration in the plant unless the soil content is expressed as extractable lead. Foliar uptake of lead occurs to a very limited extent. No specific category of food is especially high in lead content, and levels in raw edible plants are usually below 0.05 mg/kg wet weight. In a recent study, levels of lead in grain were not correlated to soil content and surface contamination introduced during grain harvest or storage was the primary source of lead in feeds using these feedstuffs (Zhao et al., 2004). Cattle grazing near lead smelters have elevated blood lead levels and the mean levels decrease with distance. However, ingestion of soil along with roots is thought to be the primary source of lead (Neuman and Dollhopf, 1992). There is some indication that dairy cattle can detect lead on pasture grasses and that they prefer to graze pastures without lead (Strojan and Phillips, 2002).

Silage can be an important contributor of lead. In one report, alfalfa, estimated to contain 25.89 mg Pb/kg dry matter due to contamination of contaminating soil, was ensiled for 3 weeks in a glass-lined silo and the haylage from the bottom of the silo contained 118.6 mg of Pb/kg. Apparently, lead migrated and concentrated in the bottom of the silo (Coppock et al., 1988).

Bioavailability

The bioavailability of lead is markedly influenced by its form and especially by its solubility. The efficiency of lead absorption is directly related to its solubility in the gastrointestinal tract. Unfortunately, most toxicity studies use lead acetate, which is one of the most soluble forms of lead and does not mimic the bioavailability of lead oxide, sulfide, sulfate, carbonate, or phosphate found in contaminated soils and waters. The bioavailability of lead sulfide is only about 10 percent that of lead acetate and lead oxide is intermediate in bioavailability (Dieter et al., 1993; Freeman et al., 1996). In growing rats, the bioavailability relative to lead acetate of lead in soil from mining wastes mixed with feed is typically 10 percent or less (Freeman et al., 1992; Dieter et al., 1993; Freeman et al., 1994; Polak et al., 1996). The bioavailability of lead in contaminated soil to growing pigs was found to be 58 to 74 percent of lead acetate, depending on the tissue examined (Casteel et al., 1997). However, the soil was dosed 2 hours before a meal and was not incorporated into food, and the absorption of lead from soil was greatly depressed by food consumption (Maddaloni et al., 1998). The bioavailability of lead in sewage and harbor sludges to lambs is only about half that of lead acetate (Van Der Veen and Vreman, 1986). The bioavailability of metallic lead is only about 15 percent of lead acetate, and particle size is inversely related to lead absorption (Barltrop and Meek, 1975, 1979).

The bioavailability of lead in animal tissues is less than that in lead acetate. For example, lead intrinsically incorporated into the soft tissues of oysters has a relative bioavailability of 72 percent (Stone et al., 1981). Lead in milk is highly available (Hallen and Oskarsson, 1995).

TOXICOSIS

Lead intoxication in humans has been documented since the second century B.C. and much of our understanding of lead toxicity comes from studies in humans and other primates. While not as extensive, studies with companion animals, poultry, and livestock have demonstrated similar health effects of lead as those observed in humans (Table 17-1). Cardiovascular, hematological, and neurodevelopmental signs of lead occur at the lowest levels of exposure, and renal, gastrointestinal, hepatic, and immunological signs occur with higher doses or lengths of exposures. The toxicity of lead does not appear to be dependent on the route of exposure and is readily predicted by blood lead levels (IPCS, 1995; ATSDR, 1999). Correlation and regression analyses of blood lead levels versus the incidence of various clinical endpoints indicate that toxic symptoms in humans begin when blood lead levels reach 10–15 μg/dL. These include effects on heme metabolism, erythrocyte pyrimidine nucleotide metabolism, serum vitamin D levels, mental and physical development, and blood pressure. Anemia, nephrotoxicity, and more overt neurological impairment occur when blood lead levels exceed 30 μg/dL.

Hematological Changes

Lead inhibits the activities of δ-aminolevulinic acid dehydratase and ferrochelatase enzymes and, consequently,

heme biosynthesis. Heme is a feedback inhibitor of δ-aminolevulinic acid synthetase activity, so lead toxicity causes an increase in plasma and urinary δ-aminolevulinic acid. Lead inhibition of ferrochelatase results in an accumulation of zinc protoporphyrin in circulating erythrocytes because of the placement of zinc, rather than iron, in the porphyrin moiety. Decreased hemoglobin production, coupled with an increase in erythrocyte destruction, results in a hypochromic, normocytic anemia with associated reticulocytosis (Lubran, 1980; Scheuhammer, 1987b).

Cardiovascular Effects

Lead toxicosis often causes hypertension in humans and other animals. Additional signs include myocarditis, electrocardiographic disturbances, heightened catecholamine arrhythmogenicity, altered myocardial contractile responsiveness to inotropic stimulation, hypercholesterolemia, atherosclerosis, degenerative structural and biochemical changes affecting the musculature of the heart and vasculature, and increased vascular reactivity to alpha-adrenergic agonists (Kopp et al., 1988). Several mechanisms for these multifactorial actions appear to be involved. Lead causes increased intracellular concentrations of calcium in capillaries and arteries, triggering smooth muscle contraction and increased vascular smooth muscle tone. Lead also causes oxidation and inactivation of nitric oxide, increasing vascular constriction. These direct effects are augmented by increased sympathetic activity and circulating noradrenaline and angiotensin-converting enzyme activity (Vaziri, 2002).

Neurological and Neurodevelopmental Effects

Lead impairs neurological functions at virtually all stages of the life cycle (Regan, 1989; Winneke et al., 1996). At low levels, lead has subtle effects on learning and IQ. Higher levels of exposure cause blindness and encephalopathy, which manifests as dullness, irritability, poor attention span, headache, muscular tremor, loss of memory, and hallucinations. Lead blocks voltage-regulated calcium channels, inhibiting the influx of calcium that triggers the release of neurotransmitters (Gill et al., 2003). Thus, lead inhibits the propagation of nerve transmission that follows the depolarization of presynaptic nerve terminals. Lead also enters the cell by the same channels as calcium and acts as a calcium agonist to increase the spontaneous release of neurotransmitters. Lead-induced synaptic noise during critical early periods of postnatal development may permanently disrupt the synaptic organization and functional processing of neurons (Johnston and Goldstein, 1998; Bressler et al., 1999; Marchetti, 2003).

Hydrocephalus may also occur during lead toxicosis, especially in younger animals and children. Lead affects the differentiation of brain endothelial cells and influences its vasculature. The resulting disruption of the blood-brain barrier allows albumin, ions, and consequently water to freely enter the brain. Because the brain lacks a well-developed lymphatic system, clearance of plasma constituents is slow, edema occurs, and intracranial pressure rises (Bressler and Goldstein, 1991). Increased capillary permeability allows more lead to enter the brain. Fetal astrocytes and neurons lack the ability to form detoxifying lead-protein complexes and are especially susceptible. Consequently, lead causes reductions or delays in the development of the hippocampus and cerebral cortex, and reductions in the number and size of axons in the optic nerve (Goyer, 1990).

The effects of lead are not limited to the central nervous system. Demyelination of peripheral nerves and decreased nerve conduction velocities cause impaired motor skills due to lead toxicosis (Araki et al., 2000). Poor coordination and erratic movements are among the first obvious clinical signs noticed in farm and companion animals.

Gastrointestinal Effects

Colic is a consistent early symptom of lead poisoning in individuals acutely exposed to high levels of lead. Colic is characterized by a combination of abdominal pain, constipation, cramps, nausea, vomiting, anorexia, and weight loss (Pagliuca et al., 1990).

Renal Effects

Impairment in kidney function occurs acutely when the lead dose is high or after chronic exposure to lower levels (Loghman-Adham, 1997; ATSDR, 1999; Brewster and Perazella, 2004). The characteristics of early or acute lead-induced nephropathy include nuclear and mitochondrial inclusion bodies and cytomegaly of the proximal tubular epithelial cells. Dysfunction of the proximal tubules is manifest as aminoaciduria, glucosuria, and phosphaturia with hypophosphatemia. These effects appear to be reversible. Chronic lead nephropathy progresses to interstitial fibrosis, dilation of tubules and atrophy or hyperplasia of the tubular epithelial cells forming microcysts, reduction in glomerular filtration rate, and azotemia. These changes are not readily reversed upon lead withdrawal. Lead's inhibition of renal cytochrome P-450 system interferes with the activation of 25-hydroxyvitamin to 1,25-dihydroxyvitamin D and secondary disruption in calcium homeostasis (Smith et al., 1981; ATSDR, 1999).

Inclusion bodies are lead-protein complexes composed of acidic non-histone proteins and are diagnostic of lead toxicosis. As much as 90 percent of lead in the kidney is contained in the inclusion bodies, suggesting that they provide a detoxification function.

Immunological Effects

Lead exposure at low to moderate levels does not produce widespread changes in the numbers of leukocytes but

does have important functional impacts on regulatory cells, including macrophages and T lymphocytes. Lead causes a pronounced shift in the balance in T helper cell function toward T helper 2 responses at the expense of T helper 1 functions. This bias alters the type of effector responses triggered by vaccinations, allergens, and infectious organisms and influences the host's susceptibility to various diseases (Dietert et al., 2004). In poults, lead disrupts eicosanoid metabolism of macrophages and influences their regulatory functions (Knowles and Donaldson, 1997).

Reproduction

Lead adversely affects both male and female reproductive functions. Decreased fertility and increased incidence of abortions and stillbirths occur in females (IPCS, 1995; ATSDR, 1999; Sallmen, 2001). In males, lead causes asthenospermia, hypospermia, and teratospermia. Reproductive effects of lead toxicosis range from indirect effects of lead on nutrition or hormonal status to more direct effects on chromatin stability and epigenetic changes. However, lead does not appear to be a major cause of congenital anomalies.

Bone

Lead impairs normal bone growth and remodeling as indicated by decreased bone density and bone calcium content, decreased trabecular bone volume, and altered growth plate morphology. Lead may affect bone indirectly by decreasing the activation of 1,25-dihydroxyvitamin D and disrupting calcium homeostasis (Pounds et al., 1991; Silbergeld et al., 1993). The appearance of lead inclusion bodies in osteoclasts is one of the most sensitive histological changes that occurs in subclinical lead toxicosis of dogs (Hamir et al., 1988).

Cancer

Data in laboratory animals indicate that lead acetate and lead phosphate at very high doses are carcinogenic, and that the most common tumor in rats is renal. The relevance of this observation to human cancer is controversial, but the U.S. Environmental Protection Agency has designated lead as a probable human carcinogen (EPA, 1999).

Toxicity to Aquatic Organisms

Lead toxicosis in fish is very common. Clinical signs include muscular atrophy, lordoscoliosis, paralysis, black tails, degeneration of the caudal fins, hyperactivity, loss of equilibrium, erratic behavior, decreased growth, and death. In many species, black tails are the first noticeable signs of high levels of lead in the water. Juvenile fish generally are more sensitive to lead toxicosis than adults. Organic compounds are usually more toxic to fish than inorganic lead salts. Disruptions in ion regulations, especially calcium, sodium, and chloride, appear to be a primary mechanism of the toxic effects of lead (Rogers et al., 2003). Lead toxicity to fish and other aquatic animals has been extensively reviewed (Eisler, 1988; IPCS, 1989; Sorenson, 1991).

Single Dose

Very large doses of soluble lead sources can cause severe intestinal pain and death due to encephalopathy in humans (ATSDR, 1999). Most studies examining the effect of single doses of lead on farm animals were published prior to 1980 and were reviewed in the previous version of this report (NRC, 1980). Goats drenched with 400 mg/kg BW as lead acetate died after 23 days and 50 mg/kg BW resulted in abortion. Ponies drenched with 1 g lead/kg BW as lead acetate were highly anorexic and lost considerable body weight (Dollahite et al., 1975). A single dose of lead acetate to calves at 200–400 mg Pb/kg BW is fatal. In adult cattle, 600–800 mg Pb/kg BW is fatal (NRC, 1980). When given in the diet, 1 g lead acetate/kg BW is fatal to sheep (Blaxter, 1950). Pigs and chickens appear to be more resistant to single dose or acute toxicosis of lead (NRC, 1980).

Acute

In humans, early neurological symptoms of acute lead poisoning include dullness, irritability, fatigue, decreased libido, dizziness, and confusion. The condition may then worsen, sometimes abruptly, to delirium, convulsions, paralysis, coma, and death. Overt signs and symptoms of neurotoxicity occur when blood lead levels reach 40–60 μg/dL (IPCS, 1995; ATSDR, 1999).

Acute exposure of animals to lead most frequently occurs from contamination of the feed; licking and chewing batteries; or consuming lead paint, lead shot, or lead sinkers. Yearling dairy heifers that consumed lead-contaminated silage developed blindness, tachypnea, foaming at the mouth, chewing, and facial fasciculations (Galey et al., 1990). Dogs with acute lead poisoning developed anorexia, salivation, vomiting, and diarrhea accompanied by spasmodic colic (Prescott, 1983). Death in dogs from acute exposure occurs at doses of 191, 1,300, and 1,366 mg Pb/kg BW for lead acetate, oxide, and sulfate, respectively (ATSDR, 1999). Chickens fed 200 mg/kg BW Pb/day from lead acetate develop anorexia, diarrhea, watery discharge from the mouth, muscular weakness, brownish-black discoloration of the comb, difficult respiration, and convulsions. Consumption of this level of lead results in death starting at about day 10 (Brar et al., 1997).

Lead shot or sinkers ingested by birds and other animals can be fatal. Depending on the amount of shot ingested, clinical signs develop within a few days and death may occur after a few weeks. In ducks, clinical signs include abnormal behavior, intense green diarrhea, anorexia, weakness, inability to fly, and eventually paralysis (De Francisco et al., 2003).

The toxicity of lead-contaminated water to fish varies considerably, depending on water hardness, pH, salinity, and organic matter. Young stages of fish are more susceptible to lead than adults or eggs. Rainbow trout are among the most sensitive species, and death occurs (LC_{50}) at 1.0 mg Pb/L from lead nitrate when the water hardness is 140 mg $CaCO_3$/L (Rogers et al., 2003). The LC_{50} value for tilapia was found to be 202 mg Pb/L using lead nitrate. At lower levels of exposure, the lability of lysosomal membranes in the gill was found to be a more sensitive indicator of toxicosis than decreases in blood hemoglobin and occurred at levels above 47 mg/L (Tabche et al., 1990).

Chronic

Subtle changes in hematological, neurological, and biochemical indices in rats, monkeys, and humans chronically exposed to lead occur at around 0.005 to 0.01 mg Pb/kg BW/day, and functional changes become apparent at around 1 mg/kg BW/day (ATSDR, 1999). For example, in a 9-year study with monkeys, evidence of functional changes in temporal visual function occurred at 2 mg Pb/kg BW/day (Rice, 1998). In most studies, lead was administered by gavage or in the water. Considerably higher levels are needed to obtain similar results when lead is provided in the feed. In a 2-year feeding study with rats, lead did not have detrimental hematological effects at 10 mg/kg diet, but caused significant inhibition of aminolevulinic acid dehydratase (ALAD) at 50 mg/kg. In this study, changes in enzyme activity did not translate into changes in hemoglobin concentration, hematocrit, or renal function at 500 mg/kg, but did at 1,000 mg/kg (Azar et al., 1973). Regardless of the route of delivery, impaired learning and memory is the most sensitive indicator of lead toxicosis and occurs at blood lead levels of about 150 µg/L in rats and primates (IPCS, 1995).

In a 2-year feeding study with dogs, 50 mg/kg diet of lead was tolerated without hematological changes. There was a significant inhibition of ALAD at 100 and 500 mg/kg, but hematocrit was normal (Azar et al., 1973). Histological lesions in the central nervous system were observed in dogs gavaged a mixture of lead salts at 5 mg Pb/kg BW/day (Hamir et al., 1984), but were not observed in dogs given slightly lower levels (Steiss et al., 1985).

Laying hens tolerate 25 mg Pb/kg diet from lead acetate, but 50 mg/kg results in decreased egg production accompanied by feather molt (Edens and Garlich, 1983). Growing quail tolerate 100 mg/kg diet of lead from lead acetate with only changes in plasma calcium levels, whereas 1,000 mg/kg diet results in decreased growth rate. Exposure of quail from hatching through reproduction resulted in decreased egg production at dietary lead levels of 10 mg/kg (Edens and Garlich, 1983). In one study, adding 1 mg Pb/kg diet as lead acetate or lead sulfate significantly decreased the growth rate of broiler chickens without significantly influencing the concentration of lead in the liver, kidney, or muscle (Bakalli et al., 1995a). The background level of lead in the control diet was 2.6 mg/kg so the total lead content was estimated to be 3.6 mg/kg. When dietary calcium levels were increased, the chickens tolerated 500 mg/kg without a decrease in growth rate, which is similar to prior experiments (Berg et al., 1980; NRC, 1980). Growing pigs are also relatively sensitive to chronic lead exposure as indicated by one experiment where feeding 25 mg Pb/kg diet as lead acetate resulted in decreased weight gain and efficiency of gain (Phillips et al., 2003).

Lead sulfate at 500 mg Pb/kg diet had no apparent effect on calves (Logner et al., 1984), but 1,000 mg/kg resulted in decreased weight gain and feed efficiency (Neathery et al., 1987). In ponies, lead toxicosis from consumption of contaminated hay resulted initially in weakness and slight incoordination followed in time by difficulty swallowing and decreased muscle control of the lips and rectal sphincter, and eventually by muscular tremors, severe incoordination, and esophageal paralysis (Burrows and Borchard, 1982). Similar clinical signs have been observed in calves and goats (Zmudski et al., 1983; Zmudzki et al., 1985; Swarup and Dwivedi, 1992).

The prominent indication of lead toxicosis via water exposure in fish is darkening of the skin extending from the caudal fin to the anal fin. In rainbow trout, blackening of the tail is a more sensitive indicator of lead exposure than liver, kidney, or brain histopathology and occurs at 120 µg Pb/L (Sippel et al., 1983). When lead is delivered as part of the diet, blackening of the tail is not a prominent clinical sign, and changes in growth rate, behavior, and histopathology are diagnostic. Rainbow trout tolerate 45 mg Pb/kg diet as lead nitrate with no apparent effect (Sippel et al., 1983).

Factors Influencing Toxicity

The dietary level at which lead becomes toxic is affected by other dietary constituents and by the physiological state of the animal (Scheuhammer, 1987a). Animals can tolerate considerably higher daily exposure levels of lead when it is consumed in the diet than when it is delivered in the water. Lead ingested in the water without simultaneous food consumption is considerably more toxic than when water is ingested with a meal. Among dietary factors, calcium is very important in modulating lead toxicity. Animals fed low calcium diets exhibit increased susceptibility to lead toxicosis as a consequence of increased lead retention associated with decreased renal excretion of lead. High levels of calcium and phosphorus decrease intestinal absorption of lead and decrease its toxicity. Lead toxicosis also impairs vitamin D metabolism and increases the apparent need for dietary calcium.

Low dietary iron increases the susceptibility of animals to lead intoxication and increases tissue deposition of lead because of enhanced gastrointestinal absorption. Lead absorption, tissue deposition, and toxicity are enhanced by

physiological factors that increase absorption of iron, such as fast growth, gestation, and lactation. High dietary zinc has been shown to have a protective effect against lead toxicosis in rats, chicks, horses, and rabbits. The protective action of zinc on lead toxicity is thought to be mediated by decreased absorption (Brewer et al., 1985). Unlike zinc, high levels of cadmium increase lead deposition and toxicity (Prasada Rao et al., 1989; Phillips et al., 2003).

Lactose promotes lead absorption in rats and in calves (Zmudzki et al., 1986). In chicks, monensin or selenium increases accumulation of lead in tissues and decreases the amount of lead needed to cause anemia (Khan et al., 1993, 1994). Addition of methionine to a deficient diet decreases the toxicity of lead to growing chicks by increasing lead excretion (Latta and Donaldson, 1986).

The toxicity of lead to fish decreases with increasing hardness of the water. Lead readily forms $PbCO3_2$ in hard water and is poorly absorbed. Calcium in hard water also competes with lead for absorption and decreases its toxicity. Lead species such as Pb^{++} and $Pb(OH)^{+}$ are more prominent in softwater and are more available and toxic.

Once lead has been absorbed, methods to minimize its toxic effects are based on chelation to reduce the body burden. The standard chelating agents currently in use are CaNa2-EDTA, meso-2,3-dimercaptosuccinic acid (DMSA), and dimercaprol (BAL). Thiamin supplementation diminishes the clinical signs of lead toxicosis to cattle (Coppock et al., 1991).

TISSUE LEVELS

Accumulation of lead in tissues is dependent upon the dose and the length of exposure. These relationships have been extensively detailed in rat models (IPCS, 1995; ATSDR, 1999). In one study, rats exposed to 50 mg Pb/L water as lead acetate for 90 days accumulated lead in tissues in the order kidney > brain > spleen > prostate > heart > testis and liver. Muscle is not a predominant site of lead accumulation. At 5 mg/L, significant lead accumulation was seen only in the brain and kidney. In most organs, the lead concentration was highest 2 weeks after dosing began and subsequently declined. However, in the brain, lead increased gradually over the 90-day dosing period (Areola et al., 1999). In sheep fed 1,000 mg/kg lead acetate, muscle levels reached a maximum in 30 days and then began to decline (Pearl et al., 1983). Similarly in fish, lead uptake reaches equilibrium after several weeks of exposure and decreases in some tissues. In fish, lead is accumulated mostly in the gill, liver, kidney, and bone (IPCS, 1989).

The relationship between lead exposure level and tissue accumulation is summarized in Table 17-2. Concentrations of lead in muscle and milk are relatively low and remain so unless high dietary levels are fed. Lead accumulates in kidney and bone even when relatively low levels of lead are added to the diet. Similar observations have been made in the field. In acutely sick cattle poisoned by licking the remains of storage batteries burned and left in a pasture, levels of lead in milk averaged 0.08 mg/L and in the muscle ranged from 0.23 to 0.5 mg/kg (wet weight basis) compared to normal levels of below 0.005 and 0.01 mg/kg, respectively. Very high concentrations were found in the kidneys, with a range of 70–330 mg/kg (Oskarsson et al., 1992). In cattle grazing near a lead smelter, the tissue lead levels were highest in cattle grazing near the smelter and decreased with distance (Neuman and Dollhopf, 1992).

Accumulation of lead in the eggs of chickens is not well characterized. In one study, hens were dosed with lead acetate starting at 5 and increasing to 100 mg Pb/kg BW/day over a period of 102 weeks. During the dosing period, the level of lead in egg yolks from controls ranged from 0.08 to 0.18 mg/kg. In the yolks from lead-dosed hens, lead ranged from 0.40 to 1.08 mg/kg. Levels in egg white were always less than 0.4 mg/kg and were not affected by dietary lead (Mazliah et al., 1989).

MAXIMUM TOLERABLE LEVELS

The maximum tolerable level of lead is defined as the dietary level that, when fed for a defined period of time, will not impair accepted indices of animal health or performance. Rainbow trout are among the most sensitive fish to lead toxicosis. Juveniles of this species can tolerate chronic consumption of 45 mg Pb/kg diet and can tolerate levels of 50 μg Pb/L water. Rats and dogs tolerate 10 mg lead/kg diet without changes in functional indices in hematopoiesis or kidney function. In chickens and quail, slight but significant changes in growth and egg production occur with the addition of 1 mg Pb/kg diet as lead acetate, and 0.5 mg/kg of highly soluble lead source appears to be a maximum tolerable dose for chronic exposure in these species when dietary calcium levels are low. However, when dietary calcium levels are high, 100 mg Pb/kg diet is tolerated. In pigs, 25 mg Pb/kg diet from lead acetate results in decreased growth, but there is insufficient information to establish a maximum tolerable dose in this species. In ruminants, 250 mg/kg lead in the diet can be tolerated for several months without significant effects on performance; however, levels of lead in kidneys and bone become of concern if consumed by humans. It should be noted that the above MTL are for highly available sources such as lead acetate. Animals are likely able to tolerate higher levels of many lead sources. As discussed in the "Bioavailability" section, the bioavailability of lead in soil and municipal sewage is about half of that in lead acetate and metallic lead is only 15 percent as available.

HUMAN HEALTH

Muscle tissue does not accumulate marked amounts of lead; however, levels in bone and kidney can be unacceptably high in animals suffering from lead toxicosis. Lead does

not migrate significantly from bone to meat under a variety of cooking regimens (Baxter et al., 1992).

FUTURE RESEARCH NEEDS

There are inadequate data to accurately define MTL for lead from dietary or water sources for most nonlaboratory animals. Few studies have included incremental dose levels adequate for determining thresholds for toxicity. Additionally, most studies utilize lead acetate, but this is not a form that animals would be exposed to via diet, water, or accidents. Studies using metallic lead and lead salts found in typical contaminants are needed. Most studies on toxicity of lead in water use distilled water and single daily doses, usually to animals with empty stomachs. Research is needed where water is provided for ad libitum consumption and the water has typical hardness characteristics.

SUMMARY

Lead is a common cause of accidental poisonings in animals. Primary sources of lead exposure to animals include contaminated soils and lead-based products, especially batteries and older paints. Most plants do not take up large amounts of lead from the soil, and plant-based feed ingredients are low in lead unless they are contaminated by soil or airborne sources. Lead in feed is not efficiently absorbed, but lead in water consumed without a meal is much more available. Young animals absorb lead more efficiently than older animals and have a lower tolerance. Most research on lead toxicity has utilized lead acetate, which is highly available. Animals are more likely to be exposed to metallic lead or lead salts that are considerably less available. Low levels of lead exposure cause subtle cardiovascular, hematological, and neurodevelopmental changes. Higher levels of exposure cause renal, gastrointestinal, hepatic, and immunological disturbances. Lead accumulates in kidney, brain, and bone. Muscle is not a major site of accumulation except at very high doses of lead.

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TABLE 17-1 Effects of Lead Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	5	21 kg	1.5–5 mg/kg BW	Pb-acetate	24–40 wk	Oral	No effect on behavior, weight, or histopathology of brain, kidneys, or liver	Steiss et al., 1985
Dogs	2	8 mo	5 mg/kg BW 15 30	Mix of $PbCl_4$, $PbBr_2$, $PbSO_4$	14–152 d	Oral	CNS lesions CNS lesions CNS lesions	Hamir et al., 1984
Chickens, layers	60	Adult hens	25 mg/kg 50	Pb-acetate	28 d	Diet	No adverse effects Decreased egg production	Edens and Garlich 1983
Chickens	14	1 d	20 mg/kg BW 40	Pb-acetate	8 wk	Water, gavage	For both groups: Decreased response to vaccines, increased mortality following infection with Newcastle disease virus	Youssef et al., 1996
Chickens, leghorns	10	1 d	500 mg/kg 1,000 1,500 2,000	$PbCO_3$	14 d	Diet	No adverse effect on weight gain No adverse effect when calcium was sufficient; decreased gain Large decrease in gain	Berg et al., 1980
Chickens, broilers	4	1 d	0.1 mg/kg 0.5 1.0	Pb-acetate	42 d	Diet	No change in weight gain, efficiency No change in weight gain, efficiency Decreased gain, but no change in tissue levels	Bakalli et al., 1995a
Quails, Japanese	20	1 d	1 mg/kg 10 100 1,000	Pb-acetate	15 wk	Diet	No apparent effect No apparent effect Decreased plasma Ca Decreased weight gain, tibia length, plasma Ca	Edens and Melvin 1989
Quails, Japanese	80	1 d	1 mg/kg 10 100	Pb-acetate	10 wk	Diet	25% decrease in egg production 40% decrease in egg production 70% decrease in egg production	Edens and Garlich, 1983
Ducks, Pekin	6	7 mo	44 mg/kg	Pb-acetate	13 wk	Diet	No effect on renal histology	Prasada Rao et el., 1989
Pigs	6	30 d	25 mg/kg	Pb-acetate	137 d	Diet	Decreased weight gain, feed efficiency	Phillips et al., 2003
Pigs	3	17–24 kg	8 mg/kg BW 16 32 64	Pb-acetate	13 wk	Oral in water	Decreased ALA-D[c] Decreased ALA-D[c] Decreased ALA-D[c] Decreased ALA-D[c]; increased mortality	Lassen and Buck, 1979
Calves	8	3 wk	1 mg/kg BW	Pb-acetate	56 d	Oral in water	Increased mortality	Zmudzki et al., 1985
Calves	4	74 d	500 mg/kg 1,500 4,500	$PbSO_4$	49 d	Diet	No apparent effect 50% mortality All died by 10 d	Logner et al., 1984

continued

TABLE 17-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Calves	6	89 kg	1,000 mg/kg	$PbSO_4$	4 wk	Diet	Decreased weight gain and feed efficiency	Neathery et al., 1987
Fish, rainbow trout	5 groups	6.5 g	3 µg/L	$Pb(NO_3)_2$	32 wk	Water	No adverse effects on tissue histology, mortality, metabolic rate, or anemia	Sippel et al., 1983
			13				" "	
			21				" "	
			35				" "	
			60				" "	
			120				Blackening of the tail, curvature of spine	
Fish, rainbow trout	5 groups	10.5 g	2 mg/kg	$Pb(NO_3)_2$	32 wk	Diet	No adverse effects on tissue histology, mortality, metabolic rate, or anemia	Sippel et al., 1983
			7				" "	
			11				" "	
			14				" "	
			23				" "	
			45				" "	
Fish, rainbow trout	2 tanks of 60	Juvenile	0.029 mg/L	$Pb(NO_3)_2$	29 d	Water	No adverse effect	Burden et al., 1998
			0.048				No adverse effect	
			0.121				Decreased ALA-D[c]	
			0.201				Decreased ALA-D[c] and weight gain	

[a]Number of animals or pools of animals per treatment group.
[b]Quantity of lead dosed. SI unit conversion: 1 mg lead equals 4.82 µmoles lead.
[c]ALA-D = aminolevulinic acid dehydratase.

TABLE 17-2 Lead Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)

Animal	Quantity	Source	Duration	Route	Muscle	Kidney	Liver	Spleen	Bone	Milk	Blood	Reference
Chickens, broiler	3.9 mg/kg 4.9 13.9 103.9	Pb-acetate	42 d	Diet		1.06[b] 2.05 3.27 7.17	1.22[b] 1.59 1.90 3.17				0.040[a] 0.134 0.177 0.496	Bakalli et al., 1995a
Chickens, broiler	0 mg/kg[c] 50	$PbSO_4$	7 d	Diet		2.1[b] 4.9	1.4[b] 3.6				0.03[a] 0.33	Bakalli et al., 1995b
Pigs	0 mg/kg[c] 5 10 25	Pb-acetate	137 d	Diet		0.012[a] 0.048 0.070 0.191	0.014[a] 0.061 0.090 0.092	0.027[a] 0.050 0.054 0.073	0.013[a] 0.052 0.141 0.160		0.006[a] 0.008 0.010 0.015	Phillips et al., 2003
Ponies	21 mg/kg 423 423[d]	Conventional hay Contaminated hay Pb-acetate	92 d	Diet Diet Gavage	ND[a] ND 0.3[a]	ND[a] 35.3 21.7	0.4[a] 50.2 82.2	ND[a] 6.0 17.7	38.8[a] 63.2 202			Burrows and Borch, 1982
Lambs	1.4 mg/kg 12.9 11.6 13.5	 Pb-acetate Harbor sludge Sewage sludge	12 wk	Diet	0.023[a] 0.017 0.013 0.022	0.26[a] 1.13 0.47 0.31	0.54[a] 1.09 0.58 0.64					Van Der Veen and Vreman, 1986
Sheep	1,000 mg/kg	Pb-acetate	75 d	Diet	0.75[b]	342[b]	10[b]	3.3[b]	70[b]		0.6[a]	Pearl, 1983
Cattle, calves	0 mg/kg BW[c] 2.7	Pb-acetate	20 d	Water by gavage	0.07[a] 0.17	0.11[a] 49.9	0.13[a] 19.0	0.08[a] 0.73			0.03[a] 0.47	Zmudski et al., 1983
Cattle, calves	1.42 mg/kg 500.00 1,500.00	$PbSO_4$	49 d	Diet	0.39[a] 0.23 1.72	0.5[a] 7.3 21.3	0.2[a] 6.7 16.7		0.55[a] 0.77 3.53		0.07[a] 0.54 0.66	Logner et al., 1984
Cattle, dairy	2.5 mg/kg 10.3 10.3 10.3	Pb-acetate Harbor sludge Sewage sludge	2–3 lactations	Diet	0.01[a] 0.02 0.02 0.02	0.42[a] 1.19 1.00 0.66	0.11[a] 0.56 0.26 0.28			0.002[a] 0.006 0.005 0.004		Vreman et al., 1986
Cattle, dairy	2.7 mg/kg 11.1	Pb-acetate	12 wk	Diet	0.051[a] 0.047	0.51[a] 0.91	0.21[a] 0.47			0.007[a] 0.015		Vreman et al., 1986
Cattle, Holsteins	3.7 mg/kg 9.2 31.5	Pb-acetate	12 wk	Diet	0.02[a] 0.06 0.03	0.63[a] 1.24 4.04	0.15[a] 0.43 0.72		1.5 1.0 6.4	< 0.02[a] < 0.02 0.06	0.02[a] 0.06 0.28	Sharma et al., 1982

[a]Data are on a fresh tissue basis. ND, not detectable.
[b]Data are on a dry tissue basis.
[c]Basal level not provided.
[d]Daily dose gavaged to give an intake similar to the group fed contaminated hay.

18

Magnesium

INTRODUCTION

Magnesium (Mg) is a divalent cation belonging to Group IIA of the periodic table of elements. In its pure form, magnesium is a silvery gray, very light metal that corrodes easily and is also highly inflammable. Magnesium is most commonly found in the Earth's crust as magnesite, primarily composed of magnesium carbonate hydroxide. Magnesium oxide is used in many industrial processes as a means of neutralizing acidity of solutions. Magnesium oxide is produced from magnesite ore $(MgCO_3)_4\ Mg(OH)_2$ by exposing the ore to high heat in a process called calcining. The temperature and duration of the calcination procedure determines the reactive properties (grades) of the magnesium oxide.

Decomposition of magnesium carbonate to form magnesium oxide and carbon dioxide begins at a temperature slightly above 400°C. Calcination temperatures of between 800°C and 1,000°C produce magnesium oxide with a relatively high surface area and remarkable reactivity. This grade reacts readily with water and fairly vigorously with diluted acid solutions (stomach secretions). Grades produced at relatively low temperatures (up to approximately 1,000°C) are called caustic calcined magnesite, and are produced for a wide variety of applications including animal feeds (Adam et al., 1996). The finer the grinding of the magnesium oxide particles, the better the availability of the magnesium for absorption by animals. In contrast, calcining at temperatures above 1,600°C produces "dead burnt" magnesite, a magnesium oxide with extremely low reactive properties that should not be used in animal feeds as the magnesium is not readily available for absorption. These grades are also called sinter or sinter magnesite and are principally used in iron foundries as a refractory brick material.

Though magnesium sulfate (Epsom salts) can be found in relatively pure form in deposits in various parts of the world, it is more commonly produced after the harvesting of common salt (halite). Magnesium sulfate is extracted from the waste waters (bitterns) of solar salt operations by further evaporation, refrigeration, and re-crystallization. Cooling the diluted bittern to between -5°C and –10°C precipitates up to 70 percent of the magnesium sulfate, which is then filtered to recover 85–96 percent pure Epsom salt that is marketed as an industrial-grade Epsom salt.

ESSENTIALITY

Magnesium is a major intracellular cation that is a necessary cofactor for enzymatic reactions vital to every major metabolic pathway. Magnesium is a cofactor of many enzymes in the body including those involved in cellular respiration and transfer of phosphate between adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP). Extracellular magnesium is vital to normal nerve conduction, muscle function, and bone mineral formation. A common sign of magnesium deficiency in all species is hyperexcitability caused by a reduction in nerve resting membrane potential closer to the level at which an action potential is triggered. Less severe signs of magnesium deficiency include reduced appetite and poor performance. Magnesium deficiency is also pro-inflammatory and is associated with increases in oxidative stress in vivo and cardiac susceptibility to ischemia/reperfusion (I/R) injury (Kramer et al., 2003). Magnesium deficiency can increase the susceptibility to toxicoses from various other metals such as aluminum (Nielsen et al., 1988). In freshwater fish, low magnesium water increases the uptake of copper (de Schamphelaere and Janssen, 2002), cadmium (Michibata et al., 1986), silver (Schwartz and Playle, 2001) and tin (Douglas et al., 1996). Magnesium appears to out-compete these toxic elements for metal binding ligands on the surface of the gills, reducing uptake of these more toxic metals.

Magnesium deficiency occurs more commonly in ruminants than nonruminants, especially those grazing in pastures. This appears to be due to the low magnesium content of lush growing pasture coupled with a relatively high content of potassium, protein, organic acids, and other antago-

nists that interfere with the transport of magnesium across the rumen wall (Martens and Schweigel, 2000). In contrast to nonruminants, adult ruminants must absorb magnesium effectively from the forestomach if they are going to meet their tissue magnesium requirements. Though the small intestine of ruminants can absorb magnesium, overall, the small intestine is actually an area of net magnesium secretion (Greene et al., 1983).

Because they are dependent on forestomach magnesium absorption, the magnesium requirements of ruminant species are higher than other species and range from 0.15–0.3 percent of the diet. For most nonruminant animals, including most freshwater fish (Gatlin et al., 1982), requirements for magnesium can be satisfied with just 0.06 percent magnesium diets (NRC, 1994, 1998, 2006). Nearly 60 percent of the magnesium in the body is located within bone mineral, and most of the rest is located within the intracellular fluids of the body.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Determination of magnesium in feeds and tissues is best accomplished by wet or dry ashing of the sample followed by resuspension of the ash in an acidic solution for analysis by atomic absorption spectrophotometry. Atomic absorption is conducted at a wavelength of 285.2 nm and can detect as little as 0.001 mg magnesium per liter. Phosphate, silicon, titanium, and aluminum that might be in the sample can interfere with magnesium absorption spectra, but their effect is masked by the addition of lanthanum to the standards and samples being analyzed. Since low magnesium values result if the pH of the sample is above 7, both standards and samples are prepared in dilute hydrochloric acid solution. Concentrations of calcium greater than 1,000 mg/L in the analyzed ash suspension can also cause low magnesium values. Concentrations of up to 500 mg/L each of sodium, potassium, and nitrate cause no interference. Anionic chemical interferences can be expected if lanthanum is not used in samples and standards (EPA, 1983). The nitrous oxide-acetylene flame will provide two to five times greater sensitivity than an air-acetylene flame, but is not necessary for routine analysis of feeds or biological samples. Near-infrared spectrophotometry is not an acceptable method of determining magnesium content of feedstuffs or forages, though it is often used for that purpose.

Magnesium concentrations can also be measured to ppb (ug/L) levels using the ICP-OES. The ICP uses radio frequency-generated plasma to excite the electrons of the magnesium atoms, which then produce photons of light unique to magnesium. Photomultiplier tubes detect and quantitate the photons emitted by the excited magnesium atoms, allowing quantification of the magnesium concentration. An inductively-coupled plasma source atomizes and excites even the most refractory magnesium atoms with high efficiency, so there is less interference and about 10-fold greater sensitivity using ICP-OES than using atomic absorption spectrometry.

REGULATION AND METABOLISM

In nonruminant animals and young (preruminant) ruminants, magnesium is absorbed primarily from the small intestine. In most cases, the dietary magnesium is readily solubilized within the acidic environment of the stomach and a large proportion remains available for absorption in the upper duodenum. In adult ruminants, small intestine secretion of magnesium generally exceeds small intestine absorption of magnesium (Cragle, 1973) so that the ruminant becomes highly dependent on absorption of magnesium across the forestomach walls to meet its magnesium needs. Magnesium is usually absorbed by an active transport process, which is especially useful when magnesium content of the diet is low. Passive diffusion across gastrointestinal and forestomach walls can occur whenever ionized magnesium concentration in the digestive fluid is greater than the ionized magnesium content of the extracellular fluids, which allows magnesium to flow down its concentration gradient into the blood.

Large amounts of magnesium are stored in bone where it is an integral part of bone mineral crystals. However, it is not readily labile and available for metabolic needs of the animal during periods of dietary magnesium insufficiency. Magnesium homeostasis essentially consists of urinary excretion of any excess dietary magnesium that is absorbed. About 72 percent of magnesium in the plasma (the portion that is not bound to plasma proteins) crosses the glomerulus and enters the renal tubular fluids. Under most circumstances, much of this magnesium is reabsorbed and passes back into the plasma. Plasma magnesium concentration for most mammals and birds is normally between 0.75 and 1.5 mmol/L or 18 and 36 mg/L. The kidneys play a key role in maintaining magnesium homeostasis, but only under conditions of hypermagnesemia. If dietary magnesium is absorbed in excess of need, plasma magnesium concentration rises above the renal threshold for reabsorption of magnesium and the excess is excreted into the urine. The renal threshold for magnesium, i.e., the plasma magnesium concentration at which all magnesium filtered across the glomerulus is reabsorbed, is approximately 0.75 mmol/L or 18 mg/L in the cow and 0.90 mmol/L or 22 mg/L in sheep, and closer to 1.0 mmol/L for most other species (Littledike and Goff, 1987). Plasma magnesium concentrations below these levels indicate that dietary magnesium absorption is not sufficient and little or no magnesium will be detected in urine. The renal threshold is responsive to parathyroid hormone, which is secreted in response to hypocalcemia, not hypomagnesemia. Under the influence of parathyroid hormone, the renal tubular absorption of both calcium and magnesium is enhanced, returning more of the filtered calcium and magnesium into

the blood, raising blood calcium and magnesium concentrations. Unfortunately, since hypomagnesemia does not elicit parathyroid hormone secretion, renal reabsorption of magnesium does not improve in response to developing hypomagnesemia to any great extent (Rude et al., 1978).

Animals have no hormonal mechanisms that allow the animal to adapt to a low magnesium diet for any length of time by increasing intestinal magnesium absorption. Since gastrointestinal magnesium absorption is not directly regulated hormonally, the animal must receive a relatively constant supply of magnesium in the diet to avoid developing hypomagnesemia and deficiency symptoms.

SOURCES AND BIOAVAILABILITY

Magnesium is most commonly supplemented using magnesium oxide. Pure magnesium oxide is 60.3 percent magnesium and 39.7 percent oxygen. The heat of calcination required to form pure MgO from magnesite ore renders the magnesium less bioavailable. Feed-grade sources of MgO are incompletely calcined and are generally between 52 and 56 percent magnesium. Magnesium sulfate is more water soluble than MgO and therefore more available for absorption than MgO. It is used less frequently because it is generally less palatable, has a stronger laxative effect, and is more expensive than MgO (Miller, 1979). Similarly magnesium chloride is available and occasionally used but is more expensive than MgO. Magnesium acetate is available but is cost prohibitive for routine formulation of diets as a magnesium supplement. Dolomitic limestone is approximately 12 percent magnesium, and when used as a source of calcium it will ordinarily supply all the magnesium needed to meet the requirements of most nonruminant species if ground finely enough to become solubilized in the stomach acids.

Plant uptake of magnesium increases with soil magnesium content. Magnesium fertilization of the soil can increase plant magnesium content. High soil potassium tends to decrease magnesium uptake from soils. Cool, wet weather can also reduce plant magnesium content, which increases susceptibility of grazing animals to development of hypomagnesemic tetany. In some forage systems, it is common practice to spray magnesium oxide slurries onto the surface of foliage that cattle or sheep are expected to graze within the next few days to help avoid hypomagnesemia.

Most major feedstuffs used in diet formulation (corn, wheat, soybean meal) contain more than 0.10 percent magnesium. Therefore it is not usually necessary to supplement diets of nonruminants with inorganic magnesium unless purified ingredients dominate the diet.

TOXICOSIS

Magnesium toxicosis presents itself as three distinct clinical pictures. These are hypermagnesemia with sedation, diarrhea, and inappetance, which all lead to reduced performance. Only with a single very large dose of magnesium is it possible to raise blood magnesium concentration to the extent necessary to observe sedation. Acute and chronic supplementation with excessive magnesium can cause diarrhea and/or inappetance. See Table 18-1 for effects of magnesium exposure on animals.

Single Dose

When blood magnesium concentration is increased above 5 mEq/L (60 mg/L), there is a general reduction in nerve activity and loss of muscle tone. Animals lose the ability to stand. At 6 mEq/L, the heart rate slows and there are characteristic changes observed in the electrocardiogram indicative of reduced ventricular contraction. At about 7 mEq/L, there is a loss of many of the reflexes of the body and there is central nervous system depression. Above 10 mEq/L, the animal is likely to go into a coma and there is a high risk of asystolic cardiac arrest (Mordes and Wacker, 1978). Prior to the 1930s and the development of suitable barbiturates for veterinary anesthesia, veterinarians rapidly administered magnesium intravenously to horses, cattle, and dogs to induce a recumbent state to provide restraint for short surgical procedures. The magnesium caused hyperpolarization of cell membranes, reducing the likelihood that an action potential could be generated in the nerves and muscles of the body. The effective dose was about 0.025–0.028 g Mg/kg BW as magnesium sulfate (Bowen et al., 1970). Smaller doses of magnesium sulfate are often incorporated into chloral hydrate anesthesia protocols for horses to improve muscle relaxation. By increasing the intravenous dose of magnesium above 0.2 g/kg BW, animals could be effectively euthanized by inducing cardiac arrest (but not humanely by today's standards because consciousness may or may not be induced first). Hypertonic magnesium sulfate administered rectally can also cause an increase in blood magnesium concentration and was once used to treat hypomagnesemic tetanies of cattle (Bacon et al., 1990). To achieve the same effects by oral administration of magnesium would require very large doses of readily soluble magnesium administered as a drench. In most cases, the kidneys would be likely to rid the body of the excess absorbed magnesium before blood magnesium concentrations could rise to deleterious levels. In fact, there are no reports of toxicosis from hypermagnesemia following oral ingestion of magnesium occurring in animals with intact renal function. Several cases of coma induced by oral magnesium have been reported in humans, but in nearly every case the person was in renal failure, which compromised the ability of the body to rid itself of the excess absorbed magnesium (Fassler et al., 1985). In newborn lambs, administration of 10 ml of a 25 percent magnesium sulfate solution rectally induced coma and loss of deep tendon reflexes. Rectal administration of 10 ml of a 50 percent magnesium sulfate solution caused cardiorespiratory failure in less than an hour (Andrews et al., 1965). Based on this re-

search, it is assumed that a single oral dose of 0.10 g Mg/kg BW (approximately one-half the dose causing sedation in lambs) administered orally would not cause intolerable hypermagnesemia in animals. Absorption of magnesium administered orally is expected to be even slower and less efficient than rectal administration and absorption across the colon mucosa. Unfortunately, unabsorbed magnesium can induce an osmotic diarrhea in animals. Cattle given drenches consisting of between 102 and 205 g magnesium as magnesium oxide developed severe scours within 48 hours. Those receiving just 68 g magnesium suffered no diarrhea (Care, 1960).

Magnesium sulfate, a saline laxative, is often used for treatment of intestinal impactions in horses. Clinical signs of hypermagnesemia are an uncommon complication following oral administration of magnesium sulfate but have been known to occur (Henninger and Horst, 1997). Renal insufficiency, hypocalcemia, or compromise of intestinal integrity were thought to have predisposed the horses in this report to magnesium toxicosis.

Acute

Care (1960) fed steers a diet that was 0.76 percent magnesium for 8 days with no adverse effects, but diets that were 1.15 percent or greater magnesium induced diarrhea within 48 hours.

Chronic

Veal calves were fed milk replacers containing 0.1 percent (NRC requirement), 0.3 percent, or 0.6 percent magnesium for 16 weeks (Petersson et al., 1988). No adverse effects were observed with the 0.3 percent magnesium diet. However, weight gain was reduced in calves fed the 0.6 percent magnesium milk replacer and upon necropsy it was discovered that 70 percent of calves fed 0.6 percent magnesium milk replacer had stones in their kidneys consisting primarily of calcium apatite and secondarily of struvite. Adding NaCl to the milk replacer reduced kidney and bladder calculi formation. When calves were allowed access to water ad libitum, the added NaCl prevented calculi formation completely.

Lactating cows were fed diets that were either 0.37 or 0.63 percent magnesium. Cows that were fed the high magnesium diet absorbed more total grams of dietary magnesium, but there were no differences in feed intake or milk production in these cows during the 16 days of the trial (Jittakhot et al., 2004). Holstein bull calves fed 1, 2, or 4 percent supplemental magnesium as magnesium oxide (added to a control diet containing 0.3 percent magnesium) experienced diarrhea with tubular mucus casts at all three supplemental magnesium levels. As dietary magnesium content increased, the extent and intensity of the diarrhea increased. High (2 and 4 percent) magnesium diets reduced feed consumption and weight gains. Plasma magnesium rose sharply in response to the increased intake of magnesium. In calves receiving the 4 percent added magnesium, the plasma values were abnormally high at 50–60 mg magnesium per liter of plasma. Within 1 week after calves were returned to the control diet, magnesium in urine and plasma declined to control levels (Gentry et al., 1978).

In growing steers, increasing diet magnesium from 0.3 to 1.4 percent with magnesium oxide reduced diet DM digestibility. Increasing diet magnesium to 2.5 or 4.7 percent caused a magnesium dose-related loss in weight gain and severe diarrhea and lethargy (Chester-Jones et al., 1990).

High magnesium chloride in the drinking water of sheep (water was 0.2–0.3 percent magnesium chloride) caused occasional diarrhea over a 16-month period and reduced the growth rate of the sheep (Pierce, 1959).

Day-old chicks fed corn-soy rations containing adequate amounts of calcium and phosphorus could be fed up to 0.51 percent magnesium in the diet with no adverse effects. However, when diet magnesium was increased to 0.71 percent, the chicks exhibited poorer growth and their tibia were lower in ash than control chicks (Chicco et al., 1967). Nugara and Edwards (1963) fed broiler chicks diets that were 0.32 percent magnesium with no ill effects but found that increasing magnesium content to 0.64 percent reduced body weight and bone ash and increased mortality. Lee et al. (1980) demonstrated that young broilers fed corn-soymeal diets with 0.9 percent added magnesium had reduced growth and diarrhea. Bones from these chicks had lesions similar to those of rickets, and bone ash was greatly reduced. When dietary phosphorus was marginal to below normal, the addition of as little as 0.3 percent magnesium to the basal diet also caused rachitic lesions in bone, suggesting that magnesium supplementation could interfere with dietary phosphorus use. The study cautioned that dietary magnesium concentrations could often reach levels of 0.4–0.7 percent if high magnesium limestones were used in the diet or if the soybeans or corn used in the ration originated from high magnesium soils.

Day-old Japanese quail (*Coturnix coturnix japonica*) were fed 11 dietary levels of magnesium ranging from 125 to 2,000 mg/kg (0.0125–0.2 percent magnesium diets) using magnesium sulfate as the source of magnesium added to highly purified diets. Diets below 0.03 percent magnesium were associated with magnesium deficiency signs including poor growth, and occasional excitability, gasping, and convulsions. The 0.2 percent diet did not affect growth of the birds, though there was an unexplained increase in mortality of the chicks in this group and was unlikely due to magnesium (Harland et al., 1976).

Inclusion of magnesium carbonate into laying hen rations for 38 weeks at levels that raised total diet magnesium to 1.12 percent reduced egg production, and the hens had lower BW at the end of the trial. When dolomitic limestone was used as the source of magnesium, no effects on egg production or shell strength were observed with diet magnesium as

high as 0.79 percent (Stillmak and Sunde, 1971). No adverse effects were observed when diet magnesium was increased to 0.77 percent in laying hens over a period of 7 weeks (Atteh and Leeson, 1983). McWard (1967) found that diets that were 1.9 percent magnesium supplied by magnesium sulfate reduced egg production.

Swine have been fed 0.24 percent magnesium diets for short periods of time immediately prior to slaughter to reduce fluid exudation by muscle postmortem, which enhances carcass characteristics (Hamilton et al., 2002). Sows are often fed 1 percent magnesium sulfate (0.20 percent magnesium) before farrowing as a stool softener with no evidence of toxicosis. There are no data that demonstrate a toxic level of dietary magnesium in this species.

In tilapia, 0.32 percent dietary magnesium concentration in a low (24 percent) protein diet caused signs of toxicosis. At higher protein diets (44 percent), this level of magnesium was not a problem (Dabrowska et al., 1989).

Guinea pigs fed diets that were 1.2 percent magnesium suffered diarrhea, lethargy, reduced growth, and increased mortality, especially if the dietary calcium to phosphorus ratio was below 0.6:1 (Morris and O'Dell, 1963). If the calcium to phosphorus ratio was greater than 1.5:1, there was no ill effect of 1.2 percent magnesium diets.

Cats fed diets consisting of 0.5 percent magnesium were found to have greater amounts of struvite stones in their bladder and urethra. In these studies, magnesium oxide was used to increase dietary magnesium content (Lewis et al., 1978; Kallfelz et al., 1980). Subsequent studies suggest that the magnesium oxide may have acted as an alkalinizing agent and that magnesium, in and of itself, was not the true cause of the struvite stones. High pH urine, regardless of magnesium status, will cause struvite stones (Taton et al., 1984; Buffington et al., 1990).

Horses often consume forages that are 0.5 percent magnesium with no ill effects (Lloyd et al., 1987). Mature ponies consuming diets that were 0.86 percent magnesium from magnesium oxide for one month suffered no ill effects of the diet (Hintz and Schryver, 1973).

Purified mouse diets supplemented with magnesium chloride to achieve dietary levels of 0, 0.036, 0.072, 0.144, 0.288, or 0.575 percent magnesium were fed to mice for 13 weeks. The 0.575 percent magnesium diet caused a decrease in BW of the mice. This diet also contained 1.725 percent chloride, which may have affected feed intake as much or more than the magnesium content of the diet. Adverse clinical signs and hematological or blood biochemistry parameters were not evident in mice fed diets lower in magnesium. Histopathologically, vacuolation of kidney tubular cells was apparent in males of the 0.288 and 0.575 percent magnesium diet groups (Tanaka et al., 1994). However, it is possible that the observed lesions could have been caused by the metabolic acidosis induced by the chloride added to the diet. Thus, the study only demonstrated that diets containing more than 2.5 percent $MgCl_2 \cdot 6H_2O$ can exert toxic effects in mice. It may not be a good study to assess magnesium toxicity.

Factors Influencing Toxicity

When animals with compromised renal function are fed a diet that contains more magnesium than they require, they are unable to excrete the excess absorbed magnesium as they normally would. They are therefore at greater risk of developing hypermagnesemia and they can become lethargic. Availability of water on an ad libitum basis will also permit excretion of excess absorbed magnesium by the kidneys. Adequate phosphorus in the diet can overcome some of the deleterious effects that magnesium can have on bone formation, especially in poultry (Lee et al., 1980).

The source of magnesium in the diet can affect feed intake and if feed intake is reduced, growth and performance will be reduced over a period of time. Magnesium oxide is generally considered to be less detrimental to feed intake, has less acidifying activity, and has less of a laxative effect than magnesium sulfate and magnesium chloride. Magnesium oxide is also used in lower amounts since it is a highly concentrated source of magnesium. The availability of the magnesium, particularly from magnesium oxide, is also dependent on the source and the particle size of the magnesium supplement (Miller, 1979; Henry and Benz, 1995).

Elevated levels of fluoride in the diet can increase the deposition of magnesium in bone tissues. This may weaken the bone if diet magnesium levels are elevated along with high dietary fluoride.

Magnesium can also act as an alkalinizing agent and is often incorporated into ruminant diets as a means of raising the pH of rumen fluid in animals fed high grain diets. Should magnesium enter the plasma unaccompanied by an anion, it could cause metabolic alkalosis in the animal (see chapter on Minerals and Acid-Base Balance).

TISSUE LEVELS

Serum magnesium is typically between 0.75 and 1.11 mM (18–27 mg/L) in most mammalian species (Table 18-2). Cerebrospinal fluid (CSF) magnesium concentrations are generally slightly lower than the concentrations in plasma. In normal cows cerebrospinal fluid magnesium concentration is 0.9–1.0 mM. During hypomagnesemic tetany of cattle and sheep, plasma and CSF magnesium concentrations are often less than 0.45 and 0.5 mM, respectively (Bohman et al., 1983; Puls, 1994). If the kidneys fail to excrete excess absorbed magnesium rapidly enough, serum and then cerebrospinal fluid magnesium concentrations can rise, inducing lethargy and sedation in the animal. Sedation would begin to become apparent once plasma magnesium concentration exceeded 1.6–2.0 mM (40–50 mg/L) (Mordes and Wacker, 1978). Diet magnesium is an extremely rare cause of hypermagnesemia in animals with fully functioning kidneys. Prolonged elevations

in dietary magnesium will increase bone magnesium deposition. Mammalian bone typically has 6–12 mg Mg/g ash (0.6–1.2 percent magnesium in the ash). Poultry have bone ash that is normally 0.5–0.8 percent magnesium. Under toxic conditions, bone ash magnesium content in chicks was reported to be as high as 1.50 percent (Lee et al., 1980). Magnesium concentration in horse muscle does not change during magnesium deficiency (Stewart et al., 2004). However, in most species liver and kidney magnesium concentrations increase with dietary magnesium, though levels rarely exceed 1,000 mg/kg tissue DM (250 mg/kg wet weight) (Puls, 1994). Chester-Jones et al. (1990) did an extensive study of the effect of high dietary magnesium concentration on the magnesium content of various tissues of steers fed the diets for 130 days. While there was a significant linear effect of diet magnesium on tissue magnesium content, the changes in magnesium content were negligible, with the exception of bone. Bone magnesium content nearly doubled with extremely high dietary magnesium: from 3.6 g/kg dry bone when fed a 0.3 percent magnesium diet to 6.4 g/kg dry bone in steers fed a 4.7 percent magnesium diet (Table 18-3).

MAXIMUM TOLERABLE LEVELS

Single Oral Dose

The maximum tolerable single dose in ruminants is estimated from the work of Care (1960) to be 0.12 g Mg/kg BW. Higher amounts risk induction of diarrhea and hypermagnesemia. In nonruminant species, where the efficiency of absorption of magnesium is usually greater, the maximum tolerable dose would be smaller. No data exist to recommend an upper tolerable single oral dose in nonruminant animals.

Acute

The absence of osmotic diarrhea is used as the criterion for determining the level of dietary magnesium that can be tolerated for short periods of time. Usually diets that are high enough in magnesium to cause adverse effects in less than 10 days also result in a drastic decrease in feed intake. In cattle, the work of Care (1960) demonstrates that diarrhea rapidly occurs in cattle fed 1.15 percent magnesium diets, but 0.76 percent diets were well tolerated for short periods. Therefore, the acute tolerable dietary level of magnesium for ruminants is set at 0.76 percent. No data exist with respect to diets between 0.7 and 1.15 percent magnesium, which would allow a more precise definition of the acute tolerable dietary magnesium level.

Chronic

The criteria for determining the maximum tolerable dietary magnesium concentration is considered to be the highest dietary magnesium level that can be fed without risk of detrimental effects on feed intake and growth or performance of the animals. In cattle, reduced diet digestibility or diarrhea has been observed only with diets that are greater than 1.15 percent magnesium. Dairy cattle have been fed diets that are between 0.50 and 0.60 percent magnesium with no observable adverse effects prior to parturition (Goff et al., 1991; Oetzel et al., 1991; Abu Damir et al., 1994) and during lactation (Jittakhot et al., 2004). Though detrimental effects of high dietary magnesium have only been reported with diet magnesium greater than 1 percent, no experimental data exist to extend the maximum tolerable dietary magnesium level beyond 0.6 percent of the diet for ruminants. In the horse, the maximum tolerable level is set at 0.8 percent magnesium, based on the work of Hintz and Schryyer (1973). In broiler diets, provided there is adequate available phosphorus in the diet, the lowest magnesium level causing significant adverse effects is 0.64 percent magnesium. The maximum tolerable level is therefore set at 0.5 percent magnesium, based largely on the work of Chicco et al. (1967) in which diets that were 0.51 percent magnesium were without adverse effects. In laying hens, adverse effects on egg production are not seen until diet magnesium is well above 0.79 percent. The maximum tolerable diet magnesium level for laying hens is therefore set at 0.75 percent.

In most of the other nonruminant species of mammals, studies to examine the tolerable limit for dietary magnesium are not available. For example, the 1980 NRC *Mineral Tolerance of Domestic Animals* suggested that the maximum tolerable level of magnesium for swine be set at 0.3 percent. This limit is not based on any reported data. The highest reported level of dietary magnesium safely fed to pigs was 0.24 percent and it was fed for just a short amount of time (Hamilton et al., 2002). If there is water available and the kidneys are functioning it is likely that no ill physiological effects will be observed from magnesium ingestion at levels higher than 0.24 percent, but there are no data to support a dietary maximum tolerable magnesium level in this species. Similarly, only one study examines the effect of dietary magnesium on fish performance and this study is confounded by dietary protein alterations at the same time. Dogs and cats can commonly consume diets that are up to 0.2 percent magnesium—well above their requirement. In cats some detrimental effects (uroliths) have been noted with high magnesium diets—but uroliths seem more highly dependent on urine pH as opposed to dietary magnesium. There are no studies of the effects of higher dietary magnesium levels in dogs and cats upon which to base a maximum tolerable level. Decreased palatability of the diet is a possibility with magnesium supplementation, but no studies with any other species have demonstrated any effect on feed intake with diets that were less than 0.5 percent magnesium.

Magnesium in the digesta can complex with phytin phosphorus, rendering the complex insoluble and reducing the availability of the phytate-bound phosphorus for absorption.

Complexing of magnesium with phytin phosphorus can occur at normal dietary magnesium levels. Therefore, feeding more magnesium than is required will have some impact on phosphorus use of nonruminant animals, but this effect was not considered in setting the maximum tolerable limits for magnesium.

FUTURE RESEARCH NEEDS

Few studies involve feeding diets with levels of magnesium that are above the required levels of magnesium in swine. This could be of great importance if magnesium supplementation just before slaughter proves a fruitful means of enhancing pork quality, but only if it can be done without other detrimental effects.

If farmed fish are to be fed large amounts of fish meal, they could be receiving relatively high amounts of dietary magnesium—fish meals are approximately 2.5–3 percent magnesium on a DM basis. If there is a negative effect of dietary magnesium on growth of fish, as suggested by Dabrowska et al. (1989), this information could be of great importance to the aquaculture industry.

SUMMARY

Magnesium is a relatively nontoxic mineral. Very high amounts can be fed to animals with no ill effects if the kidneys are functioning. Inappetance is the major practical problem encountered from excessive dietary magnesium. Because only a fraction of dietary magnesium is generally absorbed, high amounts of magnesium in the diet can cause an osmotic diarrhea in the animal. This is usually of greater concern in poultry houses where the water content of manure can be a major concern to producers but does not necessarily impact the health of the birds.

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TABLE 18-1 Effects of Magnesium Exposure on Animals

Toxicity of a Single Dose of Magnesium (per os)

Animal (N)[a]	Age	Quantity of Mg[b]	Source	Route	Effect(s)	Reference
Horses (2)	Adult	67–90 g	Mg sulfate	Drench for colic	Tremors, recumbency Severe hypermagnesemia Renal function was compromised at time of drench	Henninger and Horst, 1997
Cattle (2)	Adult	68 g	MgO	Drench	No adverse effects; increased blood Mg concentration for several hrs	Care, 1960
		102 g	MgO	Drench	Scours-osmotic diarrhea	
		205 g	MgO	Drench	Severe scours	

Acute Magnesium Toxicity (from < 10 d ingestion of the diet)

Animal (N)[a]	Age	Quantity of Mg[b]	Source	Route	Effect(s)	Reference
Cattle (4)	Steers (18 mo)	0.76%	Calcined magnesite (MgO)	Diet	No adverse effects	Care, 1960
Cattle (4)	Steers (18 mo)	1.15%	Calcined magnesite (MgO)	Diet	Osmotic diarrhea	Care, 1960

Chronic Magnesium Toxicity (from >10 d ingestion of the diet)

Animal (N)[a]	Age	Diet % Mg[b]	Source	Duration	Effect(s)	Reference
Mice (20)	Weanlings	0.036, 0.072, 0.144, 0.288, or 0.575	Mg chloride	13 wk	Decreased weight gain at 0.575% Mg only; evidence of renal edema with 0.288 and 0.575% Mg	Tanaka et al., 1994
Chickens (24)	Day-old	0.32 or 0.64	Mg carbonate	3 wk	Decreased growth rate; decreased bone calcification at 0.64% Mg only	Nugara and Edwards, 1963
Chickens (60)	Day-old	0.31, 0.51, or 0.71%	Mg carbonate	4 wk	Decreased growth rate and decreased tibial ash at 0.71% Mg	Chicco et al., 1967
Chickens (10)	Day-old	0.3, 0.6, or 0.9 supplemental Mg	MgO		When diet P and Ca were adequate adverse effects of decreased growth and bone ash and increased mortality only at 0.9% Mg	Lee et al., 1980

Chickens (8)	Laying hen	0.17 0.37 0.57 0.77	MgO	7 wk	Controls Plasma Mg = 0.86 mmol Bone ash Mg = 0.50% Plasma Mg = 1.00 mmol Bone ash Mg = 0.61% Plasma Mg = 1. 15 mmol Bone ash Mg = 0.64% Plasma Mg = 1.36 mmol Bone ash Mg = 0.69% No adverse effects observed for any diet Mg level on feed intake, egg production, or egg weights.	Atteh et al., 1983
Chickens (12)	Pullet hens	0.20, 0.47, or 0.79	Dolomitic limestone	38 wk	No adverse effects on egg production or shell quality	Stillmak and Sunde, 1971
Chickens (6)	Pullet hens	0.43, 0.66, 1.12, or 1.59	Mg carbonate	38 wk	No adverse effects at 0.43 or 0.66% Mg; decreased egg production and lower BW at 1.12 and 1.59% Mg	Stillmak and Sunde, 1971
Horses (3)	Mature ponies 181 kg	0.16, 0.31, or 0.86	MgO	31 d	No detrimental effects on feed intake or any other parameters. Serum magnesium increased with diet Mg but never reached dangerous levels; 0.91, 1.08, and 1.39 mmol, at 0.16, 0.31, and 0.86% Mg diets, respectively	Hintz and Schryver, 1973
Cattle (47)	Late gestation dairy cows	0.60	Mg chloride and Mg sulfate mix	3 wk	No diarrhea observed	Goff et al., 1991
Cattle (6)	Lactating dairy	0.37 and 0.63	MgO	16 d	No difference in feed intake or milk production	Jittakhot et al., 2004
Cattle (4)	Calves 1–3 mo	0.3, 1.3, 2.3, or 4.3	MgO	2 wk	Diarrhea at 1.3% Mg; decreased feed intake and weight gain at 2.3%; hypermagnesemia at 4.3% Mg	Gentry et al., 1978
Cattle (10)	Day-old	0.1, 0.3, or 0.6	MgO added to milk replacer	16 wk	Reduced weight gain and renal calculi with 0.6% Mg added when access to water restricted	Petersson et al., 1988
Cattle (4)	Steers <1 yr	0.3 1.4 2.5 4.7	MgO	130 d	No adverse effects Reduction in diet DM digestibility Reduced feed intake and weight gain, diarrhea Reduced feed intake and weight gain, severe scours lethargy	Chester-Jones et al., 1990

[a]Number of animals/treatment group.
[b]Quantity of magnesium dosed. SI conversion: 1 mg magnesium equals 41.1 μmoles Mg.

TABLE 18-2 Magnesium Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)[a]

Animal	Serum (mg/L)	Muscle (mg/kg)	Liver (mg/kg)	Kidney (mg/kg)	Bone (mg/g ash)	Milk (mg/L) or Egg (mg/g eggshell)
Broiler chickens	16–36	600–2,000	600–2,000	800–2,000	5–8	
Laying hens	16–36		600–2,000	800–2,000		5.3–6.4
Pigs	19–33		600–800	560–720	5.8–7.5	75–140
Cows	18–24	600–1,000	400–1,000	200–800	6–12	100–140
Sheep	19–30	800–1,000	440–800	440–920	4–8	
Fish[b]		Meal with bone 25–31 g/kg DM				

[a]Data largely adapted from Puls, 1994.
[b]Menhaden or anchovy fish meal fed to livestock.

TABLE 18-3 Tissue Magnesium Concentrations in Steers Fed Different Dietary Levels of Magnesium (mg/kg)[a,b]

Tissue	0.3% Dietary Mg	1.4% Dietary Mg	2.5% Dietary Mg	4.7% Dietary Mg
Liver	540	560	570	650
Kidney	690	730	890	970
Heart	940	880	920	900
Rib-bone	3,620	4,330	5,750	6,430
Skeletal muscle	750	770	800	800

[a]Adapted from Chester-Jones et al., 1990.
[b]Data are on a dry matter basis.

19

Manganese

INTRODUCTION

Manganese (Mn) has an atomic number of 25 and has two oxidation states: Mn (0) and Mn^{+2}. It does not occur naturally as a metal but as a component of over 100 minerals (ATSDR, 2000).

The United States has used an estimated 700,000 tons of manganese per year since 1994 (Goonan and Jones, 2003). Between 85–90 percent of the demand for manganese in the United States is for steelmaking because of its sulfur-fixing, deoxidizing, and alloying properties (Corathers, 2001). Manganese is a component of certain aluminum alloys, particularly those used in the manufacture of soft drink cans, because it increases corrosion resistance. Inorganic manganese is also used in dry cell batteries, animal feed, brick coloring, and fertilizers (Corathers, 2001).

There are three forms of organic manganese that are of toxicological interest to humans. Methylcyclopentadienyl manganese tricarbonyl (MMT) replaced lead compounds as a fuel additive that improves the antiknock properties of fuels (Kaiser, 2003). Maneb (ethylenebisdithiocarbamate manganese) and Mancozeb (a mixture of substances that contain manganese, zinc, and dothiocarbamates) are plant fungicides (ATSDR, 2000).

ESSENTIALITY

Manganese is an essential element (Hurley and Keen, 1987; NRC, 1995, 2001; Klein, 2002). The estimated manganese requirement for maintenance, growth, and reproduction in rodents (rats and mice) is 10 mg/kg diet of manganese and for guinea pigs is 40 mg/kg diet of manganese (NRC, 1995). The estimated manganese requirements of growing pigs weighing more than 20 kg is 20 mg/kg diet of manganese (NRC, 1998); of immature leghorn chickens chicks older than 6 weeks is 28 or 30 mg/kg diet of manganese (NRC, 1994); of growing and finishing beef cattle is 20 mg/kg diet of manganese (NRC, 2000); and of dairy cattle is 40 mg/kg diet of manganese (NRC, 2001).

Manganese deficiency has occurred naturally in cattle, pigs, and poultry fed practical diets. The signs of manganese deficiency include abnormal bone formation (called perosis in chickens), abnormalities in carbohydrate and lipid metabolism, growth retardation, dermatitis, and reproductive failure. The primary enzymes that have been demonstrated to be sensitive to dietary deficiency of manganese are the glycosyltransferases and xylosyltransferases (manganese-activated enzymes involved in proteoglycan synthesis and hence bone formation), as well as arginase and mitochondrial superoxide dismutase (manganese metalloenzymes).

DIFFICULTY IN METHODS OF ANALYSIS AND EVALUATION

Determination of manganese in biological samples usually requires digestion of the organic matrix (generally by an oxidizing acid mixture) prior to analysis (ATSDR, 2000). Analyses are performed by flame atomic absorption (most commonly), furnace atomic absorption (when very low levels of manganese are present, as in plasma and urine), ICP-ES (for multi-mineral analyses), or neutron activation analysis (when very low amounts of manganese are present). None of the methods distinguish between different oxidation states of manganese.

Organic forms of manganese (i.e., MMT) are present in toxicological and environmental samples at very low levels (ng/g) and require a combination of techniques (i.e., gas or liquid chromatography with atomic absorption spectrometry) (ATSDR, 2000).

REGULATION AND METABOLISM

Absorption

The gut is the primary organ preventing excess manganese accumulation in the body when excess manganese is ingested (Hurley and Keen, 1987; Davis et al., 1993;

Andersen et al., 1999; ATSDR, 2000). Apparent absorption of orally administered ^{54}Mn has been estimated to range from 1–5 percent in rats, humans, and livestock (Hurley and Keen, 1987; Davis et al., 1993). However, excretion of absorbed manganese in the bile occurs very rapidly (i.e., as soon as 1 hour after ingestion) (Malecki et al., 1996). Thus, in most studies manganese excreted in the bile was part of the manganese measured in the feces and assumed to be nonabsorbed. True absorption of manganese has been estimated to be 8 percent in young rats (Davis et al., 1993).

Manganese absorption is inversely related to dietary levels of manganese (Abrams et al., 1976). Absorption of manganese appears to occur by a low capacity saturable process (Garcia-Aranda et al., 1983) and by diffusion (ATSDR, 2000). Uptake and retention of dietary manganese was found to be greater in the suckling than postweaning stage in rats (Keen et al., 1986).

A variety of dietary factors affect apparent absorption of manganese. These factors include iron, calcium, phosphorus, phytate, and amino acids. These interactions will be discussed in the "Bioavailability" section below, because investigators generally measure tissue retention of manganese or ^{54}Mn, not apparent absorption of manganese, and rarely true absorption of manganese.

Inhalation

Inhaled manganese can be classified as respirable (generally dust particles of <5 microns) and as total dust (ATSDR, 2000). The respirable dust enters the bronchioles and alveoli. and the manganese is absorbed rapidly into the blood stream (Andersen et al., 1999). The total dust does not travel deeply into the lungs and is coughed up and swallowed (ATSDR, 2000). The percentage of this inhaled manganese that is absorbed in the gut is unknown.

Although there are very few data in animals, it is assumed that inhaled manganese is not a major source of manganese accumulation in the tissues of livestock. However, Bench et al. (2001) found that California ground squirrels took up measurable amounts of manganese from the soil by the olfactory pathway. Tjälve and Henriksson (1999) hypothesized that inhaled manganese taken up via the olfactory pathways was passed transneuronally to other parts of the brain.

Transport

Manganese absorbed in the gut is transported by α 2-macroglobulins and albumin to the liver (Andersen et al., 1999). Davis et al. (1993) demonstrated that intraportally administered ^{54}Mn complexed to albumin (but not as free ^{54}Mn or ^{54}Mn complexed to transferrin) distributed in tissues, as did orally administered ^{54}Mn. This protein-bound manganese is efficiently cleared in the liver (Andersen et al., 1999).

Manganese leaving the liver is bound to transferrin. Inhaled and injected manganese is transported by transferrin (Hurley and Keen, 1987). Hypotransferrinemic mice (produce <1 percent of normal transferrin levels) have been found to accumulate extra manganese in their livers but accumulate less manganese in other tissues (Dickinson et al., 1996). This suggests that alternatives to transferrin exist. However, transferrin is believed to be the primary transporter of manganese across the blood–brain barrier (Aschner et al., 1999).

Urinary Excretion

Urinary excretion of manganese does not appear to be sensitive to dietary intake and is a minor route of excretion of manganese (Freeland-Graves et al., 1988; Greger et al., 1990; Davis and Greger, 1992). Adult human females excreted about 11 nmoles/day of manganese (0.01 μg/kg BW/day of manganese) whether intake was 1.7 or 16.7 mg manganese daily (Davis and Greger, 1992; Davis et al., 1992).

Biliary Secretion

Bile is the major excretory route of injected manganese (Bertinchamps et al., 1966; Klaassen 1974; Thompson and Klaassen, 1982; Ballatori et al., 1987). Rats excreted 15–40 percent (Ballatori et al., 1987) and calves excreted 21 percent (Abrams et al., 1977) of injected doses of manganese in bile. Klaassen (1974) observed that rats secreted proportionately more manganese into bile than rabbits and dogs.

Bile is also the major excretory route of ingested manganese, but the effectiveness of the gut in preventing excess absorption blunts the effect of biliary secretion (Abrams et al., 1977; Davis et al., 1993; Malecki et al., 1996). Using an isotope-based model system, Davis et al. (1993) estimated that growing rats lost 37 percent of their absorbed manganese (2.8 percent of their total manganese intake when true absorption was 8.2 percent) through biliary secretion. Malecki et al. (1996) observed that biliary secretion of manganese immediately after an oral dose was proportional to the amount of manganese given and that biliary secretion of manganese by fasted rats was proportional to chronic dietary manganese intakes. Hence, the biliary secretion of manganese during a 4-hour collection period immediately after an acute dose accounted for 3.4 percent of the dose, and the biliary secretion of manganese during a 4-hour collection period by fasted rats accounted for 0.2 percent of their previous day's intake. Calves excreted 0.2 percent of a duodenal dose of manganese into bile in one study (Abrams et al., 1977) and 2.1–3.6 percent of high levels of manganese infused intraduodenally in another study (Symonds and Hall, 1983).

Thus, there are several mechanisms that account for the huge difference in toxicity of inhaled and ingested manganese. Absorption of manganese is lower in the gut than in the lungs. Absorbed manganese is transported by macroglobu-

lins and albumin to the liver, where these manganese complexes are rapidly cleared and the manganese is excreted in bile. Transferrin receptors determine the location of manganese deposition in the brain under normal conditions.

SOURCES AND BIOAVAILABILITY

Diet

Diet is the major source of ingested manganese for humans and presumably livestock (EPA, 2004). Vegetable products (grain products, tea, and vegetables) contribute almost 75 percent of the manganese consumed by the average adult human male in the Total Diet Study (Pennington and Young, 1991). Similarly, unrefined grains and forages are the primary natural sources of manganese for livestock (Schroeder et al., 1966; Hurley and Keen, 1987).

The concentration of manganese in forages can vary by 10-fold with the species of plant, type of soil, and soil treatment. Diets for poultry based on corn (sometimes sorghum and barley) are deficient in manganese unless supplemented with it (typically through addition of manganese salts or manganese-rich ingredients such as wheat bran or middlings to the diet) (Hurley and Keen, 1987).

Water

The median manganese level in surface water studied in the National Ambient Water Quality Assessment was 16 μg/L of manganese, but 1 percent of samples had concentration of 400–800 mg/L of manganese (EPA, 2004). Higher levels of manganese were usually associated with industrial pollution. However, manganese in water can also reflect erosion of soils that can contain 2 to 7,000 μg/g soil of manganese (EPA, 2004).

Generally water is a minor source of ingested manganese. If cattle consume water containing 3 mg/L of manganese, the previous NRC report (1980) estimated that the cattle could consume 3 to 6 times their requirement for manganese. However, water with such a concentration of manganese would be discolored and would be less palatable to livestock (Personal communication, Linn and Raeth-Knight, 2004).

Air

Industries (primarily manganese mines and to a lesser extent battery plants, ferroalloy production facilities, coke ovens, and power plants) are the major sources of manganese dust in the air (ATSDR, 2000; Hudnell, 1999). Air levels of manganese vary with the proximity of industrial sources. Average ambient manganese levels have been reported near industrial sources to range from 220 to 330 ng/m^3 of manganese and in urban and rural areas without industrial sources to range from 10 to 70 ng/m^3 of manganese (EPA, 2004). Lynam et al. (1999) reported little difference in ambient manganese levels in areas with and without use of MMT in gasoline.

Bioavailability

The bioavailability of manganese varies greatly with natural diets. Manganese was more available to chicks fed casein-based versus soy-based diets (Halpin et al., 1986). The addition of fishmeal to manganese-deficient diets worsened the signs in chicks more than the addition of wheat bran (Halpin and Baker, 1986). Uptake of manganese was greater among 14-day-old rats fed cow or human milk rather than soy formula (Keen et al., 1986).

These effects could reflect differences in the phytate or amino acid content of the diets. Phytate has inconsistently been found to decrease manganese absorption and retention (Davies and Nightingale, 1975; Lee and Johnson, 1989). The presence of cysteine and histidine (Garcia-Aranda et al., 1983) and lactose (Dupuis et al., 1992) in the gut has been found to enhance uptake of manganese. Henry (1995) estimated the relative bioavailability of manganese to poultry was 1.2 times as great from manganese methionine and 1.1 times as great from manganese proteinate as from manganese sulfate or manganous chloride. In sheep, manganese methionine was 1.25 times as bioavailable as manganese sulfate (Henry, 1995).

The relative bioavailability of supplemental forms of manganese differed in some studies. Ammerman and Miller (1972) observed that the manganese salts included in diets did not affect bioavailability of manganese to chicks. However, Southern and Baker (1983) observed that chicks fed excess supplemental manganese were more sensitive to manganese chloride, carbonate, and sulfate than manganese oxide. Henry (1995) estimated the relative bioavailability of manganese to poultry was 0.55 from manganese carbonate, 0.30 from manganese dioxide, and 0.75 from manganese monoxide when the bioavailability of manganese from manganese sulfate and manganese chloride was considered to be 1. Manganese from manganese carbonate, dioxide, and monoxide (relative bioavailabilities were 0.3, 0.35, and 0.6, respectively) was even less bioavailable to sheep than manganese sulfate (relative bioavailability was 1) (Henry, 1995).

Calcium and Phosphorus

Two of the dietary factors most often studied in regard to manganese bioavailability for livestock are calcium and phosphorus (Hurley and Keen, 1987). High levels of both dietary calcium and phosphorus have been found to increase the severity of signs of manganese deficiency in dairy calves (Hawkins et al., 1955), in chickens (Wilgus and Patton, 1939), and in rats (Wachtel et al., 1943).

However, high levels of calcium and phosphorus did not decrease tissue manganese levels in pigs (Kayongo-Male et al., 1977) or decrease apparent absorption of manganese in

humans (Greger et al., 1981). Wedekind et al. (1991) demonstrated that the addition of graded levels of calcium as feed-grade limestone did not affect true absorption of manganese by chickens but addition of graded levels of phosphorus as dicalcium phosphate did. Dupuis et al. (1992) observed that the addition of calcium to gut infusates negated the enhancing effect of lactose on manganese absorption. Together these data suggest that the effect of calcium and phosphorus on manganese absorption reflects not only the relative and absolute amounts of calcium, phosphorus, and manganese fed, but also the levels of other dietary factors.

Iron

In iron-deficient rats, intestinal absorption of manganese was increased (Thomson et al., 1971; Flanagan et al., 1980) and liver manganese levels were increased (Shukla et al., 1990). Mena (1981) found that anemic and normal human subjects absorbed 7.5 percent and 3.0 percent, respectively, of ingested manganese.

The effect of iron supplementation above required amounts is less clear. Iron supplementation did not affect tissue manganese concentrations in calves (Ho et al., 1984), lambs (Prabowo et al., 1988), or chicks (Baker and Halpin, 1991), but decreased tissue manganese concentrations in mice (Hurley et al., 1983), sheep (Ivan and Hidiroglou, 1980), and rats (Davis et al., 1990) and decreased manganese transfer through the gut of rats (Gruden, 1977). In humans, inorganic iron supplementation increased fecal manganese losses (Kies et al., 1987) and decreased serum manganese concentrations after 60 days and lymphocyte manganese-dependent superoxide dismutase (Mn-SOD) activity after 124 days (Davis and Greger, 1992). Heme iron intake had no consistent effect on serum manganese or lymphocyte Mn-SOD activity (Davis et al., 1993).

TOXICOSIS

There are no practically important studies reporting the toxic effects of a single oral dose of manganese in mammals (ATSDR, 2000). Thus, all toxicity studies reported here are chronic in nature (Table 19-1).

Chronic Dietary Exposure to Inorganic Manganese

Manganese is considered to be one of the least toxic of the essential elements (NRC, 1980; Hurley and Keen, 1987). Decreased growth was not observed until 500 and >2,000 mg/kg diet of manganese was consumed by swine (Grummer et al., 1950; Leibholz et al., 1962, respectively); 1,000 and ≥2,000 mg/kg diet of manganese was consumed by calves (Jenkins and Hidiroglou, 1991; Cunningham et al., 1966, respectively); 3,000 mg/kg diet of manganese was consumed by sheep (Ivan and Hidiroglou, 1980); ≥3,000 mg Mn/kg diet of manganese was consumed by chickens (Heller and Penquite, 1937; Southern and Baker, 1983; Black et al., 1985b); ≥ 4,000 mg/kg diet of manganese was consumed by turkeys (Vohra and Kratzer, 1968); and ≥ 7,000 mg/kg diet of manganese was consumed by rats (Becker and McCollum, 1938; Moinuddin and Lee, 1960).

These data suggest that swine were more sensitive to excess manganese than other livestock. However, differences in the composition of the diets, water sources, and other study conditions could create these apparent differences among species.

Generally, depressed iron status and hematological changes were the most common signs of manganese toxicosis, even in animals fed typically adequate levels of iron. Cattle consuming 1,000 mg/kg diet of manganese had decreased iron-binding capacity in serum (Ho et al., 1984), and cattle consuming 3,000 mg/kg diet of manganese had depressed hemoglobin levels (Cunningham et al., 1966). Preruminant calves were more sensitive to manganese; calves fed only 500 mg/kg of manganese in milk replacer developed low hematocrits (Jenkins and Hidiroglou, 1991). Sheep consuming 2,550 to 5,000 mg/kg diet of manganese had depressed tissue iron and hemoglobin levels (Hartman et al., 1955; Watson et al., 1973). Chickens consuming 3,000 mg/kg diet of manganese had depressed tissue iron concentrations (Southern and Baker, 1983; Black et al., 1985b). Rats consuming ≥3,550 mg/kg diet of manganese had depressed tissue iron concentrations and depressed hemoglobin levels and red blood cell counts (RBCs) (Becker and McCollum, 1938; Moinuddin and Lee, 1960; Carter et al., 1980; Rehnberg et al., 1982). Fish exposed to 2,500 mg/L water of $MnSO_4$ had decreased erythrocyte counts (Agrawal and Srivastava, 1980) and those exposed to 50 mg/L of manganese had transient decreases in hematocrits (Cossarini-Dunier et al., 1988).

Some of the differences in sensitivity of animals to the hematological effects of excess manganese reflect the iron content of the diets. Repeatedly, investigators have demonstrated that animals fed low levels of iron were more sensitive (as judged by hematological status) to excess manganese, i.e., 45 mg/kg diet of manganese in sheep (Hartman et al., 1955), 1,000 mg/kg of manganese in chickens (Baker and Halpin, 1991), and 400 mg/kg of manganese in rats (Rehnberg et al., 1982). Matrone et al. (1959) found that a supplement of 400 mg/kg diet of iron would overcome the effects of 2,000 mg/kg diet of manganese on hemoglobin formation in pigs.

To a lesser extent, manganese may interact with other trace elements. Occasionally depressed liver zinc and elevated liver copper concentrations (Hartman et al., 1955; Watson et al., 1973; Ivan and Hidiroglou, 1980; Black et al., 1985b) and depressed copper absorption (Ivan and Grieve, 1976) were observed in animals exposed to excess manganese. Excess manganese intake has also been found to counteract excess intake of cobalt and decrease tissue retention of excess cobalt (Brown and Southern, 1985). Some of these

changes could partially reflect changes in iron status induced by excess manganese.

Several groups noted depressed calcium and/or phosphorus utilization in cattle (Reid et al., 1947; Gallup et al., 1952) and depressed serum phosphorus levels among rats (Moinuddin and Lee, 1960) fed excess manganese.

Two groups have noted a potential link between manganese and magnesium. Swine fed excess manganese and low levels of magnesium had depressed heart magnesium concentrations (Miller et al., 2000). Fain et al. (1952) observed that cattle fed excess manganese had transient depression of serum magnesium levels. Miller et al. (2000) suggested that excess manganese in diets might be a contributing factor to convulsions among livestock fed insufficient magnesium.

Exposure to Inorganic Manganese in Water

Limited studies with fish suggest that fish are more sensitive to excess manganese than mammals and birds. The LC_{50} for teleost in freshwater was 2,850 mg/L water of $MnSO_4$ (Agrawal and Srivastava, 1980), but the LC_{50} for the fry of *Lates calcarifer* in brackish water was 2.2 to 2.5 mg/L of manganese (Krishnani et al., 2003) after only 90 hours of exposure. Low levels of manganese reduced hematocrits (50 mg/L of manganese: Cossarini-Dunier et al., 1988) and induced cataracts (198 mg/kg diet of manganese: Waagbo et al., 2003) in fish.

The sensitivity of fish to manganese may reflect species differences. However, mammals "appear" to be more sensitive to manganese in water rather than the dietary milieu. Chandra and Imam (1973) found gavage doses of manganese were fatal to guinea pigs or caused gut necrosis. Rehnberg et al. administered excess Mn_3O_4 to rats by diet (1982) and by a gavage solution (1981). The rats accumulated similar concentrations of manganese in their livers and kidneys but accumulated more manganese in their cerebrums when the manganese was administered by gavage solution. Umarji et al. (1969) observed neurological signs in rabbits given excess manganese in their drinking water.

Kondakis et al. (1989) observed more neurological signs in long-term (>10 years) residents who were older than age 50 in regions of Greece in which the drinking water contained 1.8 to 2.3 mg Mn/L. Unfortunately dietary manganese was not assessed in this study and another epidemiological study did not confirm human sensitivity to manganese in water (Vieregge et al., 1995). The observation of Kondakis et al. (1989) led the EPA to suggest the LOAEL for manganese in water for humans was 4.2 mg/day of manganese or 0.06 mg/kg BW of manganese (Velasquez and Du, 1994). The standard was problematic because it was lower than recommended dietary levels (Greger, 1998) and is now judged inappropriate (EPA, 2004).

The Agency for Toxic Substances and Disease Registry (ATSDR, 2000) found no reports of adverse effects in livestock due to exposure to manganese in water. Unpublished data indicate that calves provided with water containing 0.75 mg/L of manganese did not have reduced water or feed intake or decreased growth (Personal communication, Linn and Raeth-Knight, 2004).

Inhalation Exposure to Inorganic Manganese

Chronic exposure to inhaled manganese (usually but not exclusively MnO_2) causes a disabling neurological syndrome called manganism in humans (ATSDR, 2000). The clinical symptoms initially include nonspecific symptoms (e.g., fatigue, headache, muscle cramps, lumbago, insomnia, loss of memory, impotence, slowing of movements) and eventually behavioral and psychotic changes, symptoms resembling Parkinson's disease, and dystonia with severe gait disturbances (Pal et al., 1999). The extrapyramidal symptoms are often irreversible and the primary site of the neurological damage is the globus pallidus.

Accordingly, investigators have identified subclinical neurological signs through a variety of tests. These include signal hyperintensity on T1 weighted MRI (magnetic resonance imaging) scans (Pal et al., 1999) and batteries of neurobehavioral, small motor, and postural tests (Mergler et al., 1999). Neither clinical nor subclinical neurological effects of excess inhaled manganese have been reported in livestock or pets (ATSDR, 2000).

Intravenous Exposure to Inorganic Manganese

Because manganese is an essential element, some physicians have added manganese to parenteral solutions. Veterinarians may also be interested in supplementing the parenteral solutions given to pets. This may not be wise.

Malecki et al. (1995) observed that rats rapidly accumulated manganese in extrahepatic tissues when small (0.1 μmole/day) amounts of manganese were added to parenteral solutions. Furthermore, several investigators noted altered MRI scans (resembling those observed in patients with manganism from excess inhalation of manganese) among patients receiving manganese-supplemented total parenteral nutrition (TPN) solutions (Ono et al., 1995; Fell et al., 1996) and among patients with impaired liver functions (Hauser et al., 1994). Takagi et al. (2002) calculated the optimal parenteral does of manganese for adult humans to be 1 μmol/day of manganese (<0.02 μmol/kg BW/day of manganese).

Exposure to Organic Forms of Manganese

The effects of ingesting in diet or inhaling organic forms of manganese (i.e., MMT, Maneb, Mancozeb) have not been reported for livestock or pets (ATSDR, 2000). Moreover, the adverse effects of the manganese-containing pesticides on humans and rodents were assumed to result from expo-

sure to the "whole compound, not necessarily from exposure to manganese" by the ATSDR (2000).

TISSUE LEVELS

Most tissues normally contain less than 3 μg/g wet weight of manganese (ATSDR, 2000). Generally tissue concentrations of manganese are decreased during manganese deficiency (Hurley and Keene, 1987; Malecki et al., 1996).

Generally, livestock do not accumulate extremely high levels of manganese in their tissues when excess manganese was fed (Table 19-2). Chicks fed 1,000 to 4,000 mg/kg of manganese as chloride, sulfate, carbonate, and oxide salts of manganese were reported to have <2 mg/kg dry muscle of manganese and <31 mg/kg dry liver of manganese (Southern and Baker, 1983; Black et al., 1984, 1985b). Feeding hens 13 and 1,000 μg/g diet of manganese resulted in egg yolks containing 4 and 33 μg/g wet weight of manganese, respectively (NRC, 1980).

Cattle fed 1,000 mg/kg diet of manganese had ≤2 mg/kg dry muscle of manganese, 13 to 27 mg/kg dry liver of manganese, and manganese induced histochemical and histological alterations in gastrointestinal mucosa of guinea pigs ≤2 mg/kg dry bone of manganese (Ho et al., 1984; Jenkins and Hidiroglou, 1991). Sheep fed 2,000 to 4,500 mg/kg diet of manganese had <2 mg/kg dry muscle of manganese, usually <46 mg/kg dry liver of manganese, and <29 mg/kg bone ash of manganese (Watson et al., 1973; Black et al., 1985a; Wong-Valle et al., 1989).

MAXIMUM TOLERABLE LEVELS

Many of the studies with swine, cattle, and sheep are old and the diets were incompletely defined in terms of concentrations of other minerals. Generally cattle and sheep fed typically adequate levels of iron developed no adverse signs if fed <2,000 mg/kg diet of manganese and swine if fed <1,000 mg/kg diet of manganese. The exception was preruminant calves. Calves (weighing about 60 kg and consuming 0.81 kg dry matter/day at the end of the study) had depressed hematocrits when fed 500 mg/kg milk replacer of manganese (Jenkins and Hidiroglou, 1991).

Poultry generally developed signs if their diets contained ≥3,000 mg/kg diet of manganese. Accordingly, 2,000 mg/kg diet of manganese is a conservative estimate of a safe manganese intake for chickens fed adequate, but not excessive, levels of other minerals.

FUTURE RESEARCH NEEDS

There is limited need for additional data on the toxicity of dietary inorganic manganese to livestock. The impact of interactions between manganese and other elements, including magnesium, on the toxicity of manganese is probably the work most apt to produce practically important data. Future research should focus also on apparent differences in manganese toxicity from feed and water.

SUMMARY

The potential for toxicity of dietary inorganic manganese to livestock is limited except when dietary intake of other minerals is marginal. The first signs of manganese toxicosis in normal animals reflect the adverse effect of dietary manganese on iron or magnesium utilization. However, mammals are very sensitive to excess manganese in intravenous fluids. Fish may be more sensitive to manganese toxicity than mammals.

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TABLE 19-1 Effects of Manganese Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Rabbits	2	Young	24.4 mg/day	Manganese sulfate	50 d	Water	Weight loss by 180 days with transient paralysis and continuing anesthesia in extremeties	Umarji et al., 1969
Rats		Young	4,990 mg/kg diet	$MnCl_2 \cdot 4\ H_2O$	240 d	Diet	No adverse effect	Becker and McCollum, 1938
Rats		Young	9,980 mg/kg diet	$MnCl_2 \cdot 4\ H_2O$	240 d	Diet	Depressed growth; no effect on reproduction	Becker and McCollum, 1938
Rats	6	Young	3,800 mg/kg diet	$MnSO_4 \cdot H_2O$	4 wk	Diet	No adverse effect	Moinuddin and Lee, 1960
Rats	6	Young	7,586 mg/kg diet	$MnSO_4 \cdot H_2O$	4 wk	Diet	Decreased feed intake and body weight, hemoglobin, serum P; loss of incisor pigment; increased water consumption, urine volume and RBC count	Moinuddin and Lee, 1960
Rats	5	Pre-weaning to adult	3,550 mg Mn/kg diet	Mn_3O_4	40 d	Diet	Mn increased mortality and decreased RBC counts in rats fed Fe deficient diet	Carter et al., 1980
Rats	11	Young	1,100 mg Mn/kg diet	Mn_3O_4	40 d	Diet	Increased serum Ca and P	Carter et al., 1980
Rats	6–10	Young and adult	400, 1,100 and 3,550 µg Mn/kg diet	Mn_3O_4	2 generations	Diet	Depressed liver Fe in rats fed 3,550 µg Mn/kg diet and normal Fe in diet; transient decrease in liver Fe in rats fed 400 mg Mn/kg diet and low Fe	Rehnberg et al., 1982
Rats	27	Pre-weaning	71 µg Mn/µL	Mn_3O_4	27 d	Gavage	Elevated tissue (including brain) Mn	Rehnberg et al., 1981
Guinea pigs	30	350 g	4.37 mg/kg	$MnCl_2$	30 d	Gavage	Six deaths; patchy necrosis with decreased activities of some enzymes in gastric and intestinal mucosa	Chandra and Imam, 1973
Chickens	25	Young	4,779 mg/kg diet	$MnCO_3$		Diet	Decreased growth; 52% mortality	Heller and Penquite, 1937
Chickens	25	Young	1,000 mg/kg diet	$MnCO_3$	20 wk	Diet	No adverse effect	Gallup and Norris, 1939

continued

TABLE 19-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Chickens	15	Young	3,000, 4,000, and 5,000 mg/kg diet	$MnSO_4 \cdot H_2O$; $MnCl_2 \cdot 4H_2O$; MnO_2; $MnCO_3$, MnO_2	8–21 d	Diet	$MnCl_2$ depressed growth; $MnCl_2$, $MnSO_4$, and $MnCO_3$ depressed hemoglobin and hematocrits and liver Fe; MnO_2 depressed liver Fe	Southern and Baker, 1983
Chickens	12	Young	3,000 mg/kg diet	$MnSO_4 \cdot H_2O$	3 wk	Diet	Decreased feed intake; decreased liver and pancreas Fe; increased liver and plasma Cu, and plasma Zn	Black et al., 1985b
Chickens	15	Adults	1,000 and 2,500 Mg/kg diet	$MnSO_4 \cdot H_2O$		Diet	Reduced blood hemoglobin only if diet low in Fe	Baker and Halpin, 1991
Turkeys	10	Young	4,080 mg/kg diet	$MnSO_4 \cdot H_2O$	21 d	Diet	No adverse effect	Vohra and Kratzer, 1968
Turkeys	10	Young	4,800 mg/kg diet	$MnSO_4 \cdot H_2O$	21 d	Diet	Decreased growth	Vohra and Kratzer, 1968
Swine	8	Young	500 mg/kg diet			Diet	Decreased feed intake and growth; limb stiffness; stilted gait	Grummer et al., 1950
Swine	6	Young	675 mg/kg diet	Manganese sulfate	2–8 wk	Diet	No adverse effect	Leibholz et al., 1962
Swine	6	Young	2,025 mg/kg diet	Manganese sulfate	2–8 wk	Diet	Decreased growth; depressed hemoglobin	Leibholz et al., 1962
Swine	15	Young	4,000 mg/kg diet	Manganese sulfate	2–12 wk	Diet	Decreased growth	Leibholz et al., 1962
Swine	6	Young	51 mg/kg diet	$MnSO_4 \cdot H_2O$	5 wk	Diet	Decreased heart Mg	Miller et al., 2000
Cattle	4	Adult	70 mg/kg diet	$MnSO_4 \cdot H_2O$	Three - 1 wk balances	Diet	Negative Ca balance; decreased Ca utilization	Reid et al., 1947
Cattle	5	Adult	100, 150, and 200 mg/kg diet	Manganous sulfate	4.5 mo	Diet	No adverse effect; Transient decrease serum Mg when diet 100 mg Mn/kg	Fain et al., 1952
Cattle	8	Young	250 and 500 mg/kg diet	Manganese sulfate		Diet	Increased fecal P	Gallup et al., 1952

Cattle	8	Young	1,000 and 2,000 mg/kg diet	Manganese sulfate		Diet	Increased fecal Ca	Gallup et al., 1952
Cattle	7	Young	820 mg/kg diet	Manganese sulfate	84 d	Diet	No adverse effect	Cunningham et al., 1966
Cattle	7	Young	2,460 and 4,920 mg/kg diet	Manganese sulfate	84 d	Diet	Decreased growth and feed intake	Cunningham et al., 1966
Cattle	6	Young	2,000 mg/kg diet	Manganese sulfate	100 d	Diet	No adverse effect	Cunningham et al., 1966
Cattle	6	Young	3,000 mg/kg diet	Manganese sulfate	100 d	Diet	Decreased hemoglobin	Cunningham et al., 1966
Cattle	8	Young	50 mg/kg diet	Manganous sulfate	11 wk	Diet	Decreased net absorption of Cu	Ivan and Grieve, 1976
Cattle	4	Young	1,000 mg/kg diet	Manganese carbonate	18 d	Diet	Decrease unsaturated and total iron binding capacity in serum	Ho et al., 1984
Cattle	7	Young	500 mg/kg diet	$MnSO_4 \cdot H_2O$	5 wk	Milk replacer	Lowered hematocrit	Jenkins and Hidiroglou, 1991
Cattle	7	Young	1,000 mg/kg diet	$MnSO_4 \cdot H_2O$	5 wk	Milk replacer	Decreased weight gain	Jenkins and Hidiroglou, 1991
Cattle	7	Young	5,000 mg/kg diet	$MnSO_4 \cdot H_2O$	5 wk	Milk replacer	None survived	Jenkins and Hidiroglou, 1991
Cattle	7	Young	1,000 mg/kg diet	$MnSO_4 \cdot H_2O$	5 wk	Milk replacer	Increased serum lipids and liver triglyceride	Jenkins and Hidiroglou, 1991
Sheep	4	Young	2,500 mg/kg diet	$MnSO_4 \cdot H_2O$	11 wk	Diet	Decreased Fe in liver, kidney, and spleen	Hartman et al., 1955
Sheep	4	Young	45 mg/kg diet	$MnSO_4 \cdot H_2O$	12 wk	Diet	Decreased hemoglobin and serum Fe when diet low in Fe	Hartman et al., 1955
Sheep	4	Young	5,000 mg/kg diet	$MnSO_4 \cdot H_2O$	11 wk	Diet	Decreased hemoglobin and Fe in serum, liver, kidney and spleen; increased Cu in liver	Hartman et al., 1955
Sheep	4	34 kg	4,030 mg/kg diet	Manganese carbonate	11 wk	Diet	Decreased Fe and Zn in liver and increased Cu in liver	Watson et al., 1973
Sheep	5	Adult	3,000 Mn/kg diet	$MnSO_4 \cdot H_2O$	8 wk	Diet	Decreased weight gain; decreased liver Zn and increased liver Cu	Ivan and Hidiroglou, 1980
Fish, teleost	120	Adult female	2,500 mg $MnSO_4$/L	$MnSO_4$	90 h	Freshwater	LC_{50} = 2,850 mg Mn/L in fresh water	Agrawal and Srivastava, 1980

continued

TABLE 19-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Fish, *Cyprinus carpio*	4–10	40–80 g	50 mg Mn/L	$MnCl_2$	2.5 mo	Freshwater	Increased Mn in spleen and kidney; decreased hematocrits/vary with time	Cossarini-Dunier et al., 1988
Fish, *Lates calcarifer*		Fry	50–300 mg Mn/L	$MnCl_2$	96 h	Brackish water	LC_{50} = 220–250 mg Mn/L	Krishnani et al., 2003
Fish, *Salmo salar*	16	Smolts	198 mgMn/kg diet	$MnSO_4 \cdot H_2O$	23 wk	Diet	Increased cataracts	Waagbo et al., 2003

[a]Number per treatment.
[b]Quantity of manganese dosed. SI conversion: 1 mg manganese equals 18.2 μmoles manganese.

TABLE 19-2 Manganese Concentrations in Fluids and Tissues of Animals (mg/kg)

Animal	Quantity[a]	Source	Duration	Route	Tissue Concentrations[b]	Reference
Cattle	55	Unknown	18 d	Diet	Muscle: 4; liver: 9; bone: 4 (dry basis)	Ho et al., 1984
	≈1,000	Mn carbonate	18 d	Diet	Muscle: 2; liver: 13; bone: 2 (dry basis)	
Cattle	40	Mn sulfate	5 wk	Milk	Muscle: 3; liver: 7	Jenkins and Hidiroglou, 1991
	1,000	Mn sulfate	5 wk	Replacer	Muscle: 2; liver: 27	
Sheep	30	Unknown	≈56 d	Diet	Muscle: 1; liver: 10; bone: 10	Watson, 1973
	4,000	Mn carbonate	≈56 d	Diet	Muscle: 2; liver: 44; bone: 19	
Sheep	31	Unknown	84 d	Diet	Muscle: <1; liver: <9; bone: ≈3	Black et al., 1985a
	2,000	Mn oxide and carbonate	84 d	Diet	Muscle: <1; liver: 19–46; bone: 9–11	
	4,000	Mn oxide and carbonate	84 d	Diet	Muscle: ≈1; liver: 39–232; bone: 22–29	
Sheep	38	Unknown	21 d	Diet	Liver: <10; bone: <1	Wong-Valle et al., 1989
	3,000 and 4,500	Mn sulfate	21 d	Diet	Liver: ≈44; bone: [a]5	
	3,000	Mn oxide and carbonate	21 d	Diet	Liver: 24–33; bone: 1–3	
Chickens	168	Unknown	21 d	Diet	Liver: 13; bone: 17	Southern and Baker, 1983
	3,000	Mn chloride and sulfate	21 d	Diet	Liver: 24–26; bone: 95–111	
Chickens	116	Unknown	26 d	Diet	Muscle: <1; liver: <12; bone: 12	Black et al., 1984
	1,000	Mn sulfate, carbonate, and oxide	26 d	Diet	Muscle: ≈1; liver: 16–21; bone: 38–65	
	2,000	Mn sulfate, carbonate, and oxide	26 d	Diet	Muscle: 1–2; liver:19–24; bone: 56–117	
	4,000	Mn sulfate, carbonate, and oxide	26 d	Diet	Muscle: ≈2; liver: 20–31; bone: 79–207	
Chickens	112	Unknown	21 d	Diet	Muscle: 1; liver: 10	Black et al., 1985b
	1,000–3,000	Mn sulfate	21 d	Diet	Muscle: 1–2; liver: 15–18	

[a]Quantity of exposure reported as mg/kg diet of manganese unless noted otherwise.
[b]Concentrations reported as mg/kg dry weight of soft tissue and mg/kg bone ash unless noted otherwise.

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Mercury

INTRODUCTION

Mercury (Hg, with atomic number, 80; atomic weight, 200.59; specific gravity, 13.55) exists in three oxidation states: Hg^0 (metallic), Hg^+ (mercurous), and Hg^{++} (mercuric) mercury. Each form has a different solubility, reactivity, and toxicity profile. The metal is dense, silvery-white, shiny, and a liquid at room temperature. Mercuric compounds are much more common than mercurous compounds and include simple salts, such as mercuric chloride, nitrate, and sulfate. Mercuric chloride ($HgCl_2$) and acetate are soluble in water in toxicologically relevant amounts, whereas mercurous chloride (Hg_2Cl_2) is only marginally soluble (2 mg/L) and HgS is highly insoluble. Hg^{++} can also form organometallic derivatives in which the mercury atom is covalently bound to one or two carbon atoms. The carbon–mercury bond is chemically stable and is not normally split in water or by weak acids or bases. Methylmercury salts are highly soluble in water. The metabolism and biological effects of mercury are intimately related to its high affinity constant (10^{15}– 10^{20}) for thiol-containing molecules.

Naturally occurring mercury is usually found in cinnabar ore, which contains mercuric sulfide. Algeria, China, Kyrgyzstan, and Spain are currently the leading countries that mine cinnabar for mercury production. Active mining of mercury in North America has diminished, and mercury is now produced predominantly as a by-product from gold mining operations in the states of California, Nevada, and Utah, and in Canada.

Mercury has many unique metallic properties including fluidity at room temperature, uniform volume expansion over the entire liquid temperature range, high surface tension, good electrical conductivity, and ability to alloy with other metals. These physical characteristics make it valuable for a wide variety of applications such as thermometers, barometers, switching devices, batteries, and dental restorations. Amalgams used in dentistry contain approximately 50 percent metallic mercury. Mercury's ability to amalgamate with gold and silver is used in the mining of these precious metals. Most of the mercury used in the United States is for chlorine-caustic soda production and electronic applications such as switches (USGS, 1992). Due to their toxic properties, mercuric compounds were routinely used as bactericides and fungicides in paints and agricultural fumigants and as worming medications. Many of these applications have been banned due to the persistence of mercury in the environment. In human and veterinary medicine, mercurochrome, thimerosal, and phenylmercuric nitrate are used as antiseptics and preservatives, although use is declining.

Human activities are a major source of mercury release into the environment. Volcanic activity is the predominant natural source. When mercury is released into the environment, some of it is transformed into methylmercury by bacteria and fungi. The methylation is believed to involve a nonenzymatic reaction between Hg^{++} and a methylcobalamin compound that is produced by bacteria (Wood and Wang, 1983). This reaction takes place primarily in aquatic systems. Thus, it is methylmercury of microbial origin that enters the food chain and accumulates in animals. Consequently, most of the research on mercury toxicity has examined the organic form. Toxicities due to inorganic mercury are typically due to accidental consumption of medicinals. Metallic mercury toxicities occur only following inhalation exposure during various industrial processes, but would not likely occur in animal husbandry. Metallic mercury consumed orally has a very low toxicity profile and will not be considered here. Several excellent reviews are available on the toxicity of metallic mercury (ATSDR, 1999; IPCS, 2003).

ESSENTIALITY

Mercury is not known to be an essential element for animals. In several experiments in rodents, pigs, and chicks, low levels of inorganic mercury increased growth rate; however, this effect was not seen in all experiments (Johnston and Savage, 1991).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

The analysis of total mercury in biological samples is useful as an initial screening, but due to the vastly differing toxicological profiles of inorganic and organic forms, quantification of each species is useful. Mercury is relatively volatile and easily lost during sample storage, preparation, and analysis if appropriate methods are not used. Oxidizing properties of lab ware can cause the loss of methylmercury, whereas methylmercury may be produced from inorganic mercury by microbial action in aqueous samples. As with all minerals, careful attention must be paid to inadvertent contamination of the sample with mercury, especially when determining trace concentrations. Glass or Teflon® should be thoroughly cleaned, acid-leached, and given a final soaking in hot (70°C) 1 percent HCl to remove any traces of oxidizing compounds (e.g., chlorine) that may subsequently destroy methylmercury (ATSDR, 1999). Repeated freezing and thawing of wet, biological samples can cause the loss of methylmercury (Horvat and Byrne, 1992). Tissue samples may be freeze-dried without loss of methylmercury. Standard or certified reference materials are useful to validate sample preparation, storage, and analytical methods. Standards should have a physical and chemical composition that reflects the samples being analyzed (Horvat, 1999).

Total mercury may be determined following reduction of all the mercury in the sample to its elemental state using harsh (nitric acid/perchloric acid, bromate/bromide) digestions. Inorganic mercury can be determined after milder digestions (HCl, sulfuric acid) and reduction. Quantification may be accomplished using a variety of methods including AAS, atomic fluorescence spectrometry (AFS), electrothermal atomic absorption (ETAAS), neutron activation analysis (NAA), mass spectrometry (MS), and anodic stripping voltammetry (ASV). Of the available methods, cold vapor (CV) AAS is the most widely used to determine total mercury in plant and animal tissues. Using standard techniques, levels of around one ppb can be reliably measured (ATSDR, 1999).

Independent quantification of inorganic and organic forms of mercury requires isolation or separation of the species prior to detection. Extraction, chromatography, distillation, acid leaching, and alkaline digestion have been routinely employed for separating species followed by quantification using CV AAS, CV AFS, or other detection techniques. Capillary gas-liquid chromatography with electron capture detection is often used for determining methylmercury levels in biological samples.

REGULATION AND METABOLISM

Absorption of mercury is highly dependent on its chemical form. Gastrointestinal absorption of metallic mercury is only about 0.01 percent of the dose in both humans and animals. Dermal absorption is also low. Absorption of inorganic mercury from the GI tract ranges between 1 and 40 percent depending on species, age, diet, intestinal pH, and the solubility of the source. Absorption of organic mercury compounds is very efficient; the efficiency of methylmercury absorption is 90 percent or greater in mammals and chickens (March et al., 1983; ATSDR, 1999). In ruminants, methylmercury is demethylated in the rumen to inorganic mercury, markedly lowering its absorption (Kozak and Forsberg, 1979). Fish are able to absorb methylmercury from water 100 times as fast as inorganic mercury and they absorb methylmercury from food 5 times more efficiently (Johnston and Savage, 1991).

Age is a primary factor that influences the absorption of inorganic mercury. For example, 1-week-old suckling mice absorbed 38 percent mercuric chloride, whereas adult mice absorbed only 1 percent of a dose in standard diets and 7 percent in a milk-based diet (Kostial et al., 1979). Absorption of mercurous compounds is lower than mercuric forms, probably because of lower solubility. Bacteria flora in the gastrointestinal tract may convert some ionic mercury into methylmercuric compounds prior to absorption. Presumably, species with active microbial conversion absorb inorganic mercury most efficiently.

Once absorbed, mercury is transported to tissues via the blood. Inorganic mercury in the blood is divided about equally between plasma and red blood cells (Zalups and Lash, 1994). In plasma, mercury is bound to sulfhydryl groups on proteins, especially albumin. In erythrocytes, it is bound to hemoglobin and glutathione. About 90 percent of the methylmercury in blood is found in the red blood cells. Methylmercury associates with thiol-containing amino acids because of the high affinity of the methylmercuric cation for sulfhydryl groups. Binding of the methylmercury cation to the thiol group of cysteine creates a chemical structure similar to that of the essential amino acid methionine. Thus, methylmercury can cross cell membranes via a carrier-mediated amino acid transport system. Inorganic mercury does not readily cross cell membranes, but the ionic form may form complexes with selenium that are more lipophilic and better able to cross membranes.

The three forms of mercury can be interconverted in the tissues. Metallic mercury can be oxidized by the hydrogen peroxide–catalase pathway in the body to its inorganic divalent form. Conversely, absorbed divalent mercury compounds can be reduced to the metallic or monovalent form. In the presence of protein sulfhydryl groups, mercurous mercury (Hg+) disproportionates to one divalent cation (Hg^{++}) and one molecule at the zero oxidation state (Hg^0). Methylmercury and phenylmercury can be converted into divalent inorganic mercury in the microsomes of liver and other tissues (Suda and Hirayama, 1992). Hydroxyl radicals produced by cytochrome P-450 reductase appear to be a primary source of reactive species that induces alkyl mercury degradation.

The tissue distribution of mercury differs depending upon the form of mercury consumed. Following absorption of mercuric chloride, the liver and kidneys have the highest mercury levels, whereas the brain and muscle have substantially lower levels. Methylmercury distributes readily to all tissues. The liver, kidney, and spleen have the highest levels, but the brain and muscle also accumulate substantial amounts of methylmercury. This relatively uniform tissue distribution is due to methylmercury's ability to cross cell membranes without difficulty. However, its continual demethylation in tissues over time results in a shift in distribution because inorganic mercury accumulates in the kidney and liver. In fish, methylmercury is found predominantly in the muscle, whereas inorganic mercury is found in highest levels in the gastrointestinal epithelium.

Mercury can accumulate in hair following oral exposure to either organic or inorganic mercury. Hair mercury levels, determined using segmental hair analysis, can be used to monitor the historical record of exposure to mercury. The incorporation of mercury into hair is irreversible; the loss of hair mercury occurs only as the result of hair loss. Following methylmercury exposure, the concentration of mercury in the hair is proportional to the concentration of mercury in the blood. However, hair mercury levels do not reliably reflect the level of exposure to elemental mercury or inorganic mercury compounds (IPCS, 2003). Feather mercury concentrations in chickens approximate tissue concentrations on a dry matter basis following consumption of either organic or inorganic mercury (March et al., 1983).

Methylmercury effectively crosses both the blood–brain barrier and the placenta, resulting in higher levels of mercury in the fetal than the maternal brain. Mercuric mercury penetrates the blood–brain and placental barriers only to a limited extent, although it does slowly accumulate in the fetus. Mercury from either organic or ionic sources is also transported into milk (Yoshida et al., 1994).

Tissues are protected against the toxic effects of inorganic mercury in two ways. Divalent mercury can induce metallothionein, and a large proportion of the inorganic mercury in the liver and kidneys is bound to this sulfhydryl-rich protein. Mice that are genetically unable to express metallothionein are more susceptible to inorganic mercury toxicity (Satoh, 1997; Stankovic et al., 2003). In the liver, mercury forms complexes with selenium. Divalent mercury forms insoluble mercuric selenide in the liver, which is thought to be a detoxification mechanism in fish and marine mammals (Storelli and Marcotrigiano, 2002).

Methylmercury has little affinity for metallothionein. It interacts with selenium to form bismethylmercury selenide, which is soluble and not stable and thus not likely to be a good detoxification mechanism. Methylmercury is thought to be detoxified in two stages (Palmisano et al., 1995). In the first stage, mercury is stored in the liver as methylmercury. Above a threshold concentration, a demethylation process takes place and inorganic mercury forms mercury selenide.

Excretion of inorganic mercury is predominantly via the urine and feces. High doses increase the percentage of excretion via the urine. Elimination of metallic mercury also occurs through the urine and feces, but significant amounts are also lost through expired air. Methylmercury is excreted more slowly than inorganic mercury, and the major route of excretion is the feces via the bile. In bile, methylmercury is complexed to nonprotein sulfhydryl compounds like glutathione and secreted into the lumen of the intestines (Ballatori and Clarkson, 1984; Naganuma and Imura, 1984). Methylmercury is slowly converted into its inorganic form by intestinal flora, and most of the mercury excreted is in the inorganic form. Methylmercury that is not demethylated is resorbed via the enterohepatic circulation and retained. Elimination of methylmercury compounds generally follows first-order kinetics. The whole-body half-life of methylmercury and mercuric chloride in humans is about 70 and 40 days, respectively (IPCS, 2003). Neonatal animals have a lower excretory capacity than adults. In fish, the half-life of methylmercury is 700 days (Sweet and Zelikoff, 2001), and methylmercury is retained in the body two to five times longer than inorganic mercury (Johnston and Savage, 1991). In chickens, the egg is a major excretory pathway for methylmercury (Kambamanoli-Dimou et al., 1991).

Mechanism of Toxicity

Cellular mechanisms for the toxic effects of inorganic and organic mercury are believed to be similar, with the differences in toxic symptoms caused by these two forms resulting from differences in their tissue distribution (ATSDR, 1999). High-affinity binding of divalent mercuric ions to thiol or sulfhydryl groups of proteins is believed to be the major mechanism of mercury toxicity. Binding to hydroxyl, carboxyl, and phosphoryl groups may also contribute to toxicity (ATSDR, 1999). Sulfhydryl groups play an integral part in the structure and function of most proteins, and binding by mercury results in decreased enzyme activities, impaired structural functionality, and disruption of transport processes (Zalups and Lash, 1994). Through alterations in intracellular thiol status, mercury can promote oxidative stress, lipid peroxidation, mitochondrial dysfunction, and changes in heme metabolism. Mercury-thiol complexes also possess redox activity that promotes the oxidation of many molecules, including nucleotides. The selenium-dependent form of glutathione peroxidase is highly sensitive to inhibition by inorganic mercury, and it has been proposed that mercury's interactions with selenium limits the amount of selenium available for this enzyme (Nielsen and Andersen, 1991). Mercury also disrupts intracellular calcium homeostasis leading to dysregulation of a wide variety of cellular functions.

The cytotoxic effects of mercury exhibit a threshold phenomenon. No cellular necrosis is observed up to a certain dose, but at higher levels necrosis progresses rapidly, some-

times in an all-or-none relationship. This is thought to be due to the buffering effect of endogenous ligands like metallothionein and glutathione. Once the buffer becomes saturated, additional mercury binds to critical nucleophilic groups in the cell and causes functional impairment (Zalups and Lash, 1994).

SOURCES AND BIOAVAILABILITY

Animals are exposed to mercury primarily by consuming high levels of mercury-containing foods. Consumption of mercury-laden soils while grazing may occur occasionally, as does accidental consumption of liquid metallic mercury. A variety of mercuric and mercurous-based medications are approved for veterinary use, primarily as antiseptics. Excessive dermal application or accidental consumption of these products has occasionally resulted in toxicities in animals. Thimerosal (ethylmercury) is used as a preservative in vaccines and pharmaceuticals. Its toxicity is similar to that of methylmercury (Magos, 2001). Most foods and environments have relatively low levels of mercury, but point sources in the environment and contaminated feedstuffs remain a problem.

Mercury occurs in the Earth's crust at levels averaging 80 µg/kg, but the actual concentration varies considerably depending on location. Certain shales have mercury up to 10 mg/kg. The major source of mercury is the natural degassing of the Earth's crust by volcanoes and volatilization from the ocean. Anthropogenic activities also account for substantial releases into the environment. These include the burning of fossil fuel; the production of steel, cement, and phosphate; alkali processing; smelting of metals; and mining of gold and mercury. Once in the atmosphere, mercury is widely disseminated and can circulate for years, accounting for its widespread distribution.

Concentrations of dissolved mercury in aquatic environments are open oceans, 0.5–3 ng/L; coastal sea waters, 2–15 ng/L; rivers and lakes, 1– 3 ng/L (IPCS, 1986). Local variation is considerable because suspended material may also contribute to the total load.

Organic and inorganic forms of mercury differ greatly in their environmental fate and potential to become toxic. Much of the inorganic mercury in natural waters and in soil is strongly bound to sediment or organic material and is unavailable to organisms. The methylation of inorganic mercury by bacteria in the sediment of aquatic environments is the limiting step in the transport of mercury into food chains. Low pH and high levels of dissolved organic compounds increase the rate of methylation. Once produced by bacteria, methylmercury enters the food chain. Because animals accumulate methylmercury faster than they can eliminate it, animals consume higher concentrations of mercury at each successive level of the food chain. Thus, low environmental concentrations of methylmercury can bioaccumulate to potentially harmful concentrations in fish, fish-eating wildlife, and people. The highest levels are found in long-lived predatory fish, such as swordfish and sharks in the oceans and pike and bass in freshwater.

Even at locations remote from point sources, mercury biomagnification can result in toxic effects in consumers at the top of aquatic food chains. Bioconcentration factors, which are the ratio between the concentration of mercury in an organism and the concentration in the medium to which the organism was exposed, are as follows: algae, about 1,000–8,000; vegetables, <0.1; invertebrates, often >1,000; fish, often >1,000; birds, about 2 (IPCS, 1986).

In soil and in water, the monovalent or divalent forms of inorganic mercury predominate and bind strongly to humic materials and sesquioxides. Mercury sorption to soils generally decreases with increasing pH and/or chloride ion concentration. Soils in proximity to mercury mines may contain high levels of mercury. For example, soil in a sheep pasture in Germany near a mercury mining area that was operated since the 15th century contains 435 mg Hg/kg (Gebel et al., 1996). The accumulation of mercury in plants generally increases with increasing soil mercury concentration, but is highly dependent on soil characteristics. High organic matter content and pH decrease the uptake. Generally, the highest concentrations of mercury are found at the roots, but translocation to other organs (e.g., leaves) occurs (IPCS, 1986).

Concentrations of mercury in most foodstuffs are often below the detection limit (usually 20 ng/g fresh weight). Fish and marine mammals are the dominant sources. The typical concentration in edible tissues of various species of fish range from 50–1,400 µg/kg fresh weight; however, fish from contaminated aquatic environments can have 10 mg/kg (IPCS, 2003). Liver typically has the highest concentrations, followed by kidney, and muscle has lower levels. The chemical form of the mercury is tissue dependent. In skeletal muscle, most (70–90 percent of the total) is methylmercury compounds, especially methylmercury-cysteine or a chemically related species (Harris et al., 2003). The liver contains variable amounts of mercury-selenium complexes. When mercury levels are high, these insoluble complexes dominate and methylmercury levels account for less than 50 percent of the mercury. Fish typically used for the production of fishmeal, such as anchovy, herring, and menhaden, occupy a low level in the food chain and typically have relatively low levels of mercury. When fishmeals are made from the offal of fish at higher levels of the food chain (e.g., dogfish or orange roughy), the meal can contain mercury at 1–2.4 mg/kg wet weight. Whalemeals have been found to contain in excess of 10 mg/kg. In most fishmeals, greater than 80 percent of the mercury is in the form of methylmercury (Johnston and Savage, 1991).

Bioavailability

The bioavailability of different forms of mercury is dependent on the efficiency of absorption from the gas-

trointestinal tract. Consequently, the bioavailability of methylmercury is 3–10 times greater than that of mercuric salts, and roughly a 1,000-fold higher than elemental metallic mercury.

Mercury in soil is largely immobile and insoluble, suggesting that it has a bioavailability that is 3- to 10-fold lower than mercuric chloride (Paustenbach et al., 1997). Mercuric sulfide, which is the predominant form naturally found in many soils, is highly insoluble. Its bioavailability is less than that of mercuric chloride (Sin et al., 1983). The mercury in harbor sludge and in sewage sludge is also relatively unavailable (Van Der Veen and Vreman, 1986). Milk proteins appear to increase the bioavailability of inorganic mercury through forming complexes and increasing absorption (Mata et al., 1997).

The bioavailability of methylmercury is decreased by phytate, some types of fiber, and complexing with selenium (Chapman and Chan, 2000; Tchounwou et al., 2003). Dietary fibers, such as pectin and cellulose, can alter the ability of microflora to demethylate methylmercury and therefore affect its reabsorption rate. For example, wheat bran enhances fecal excretion of mercury after methylmercury exposure by increasing its demethylation rate by intestinal flora (Rowland et al., 1986). Using neurotoxicity as an endpoint, the bioavailability of mercury in fish or seal liver is somewhat lower than methylmercury chloride in cats, rats, and quail (Ganther and Sunde, 1974; Ohi et al., 1976; Eaton et al., 1980). This may be due to the presence of mercury-selenium complexes that are of low bioavailability. However, methylmercury-cysteine found in fish has a higher rate of fecal excretion and lower rate of tissue accumulation than methylmercury chloride (Berntssen et al., 2004).

TOXICOSIS

Because of their differing bioavailabilities and tissue distributions, the toxicity profiles of organic mercury and inorganic mercury differ (Table 20-1). Accumulation of inorganic mercury in the kidneys causes changes in renal function, which are one of the most sensitive indications of its toxicity. The easy transport of methylmercury into the brain and across the placenta makes the nervous system and the fetus sensitive indicators for the organic form. The following discussion treats these two forms of mercury separately. The bulk of the information regarding toxicity resulting from oral exposure to inorganic mercury comes from studies of mercuric chloride, whereas methylmercuric chloride has served as the model for studying organic mercury toxicity. The extensive literature on the toxicology of mercury exposure in humans and animal models, primarily primate and rodent, has been reviewed by the National Research Council, the U.S. Environmental Protection Agency, and the World Health Organization (ATSDR, 1999; NRC, 2000; IPCS, 2003).

Single Dose

Inorganic Mercury

Ingestion of mercuric chloride is highly irritating to the tissues of the mucosa of the mouth and the gastrointestinal tract. Blisters and ulcers on the lips and tongue and vomiting occur quickly after consumption. Necrotizing ulceration and hemorrhages develop throughout the gastrointestinal tract. Death from oral exposure to inorganic mercury is usually caused by shock, cardiovascular collapse, acute renal failure, and severe gastrointestinal damage. In human adults, a lethal dose of mercuric chloride is estimated to be 10–42 mg Hg/kg BW. In rats, the oral LD_{50} for a single dose of mercuric chloride ranges from 26–78 mg Hg/kg BW depending on age, with younger animals being most sensitive (Kostial et al., 1978). A single gavage dose of mercuric chloride at 7.4 mg Hg/kg BW in water caused significant decreases in blood lactate dehydrogenase hemoglobin, erythrocytes, and hematocrit and renal pathology (Nielsen and Andersen, 1995). In quail, the LD_{50} for a single dose of mercuric chloride ranges from 26–54 mg Hg/kg BW from 3–30 days of age, respectively (Hill and Soares, 1987). The quail rapidly (15 minutes–1 hour) developed extreme neurological dysfunction and usually died within 6–24 hours.

Organic Mercury

In quail, the LD_{50} for a single dose of methylmercury chloride ranges from 11–26 mg Hg/kg BW from 3–30 days of age, respectively (Hill and Soares, 1987). The quail slowly developed clinical signs and most deaths occurred from 3–7 days after dosing (Hill and Soares, 1987).

Acute

Inorganic Mercury

Chickens given water containing 500 mg Hg/L as $HgCl_2$ had decreased growth rates and hematological changes within 3 days, and mortality increased within 9 days. One of the major signs of toxicity was dehydration due to refusal to drink the mercury-containing water (Grissom and Thaxton, 1985, 1986). The LD_{50} for $HgCl_2$ in quail chicks exposed via the diet for 5 days ranged between 2,956 and 5,086 mg Hg/kg diet (Hill and Soares, 1987). Symptoms included ruffled feathers, tremors, and lethargy.

The NOAEL for rats administered mercuric chloride 5 days a week for 2 weeks is 0.93 mg Hg/kg/day using a change in kidney weight as the endpoint. Renal pathology and changes in renal function occur at 3.7 mg Hg/kg/day (ATSDR, 1999).

Inorganic mercury is toxic to fish at low concentrations. The gills are a primary site of pathology, which is characterized as apoptosis of laminar cells and laminar fussion within the branchial tissue (Daoust et al., 1984). The LC_{50} values

(μg Hg/L) for freshwater fish exposed for 96 hours are tilapia, 350; rainbow trout, 220; striped bass, 90; carp, 180 (IPCS, 1986); and catfish, 570 (Elia et al., 2003). Toxicity is affected by temperature, salinity, dissolved oxygen, and water hardness. Embryonic and larval stages are considerably more sensitive, and LC_{50} values (μg Hg/L) for the embryo through the larval period are 30 and 4.7 for channel catfish and rainbow trout, respectively.

Organic Mercury

Laying hens gavaged with methylmercury chloride at 2.7 mg Hg/kg BW for 6 days had a marked decrease in egg production and shell quality (Lundholm, 1995). This level of exposure is equal to 27 mg Hg/kg diet at a food intake of 100 g/day. The LD_{50} for methylmercury chloride in quail chicks exposed via the diet for 5 days ranged between 32 and 47 mg Hg/kg diet, for hatchlings and 2-week-old chicks, respectively (Hill and Soares, 1987).

Rats exposed to methylmercury at 4 mg Hg/kg BW/day for 8 days develop overt signs of neurotoxicity. These symptoms may not be observed until several days after cessation of dosing (Magos et al., 1985). Methylmercury at levels below 0.1 mg Hg/kg BW/day appear to be tolerated over short periods of time by rodents (ATSDR, 1999).

Chronic

Inorganic Mercury

Chronic exposure to inorganic mercury results in progressive anemia, nephrotoxicity, gastric disorders, salivation, metallic taste in the mouth, inflammation, tenderness of gums, tremors, inactivity, and an abnormal gait. The kidney, particularly the renal proximal tubules and glomerulus, is particularly sensitive to inorganic mercury (Zalups and Lash, 1994). Histopathology of mercury-induced nephropathy in humans and rats includes dilated tubules with hyaline casts, degeneration and atrophy of tubular epithelium, and thickened tubular and glomerular basement membranes. In some cases accumulation of inflammatory cells may occur. Markers of renal toxicity include proteinuria, oliguria, increases in urinary excretion of tubular enzymes, decreased ability to concentrate the urine, and increased plasma creatinine (Zalups and Lash, 1994). In rabbits, low levels of mercury cause the production of antibodies against the glomerular basement membrane resulting in immunologically mediated membranous glomerulonephropathy. This can occur in the absence of significant tubular damage. In rodents, decreases in body weight or rate of gain after ingestion of mercuric chloride require a larger dose than nephropathy (IPCS, 2003). Although inorganic mercury does not readily cross the blood–brain barrier, a broad range of neurotoxic symptoms occur following chronic exposure and are qualitatively similar to those induced by organic mercury compounds (see below).

Dose–response studies designed to accurately determine safe levels of inorganic mercury for poultry, pigs, ruminants, and companion animals are generally lacking. However, studies designed to determine symptoms of toxicity, mechanisms of toxicity, and tissue accumulation of mercury are relevant. The insoluble HgO and $HgSO_4$ are tolerated at 100 mg Hg/kg diet in chickens with no loss in growth or egg production, respectively (NRC, 1980; Hill et al., 1987). However, the more soluble $HgCl_2$ reduces the fertility and growth of quail at 8 and 25 mg Hg/kg diet, respectively (Hill and Shaffner, 1976; El-Begearmi, 1980). Quail tolerate 4 mg Hg/kg diet for 1 year without adverse effects on egg production or fertility (Hill and Shaffner, 1976). Ducks tolerate 0.5 mg Hg/kg diet as $HgCl_2$, but 5 mg/kg causes histopathology in the seminiferous tubules (McNeil and Bhatnagar, 1985). In pigs fed $HgCl_2$, 50 but not 5 mg Hg/kg diet causes hepatic steatosis and enlarged lymph nodes (Chang et al., 1977).

In rats, the 6-month NOAEL for mercuric chloride administered in water by gavage is 0.23 mg Hg/kg BW/day using nephropathy as the endpoint (ATSDR, 1999). Assuming a food intake of 10g/100 g BW, this dose is equivalent to 2.3 mg/kg diet. In a 2-year study giving mercuric acetate to rats in their feed, renal damage occurred at levels as low as 2 mg Hg/kg BW/day (Fitzhugh et al., 1950). Loss of body weight required higher doses.

In chickens, chronic consumption of water containing $HgCl_2$ at 125 mg Hg/L causes a depression in growth, but 25 mg/L is tolerated (Thaxton et al., 1975). At 300 mg/kg, $HgCl_2$ results in growth depression, increased adrenal weights, decreased bursal weights, bursal pathology, and impaired humoral immune responses to antigens (Thaxton et al., 1982; Bridger and Thaxton, 1983).

In Atlantic salmon, a diet containing 5 mg Hg/kg of $HgCl_2$ results in pathological changes in the brain that are indicative of mercury toxicity (Berntssen et al., 2003). Water containing 64 μg/L of mercury from $HgCl_2$ causes mortality after 3 months in rainbow trout (Niimi and Kissoon, 1994).

Organic Mercury

The most sensitive endpoint for oral exposure to organic forms of mercury is the nervous system. The nature and severity of symptoms are dependent on dose and duration of exposure, as well as developmental stage during the exposure. A developing nervous system is considerably more sensitive than an adult's. Both the central and peripheral nervous systems can be damaged. Ataxia, muscle spasms, paralysis, impaired vision, loss of coordination, and hind limb crossing are common neurological signs of methylmercury exposure in animals. Changes in behavior, decreased activity, and deficiencies in learning and memory also occur. In monkeys, neurotoxicity may not be expressed until years after cessation of exposure to methylmercury.

Mercury-induced damage is selective to certain areas of the brain associated with sensory and coordination functions,

particularly neurons in the visual cortex and granule cells of the cerebellum. Neuronal degeneration, loss of astrocytes, and glial proliferation in the cortical and cerebellar gray matter and basal ganglia are evident in histological sections (Magos et al., 1985). This damage is usually irreversible. Cholinergic and GABA neurotransmitter systems are affected by mercury exposure, but it is unclear whether changes in neurochemical parameters are primary targets of mercury or whether the changes are secondary to degenerative changes in neurons.

The effects of methylmercury on neurodevelopment and tissue pathology in nonreproducing rodents have been examined in a very large number of studies and several excellent reviews of this literature are available (ATSDR, 1999; NRC, 2000; IPCS, 2003). In most studies, the NOAEL was 0.1 mg Hg/kg BW/day or greater, although a few studies found lower thresholds of toxicity. At a food intake of 150 g/kg BW for growing rats, this would be equivalent to a level of 6.7 mg/kg diet. However, levels that cause behavioral changes in offspring are often lower (see "Effect of Mercury on Reproduction" below).

Cats and monkeys are generally more sensitive to methylmercury than rodents. The NOAEL of methylmercury using neurobehavioral and renal pathology endpoints in monkeys is about 0.04 mg Hg/kg/day following long-term exposure (ATSDR, 1999; NRC, 2000). Cats fed contaminated fish at doses as low as 0.046 mg Hg/kg/day began to exhibit neurobehavioral changes after 60 weeks, including mild impairment of motor activity and diminished sensitivity to pain (Charbonneau et al., 1976). At 0.074 mg Hg/kg/day, cats displayed neurological signs and convulsions. The NOAEL in this study was estimated at 0.02 mg/kg BW. Assuming a food intake of 75 g/kg BW of dry diet, chronic consumption of a diet with 0.27 mg Hg/kg should be safe for cats.

When exposure is high enough, methylmercury also affects the kidney and causes nephritis in a manner very similar to inorganic mercury (see above), suggesting that its toxicity results from metabolism to inorganic mercury (Magos et al., 1985).

A variety of studies have shown decreased growth, egg production, and fertility in chickens when methylmercury was fed at levels above 4 mg Hg/kg diet (NRC, 1980; Bhatnagar et al., 1982; McNeil and Bhatnagar, 1985; Prasada Rao et al., 1989; Lundholm, 1995; Maretta et al., 1995; Pribilincova et al., 1996). Young chickens tolerate 1.35 mg/kg diet without decreased growth (March et al,, 1983), but 5 mg/kg greatly increases mortality (Soares, 1973). In ducks, 0.5 mg Hg/kg diet as methylmercury did not affect behavioral endpoints, although 3.8 mg/kg had adverse effects (Bhatnagar et al., 1982).

Studies useful for determining the threshold for toxicity of organic mercury sources to ruminants have not been conducted since 1980. The previous NRC publication (NRC, 1980) reviewed five studies in ruminants and arrived at a dietary level of 2 mg/kg as safe for all species.

A study was conducted in which brook trout were raised for three generations over a 144-week period and exposed to six levels of methylmercury ranging from 0.01 to 2.93 µg Hg/L (McKim et al., 1976). At 0.93 µg Hg/L, second generation trout developed deformities and most females eventually died. At mercury levels of 0.29 µg/L and below, survival, growth, and reproduction were normal. In general, fish at higher trophic levels are more tolerant to mercury than those at lower trophic levels (Johnston and Savage, 1991).

Effect of Mercury on Reproduction

Methylmercury alters reproductive success in both males and females. In males, mercury exposure results primarily in impaired spermatogenesis, decreased sperm motility, and degeneration of seminiferous tubules. In females, mercury exposure induces abortions, increases fetal resorption and malformations, and impairs neurodevelopment.

The placenta acts as a barrier for the transport of inorganic mercury to the fetus, but organic mercury is transported efficiently. The developing fetal brain is the most sensitive target for the toxic effects of methylmercury (Yoshida, 2002). Methylmercury toxicity in the fetus is often referred to as "fetal Minamata disease" because the syndrome was first described following a mercury contamination disaster in Minamata, Japan, that affected thousands of people (NRC, 2000). This syndrome is characterized by microcephaly, degeneration and trophy of cortical structures, loss of cellularity in the cerebrum and cerebellum, a reduction in myelin, ventricular dilation, gliosis, and disorganization of the brain layers. Newborns suffer from seizures, spasticity, blindness, and severe learning deficits. A similar syndrome has been described in rats, mice, hamsters, guinea pigs, cats, and monkeys.

Methylmercury causes developmental effects following oral exposure during gestation, lactation, or postweaning, but the outcome of prenatal exposure is most severe (Nielsen and Andersen, 1995). In rodents, one study found subtle behavioral changes in the offspring of rats at a dose of 0.008 mg Hg/kg BW/day. The NOAEL in this study was 0.004 mg/kg/day (Bornhausen et al., 1980). The reference dose for safe maternal daily dietary intake of methylmercury has been set at 0.0001 mg/kg BW (EPA, 2001).

Factors Influencing Toxicity

The fetus and neonate are most sensitive to the toxic effects of methylmercury. Males of some species are more sensitive to mercury toxicity than females. Animals with reduced renal capacity due to aging or renal disease are also more susceptible (Zalups and Lash, 1994).

Selenium protects against acute nephrotoxicity induced by inorganic or organic mercury. Inorganic forms of selenium appear to be more effective than organic forms. In chickens, selenium also protects against toxicity of organic

mercury to the seminiferous epithelium (Maretta et al., 1995). Possible mechanisms include redistribution of mercury, competition by selenium for mercury-binding sites, formation of a mercury-selenium complex that diverts mercury from sensitive targets, and prevention of oxidative damage by increasing selenium available for the selenium-dependent glutathione peroxidase (Chapman and Chan, 2000).

High levels of dietary zinc protect against the nephrotoxic effects of mercury. Zinc-induced metallothionein binds mercury in the renal cortex and shifts the distribution of mercury away from the more sensitive epithelial cells in the proximal tubules (Zalups and Lash, 1994). Conversely, the nephrotoxicity of mercury is exacerbated in zinc-deficient animals. In the zinc-deficient state, less mercury accumulates in the kidneys, but the toxicity is greater.

Vitamin E decreases the toxicity of methylmercury, probably by protection against oxidation (Kling et al., 1987). Vitamin C has been shown to be protective against methylmercury toxicity in some studies, but to enhance toxicity in others (Chapman and Chan, 2000).

TISSUE LEVELS

The enrichment of different tissues in mercury depends on its form. Inorganic forms of mercury accumulate in kidney and liver, with considerably lower levels in muscle. Most organic forms of mercury distribute uniformly across tissues, including muscle. Phenylmercury distributes similarly to mercuric sources, being highest in kidney and liver, but low in muscle (Kosutzka et al., 2002; Marettova et al., 2003).

Regardless of the form, accumulation of mercury in the tissues of animals is relatively linear over the range from background levels to levels that are toxic for the animal (Table 20-2). However, the rate of accumulation of mercury in muscle of poultry and livestock is much greater for methylmercury than inorganic forms of mercury. Accumulation of mercury in tissues takes many months to plateau, and older animals usually have higher levels than younger animals. Once mercury has accumulated in tissues, its depletion occurs very slowly so that depuration by feeding clean feed and providing clean water has little value. Regardless of the form of mercury, dietary levels that are safe for the animal result in levels in muscle that would cause toxicity in humans.

The accumulation of mercury in the eggs of hens fed methylmercury reaches a plateau in about 4 weeks, and higher concentrations are found in the albumen than in the yolk (March et al., 1983). The shell contains relatively low amounts of mercury.

In fish, the accumulation of mercury increases with age and size of the fish. The kidney, spleen, and liver accumulate the highest level of mercury, followed by the gill, gonad, brain, and then muscle (McKim et al., 1976; Sweet and Zelikoff, 2001).

Chelation therapy is presently the treatment of choice for reducing the body burden of inorganic mercury, but is largely ineffective for organic mercury. Efficacious chelators contain sulfhydryl groups that can bind mercury and compete with its binding to sulfhydryl groups in body tissues. BAL, DMPS, D-penicillamine, and DMSA have been used to mobilize tissue stores (NRC, 2000).

MAXIMUM TOLERABLE LEVELS

The maximum tolerable level of mercury is defined as the dietary level that, when fed for a defined period of time, will not impair accepted indices of animal health or performance.

Inorganic Mercury

Acute exposure to soluble forms of inorganic mercury at 1 mg/kg BW is tolerated without morbidity in those nonruminant species examined, but future development of neurological signs cannot be excluded. Chronic consumption of diets containing soluble forms of inorganic mercury of 0.2 mg/kg is tolerated by rodents, poultry, and pigs. Insoluble forms of mercury are tolerated at considerably higher levels. Given the lack of studies using ruminant species and the possibility of greater bioavailability of inorganic mercury due to methylation in the rumen, no recommendation on safe levels of inorganic mercury for ruminants can be given at this time.

Organic Mercury

Methylmercury at 0.5 mg Hg/kg BW/day is tolerated over short periods of time by rodents. Assuming a food intake of 10g/100g BW, this dose is equivalent to 5.0 mg Hg/kg diet. Because information is not generally available for other species, 5.0 mg Hg/kg diet is suggested as a safe level for acute exposure (10 days or less) for other mammals and birds, although future development of neurological signs cannot be excluded.

Chronic consumption of methylmercury at 1 mg Hg/kg diet is tolerated by poultry and salmon. The 1980 Mineral Tolerances publication (NRC, 1980) established a dietary level of 2 mg/kg as safe for swine and ruminants. Given that little relevant research on these species has been conducted since the previous report, no changes are made to this recommendation.

Methylmercury at 0.1 mg Hg/kg BW is tolerated in nonreproducing rodents and cats when consumed chronically. In all studies reviewed, rodents, nonhuman primates, and cats tolerated 0.005 mg HG/kg BW/day during reproduction.

Trout tolerate water containing 0.29 μg/L of methylmercury; however, fish at lower trophic levels may be sensitive to the toxic effects of mercury at this level.

HUMAN HEALTH

The MRL is the dose that can be ingested daily for a lifetime without a significant risk of adverse effects. The MRL

for mercury was set at 0.0003 mg Hg/kg/day for a 70-kg person, based on neurodevelopmental outcomes in children exposed in utero to methylmercury from maternal fish ingestion. For pregnant women, the suggestion is not to consume fish containing greater than 0.25 mg/kg (ATSDR, 1999). The UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO) set the maximum mercury intake at 0.23 μg/kg BW/day in order to sufficiently protect the developing fetus. The reports stressed that public health authorities should keep in mind that fish play a key role in meeting nutritional needs in many countries. Commercial fish sold through interstate commerce that are found to have levels of methylmercury above an "action level" of 0.5 mg/kg (established by the FDA) cannot be sold to the public. Maximum mercury levels in meats and eggs have been set at 0.05 mg/kg in many countries. The levels of dietary and water mercury that are tolerated by fish, poultry, and livestock would result in tissue levels that exceed these limits. Consequently, standards for mercury levels in feed and water supplied to animals intended for human consumption should be based on tissue residue levels and not animal health concerns.

FUTURE RESEARCH NEEDS

Currently, there is insufficient information on the dose–response relationship between mercury in feedstuffs versus levels in meat, milk, or eggs at dietary concentrations that result in food mercury levels of 0.01 to 1.0 mg/kg. Research that identifies the level of mercury in feeds that result in mercury residue levels of concern for human health are greatly needed. Though little research has been conducted to determine safe intake levels of organic mercury for poultry and livestock, this is not of great concern because tissue residues should limit the maximum permissible mercury levels in animal feeds. However, there is a need for additional research on safe levels of organic mercury for companion animals, especially cats because of their high consumption of fish.

SUMMARY

Mercury exists in numerous chemical forms, including metallic, mercuric, and organic forms. Mercury released into the environment from natural and anthropogenic sources is in the inorganic form, but it is metabolized by bacteria in aquatic environments to organic forms, primarily methylmercury. Methylmercury is concentrated at each level of the food chain and is found at high levels in carnivorous fish. Animals are exposed to organic mercury via their diet and mercuric forms via accidental consumption of mercury-based medicinals or from consumption of soil. Methylmercury is more toxic than inorganic forms because its bioavailability is considerably greater. The primary dietary source of methylmercury is fishmeal and other fish products; plant sources contribute little mercury to the diet. The nervous system is the most sensitive indication of methylmercury toxicity, whereas the kidney is most sensitive to mercuric complexes. Levels of mercury in the diet and water that are tolerated by animals with no apparent effect result in unacceptably high levels of mercury in meat and eggs for human consumption.

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TABLE 20-1 Effects of Mercury Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Cats	8	Adult	0.12 mg/kg/d 0.24 0.25 0.49	Seal liver Seal liver $HgCl_2$ Seal liver	90 d	Diet	No adverse effect No adverse effect Hindleg weakness, neurological symptoms No adverse effect	Eaton et al., 1980
Dogs, beagles	11	13 mo	0.5 mg/kg/d BW	MeHgCl	55 d	Oral	Changes in behavior and visual evoked responses	Mattsson et al., 1981
Chickens, laying hens	6–8	1.5 kg	0.53 mg/kg BW/d	MeHg	50 d	Oral, by gavage	Decrease in egg production and shell quality	Lundholm, 1995
Chickens, laying hens	6–8	1.5 kg	2.7 mg/kg BW/d	MeHg	6 d	Oral, by gavage	Decrease in egg production, egg size, and shell quality	Lundholm, 1995
Chickens, broilers	3 pens of 10	1 d	100 mg/kg 200 400	HgO	19 d	Diet	No effect on gain or efficiency of gain No effect on gain or efficiency of gain; decreased gain	Hill, 1990
Chickens, layers	12	Adult	5 mg/kg 30	Phenylmercuric Cl	8 wk	Diet	Decreased egg size, normal production rate Decreased egg size, normal production rate	Pribilincova et al., 1996
Chickens, layers	4	Adult	5 mg/kg 30	Phenylmercuric Cl	8 wk	Diet	Minor changes in seminiferous epithelium Degeneration of seminiferous epithelium, abnormal spermatids	Maretta et al., 1995
Chickens, leghorns	18	1 d	0.05 mg/kg 0.15 0.45 1.35	MeHgCl	8 wk	Diet	No adverse effect on weight gain No adverse effect on weight gain No adverse effect on weight gain No adverse effect on weight gain	March et al., 1983
Quails, Japanese	40–46	1 d	10 mg/kg	MeHg	16 wk	Diet	Decreased egg production, fertility and survival	El-Begearmi et al., 1982
Quails, Japanese	20–40	1 d	25 mg/kg 50	$HgCl_2$	4 wk	Diet	Decreased growth, increased mortality Decreased growth, increased mortality	El-Begearmi et al., 1980
Ducks, Pekin	6	7 d	0.5 mg/kg 5.0 15	MeHgCl	12 wk	Diet	No adverse effect on testes histology Histopathologyof seminiferous tubules Histopathologyof seminiferous tubules and spermatogonia	McNeil and Bhatnagar, 1985
Ducks, Pekin	12	7d	0.48 mg/kg 3.76 13.43	MeHgCl	12 wk	Diet	No effect on behavior, gain, or liver histology Decreased weight gain Paralysis, convultions, hepatic pathology	Bhatnagar et al., 1982
Ducks, Pekin	6	6 mo	8 mg/kg	MeHgCl	13 wk	Diet	Pathological changes in proximal tubules of kidney	Prasada Rao et al., 1989

continued

TABLE 20-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Goats	7	8 mo	100 mg/L	$HgCl_2$	91 d	Water	Pathology in kidney, liver, and brain	Pathak and Bhowmik, 1998
Fish, Atlantic salmon	100	15 g	5 mg/kg 10 mg/kg	MeHgCl	16 wk	Diet	No effect on growth rate, pathology in brain Severe pathology in brain, decreased activity	Berntssen et al., 2003
Fish, Atlantic salmon	100	15 g	5 mg/kg 10 mg/kg	$HgCl_2$	16 wk	Diet	No effect on growth rate, mild pathology in brain No effect on growth rate, mild pathology in brain	Berntssen et al., 2003

[a]Number of animals or pools of animals per treatment group.
[b]Quantity of mercury dosed. SI unit conversion: 1 mg mercury equals 4.99 µmoles mercury.

TABLE 20-2 Mercury Concentrations in Fluids and Tissues of Animals (mg/kg or mg/L)

Animal	Quantity	Source	Duration	Route	Muscle	Kidney	Liver	Spleen	Bone	Egg	Blood	Reference
Chickens, layers	0.05 mg/kg 0.15 0.45 1.35	MeHgCl	192 d	Diet	0.86[b] 1.48 3.27 6.35	1.95[b] 3.64 7.13 12.34	1.89[b] 3.71 7.17 16.29					March et al., 1983
Chickens, layers	5 mg/kg 30	Phenylmercuric Cl	8 wk	Diet						1.7, 0.05[a,d] 8.5, 0.25		Pribilincova et al., 1996
Chickens, leghorn chicks	0[c] mg/kg 0.05 0.15 0.45 1.35	MeHgCl	8 wk	Diet	0.42[b] 0.69 1.15 2.74 5.47	0.66[b] 1.64 2.84 5.14 7.14	0.56[b] 1.51 2.68 5.12 8.51					March et al., 1983
Ducks, Pekin	0.03 mg/kg 0.48 3.76 13.43	MeHgCl	12 wk	Diet	0.01[a] 1.32 11.6 21.83	0.03[a] 5.5 35.9 89.4	0.04[a] 5.95 42.03 88.1				0.003[a] 0.9 10.4 43.06	Bhatnagar et al., 1982
Pigs, large white	<0.01 mg/kg 0.044 0.088	MeHgCl	20–45 kg	Diet	<0.0l[b] <0.01 0.02							Batterham et al., 1983
Goats	0[c] 100 mg/L	$HgCl_2$	90 d	Water	0.003[a] 0.031	0.005[a] 0.106	0.004[a] 0.080	0.003[a] 0.050				Pathak and Bhowmik, 1998
Sheep, ram-lambs	<0.02 mg/kg 0.14 0.17 0.27	None Hg-acetate Harbor sludge Sewage sludge	12 wk	Diet	0.002[a] 0.001 0.001 0.001	0.238[a] 0.360 0.265 0.254	0.016[a] 0.028 0.037 0.034					Van Der Veen and Vreman, 1986
Cattle, Hereford	<0.02 mg/kg 2.60	Sewage sludge	106 d	Diet	<0.01[b] 0.02	0.09[b] 2.04	<0.01 0.27[b]	<0.01[b] 0.08	<0.01[b] <0.01			Johnson et al., 1981
Fish, Catfish	0 mg/L 0.035 0.070 0.140	$HgCl_2$	10 d	Water	0.09[a] 0.74 0.61 0.80	0.14[a] 11.78 9.82 17.82	0.15[a] 6.42 4.82 8.06					Elia et al., 2003
Fish, rainbow trout	0 mg/L 0.064 0.004	 $HgCl_2$ MeHgCl	60 d	Water	0.033[a] 6.2 31	0.042[a] 262 74	0.037[a] 202 76	0.034[a] 102 89				Niimi and Kissoon, 1994
Fish, tilapia	0 mg/L 0.05	HgC12	45 d	Water	0.01[a] 3.69		0.04[a] 43.97					Allen, 1994

[a]Data are on a fresh tissue basis.
[b]Data are on a dry tissue basis.
[c]Basal level not provided.
[d]Amount in yolk and white, respectively.

21

Molybdenum

INTRODUCTION

Molybdenum (Mo) has an atomic number of 42 and an atomic weight of 95.94. Although molybdenum is grouped with tungsten and chromium in the Periodic Table, its chemical properties resemble only those of tungsten. As a highly versatile element, molybdenum has various oxidation states (IMOA, 2002). While the low oxidation states of molybdenum (2– to 2+) do not occur in biological systems, the 3+ to 6+ states of molybdenum form an array of complexes with oxygen- or nitrogen-donor ligands and with the halogens. Molybdate complexes with sulfur-donor ligands are also common, but complexes with phosphorus- or arsenic-donor ligands are rare. The 4+ Mo is strongly stabilized by cyanide, and forms the most stable sulfide with sulfur (S). The 5+ or 6+ Mo is found mainly in oxomolybdenum species. Molybdenum is widely distributed in nature as molybdenite, wulfenite, ferrimolybdate, jordisite, and powellite. The United States is the largest producer of molybdenum in the world. Most molybdenum compounds are derived from molybdenum trioxide that is generated by roasting molybdenum disulfide ores. In industry, molybdenum is mainly used in manufacturing alloys and electronic devices. In human medicine, molybdenum-containing compounds may be used to treat dental caries and Wilson's disease, and to lower blood glucose and free fatty acids (Haywood et al., 1998).

ESSENTIALITY

Molybdenum is required for nitrogen fixation and for the reduction of nitrate to nitrite in bacteria (Williams and Fraústo da Silva, 2002). As a component of aldehyde oxidase, sulfite oxidase, and xanthine oxidase, molybdenum is probably essential for all higher animals. However, molybdenum requirements are extremely low, and clear signs of deficiency have been demonstrated in very few species. In the three enzymes where it serves as a cofactor, molybdenum is present as molydopterin, a mononuclear molybdenum atom coordinated to the sulfur atoms of a pterin derivative (Johnson et al., 1980). It helps in catalyzing the oxidation or metabolism of aldehydes, sulfite, sulfur-containing amino acids, purines, pyrimidines, and pteridines (Kisker et al., 1997). Deficiency of these enzymes or the molybdenum cofactors (Johnson, 1997) causes severe metabolic disorders or death in humans (Turnlund et al., 1995). Amino acid intolerances and irritability were shown in a patient with Crohn's disease receiving total parenteral nutrition, and the condition was improved by treating the person with ammonium molybdate (Abumrad et al., 1981). Primary molybdenum deficiency was reported by Anke et al. (1985) in goats fed a semi-purified diet containing 24 μg of Mo/kg. These goats showed depressed growth, impaired reproduction, and increased mortality of kids and mothers. Secondary molybdenum deficiency was produced in chicks fed low molybdenum diets containing high levels of tungsten (Nell et al., 1980; Topham et al., 1982a,b). The signs were anemia, growth retardation, and decreased tissue molybdenum levels; xanthine dehydrogenase activity; and the conversion of xanthine to uric acid. The anemia was probably related to the ferroxidase activity of xanthine oxidase in the intestinal mucosa and liver. Both pigs (Anke et al., 1978) and calves (Gengelbach and Spears, 1998) fed semi-purified diets have responded to molybdenum supplements. The effect of molybdenum on protein synthesis was positive in trout, but negative in rats (IMOA, 2002). Because of the ability of molybdenum to change between 4+ and 6+ and the redox potential link to electron acceptors such as cytochrome C and NAD, the molybdenum-containing enzymes may be important in regulating cellular peroxide and superoxide radicals. In turn, the free radicals are related to oxidative injuries in the body and inflammatory response to trauma such as invasion of nematodes in grazing animals (Suttle et al., 1992). As requirements for molybdenum by goats, rats, chicks, and perhaps other species are no higher than 0.2 mg/kg of diets (McDowell, 2003), molybdenum deficiency is rare in animals fed practical diets. The molybdenum require-

ment of adult humans is approximately 25 μg/day, but the actual daily molybdenum intakes of German and Mexican adults are 3 to 8 times higher than that level (Holzinger et al., 1998).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Total molybdenum contents in feeds, feces, water, urine, and tissues are often determined using colorimetric methods or atomic absorption spectroscopy. After the samples are dry ashed in a muffle furnace or wet ashed in heated, concentrated sulfuric acid, 4-methyl-1,2-dimercaptobenzene is used to produce a molybdenum-mercaptide complex under acidic conditions (Johnson, 1988). After the complex is extracted into an organic solvent, it is quantified by absorbance at 680 nm. To reduce the interferences from ferric iron and tungsten, KI and tartrate are added into the mixture, respectively. Ahmed and Haque (2002) have recently reported a rapid, ultra-sensitive, and highly selective spectrophotometric method for the determination of trace amounts of molybdenum, using 5,7-dibromo-8-hydroxyquinoline. Their procedure does not require an extraction step. Alternatively, molybdenum can be detected at picomole levels by graphite furnace atomic absorption spectroscopy (Johnson, 1988). Because molybdenum carbides may accumulate on the walls of the graphite furnace and thus cause memory effects (artificially elevated levels), it is better to ash the samples at low temperature and to add a burnoff cycle between analyses. In addition, molybdenum can be determined by inductively coupled plasma atomic emission spectrometry after the samples are dry-ashed at 450°C and diluted in 2.5 percent HCl (Holzinger et al., 1998).

REGULATION AND METABOLISM

Absorption and Metabolism

Absorption of molybdenum takes place in the stomach and throughout the small intestine of rats (Nielsen, 1996). The water-soluble molybdenum, such as the sodium or ammonium salts of 6+ Mo and the molybdenum in high molybdenum herbage, is readily absorbed by ruminants (Grace and Suttle, 1979). But, the disulfide form (4+ Mo) is poorly absorbed. After an oral dose of ^{99}Mo, the peak blood levels of molybdenum were detected within 4 hours in pigs, but not until 96 hours in cattle (Bell et al., 1964). Although the average absorption coefficient of stable or radioactive isotopes of molybdenum is 20 to 30 percent, the actual absorption rate of dietary molybdenum can be affected by species, age of animals, and levels of molybdenum and other nutrients in diets (Miller et al., 1972; Friberg and Lener, 1986; Turnlund et al., 1995). In the small intestine, the absorption of molybdenum across the mucosa is an active, carrier-mediated process that is also used by sulfate (Mason and Cardin, 1977). This gives rise to a possible antagonism between molybdenum and sulfate in their intestinal absorption and/or renal reabsorption. The interaction may be used to explain why increasing dietary sulfur decreased absorption or retention of molybdenum in sheep (Dick, 1956; NRC, 1980). It may also explain why ruminants are susceptible to molybdenum toxicosis because dietary sulfate is reduced to sulfide in the ruminal environment (Underwood and Suttle, 1999) so that essentially no sulfur leaves the rumen as sulfate to interfere with molybdenum absorption. However, the extremely high absorption coefficients of molybdenum in humans, up to >90 percent, at both low and high dietary intakes (22 versus 467 mg/day) (Turnlund et al., 1995) suggest a largely passive and nonsaturable mechanism for molybdenum absorption.

Absorbed molybdenum is transported in the blood, attached to proteins in the red blood cells and as free ionic molybdate in the plasma (Versieck et al., 1981). In the liver of preruminant calves, 40, 30, 23, and 7 percent of total tissue molybdenum was distributed in the nuclei, cytosol, large granule, and microsome, respectively (Jenkins, 1989). Increasing dietary copper from 10 to 1,000 mg/kg of diet resulted in a decrease in liver molybdenum from 2.7 to 0.7 mg/kg of dry matter, mainly in the nuclei. In laboratory species, approximately 36 to 90 percent of molybdenum was excreted through urine, and the output increased with the exposure (Vyskocil and Viau, 1999). Feces may serve as a major excretion pathway in ruminants fed diets with high sulfur and high copper:molybdenum ratios (Grace and Suttle, 1979) or diets with high molybdenum and "normal" levels of sulfur and copper (Pott et al., 1999). Milk is an additional molybdenum excretion pathway in lactating animals. The reported biological half-lives of molybdenum vary considerably: 0.4 to 0.6 hour in liver and kidney of rabbits, 20 hours in the whole body of cattle, several days in the tissues of rats, and several weeks in humans (Vyskocil and Viau, 1999; IMOA, 2002). According to Lesperance et al. (1985), plasma molybdenum is a better indicator of molybdenum intake than tissue copper levels, and urinary molybdenum may be used to estimate molybdenum intake in the field. However, urinary excretion of molybdenum may not remain linear with high levels of dietary molybdenum intake (Pott et al., 1999).

Metabolic Interactions, Regulations, and Mechanism of Toxicities

As there is little or no regulation of molybdenum uptake in simple-stomached species, body molybdenum balance is controlled primarily by urinary excretion (Johnson, 1997). Due to the chemical similarities, tungsten can compete with molybdenum at sites of transport, uptake, and utilization. Thus, tungsten can interrupt or replace the incorporation of molybdenum into target proteins, producing nonfunctional enzymes. Because concentrations of tungsten in water, feeds, and the environment are low, this antagonism has been shown only under experimental conditions (Nell et al., 1980).

In contrast, the interaction among molybdenum, copper, and sulfur is of great practical significance in grazing ruminants (Spears, 2003). When these animals ingest moderate to high levels of molybdenum from pasture, a concomitant high intake of sulfur induces copper deficiency (Ward, 1978). The disorder was initially attributed to the formation of insoluble copper-molybdenum-sulfur complexes (mono-, di, tri-, and tetra-thiomolybdates) in the rumen (Gooneratne et al., 1989; Suttle, 1991). However, Allen and Gawthorne (1987) argued for the importance of an association of thiomolybdates and copper with proteins in the solid digesta, suggesting that the molybdenum-copper antagonism was not a direct action, but a consequence of molybdenum affinity for sulfide generated within the rumen. After ruminal administration of ^{99}Mo-labeled compounds, tri- and tetra-thiomolybdates were found in the solid phase of ruminal, duodenal, and ileal digesta, whereas di- and tri-thiomolybdates were detected in the plasma of sheep (Price et al., 1987). Likely, the inhibition of copper absorption was mediated by tri- and tetra-thiomolybdates, and the postabsorptive effect on copper metabolism was exerted by di- and tri-thiomolybdates. In summary, absorbed thiomolybdates may affect copper metabolism in the following ways: (1) enhance biliary excretion of copper from liver stores; (2) reduce transport of available copper for biochemical synthesis by binding copper to plasma albumin; and (3) remove copper from cupro-enzymes (Suttle, 1991; Spears, 2003). Obviously, the presence of the sulfide-generating microbes in the rumen renders cattle and sheep susceptible to the molybdenum-copper-sulfur imbalance.

In the presence of adequate dietary copper, liver copper or plasma ceruloplasmin of rats was not decreased by feeding 500 mg Mo/kg of diet for 70 days (Igarza et al., 1999). High levels of dietary sulfur increase urinary excretion of molybdenum and decrease tissue molybdenum deposition. Furthermore, molybdenum can also interact with phosphorus, iron, and other elements (IMOA, 2002). There was only a slight effect of supplemental molybdenum (10 mg/kg as sodium molybdate) on the metabolism of ^{75}Se-selenomethionine in ram lambs (White et al., 1989).

Generally speaking, molybdenum toxicosis may be caused by either high levels of molybdenum intake or moderate levels of molybdenum intake combined with low levels of copper or low copper:molybdenum ratios. In most cases, molybdenosis resembles secondary copper deficiency. Some of the clinical signs may be explained by the deficiency of copper-containing enzymes: low tyrosinase activity causing rough hair coat, achromotrichia, and the loss of crimp in the wool (steely wool); low ferroxidase activity causing anemia; and low lysine oxidase activity causing skeletal and/or collagenous disorders (NRC, 1980). The biochemical changes of molybdenosis, such as reduced plasma ceruloplasmin, are related to impaired copper metabolism.

SOURCES AND BIOAVAILABILITY

Sandy soil contains low molybdenum, whereas marine origin soil contains high molybdenum, ranging from 0.1 to 20 mg/kg on a dry basis (Underwood and Suttle, 1999). The extractable molybdenum in the soil, normally 10 percent of total molybdenum, increases with increasing soil pH. As excessively high pasture molybdenum concentrations (up to 200 mg/kg) occur only on alkaline soils, molybdenum toxicosis is not normally seen in animals grazing pasture on acid (pH <6.5) and well-drained soils (McDowell, 2003). The median value of molybdenum in pasture was 1.1 mg/kg (dry basis) for 20 improved hill pasture sites in Scotland, with the highest concentration at 60 mg/kg (Suttle and Small, 1993). In areas with industrial contamination, herbage values of molybdenum were up to 231 mg/kg (Gardner and Hall-Patch, 1962). In some areas of Northern California, alfalfa and other legumes contain low levels of molybdenum and often do not contain sufficient molybdenum to maximize forage yield, whereas forages grown in much of California south of a line between San Francisco and Lake Tahoe may accumulate too much molybdenum and can produce molybdenosis in livestock (Meyer et al., 1999). The commonly detected concentrations of molybdenum (mg/kg DM) in various plant sources are as follows: cereal grains and straws, 0.2 to 0.5; grass, 0.2 to 0.8; clovers and other legumes, 0.5 to 1.5; and vegetable protein concentrates, 0.5 to 2.0 (McDowell, 2003). Apples and bilberry heather seem to contain molybdenum <0.1 mg/kg (Anke et al., 1985). In plant feeds, molybdenum exists as water-soluble sodium and ammonium salts and water-insoluble molybdenum oxide, calcium molybdate, and molybdenum sulfide (MoS_2) (NRC, 1980). Animal tissues and milk usually contain low levels of molybdenum, mainly as molybdopeterin, but can be elevated by high dietary concentrations (Anke et al., 1985). In mixed diets for humans, molybdenum concentrations range from 0.19 to 0.63 mg/kg dry matter (Holzinger et al., 1998). Water from different parts of the world contains 0.1 to 4 μg of Mo/L, but the level can be as high as 25 mg/L in the groundwater of Colorado (IMOA, 2002). Seawater contains approximately 8 μg of Mo/L. Sodium molybdate is the only accepted source of molybdenum by the U.S. animal feed industry to be used when copper toxicosis is suspected.

TOXICOSIS

Molybdenosis is produced by either high molybdenum intakes alone (>100 mg/kg) or moderately high molybdenum intakes concomitant with low dietary copper levels (<5 mg/kg) or low dietary copper:molybdenum ratios (<2:1). Mild molybdenosis may be identified by only biochemical changes such as increases in xanthine oxidase activity or blood uric acid. Severe molybdenosis is manifested by clinical signs, and even death. In most cases, the molybdenum toxicosis is largely secondary to copper deficiency or

hypocuprosis, and it may be reversed by supplemental copper. Because of the interactions of molybdenum, sulfur, and copper, it is necessary to consider dietary levels and body status of both copper and sulfur in defining or comparing molybdenum toxicity in various species (Ward, 1978).

Single Dose and Acute

When administrated orally, the LD_{50} of molybdenum trioxide and ammonium molybdate was 125 and 370 mg of Mo/kg of body weight for rats, respectively. The LD_{100} of ammonium molybdate was 1,200, 1,020, and 1,310 mg of Mo/kg for guinea pigs, rabbits, and cats, respectively (Venugopal and Luckey, 1978). For soluble molybdenum compounds, the lethal doses for repeated oral administrations ranged from 60 to 333 mg of Mo/kg of BW/day for rats, mice, guinea pigs, and rabbits (Vyskocil and Viau, 1999), but only 3 mg of Mo/kg of BW/day for steers (Cook et al., 1966). Signs of acute molybdenosis include gastrointestinal irritation, diarrhea, coma, and death from cardiac failure (Opresko, 1993). Injuries in the liver and kidney, sometimes in the adrenals and spleen, may also occur in the intoxicated animals.

Reid (2002) found that molybdenum was relatively nontoxic to juvenile Kokanee salmon, as the 96-hour LC_{50} was greater than 2,000 mg of Mo/L (sodium molybdate). Acute sublethal molybdenum exposure had little effect on oxygen consumption or plasma lactate, sodium, and cortisol concentrations at rest or active states in kokanee. Similarly, Hamilton and Buhl (1990) observed no mortality or any overt sign of stress in Chinook salmon and Coho salmon exposed to 78 to 1,000 mg of Mo/L. They suggested that the 96-hour LC_{50} exceeded 1,000 mg of Mo/L, regardless of the water dilution quality or the life stage for these species. Little acute toxicosis of sodium molybdate was shown in selected saltwater organisms including the pink shrimp, the mysid shrimp, and the sheepshead minnow, and the calculated 96-hour LC_{50} exceeded 1,000 mg of Mo/L (Knothe and Van Riper, 1988). For bluegill and rainbow trout, the 96-hour LD_{50} (mg/L) was 65 to 87 for MoO_3, 120 to 157 for ammonium molybdate, and 6,790 to 7,340 for sodium molybdate (IMOA, 2002).

Chronic

Anorexia and body weight loss are typical signs of chronic molybdenosis in cattle. When these animals graze on "teart" pasture containing 20 to 100 mg of Mo/kg dry matter (normal: 3 to 5 mg of Mo/kg)(Underwood and Suttle, 1999), scours may occur within 24 hours (Lloyd et al., 1976). Prolonged high molybdenum intake in cattle also produced anemia, achromotrichia, posterior weakness, skeletal deformities, and reproductive abnormalities (Venugopal and Luckey, 1978). These signs were shown in early studies with various types of cattle fed molybdenum from 6.2 to 400 mg/kg of diets (NRC, 1980). Unless indicated otherwise, sodium molybdate was used as the source of molybdenum in the following toxicity studies.

Severe molybdenum toxicosis in weanling heifers fed 100 mg of Mo/kg of diet (Table 21-1) for 336 days was manifested as scouring, achromotrichia, anemia, weight loss, and 31 percent mortality within 2 weeks after the study began (Lesperance et al., 1985). Secondary copper deficiency was induced in beef heifers by feeding 7 to 16 mg of Mo/kg of diet (as molybdenum:copper ratio, 2.5:1) in the presence of 0.3 percent of sulfur and 3 to 6 mg of Cu/kg (Arthington et al., 1996a,b). The molybdenum-supplemented animals also showed decreased plasma copper and ceruloplasmin and increased plasma fibrinogen and blood neutrophil numbers. Supplementing 5 mg of Mo/kg of dry matter in either copper-adequate or -deficient diets did not dramatically alter the specific immunity of the stressed cattle (Ward and Spears, 1999). When 5-week-old Holstein calves were given water containing 1, 10, or 50 mg Mo/L (as ammonium molybdate) for 21 days, liver copper content was reduced in the calves receiving the highest level of molybdenum (Kincaid, 1980). The calculated proportion of plasma copper as ceruloplasmin was reduced from 61 to 43 percent with increases in water molybdenum levels, indicating a reduced copper uptake by tissues from the plasma. In a semi-purified diet that contained 1.1 mg of copper and 1.1 mg of Mo/kg of dry matter for calves, supplementing 5 mg of Mo/kg depressed humoral immune responses and erythrocyte superoxide dismutase activity, compared with those supplemented with 10 mg of Cu/kg (Gengelbach and Spears, 1998). However, these effects were not significant in the presence of 5 mg of Cu/kg. Supplementing 5 mg of Mo/kg of diet to 7-month-old steers for 245 days reduced plasma copper and ceruloplasmin concentrations, and erythrocyte superoxide dismutase activity in the non-copper-supplemented steers, but had no effect on performance or carcass quality (Ward and Spears, 1997). Steers exhibited no further changes in copper status when dietary molybdenum was increased from 5 to 10 mg/kg of diet in the presence of 2.7 g of S/kg of diet (Gengelbach, 1994). Compared with the controls, heifers or steers showed no adverse response after grazing bahiagrass pasture treated with high molybdenum biosolids (molybdenum loads from 0.27 to 2.56 kg/ha) for 6 months (Tiffany et al., 2000, 2002).

Sheep are also susceptible to molybdenosis (NRC, 1980). Their clinical signs of chronic molybdenum toxicosis are essentially secondary hypocuprosis: reduced crimp and pigmentation of wool, anemia, alopecia, and depressed weight gain. When sheep were fed a basal diet containing 1.0 g of sulfur and 0.5 mg of molybdenum per kg of diet, supplementing 4 mg of molybdenum and 3 g of sulfur per kg of diet reduced copper bioavailability by 40 to 70 percent, but the addition of only molybdenum had no effect at all (Suttle, 1975). Increasing molybdenum (as ammonium molybdate) from 0.4 to 8.4 mg/kg of dry matter in the diets for wethers

resulted in reduced daily gains, poor feed efficiency, and decreased solubility of copper and molybdenum in the rumen, along with increased liver and kidney molybdenum contents (Ivan and Veira, 1985). There was no effect on liver copper content. Supplementing molybdenum (as tetrathiomolybdate) at 10 to 40 mg of Mo/kg of dry matter into a copper- and chromium-deficient diet for male goats exacerbated the copper deficiency (Aupperle et al., 2001). In contrast, Anke et al. (1985) reported that goats tolerated diets with 1 g of Mo/kg of diet, and they suggested that this high tolerance was not due to insufficient molybdenum absorption or related to copper metabolism.

Other ruminants and most nonruminants are resistant to molybdenum toxicity (NRC, 1980). Mule deer fed molybdenum up to 1 g/day showed no clinical signs (Nagy et al., 1975). Growing swine showed no apparent adverse response to 27 mg of Mo/kg of feed (Gipp et al., 1967), 50 mg of Mo/kg of feed (Kline et al., 1973), or 1 g of Mo/kg of diet (Davis, 1950), whereas the growth retardation effect of 1.5 g of Mo/kg of diet (in the presence of 17.8 mg of Cu/kg of diet) was reversed by addition of 0.4 percent sulfate (Standish et al., 1975). No deleterious effects were observed in horses grazing the "teart" pasture that caused diarrhea in cattle (McDowell, 2003), but an early study indicated an association with rachitis in foals and yearlings grazing on pasture containing 5 to 22 mg of Mo/kg (Walsh and O'Moore, 1953). Several chick studies indicated that 100 mg of Mo/kg of feed was safe (Davies et al., 1960), 200 to 300 mg of Mo/kg of feed caused growth depression (Kratzer, 1952), 4 g of Mo/kg of feed caused anemia (Arthur et al., 1958), and 8 g of Mo/kg of feed caused 61 percent mortality (Davies et al., 1960). In laying hens, 500 mg of Mo/kg of feed caused decreased hatchability and 1 g of Mo/kg of feed reduced egg production (Lepore and Miller, 1965).

Rabbits fed molybdenum at 1g/kg of feed or higher levels may show anorexia, loss of weight, alopecia, a slight dermatosis, anemia, splayed front legs, and premature deaths (Arrington and Davis, 1953). The adverse hematological effects of high molybdenum intakes in rabbits are fairly consistent (Vyskocil and Viau, 1999). Feeding male New Zealand rabbits (1.5 to 2.7 kg) a diet containing 4.5 g of sodium molybdate/kg or 0.3 percent of molybdenum ion for 25 to 31 days resulted in thyroidal hypofunction (Widjajakusuma et al., 1973). The signs include reduced plasma thyroxine concentration, thyroxine secretion rate, and follicular epithelial cells in the gland, and these impairments may be the major causal factor for the reduced feed intake in the molybdenotic rabbits. There was also a direct role of the molybdate ion in the degeneration of the thyroid gland.

Given ammonium molybdate in drinking water at 500 mg/kg of BW/day, male albino rats (60–70 g) showed growth retardation, altered activities of phosphatases in tissues, and increases in the basophilic substances in the cytoplasm of the liver cells (Bandyopadhyay et al., 1981). A high protein diet partially reversed these changes. However, serum ceruloplasmin activity in rats was not affected by 500 mg of Mo/kg diet, with supplemental copper at 40 mg/kg of diet (Igarza et al., 1999). When juvenile Kokanee salmon (20–70 g) were given molybdenum at 25 mg/L or higher levels for 7 days, there were increases in ventilation, post exercise loss of equilibrium, exercised-induced delayed mortality, and accumulation of molybdenum in gills and liver (Reid, 2002).

Reproduction

After male rats were administrated 30 or 50 mg of sodium molybdate/kg BW/day for 60 days, there were significant decreases in the absolute or the relative weights of epididymides, seminal vesicles and ventral prostate, sperm motility and count, and testicular sorbitol dehydrogenase, but increases in testicular lactate dehydrogenase and γ-glutamyl transpeptidase, tissue molybdenum accumulation, spermoatozoa abnormalities, and male-mediated embryotoxicity (Pandey and Singh, 2002). Given ammonium molybdate in drinking water at 500 mg/kg BW, male albino rats (60–70 g) showed elevated serum levels of luteinizing hormone, follicle stimulating hormone, prolactin, and cortisol (Bandyopadhyay et al., 1981). Given deionized water containing 10 or 100 mg of Mo/L from weaning to 21 days of gestation, female rats showed a prolonged estrous cycle, lower body weight gain, delayed fetal esophageal development, lower transfer of fetal hemopoiesis to bone marrow, and delayed myelination in the spinal cord, along with increased fetal resorption (Fungwe et al., 1990). As mentioned earlier, heifers fed a low copper (4.5 mg/kg) diet supplemented with 5 mg of Mo/kg produced calves that became more susceptible to diseases than those born to heifers that were not supplemented with molybdenum (Gengelbach et al., 1997).

Factors Influencing Toxicity

Toxicity of molybdenum, at a given level of exposure, depends on species, body status or dietary levels of copper and sulfur, and the chemical form of molybdenum. Both cattle and sheep are most sensitive to molybdenum toxicity, whereas other ruminants, nonruminants, and fish are fairly resistant to molybdenosis. The low tolerance to molybdenum in cattle and sheep may be partially explained by the reduction of digesta sulfate to sulfide in the rumen and the formation of thiomolybdates that are more toxic than molybdate (Underwood and Suttle, 1999; Spears, 2003). Comparatively, sheep are somewhat less susceptible to molybdenum toxicity than cattle, probably due to a lower ceruloplasmin turnover (NRC, 1980).

As molybdenosis is associated with the dysfunctions of several copper-containing enzymes such as tyrosinase, ferroxidase, and dopamine beta hydroxylase (NRC, 1980), dietary copper levels and body copper status are critical to

the incidence and severity of molybdenum toxicosis. Given adequate or high dietary copper, many species, even cattle, can tolerate relatively high levels of molybdenum. On the other hand, the antagonistic interaction between molybdenum and copper can be used to prevent copper toxicosis. Supplemental or intravenous administration of molybdate compounds, along with SO_4, has been used to prevent copper poisoning in sheep (MacLachlan and Johnston, 1982; Olson et al., 1984; Humphries et al., 1986).

Inorganic sulfate supplements seem to protect against molybdenum toxicity. However, Underwood and Suttle (1999) suggested that this protection might be confined to nonruminants, via reducing intestinal molybdenum absorption and increasing urinary molybdenum excretion. They considered the reported molybdenum reduction in sheep given sulfur supplement an artifact and argued that the simultaneous addition of molybdenum and sulfur actually exacerbated the molybdenum-induced impairment of copper metabolism (Suttle, 1975). A high protein diet can partially alleviate molybdenum toxicosis, probably via the metabolism of sulfur-containing amino acids (Igarza et al., 1999).

Highly soluble trioxide and ammonium molybdates are much more toxic than the insoluble compounds such as molybdenum disulphide, metal, and dioxide (Vyskocil and Viau, 1999). Ammonium molybdate seems to be more toxic to chickens than sodium molybdate (Davies et al., 1960). Male rats seem to tolerate less molybdenum than female rats (Vyskocil and Viau, 1999). Other dietary factors such as manganese, zinc, iron, lead, and tungstate and the chemical forms of molybdenum also affect molybdenum toxicity (NRC, 1980).

TISSUE LEVELS

Concentrations of molybdenum in tissues, blood, eggs, and milk vary with molybdenum intakes (Table 21-2), and the fluctuations are modulated by concomitant inorganic sulfate and copper levels (Arthur et al., 1958; NRC, 1980). Under normal conditions, liver molybdenum concentrations of Fallow deer, sheep, pig, cow, and humans fall between 2 and 3 mg/kg of dry matter, whereas the concentration is lower (0.62 mg/kg) for Roe deer and much higher for horses (8 mg/kg) (Anke et al., 1985). In many species, kidney contains approximately half the molybdenum of liver, but chicken liver and kidney have similar molybdenum contents (~ 4 mg/kg) that are higher than the molybdenum content in muscle (0.14 mg/kg; IMOA, 2002). In adult humans, brain, lung, muscle, and spleen have similar molybdenum contents as well (0.14 to 0.20 mg/kg). High levels of tungsten (up to 1 percent) reduced liver molybdenum contents in chicks fed diets containing 0.5, but not 4 to 5 mg of Mo/kg of feed (Nell et al., 1980). In wethers, liver or kidney molybdenum contents were more than doubled by increasing the dietary molybdenum level from 0.4 to 8.4 mg/kg of dry matter (Ivan and Veira, 1985). Similarly, molybdenum concentrations of seven different bones were elevated approximately 30-fold by increasing the molybdenum level of corn silage diets from 0.4 to 11 mg/kg of dry matter (Hidiroglou et al., 1982). While the molybdenum-supplemented wethers also had elevated Zn concentrations in their bones, the contents of Cu, Mg, P, Ca, and total ash were unaffected by dietary molybdenum. After fish were exposed to molybdenum in water ranging from 0 to 250 mg/L for 3 days, both gill and liver accumulated molybdenum in a dose-dependent fashion (Reid, 2002). The relationship between the tissue and exposure levels of molybdenum was linear on a double-reciprocal plot. Although both organs accumulated similar amounts of molybdenum on a weight basis, the estimated maximal binding capacity and the apparent dissociation constant for liver were 4 and 25 times higher than gill, respectively (Reid, 2002). Both liver and bone molybdenum contents in cattle can be increased up to 10 to 20 times by feeding high molybdenum diets over control diets (Ward, 1978).

The whole blood molybdenum concentrations in sheep and cattle fed low molybdenum diets range from 10 to 60 mg/L, and the mean serum molybdenum concentration of 110 healthy humans was 0.44 μg/L (Forrer et al., 2001). Plasma molybdenum concentrations in cattle fed high molybdenum diets may be 60- to 260-fold higher than those fed "normal" control diets (Ward, 1978). Cow milk molybdenum concentrations range from 18 to 120 μg/L, and may exceed 1 mg/L from cattle grazing on high molybdenum pasture or ingesting diets supplemented with molybdenum at 53–173 mg/kg of dry feed (Archibald, 1951; Huber et al., 1971; NRC, 1980). Eggs from hens fed commercial rations had average molybdenum values (wet weight basis) of 0.5, 0.4, and 0.8 mg/kg for whole eggs, white, and yolk, respectively (Arthur et al., 1958).

MAXIMUM TOLERABLE LEVELS

The maximum tolerable level of a mineral is the dietary level that, when fed for a defined period of time, will not impair animal health and performance. With the lowest tolerance to molybdenum toxicity among all species studied, cattle definitely show overt toxicosis when the dietary molybdenum level is at 100 mg/kg of dry matter or higher, regardless of dietary copper or sulfur levels. Toxicosis may also be produced in cattle by 25 to 50 mg Mo/kg of dry matter (Ward, 1994). However, the toxicosis caused by <25 mg Mo/kg is often associated with inadequate available copper. In addition, 5 mg Mo/kg may be detrimental to copper-deficient cattle (Gengelbach et al., 1997) and has been demonstrated to cause copper depletion in heifers (Bremner et al., 1987). Based on the responses of growth, liver copper concentration, and plasma copper distribution, Kincaid (1980) suggested the minimal toxic concentration of molybdenum in drinking water for calves was between 10 and 50 mg/L, and the critical copper:molybdenum ratio is <0.5 when the animals were given diets containing 13 mg

Cu/kg and 0.29 percent sulfur. Thus, the maximum tolerable level of molybdenum, based on the above-mentioned definition, is suggested as 5 to 10 mg/kg of dry matter for copper-adequate cattle. However, unless dietary copper is increased above requirement, this concentration of molybdenum may lead to copper deficiency over time. It might also be safe to suggest the same level for sheep and horses. Comparatively, swine and poultry are more resistant to molybdenosis than ruminants. As chicks performed normally at 100 mg of Mo/kg of feed (Davies et al., 1960) and 200 mg of Mo/kg of feed was the lowest level that caused growth depression (Kratzer, 1952), the maximum tolerable level of molybdenum for poultry is suggested as 100 mg of Mo/kg of feed. Two independent studies indicated that growing swine showed no apparent adverse response to 27 (Gipp et al., 1967) or 50 mg (Kline et al., 1973) of Mo/kg of feed. Although another old study showed that pigs tolerated 1 g of Mo/kg of diet (Davis, 1950), the level is unrealistically high. Based on the chick data and physiological similarities between these two species, the maximum tolerable level of molybdenum for swine is suggested as 150 mg of Mo/kg of feed. For all species of fish studied, a level of 10 mg of Mo/L seems to be tolerable.

Vyskocil and Viau (1999) reviewed 14 selected earlier studies in laboratory species based on the "quality" of the experimental design. They summarized the NOAEL and the LOAEL as follows (mg of Mo/kg BW/day): rats: 0.9 to 40 (NOAEL), 1.6 to 80 (LOAEL); rabbits: 0.5 to 23 (NOAEL), 5 to 46 (LOAEL); guinea pigs: 75 (LOAEL); and mice: 1.5 (LOAEL). Assuming a 200-g rat eats 15 g diet/day and a NOAEL of 0.5 mg Mo/kg BW, the tolerable dietary molybdenum level is 7 mg/kg diet. An intake of 0.15 mg/kg BW may be toxic to humans (Holzinger et al., 1998). The tolerable upper intake level of molybdenum ranges from 0.3 to 0.6 mg/day for children to 2 mg/day for adults, approximately 40-fold higher than the recommended dietary allowance (IMOA, 2002).

FUTURE RESEARCH NEEDS

Past studies of molybdenum toxicity were mainly conducted under conditions of low or deficient levels of copper. Future research is needed to distinguish primary molybdenum toxicosis at adequate or relatively high levels of dietary copper from the molybdenum-induced copper deficiency. The biochemical mechanism of the interactions of molybdenum with sulfur and copper, and the subsequent metabolic impact are still unclear. Data on bioavailability of dietary molybdenum of various sources will be useful for both nutritional and toxicological research on molybdenum.

SUMMARY

Molybdenum is a component of three enzymes involved in catalyzing the metabolism of aldehydes, sulfite, sulfur-containing amino acids, purines, pyrimidines, and peteridines in animals. As an essential element, its nutrient requirements for various species are easily met by feeding practical diets. Thus, molybdenum deficiency is normally produced under experimental conditions with semi-purified diets or high levels of tungstate. However, molybdenum toxicosis can be a practical problem in cattle or sheep grazing pasture on alkaline soil or contaminated with industrial sources of molybdenum. The clinical signs of molybdenosis are essentially secondary copper deficiency manifested by diarrhea, anorexia, depigmentation of hair or wool, anemia, neurologic disturbances, impaired reproduction, and premature death. Other metabolic disorders caused by molybdenum toxicosis include hypothyroidism, bone and joint deformities, impaired immunity, and liver and kidney injuries. The biochemical alterations include decreases in plasma ceruloplasmin, increases in molybdenum-containing enzyme activities, and alterations of tissue copper and molybdenum concentrations. Nonruminants and fish are relatively resistant to molybdenum toxicity. The actual tolerable levels of molybdenum depend on species, dietary levels of copper and sulfate or protein, and chemical forms of molybdenum. Molybdenum is not classified as a carcinogen. The teratogenic effect of molybdenum has yet to be observed in mammals, but molybdenum is embryotoxic.

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TABLE 21-1 Effects of Molybdenum Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Rabbits	5–11	1.5–2.7 kg (New Zealand)	4.5 g of compound/kg or 0.3% Mo	Sodium molybdate	25–31 d	Diet	Thyroidal hypofunction	Widjajakusuma et al., 1973
Rats		20 g male Druckery	0 and 10 30 and 50 mg compound/kg BW	Sodium molybdate	5 times/wk for 60 d	Oral (water)	 Caused abnormalities in testis histoarchitecture and sperm morphology	Pandey and Singh, 2002
Rats		60–70 g male albino	0 500 mg compound/kg BW/d	Ammonium molybdate	28 d	Oral (water)	 Growth retardation, alteration of enzyme activities in liver and hormones in serum	Bandyopadhyay et al., 1981
Rats	21	21-d-old female	0 and 5 10 and 100 mg of Mo/L (0.025 mg Mo/kg of diet)	Sodium molybdate	Weanling to d 21 of gestation	Water	No adverse effect Abnormal reproduction and development	Fungwe et al., 1990
Chickens	20	Young	500 mg of Mo/kg of diet 4,000 mg of Mo/kg of diet 6,000 mg of Mo/kg of diet 8,000 mg of Mo/kg of diet	Sodium molybdate Sodium molybdate and ammonium molybdate	4 wk	Diet	Decreased growth Depressed growth and anemia 33% mortality 61% mortality, ammonium molybdate more toxic	Davies et al., 1960
Swine	4	12 kg barrows	1,500 mg of Mo/kg of diet (17.8 mg of Cu/kg and 0.4% S)	Sodium molybdate	69 d	Diet	Growth retardation with elevated plasma Cu clearance	Standish et al., 1975
Horses		Foals and yearlings	5–22 mg of Mo/kg DM	Pasture	Daily	Diet	Associated with rachitis	Walsh and O'Moore, 1953
Cattle	4	160 kg weanling heifers	0 mg Mo/kg diet 100 mg Mo/kg diet (0 or 0.5% added inorganic S04)	Sodium molybdate	336 d	Diet	 Scouring, achromotrichia, anemia, weight loss, and 31% mortality post the study	Lesperance et al., 1985

continued

TABLE 21-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Cattle	3	5-wk Holstein calves	0, 1, and 10 mg Mo/L 50 mg Mo/L (13 mg Cu/kg of diet and 0.3% S)	Ammonium molybdate	21 d	Water	No adverse effect Decreased liver Cu and the percentage of plasma Cu as ceruloplasmin Cu; reduced Cu uptake by tissues from plasma	Kincaid, 1980
Cattle	6	Heifers	0 mg 15.5 mg/kg DM (0.3% S and 6.2 mg Cu/kg)	Sodium molybdate	129 d	Diet	Caused Cu deficiency; increased plasma fibrinogen and blood neutrophil number	Arthington et al., 1996a
Cattle	6	163–198 kg heifers	0 6.9 mg/kg DM (2.74 mg Cu/kg and 0.3% S)	Sodium molybdate	120 d	Diet	Induced Cu deficiency and increased peripheral blood neutrophil number	Arthington et al., 1996b
Cattle	9–10	2-y-old heifers and their calves	0 5 mg Mo/kg DM	Sodium molybdate	~9 mo since the last 1/3 of gestation	Diet	Reduced disease resistance or immune responses	Gengelbach et al., 1997
Cattle	6	2- to 4-d-old calves	0 5 mg/kg DM (1.1 or 6.6 mg Cu/kg)	Sodium molybdate	112 d	Diet	Caused more severe Cu deficiency, depressed humoral immune response, and reduced erythrocyte superoxide dismutase activity	Gengelbach and Spears, 1998
Cattle	8–9	7-mo-old steers	0 5 mg/kg DM (0 or 5–7.5 mg Cu/kg)	Sodium molybdate	196 d of growing and 49 d of finishing phases	Diet	Affected Cu status in the non-Cu supplemented steers, but had no effect on performance or carcass quality	Ward and Spears, 1997
Sheep	6	19–25 kg wethers	0.4 8.4 mg Mo/kg DM	Ammonium molybdate	221 d	Diet	Reduced daily gain and/or feed use efficiency; increased liver Mo and kidney Mo and Cu	Ivan and Veira, 1985

Goats	3	3-mo-old	Basal diet contained (per kg) 0.54 mg Mo, 0.96 mg Cu, and 0.31 mg Cr + 10–40 mg Mo/d	Tetrathio-molybdate	10 wk	Diet	Induced more severe lesions in liver, pancreas, and skin, compared with the only Cu-deficient diet	Aupperle et al., 2001
Fish, juvenile Kokanee salmon	5–10	20–70 g	0, 5, or 10, 25, 250, 500, 1,000, 1,500, and 2,000 mg Mo/L	Sodium molybdate	96 hr–7 d	Water	No adverse effect Increased in ventilation, post-exercise loss of equilibrium, exercised-induced delayed mortality, and Mo accumulation in gill and liver; but the 96-h LC_{50} was > 2,000 mg Mo/L	Reid, 2002
Fish, fry of Chinook and Coho salmons	5–10	Various stages (0.5–2.6 g)	78–1,000 mg Mo/L	Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)	96 hr	Water	No mortality or any overt signs of stress	Hamilton and Buhl, 1990

[a]Number of animals per treatment.
[b]Quantity of molybdenum dosed. SI conversion: 1 mg molybdenum equals 10.4 μmoles molybdenum.

TABLE 21-2 Molybdenum Concentrations in Fluids and Tissues of Animals

Animals	Quantity	Source	Plasma	Whole Blood	Milk	Bone	Soft Tissues (dry basis) L-liver, K-kidney, M-muscle	Reference
Rats, male druckery (120 g)	0 mg Mo/kg BW 50 mg Mo/kg BW	Sodium molybdate					Testis: 230; epididymis: 315; seminal vesicle: 283; prostate gland: 32 ng/g tissue Testis: 246; epididymis: 418; seminal vesicle: 427; and prostate gland: 242 ng/g tissue	Pandey and Singh, 2002
Chickens, broilers	0.3 mg Mo/kg of feed 4.0 mg Mo/kg of feed	Sodium molybdate		60 µg/L			0.47 mg Mo/kg (L) 0.81 mg Mo/kg (L)	Nell et al., 1980
209-kg heifers on a 176-d grazing study	0.27 kg Mo/ha 1.11 kg Mo/ha	Biosolids—treated pasture					0.95 mg Mo/kg (L)) 2.18 mg Mo/kg (L)	Tiffany et al., 2000
7 Holstein cows and 1 yearling heifer for 4 months	Basal 53 mg of Mo/kg of air dry feed 173 mg of Mo/kg of air dry feed	An orchardgrass hay and concentrate added with sodium molybdate		trace 2.66 mg/kg 7.47 mg/kg	0.03 mg/kg 1.03 mg/kg 1.83 mg/kg		3.6 (L); 1.7 (K); 0.5 (M) mg/kg 10.4 (L); 42.3 (K); 6.4 (M) mg/kg 32.4 (L); 62.6 (K); 14.0 (M) mg/kg	Huber et al., 1971
260-kg yearling steers on 180-d grazing study	0.27 kg Mo/ha 2.56 kg Mo/ha	Biosolids—treated pasture					1.86 mg Mo/kg (L) 3.11 mg Mo/kg (L)	Tiffany et al., 2002
1-year-old wethers (45–52 kg) on a 7-mo study	0.4 mg Mo/kg DM 10–12 mg Mo/kg DM	Ammonium molybdate				1.7 mg Mo/kg 48 mg Mo/kg (DM basis)		Hidiroglou et al., 1982
19–25 kg wethers on a corn-silage basal diet for 221 d	0.4 mg Mo/kg 8.4 mg Mo/kg	Ammonium molybdate					1.3 (L); 1.5 (K) mg Mo/kg 3.1 (L); 7.7 (K) mg Mo/kg	Ivan and Veira, 1985

Hypocupraemic ewes repleted for 35 d with a basal semi-purified diet (6 mg of Cu/kg) and then fed Mo supplemented diets for 21 d	0 mg Mo/kg of feed	Ammonium molybdate	0.06–0.9 mg Mo/L		Suttle, 1975
	4 mg Mo/kg offeed		1.1–2.8 mg Mo/L (approximation from figures)		

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Nickel

INTRODUCTION

Nickel (Ni) is a transition element that exhibits a mixture of ferrous and nonferrous metal properties. Metallic nickel is lustrous silver-white, malleable, and resistant to corrosion (Smialowicz, 1998). Nickel is both siderophilic (i.e., associates with iron) and chalcophilic (i.e., associates with sulfur) and constitutes 0.008 percent of the Earth's crust, which makes it the 24th element in order of natural abundance. It is mostly mined as the laterites nickeliferous limonite ((Fe,Ni)O(OH)) and garnierite (a hydrous nickel silicate), or the magmatic sulfide pentiandite ($(Ni,Fe)_9S_8$).

In the Western world, about 65 percent of the nickel is used for making stainless steel and 12 percent for making superalloys. The remaining usage is divided among alloy steels, rechargeable batteries, catalysts and other chemicals, coinage, foundry products, and plating (USGS, 2003). The principal commercial chemicals are $NiCO_3$, $NiCl_2$, NiO, and $NiSO_4$.

Nickel in compounds is usually divalent, but can exist in oxidation forms –1, 0, +1, +2, +3, and +4. The acetate, nitrate, sulfate, and halogen salts of nickel are water soluble, whereas the oxides, sulfides, carbonates, phosphate, and elemental forms of nickel are insoluble in water. In biological systems, Ni^{2+} predominates and coordinates with water or other soluble ligands (Sutherland and Costa, 2002). Proteins containing the amino acid histidine (Sarkar, 1984) are the apparent key biological ligands for nickel.

ESSENTIALITY

Nickel is essential for some lower forms of life where it participates in hydrolysis and redox reactions, regulates gene expression, and stabilizes certain structures (Nielsen, 1998). In these roles, nickel forms ligands with sulfur, nitrogen, and oxygen, and exists in oxidation states +3, +2, and +1. Among the enzymes requiring nickel for activity are hydrogenases that have been identified in >35 species of bacteria, including methanogenic, hydrogen-oxidizing, sulfate-reducing, phototrophic, and aerobic nitrogen-fixing bacteria. Nickel is a component of ureases from bacteria, mycoplasma, fungi, yeast, algae, and invertebrates. A nickel-containing superoxide dismutase has been identified in *Streptomyces* (Kim et al., 1998).

Nickel is essential for nitrogen metabolism in plants where it is a component of urease (Welch, 1981). In soybeans, urea accumulates to toxic levels as a result of depressed urease activity (Eskew et al., 1983). The mechanism for nickel deficiency disrupting nitrogen metabolism, and altering malate and amino acid concentrations in grains, is not fully understood. These disruptions result in growth depression, premature senescence, decreased tissue iron concentrations, inhibited grain development, and decreased grain viability (Brown et al., 1987, 1990).

Nickel is generally not accepted as an essential nutrient for higher animals, apparently because of the lack of a clearly defined specific biochemical function. However, under experimental conditions, nickel deprivation resulted in several subnormal functions in higher animals. Nickel deprivation (<100 µg/kg diet) in goats depressed growth (Anke et al., 1984) and in rats (27 µg/kg diet) increased blood pressure (Nielsen, 2001). Reproductive function was impaired in both goats and rats. In breeding goats, the rate of success of first insemination and conception was decreased, and number of breeding attempts to achieve pregnancy was increased (Anke et al., 1984). In rats, sperm production and motility were decreased (Yokoi et al., 2003). The biochemical changes reported to occur in nickel-deprived pigs fed <100 µg Ni/kg dry diet included increased urinary calcium excretion and decreased skeletal calcium content (Anke et al., 1984). Rats fed 2–30 µg Ni/kg diet exhibited changes in blood and iron indexes that suggested an impairment in iron metabolism (Schnegg and Kirchgessner, 1975; Nielsen et al., 1984; Stangl and Kirchgessner, 1997). In addition, nickel-deficient (13 µg/kg diet) rats accumulated triacylglycerol in liver with

increased concentrations of saturated, monosaturated, and polyunsaturated fatty acids; decreased liver activities of lipogenic enzymes glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme, and fatty acid synthase (Stangl and Kirchgessner, 1996); and altered brain and erythrocyte fatty acid composition of total lipids and individual phospholipids (Stangl and Kirchgessner, 1997). Nickel might have a function that is associated with vitamin B_{12}, because lack of this vitamin inhibits the response to nickel supplementation when dietary nickel is low (Nielsen et al., 1989), and nickel can alleviate vitamin B_{12} deficiency in higher animals (Stangl et al., 2000).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Electrothermal atomic absorption spectrometry (EAAS) with Zeeman background correction is currently the technique most often used to determine the concentration of nickel in biological materials (Sunderman et al., 1988b). The use of ICP-MS for the analysis of nickel is hampered by matrix interference by calcium, sodium, and potassium.

Four methods are commonly used for the preparation of samples for analysis by EAAS. These are acid digestion, protein precipitation, acidification (Sunderman et al., 1988b), and microwave digestion (Benson et al., 1989). Acid digestion is the oxidation of the sample with nitric acid and an oxidizer such as hydrogen peroxide. The protein precipitation method is used for fluids (serum, plasma, whole blood, saliva, bile, and cerebrospinal fluid) and involves the precipitation of protein from the fluid by nitric acid and heat. The direct technique in which the sample is just acidified with nitric acid prior to analysis, is used to determine nickel in urine, water, and protein-free aqueous samples. The microwave digestion procedure uses a microwave oven for irradiating samples in Teflon digesters containing nitric acid, hydrochloric acid, and hydrogen peroxide.

Contamination is a major concern when determining the presence of nickel in a sample. Careful handling of samples is required to prevent contamination from ambient air, skin (sweat) of the handler, instruments (e.g., stainless steel knives, scissors, needles, etc.) used to collect samples, and reagents used in the analysis. Because of contamination concerns, the validity of any nickel analysis can only be assured by the use of quality control procedures.

REGULATION AND METABOLISM

Absorption and Metabolism

It is generally accepted that <10 percent of the nickel that humans and animals ingest with food is absorbed (Nieboer et al., 1988). Nickel absorption is heightened by iron deficiency (Tallkvist and Tjälve, 1997), pregnancy (Kirchgessner et al., 1980b), and lactation (Kirchgessner et al., 1983). The mechanisms involved in the transport of nickel through the gut are not conclusively established, but both active and passive processes are thought to be involved (Foulkes and McMullen, 1986; Tallkvist and Tjälve, 1998). It has been suggested that some nickel is transported through an iron-transport system (Tallkvist and Tjälve, 1997), and cobalt can compete with these elements for transport (Eidelsburger et al., 1996). Nickel homeostasis may be regulated by absorption from the gut. The rate of nickel transfer was greater in everted jejunal sacs from nickel-deprived rats than from nickel-adequate rats (Stangl et al., 1998).

Nickel is transported in blood principally bound to serum albumin. Small amounts of nickel in serum are associated with the amino acids histidine and aspartic acid, and with α-2-macroglobulin (nickeloplasmin) (Nomoto and Sunderman, 1988; Tabata and Sarkar, 1992). Uptake of soluble nickel from serum into tissues is believed to be governed by ligand exchange reactions (Sutherland and Costa, 2002). It has been suggested that histidine removes nickel from serum albumin and mediates its entry into cells. The transfer of nickel across plasma membranes apparently involves both active and diffusion mechanisms, which have not been defined. Soluble nickel may share a common transport system with magnesium and/or iron (e.g., transported into the cell bound to transferrin) and some soluble nickel probably enters cells via calcium channels (Sutherland and Costa, 2002). Insoluble nickel compounds enter the cell via phagocytosis (Sutherland and Costa, 2002).

Although fecal nickel excretion (mostly unabsorbed nickel) is 10–100 times as great as urinary excretion, most of the small fraction of absorbed nickel is rapidly and efficiently excreted through the kidney as urinary low-molecular-weight complexes (Predki et al., 1992). In healthy humans, urinary nickel concentrations generally range from 0.1 to 13.3 μg/L (Sutherland and Costa, 2002). The nickel content in human sweat is high (~70 μg/L), which points to active secretion of nickel by the sweat glands (Omokhodion and Howard, 1994). Based on isotopic studies in which nickel was administered intravenously, excretion of exogenous nickel through the bile or gut is insignificant (Marzouk and Sunderman, 1985; Patriarca et al., 1997).

Metabolic Interactions and Mechanisms of Toxicity

Because there apparently are mechanisms for the homeostatic regulation of nickel, life-threatening toxicity of nickel through oral intake is low, ranking with elements such as zinc, chromium, and manganese. Generally, a diet of 100 mg Ni/kg supplemented as a water soluble salt is required to produce signs of nickel toxicity in rats, mice, chicks, dogs, cows, rabbits, pigs, ducks, and monkeys. Initial signs of nickel toxicity apparently are the result of reduced food intake (partially caused by reduced palatability) and gastrointestinal irritation. Some responses to excessive intake of nickel may be the result of nickel interfering with the

absorption or use of essential elements, especially copper, iron, and zinc; these responses become more evident when the intake of these elements is deficient. For example, excessive amounts of nickel as nickel chloride or nickel sulfate exacerbate signs of severe iron, copper, and zinc deficiencies in rats (Nielsen et al., 1979; Nielsen and Zimmerman, 1981; and Mathur et al., 1982, respectively). Studies using injected nickel or isolated cells suggest that nickel may have toxic effects through altering cellular redox status, which can cause DNA and cellular membrane and protein damage, impaired cell cycle progression, and abnormal cytoskeletal structure (Sutherland and Costa, 2002). The oxidation potential of nickel is lowered upon binding to certain cellular ligands; this increases its reactivity towards cellular oxidants such as molecular oxygen, hydrogen peroxide, and lipid peroxides. Oxidation of Ni^{2+} to Ni^{3+} may result in the formation of reactive oxygen species that can cause cytotoxic damage and decrease cellular antioxidant capacity (Sutherland and Costa, 2002). Nickel also may be toxic through inhibiting enzymes involved in glucose, energy, and oxidative metabolism. In mice, 1,600 mg Ni/kg diet as nickel acetate inhibited the activities of liver and heart cytochrome C oxidase, kidney malic dehydrogenase, liver, kidney and heart isocitric dehydrogenase, kidney succinic dehydrogenase, and liver NADH cytochrome C reductase (Weber and Reid, 1969). Pandey et al. (1999) found that 5 and 10 mg Ni/kg BW administered orally as nickel sulfate 5 days a week for 35 days increased the activities of lactate dehydrogenase and γ-glutamyl transpeptidase and decreased the activity of sorbitol dehydrogenase in the testes of mice. In rats, 1,000 mg Ni/kg diet as nickel chloride or nickel sulfate increased blood glucose, serum and liver protein, liver urea and liver glutamate dehydrogenase activity, and inhibited the activities of liver and heart cytochrome C oxidase, liver succinic dehydrogenase and glucose-6-phosphate dehydrogenase, and plasma alkaline phosphatase (Whanger, 1973; Schnegg and Kirchgessner, 1976; Kirchgessner et al., 1980a; Mathur, 1983).

SOURCES AND BIOAVAILABILITY

Most animal feeds, because they are plant-based, contain relatively high amounts of nickel (Nielsen, 1987). Common pasture plants contain 0.5–3.5 mg Ni/kg DM. Nickel content has been reported in some feed grains and protein sources, including wheat (0.08–0.3 mg/kg); corn (0.20 mg/kg); oats (0.71–2.09 mg/kg); linseed meal (5.24 mg/kg); soybean meal (7.91 mg/kg); and sunflower meal (7.78 mg/kg). Exposure to emissions from industry such as nickel-processing plants can increase the nickel content of plants 10-fold (Anke et al., 1995). Because nickel concentrations are low in animal tissues, milk products and meat meals used as protein supplements contain relatively low amounts of nickel. However, fish protein concentrate was found to contain 0.7–2.8 mg Ni/kg (Langmyhr and Orre, 1980). The nickel content of water is typically low. The concentrations in the major river basins and water supplies of the United States were determined to be usually <10 μg/L (NRC, 1975).

In humans, when nickel in water is ingested after an overnight fast, as much as 50 percent, but usually closer to 20–25 percent, of the dose is absorbed (Solomons et al., 1982; Sunderman et al., 1989). Foods, drinks, and specific biochemicals (e.g., ascorbic acid) depress this high absorption, often to <1 percent. The form of nickel in foods and feeds and its bioavailability has not been determined.

TOXICOSIS

The toxicity of nickel in laboratory animals exposed to nickel through dermal application, injection, or inhalation, and in isolated cells exposed to high doses of nickel, has been extensively studied. This study has been prompted by findings showing that nickel is a carcinogen and an allergen for humans. Exposure to nickel oxides and nickel subsulfide has been consistently associated with lung and nasopharyngeal cancer among nickel refinery workers in Wales, Canada, Norway, and the United States (Kasprzak, 1987). Nickel is a powerful sensitizing agent that elicits hypersensitivity reactions manifested by contact dermatitis and asthma (Smialowicz, 1998). The many toxic manifestations of nickel by routes of exposure not relevant to the mission of this document have been recently reviewed (Sutherland and Costa, 2002), and thus will not be presented here. Also, unlike the predecessor of this document (NRC, 1980), this review will not tabulate studies showing relatively high amounts of orally ingested nickel (generally between 20 and 1,000 mg Ni/kg diet) having no or beneficial effects. For example, no adverse effects were observed in rats fed 250, 500, or 1,000 mg Ni/kg diet as nickel carbonate, nickel soap, and nickel catalyst for 3 to 4 months, or in monkeys fed in a similar manner for 6 months (Phatak and Patwarhan, 1950). An example of a beneficial effect is the finding that supplementing 25 mg Ni/kg diet in chicks improved their bone strength (Wilson et al., 2001).

Acute

The acute toxicity of nickel is low. It has been suggested that low oral toxicity may be the result of nickel binding to a basolateral population of metal carriers in the gastrointestinal tract, which blocks its own basolateral transfer in a concentration-dependent manner (Müller-Fassbender et al., 2003). Nonetheless, acute lethal doses have been administered to animals. The first published study of nickel using experimental animals determined the effects of acute high doses of nickel. Gmelin (1826) found that the administration of nickel sulfate to rabbits and dogs by stomach tube produced severe gastritis and fatal convulsions; sublethal doses of nickel sulfate in dogs induced cachexia and conjunctivitis. The acute oral LD_{50} dose of nickel (as nickel acetate)

was determined to be 136 mg/kg BW for mice and 116 mg/kg BW for rats (Fairchild et al., 1977). The 48-hour LC_{50} values for carp (*Puntius conchonius)* in soft and hard water for nickel sulfate were 158.4 and 397.9 mg/L, respectively; for nickel chloride, the values were 295.4 and 586.2 mg/L, respectively (Gill and Pant, 1981). The 48-hour LC_{50} for *Channa punctatus* was found to be 30.7 mg/L of water (Khangarot and Durve, 1982).

Reports of human nickel toxicosis through oral intake are limited to a few case reports of acute effects caused by the ingestion of high doses of soluble nickel salts. The most prominent case report was of 20 people who accidentally ingested 0.5–2.5 g of nickel as the sulfate and chloride; they developed nausea, abdominal pain, diarrhea, vomiting, and shortness of breath (Sunderman et al., 1988a). A 0.6 mg oral dose of nickel as nickel sulfate in water drunk by fasting (thus nickel was highly available) nickel-sensitive individuals produced a contact dermatitis-like reaction (Cronin et al., 1980).

Chronic

Table 22-1 summarizes the doses and effects (mostly physical signs) of chronic consumption of high amounts of nickel by various animals. No chronic toxicosis signs caused by oral intake have been reported for humans. Extended periods of time consuming relatively high amounts of nickel are required before signs of chronic toxicosis are seen in animals. The most commonly reported signs of toxicosis include depressed growth, feed intake, and feed efficiency; hematological changes; kidney damage; and impaired reproductive performance characterized by increased deaths of offspring.

As indicated by Table 22-1, high oral nickel intakes may be toxic to the developing embryo. Schroeder and Mitchener (1971) reported that 5 mg Ni/L of water as nickel chloride as a soluble salt through three generations increased the number of runts born and the number of perinatal deaths in rats. Smith et al. (1993) fed 10, 50, and 250 mg/L water to rats through two generations and found increased perinatal mortality only with the two higher intakes. Ambrose et al. (1976) fed 250, 500, or 1,000 mg Ni/kg diet as nickel sulfate through three generations and observed increased stillborns only in the F1 generation. However, a decreased number of pups were weaned in all generations from rats fed 500 or 1,000 mg Ni/kg diet. These reproduction findings need to be repeated or confirmed, because all of them were obtained from experiments that were either flawed or subject to misinterpretation by their statistical design and inconsistencies in the reported quantity–response relationships. Trüpschuch et al. (1996) found that 250 or 500 mg Ni/kg as nickel sulfate in the diet of hens increased the mortality of hatched chicks; 1,000 mg Ni/kg diet increased the number of dead, malformed, and nonviable chicks in eggs and increased mortality after hatching.

Factors Influencing Toxicity

Iron, magnesium, zinc, vitamin C, cysteine, protein, and some pesticides may influence nickel toxicity (400 mg/kg diet as nickel chloride). Iron-deficient chicks were found to be more susceptible to nickel toxicity, as judged by growth depression, than iron-adequate chicks (Blalock and Hill, 1985). A review (McCoy and Kenny, 1992) summarized findings suggesting that magnesium can antagonize toxic effects of nickel, especially those induced in vitro or by injected nickel. Mathur et al. (1982) observed that high dietary zinc enhanced signs of nickel toxicosis such as depressed growth, feed efficiency, and feed intake. It has been reported that increasing dietary vitamin C (Chatterjee et al., 1979) and cysteine (Griffith et al., 1942) alleviated nickel toxicosis in rats, and increasing dietary protein from 10 percent to 30 percent decreased nickel toxicosis in chicks (Hill, 1979). Oral administration of dimethyldithiocarbamate pesticides (ferbam, ziram, or sodium dimethyldithiocarbamate) or thiram together with nickel as nickel chloride increased the concentration of nickel in several tissues of rats (Borg and Tjälve, 1988). The increase in tissue nickel apparently occurred through the formation of lipophilic nickel-pesticide chelates that facilitate the transfer of nickel through the gastrointestinal tract. This suggests that some pesticides can facilitate nickel toxicity (Hopfer et al., 1987).

TISSUE LEVELS

Nickel is widely distributed in tissues in concentrations generally between 0.01 and 0.2 mg/kg WW (see Table 22-2) when dietary nickel is not excessive (<25 mg/kg). Nickel does not accumulate with age in any organ, but, as with other mineral elements, overcoming homeostatic mechanisms by the addition of soluble nickel salts to drinking water or diet elevates tissue and blood nickel concentrations. Kidney apparently is the organ most sensitive to an increased ingestion of nickel. A kidney nickel concentration >1 mg/kg WW may be an indicator of nickel toxicosis. The highest kidney nickel concentration reported has been about 29 mg/kg WW for rats fed 1,000 mg/kg diet (Schnegg and Kirchgessner, 1976). Because monkeys, which probably respond similarly to humans, exhibited no signs of toxicosis when fed 250, 500, and 1,000 mg Ni/kg diet as nickel catalyst, nickel soap, and nickel carbonate for 24 weeks (Phatak and Patwardhan, 1950), the data in Table 22-2 indicate that no animal tissue or fluid used as a food will contain enough nickel to be of toxicological concern for humans.

MAXIMUM TOLERABLE LEVELS

The highest dietary level at which nickel has no adverse effect, or the lowest level that induces signs of toxicosis, varies with species. For example, no adverse effects were seen in dogs or monkeys fed 1,000 mg Ni/kg diet as water

insoluble nickel compounds (Phatak and Patwardhan, 1950), but signs of nickel toxicosis were observed in chicks fed 300, pigs fed 375, and rats fed 100 mg Ni/kg diet as water soluble compounds (see Table 22-1). The rat findings suggest that to assure safety, the maximum tolerable level for animals without toxicity data should be no more than 100 mg/kg diet. In the previous edition of this document (NRC, 1980), 50 mg Ni/kg diet was suggested as a maximum tolerable level for cattle. This suggestion seems conservative because steers fed 50 mg Ni/kg diet (Oscar and Spears, 1988), chicks and pigs fed 250 mg Ni/kg diet, and dogs fed 1,000 mg Ni/kg diet (Ambrose et al., 1976) for extended periods of time were not adversely affected (see Table 22-1). Thus, a maximum tolerable level for cattle may be near 100 mg Ni/kg diet, for chicks and pigs may be near 250 mg Ni/kg diet, and for dogs may be near 1,000 mg Ni/kg diet. Because most animal feed contains <10 mg/kg, nickel toxicity under normal environmental conditions is not a concern for domestic animals. However, emissions from industry such as nickel refineries can increase plant nickel concentrations 10-fold (Anke et al., 1995). In these localized environments, nickel toxicity may be a concern for animals.

As stated above, nickel is considered a carcinogen when inhaled or injected, and an allergen when inhaled or upon dermal contact by sensitive individuals. Except for the possibility that individuals with a nickel allergy may be sensitive to diets high in dietary nickel, there is no evidence for humans of adverse effects associated with exposure to nickel through consumption of a normal diet. The Institute of Medicine (2001) used two rat studies to obtain the NOAEL of 5 mg/kg BW/day to use for the calculation of the tolerable upper intake level (UL) for humans. Using this value and an uncertainty factor of 300 resulted in a UL of 0.017 mg/kg BW/day for adults; this translates to about 1.0 mg/day of soluble nickel salts.

FUTURE RESEARCH NEEDS

There are no apparent pressing research needs in regard to nickel toxicity through the oral route. However, establishing a biochemical function for nickel, the form and bioavailability of nickel found naturally in feed, and a clearer understanding of the mechanisms involved in the absorption of nickel from the gastrointestinal tract, would be helpful in clarifying the limits at which nickel is beneficial and detrimental to animals.

SUMMARY

Nickel is essential for lower forms of life where it participates in hydrolysis and redox reactions, regulates gene expression, and stabilizes certain structures. Nickel generally is not accepted as essential for higher animals and humans because it lacks a defined biochemical function. Electrothermal atomic absorption spectrometry with Zeeman background correction is the method of choice for the analysis of nickel in biological samples that have been acid-digested. Because of its ubiquity, contamination is a major problem in nickel analyses. It is generally accepted that <10 percent of nickel ingested is absorbed by mechanisms not completely understood. The small amount of nickel absorbed is excreted mainly in the urine. Because there apparently are mechanisms for the homeostatic regulation of nickel, life-threatening toxicosis through oral intake is low. Extended periods of time consuming relatively high amounts of soluble nickel (i.e., >100 mg/kg diet) are required to induce signs of chronic nickel toxicosis in animals. The toxic dietary concentration is 10–100 times greater than the concentration normally found in animal feeds. The most commonly reported signs of toxicosis observed under experimental conditions are depressed growth, feed intake and feed efficiency, hematological changes, and perhaps kidney damage. Suggested mechanisms involved in nickel toxicity include reduced palatability of the diet, interference with the absorption or use of other essential nutrients (e.g., copper, iron, and zinc), and alteration of cellular redox status. A suggested maximum tolerable limit for cattle is 100 mg/kg diet, and for chicks and pigs is 250 mg Ni/kg diet. Except for a few localized areas of the world where industry has increased nickel in the environment, nickel toxicity is not a concern for domestic animals.

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TABLE 22-1 Effects of Nickel Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	6	2 y	1,000 mg/kg	Nickel sulfate	2 yr	Diet	No adverse effect	Ambrose et al., 1976
			2,500 mg/kg[c]		3 d		Emesis, excessive salivation, gastrointestinal irritation	
			2,500 mg/kg		2 yr		Decreased BW and hemoglobin; increased urine volume, and liver and kidney weight; granulocytic hyperplasia of bone marrow; lung granulomas; bronchiolectasis; emphysema; pneumonia	
Mice	12	Young	1,100 mg/kg	Nickel acetate	4 wk	Diet	Decreased growth, females	Weber and Reid, 1969
			1,600 mg/kg				Decreased growth	
Mice	8	Young	1,100 mg/kg	Nickel acetate	1 reproductive cycle	Diet	No adverse effect	Weber and Reid, 1969
			1,600 mg/kg				Decreased number of pups born and weaned	
Mice	10	6–8 wk	1 g/L	Nickel sulfate	180 d	Water	No adverse effect	Dieter et al., 1988
			5 g/L				Decreased water consumption and thymus size	
			10 g/L				Decreased water consumption and body and organ weights; myeloid histopathology (e.g., decreased bone marrow cellularity, and granulocyte-macrophage and pluripotent stem-cell proliferative responses)	
Mice		Adult	10 mg/kg BW sulfate	Nickel	35 d	Oral dose, 5 d/wk	Decreased testes, epididymides, seminal vesicles, and prostate glands weights; decreased sperm count and motility; histopathological changes in testes, epididymides, and seminal vesicles	Pandey et al., 1999
Mice	10	Young	8 mg/kg BW	Nickel sulfate	6 mo	Oral dose, 5d/wk	Decreased seminal vesicle weight, diameter, and activity of epithelium	Pandey and Singh, 2001

Rats	10	Weanling	5 mg/L	Soluble salt	3 generations	Water	Increased perinatal deaths and runts	Schroeder and Mitchener, 1971
Rats	6	Weanling	100 mg/kg 500 mg/kg 1,000 mg/kg	Nickel acetate	6 wk	Diet	No adverse effect Decreased growth; increased tissue nickel and iron Weight loss, decreased hemoglobin, increased tissue nickel, iron, and zinc	Whanger, 1973
Rats	10	150 g	225 mg/L	Nickel chloride	4 mo	Water	Decreased growth, serum lipid and cholesterol, and urinary volume, zinc, and calcium	Clary, 1975
Rats	10	Weanling	1,000 mg/kg	Nickel chloride	13 d	Diet	Growth cessation and weight loss; increased erythrocytes, hematocrit, hemoglobin, serum protein, and tissue nickel, copper, iron, and zinc	Schnegg and Kirchgessner, 1976; Kirchgessner et al., 1980a
Rats	50	28 d	100 mg/kg 1,000 mg/kg 2,500 mg/kg	Nickel sulfate	2 yr	Diet	No adverse effect Decreased body and liver weights; increased heart weight (females) Decreased BW (both sexes); Decreased liver and increased heart weight (females)	Ambrose et al., 1976
Rats	60	28 d	250 mg/kg 500 mg/kg 1,000 mg/kg	Nickel sulfate	3 generations	Diet	Increased F1 stillborns Increased F1 stillborns; decreased pups weaned (all generations) Decreased BWs; increased F1 stillborns; decreased pups weaned (all generations)	Ambrose et al., 1976
Rats	8	30–35 g	15 mg/100 g BW	Nickel sulfate	3 wk	Daily oral dose	Depressed growth; inhibition of vitamin C metabolism; gross degenerative changes in kidney	Chatterjee et al., 1979
Rats	20	Weanling	1,000 mg/kg	Nickel sulfate	15 d	Diet	Decreased growth and activity of cytochrome, C oxidase and succinic dehydrogenase; increased blood glucose, hemoglobin, and protein	Mathur, 1983
Rats	9	35 g	100 mg/kg 200, 400 and 600 mg/kg	Soluble salt	20 d	Diet	Depressed growth, food intake, and feed efficiency; increased nickel retention	Kirchgessner et al., 1984a
Rats	6	80 d	10 mg/kg BW 20 mg/kg BW	Nickel chloride	81 d	Diet	No adverse effect Disturbed operant lever press response (behavior)	Nation et al., 1985

continued

TABLE 22-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Rats		Adults and offspring	10 mg/L 50 mg/L 250 mg/L	Nickel chloride	11 wk plus 2 gestations	Water	No significant effects Inconsistent reduced weight gain Perinatal mortality	Smith et al., 1993
Rats			100 mg/L	Nickel sulfate	6 mo	Water	Increased kidney weight and urinary albumin excretion	Vyskocil et al., 1994
Chickens	24	1 d	300 mg/kg 500–700 mg/kg 900–1,300 mg/kg	Nickel sulfate	4 wk	Diet	No adverse effect Decreased growth and nitrogen retention Decreased growth, nitrogen retention, and feed efficiency	Weber and Reid, 1968
Chickens	24	1 d	300 mg/kg 500–700 mg/kg 900–1,300 mg/kg	Nickel acetate	4 wk	Diet	No adverse effect Decreased growth and nitrogen retention Decreased growth, nitrogen retention, and feed efficiency	Weber and Reid, 1968
Chickens	16	1 d	300 mg/kg 500–900 mg/kg 1,100 mg/kg	Nickel chloride	3 wk	Diet	Decreased growth Decreased growth; increased kidney nickel Decreased growth; anemia; increased organ nickel	Ling and Leach, 1979
Chickens	—	Adult; chicks from eggs of adults	250–500 mg/kg 1,000 mg/kg	Nickel sulfate	42 d	Diet	Increased mortality of hatchlings Decreased food consumption, growth, egg production, and egg weight; increased dead, malformed, and nonviable chicks in hatching eggs; increased mortality and decreased growth of hatchlings	Trüpschuch et al., 1996
Ducks	36	1 d	200 mg/kg 800 mg/kg 1,200 mg/kg	Nickel sulfate	90 d	Diet	No adverse effect Decreased bone density Tremor and paresis; decreased weight and bone density; increased tissue nickel; death	Cain and Pafford, 1981

Pigs	18	17–23 d	125–250 mg/kg 375–500 mg/kg	Nickel sulfate	6 wk	Diet	No adverse effects Depressed appetite, rate of gain, and feed efficiency; increased blood erythrocytes, hematocrit, hemoglobin, and nickel; coarse, shaggy hair, diarrhea, and dark colored feces	Kirchgessner and Roth, 1977; Kirchgessner et al., 1980a
Cattle	6	13 wk	250 mg/kg 1,000 mg/kg	Nickel carbonate	8 wk	Diet	No adverse effect Decreased food intake, growth rate, organ size, and nitrogen retention; increased tissue nickel; kidney nephritis	O'Dell et al., 1970, 1971
Fish, *Colisa fasciatus*	42	Adult	64 mg/L sulfate	Nickel	96 hr	Water	Increase blood glucose	Chaudhry and Nath, 1985
Fish, lake trout	24	Adult, 2 yr	1,000 mg/kg 10,000 mg/kg	Nickel sulfate	18 d	Diet	Increased tissue nickel and kidney metallothionein Arrested eating; decreased weight and blood potassium; increased tissue nickel and kidney metallothionein	Ptashynski et al., 2001
Fish, lake whitefish	24	Adult, 3 yr	1,000 mg/kg 10,000 mg/kg	Nickel sulfate	18 d	Diet	Increased tissue nickel; decreased blood glucose Arrested eating; increased tissue nickel and liver and kidney metallothionein; decreased blood glucose and potassium; kidney, liver, and intestinal histopathology (e.g., cell necrosis)	Ptashynski et al., 2001
Fish, lake whitefish	6 yr	Adult, 3r	10 mg/kg 100 mg/kg 1,000 mg/kg	Nickel sulfate	104 d	Diet	No adverse effects Minor kidney and liver histopathology Kidney and liver histopathology (e.g., focal necrosis), and altered bile ducts	Ptashynski et al., 2002

[a]Number of animals per treatment.
[b]Quantity of nickel dosed. SI conversion: 1 mg nickel equals 17.0 µmoles nickel.
[c]After 3 days of distress, dogs were returned to control diet, then nickel was gradually increased for about 8 weeks to 2,500 mg/kg diet without acute problems for the remainder of the 2-year study.

TABLE 22-2 Nickel Concentrations in Fluids and Tissues of Animals

Animal	Nickel Intake	Plasma/Serum/ Blood	Skeletal Muscle	Liver	Heart	Kidney	Bone	Reference
Humans	Usual	2.6 (S) 4.8 (B)		0.0082	0.0064	0.011	0.333	Sunderman, 1980
Mice	NIH-07 diet	130		ND[a]		ND[a]		Dieter et al., 1988
	1 g/L water	560		0.13		1.29		
	5 g/L water	591		0.30		2.86		
	10 g/L water	1,020		0.89		4.80		
Rats	20 mg/kg diet	26.2		0.18		0.47		Schnegg and Kirchgessner, 1976
	1,000 mg/kg diet	3,820–5,680		1.76–3.30		21.25-29.28		
Rats	15 µg/kg diet		0.057	0.061–0.062		0.110–0.316	0.027–0.077	Schnegg and Kirchgessner, 1977
	20 mg/kg diet		0.170	0.181–0.264		0.317–0.352	0.085-0.120	
Rats	0.06–1.0 mg/kg	ND[a]	0.013– 0.035	0.009–0.033		0.013–0.039		Kirchgessner et al., 1984b
	10–25 mg/kg	29–54	0.009–0.013	0.039–0.078		0.065-0.067		
	50 mg/kg diet	116	0.020	0.032		0.067		
	100 mg/kg diet	234	0.025	0.106		0.130		
	200 mg/kg diet	483	0.035	0.108		0.216		
	400 mg/kg diet	1,476	0.086	0.211		0.557		
	600 mg/kg diet	4,561	0.222	0.909		1.834		
Chickens	Low		0.12–0.14	0.10	0.13–0.14	0.13–0.14	0.10	Ling and Leach, 197
	300 mg/kg diet		0.26	0.31	0.31	4.23	0.97	
	500 mg/kg diet		0.45–1.01	0.26–0.57	0.26–0.57	6.97–12.58	1.88	
	700 mg/kg diet		0.99	0.80	0.80	9.73	3.75	
	900 mg/kg diet		2.04	1.39	1.39	11.15	4.93	
	1,100 mg/kg diet		1.43	1.50	1.50	11.48	5.91	
Ducks	Mash diet	ND[a]		0.12		0.09	0.07	Eastin and O'Shea, 1981
	+ 12.5 mg/kg diet	ND[a]		0.09		0.16	0.07	
	+ 50.0 mg/kg diet	7		0.08		0.39	0.07	
	+ 200.0 mg/kg diet	23		0.21		0.74	0.07	
	+ 800.0 mg/kg diet	139		0.52		1.94	0.10	
Pigs, DW[b]	0.16 mg/kg diet	3.3	0.125	0.190	0.045	0.138		Spears et al., 1984
	5 mg/kg diet	5.1	0.118	0.245	0.073	0.218		
	25 mg/kg diet	11.0	0.215	0.248	0.092	0.808		
Horses, DW	Usual-Germany			0.699		0.579	0.997	Grün et al., 1980
	Usual-Hungary			1.631		1.023	1.683	

Cattle, DW (except serum)	0.5 mg/kg diet 5.0 mg/kg diet	2.4–2.8 3.6–5.6	0.046–0.054 0.049–0.065	0.038–0.046 0.056–0.090	0.064–0.075 0.076–0.079	0.046–0.051 0.286–0.654		Spears et al., 1986
Cattle, DW	630 µg/kg diet 5.63 mg/kg diet			0.284–0.619 0.600–0.656		0.350–0.583 0.580-0.624		Regius et al., 1983
Cattle, DW	~1.0 mg/kg diet 62.5 mg/kg diet 250.0 mg/kg diet 1,000.0 mg/kg diet	0.0 0.0 0.25 2.88		0.76 0.88 0.37 0.53	1.11 0.0 0.32 0.50	2.08 1.85 2.26 22.83	0.25 0.39 0.90 2.32	O'Dell et al., 1971
Goats, DW	0.137 mg/kg diet 4.36 mg/kg diet		0.350 0.526	0.548 1.115	0.288 0.591	0.438 1.209	0. 174–0.328 0.907–1.124	Anke et al., 1980a
Goats, DW	0.1–4.4 mg/kg diet		1.1–1.3	1.1–1.2		1.5		Anke et al., 1980b
Sheep, DW	Usual-Germany Usual-Hungary		0.526		0.666	0.441 1.266		Grun et al., 1980
Fish, catfish, *Clarias batrachus*	Low 5 mg/mL water 10 mg/mL water 15 mg/mL water 20 mg/mL water 30 mg/mL water			1.3 5.5 8.5 10.7 15.1 18.1		2.3 6.9 11.3 20.9 33.4 45.7		Ray et al., 1990

NOTE: Reported here as mg/kg fresh tissue and µg/L fluid unless noted otherwise. Reported concentrations of nickel in milk are limited. Human and cow milk was found to contain 0.02 and 0.011 mg/L, respectively (Casey, 1976), and sow milk was found to contain 0.16–0.23 mg/kg (Kirchgessner et al., 1982).

[a]ND = not detected.

[b]Dry weight

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Phosphorus

INTRODUCTION

Phosphorus (P) represents 0.12 percent of the Earth's crust and has an atomic number of 15 and an atomic weight of 30.97. In nature, the free elemental form of phosphorus is too reactive to exist. Thus, phosphorus is often found combined with oxygen as inorganic or organic phosphates (Berner, 1997). The organic phosphates used as insecticides or herbicides are highly toxic, and are beyond the scope of this review. The inorganic phosphates share the same basic anionic unit of orthophosphate: a tetrahedron structure of one phosphorus atom surrounded by four oxygen atoms, and the chemical forms of these salts can be simply classified as monovalent (sodium, potassium, and hydrogen); divalent (calcium and magnesium); ammonium; and aluminum (Weiner et al., 2001). The inorganic phosphates are widely used as chemical fertilizers, food and feed supplements, and industrial compounds including detergents, fire extinguishers, toothpaste, and textile processors. Igneous rocks are the ultimate source of phosphorus, and pellet phosphorite and guano are the two major sedimentary deposits for the production of feed phosphates (McDowell, 2003). In 2002, approximately 36 million tons of marketable phosphate rock ore were mined in the United States, mainly in Florida and North Carolina (USGS, 2003). Morocco and Western Sahara, China, and Russia are the next three largest producers and reserves of phosphate rock in the world.

ESSENTIALITY

As the sixth most abundant element in the body, phosphorus is involved in virtually every aspect of metabolism. In bone, phosphorus serves as a structural component of crystalline hydroxyapatite: $Ca_{10}(PO_4)_6(OH)_2$, and is deposited in the organic matrix during mineralization. Therefore, phosphorus deficiency causes rickets in young animals and osteomalacia in adult animals. In soft tissues, phosphorus plays both structural and metabolic roles as a component of phospholipids, DNA, RNA, nucleotides (e.g., ATP, cAMP, uridine di-P-glucose), and enzyme cofactors. In extracellular fluids, approximately 30 percent of phosphorus exists as inorganic phosphate ions that help maintain osmotic pressure, acid-base balance, neuron activity, and appetite (Berner, 1997). In ruminants, phosphorus is essential for proper functioning of rumen microorganisms (NRC, 2001; Guyton et al., 2003). Dietary nutrient requirements of phosphorus (nonphytate) by various species range from 0.2 to 0.8 percent, and may be affected by age, physiological stage, performance, and dietary levels of calcium and vitamin D (McDowell, 2003). Based on performance results, Erickson et al. (2002) suggested that the phosphorus requirement for finishing calves was <0.16 percent of diet DM and could be met by typical grass-based feedlot cattle diets without supplemental inorganic phosphorus.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Total phosphorus contents in feeds, feces, urine, and tissues are readily determined using colorimetric methods (Garcia-Bojalil et al., 1988; Cymbaluk and Christison, 1989; AOAC, 1995), a gravimetric procedure (AOAC, 1995), or an inductive coupling plasma atomic emission spectrometry instrument (Hutcheson et al., 1992). Initially, feed or fecal samples are ground, urinary samples are evaporated, and bone samples are crushed and the fat is extracted. These samples can then be dry-ashed in a muffle furnace (550°–600°C) (Boling et al., 2000) or wet-digested by either sulfuric acid-hydrogen peroxide or perchloric-nitric acid (Budde and Crenshaw, 2003) and diluted to appropriate concentrations for analysis. Inorganic phosphorus concentrations in serum or plasma samples are assayed directly using sodium molybdate and Elon (*p*-methylaminophenol sulfate) solution (Gomori, 1942), after the samples are deproteinated with trichloroacetic acid (Gentile et al., 2003). This method does not measure phosphorus associated with phospholipids.

There are two challenges related to phosphorus analysis. One is the lack of simple and reliable methods to determine various chemical forms of phosphorus, such as phytate-P and its intermediate metabolites in feed and digesta (Rapp et al., 2001; Applegate et al., 2003). Phytate-P represents a large portion of phosphorus in plant-based diets that is poorly available to nonruminant species (Lei and Stahl, 2001). In addition, dietary phytate-P intake by certain species may affect their actual tolerable levels of dietary phosphorus. Thus, an effective analysis of phytate and its hydrolytic products will help cope with these problems. The other challenge is the need for accurate and sensitive assessments of phosphorus status in vivo, Although biopsies of ribs, toes, or tailbones (Little and Minson, 1977; Combs et al., 1991a) as well as x-ray equipment, dual photon absorptiometry, radiographic photometry, and ultrasound (Ternouth, 1990; Williams et al., 1991b) have been applied to assay for body phosphorus status, ash contents in selected entire bones postmortem are still the commonly used criterion for that purpose (Williams et al., 1991a; Boling et al., 2000; Erickson et al., 2002). Urinary hydroxyproline concentrations and pyridinium X-links (McLean et al., 1990) have been suggested as a prognostic tool to determine bone demineralization in racing horses (Price et al., 1995). Serum concentrations of osteocalcin, a small noncollagenous bone protein, may be a good indicator of bone turnover in growing pigs (Carter et al., 1996).

REGULATION AND METABOLISM

Absorption and Metabolism

Both active transport and passive diffusion are suggested as the mechanisms of phosphate absorption in small intestines (Berner, 1997). Active transport occurs in the proximal small intestine and is a sodium-dependent event that is promoted by the active metabolite of vitamin D_3 (Danisi and Straub, 1980). Passive diffusion takes place mainly in the jejunum and ileum, and is directly related to the dietary phosphorus intake and the lumen phosphorus concentrations. Using growing pigs (40–58 kg) fitted with a simple T-cannula at the distal ileum, Ajakaiye et al. (2003) demonstrated that the large intestine played no major role in the digestion of phosphorus associated with soybean meal. In contrast, Schryver et al. (1972) showed that the dorsal large colon and the small colon were the major sites of net phosphorus absorption from various feed sources in ponies. They found no effect of either the calcium content or the source of feedstuff on the site of absorption.

The apparent absorption coefficients of dietary supplemental inorganic phosphates normally fall between 70 and 90 percent for both ruminants and nonruminants (Braithwaite, 1986; Challa et al., 1989; Cromwell, 1992). However, phosphorus in plant feeds, mainly as phytate-P, can be efficiently digested by ruminants, due to their rumen microorganisms (Field et al., 1984; NRC, 2001). The apparent absorption coefficients of phosphorus in total rations for dairy cows range from approximately 30 to 60 percent (Knowlton et al., 2001; Valk et al., 2002; Wu et al., 2003; Borucki Castro et al., 2004; Weiss and Wyatt, 2004). Wether lambs fed concentrate-based diets containing 9 percent highly weathered soil with high phosphorus-fixation capacity had apparent phosphorus absorption between 4 and 19 percent, but true absorption up to 54 percent (Garcia-Bojalil et al., 1988). Pregnant and lactating does absorbed (apparent) 23–42 percent of phosphorus in a diet of alfalfa, concentrates, and minerals (Fredeen et al., 1988). Growing pigs absorbed (apparent) 24–51 percent of phosphorus in soybean meal (Ajakaiye et al., 2003), whereas weanling pigs absorbed only 13 percent of phosphorus in a corn-soy diet (Spencer et al., 2000). Growing horses fed high forage diets (73–77 percent alfalfa) or high concentrate diets (63–65 percent grain and grain by-products) absorbed (apparent) 17–39 percent of total dietary phosphorus, and the apparent absorption coefficient decreased as dietary phosphorus rose from 0.68 to 1.06 percent (Cymbaluk and Christison, 1989). Likewise, dietary phosphorus level and body phosphorus status affected the apparent absorption of phosphorus in fish (Vielma and Lall, 1998). When dietary phosphorus was increased from 0.4 to 1.33 percent, the pooled apparent absorption coefficient was reduced from 73.8 to 63.6 percent. The phosphorus-replete fish had lower phosphorus absorption than the phosphorus-deficient ones.

The absorbed phosphorus may be retained in the body, used for milk or egg production, or excreted into feces and urine. The circulating blood phosphorus is present as both phospholipids and inorganic phosphates. In lactating cows, milk phosphorus as a percentage of dietary phosphorus intake decreased from approximately 65 to 22 percent when dietary phosphorus increased from 0.33 to 0.67 percent (Knowlton and Herbein, 2002; Guyton et al., 2003). However, milk phosphorus concentrations or milk yields were not affected by those levels of dietary phosphorus intake (Knowlton and Herbein, 2002; Dou et al., 2003). Large quantities of phosphorus (30–90 g/day) are secreted in saliva during rumination, serving as the major source of phosphorus flowing into the rumen of cattle and sheep (Challa and Braithwaite, 1989; Valk et al., 2002; Guyton et al., 2003). Approximately 68–81 percent of the salivary phosphorus, in the form of sodium or potassium phosphates, is absorbable after being recycled to the small intestine in bull calves (Challa et al., 1989). Excessive phosphate is excreted primarily via feces (Lei et al., 1993b; Knowlton et al., 2001; Dou et al., 2002, 2003; Bravo et al., 2003; Weiss and Wyatt, 2004). Kidney (urine) plays an important role in phosphorus excretion in nonruminants and ruminants fed high levels of concentrate (Underwood and Suttle, 1999). In all cases, there is a certain amount of endogenous phosphorus loss, even in phosphorus deficiency. That phosphorus loss in feces of growing goats was extrapolated to be 0.067 g/day (Vitti et al.,

2000). In growing pigs, the endogenous phosphorus loss in feces accounted for 8 percent and 18 percent of the recommended total and available phosphorus requirements, respectively (Ajakaiye et al., 2003). Urinary phosphorus excretion was increased in weanling pigs fed an alkalinogenic diet over those fed an acidogenic diet (Budde and Crenshaw, 2003).

Metabolic Interactions, Regulations, and Mechanism of Toxicities

Body phosphate homeostasis in nonruminant species is maintained primarily by three organs/tissues—intestine, kidney, and bone—and three hormones—parathyroid hormone (PTH), 1,25-$(OH)_2D_3$, and calcitonin. These organs and hormones function cooperatively in regulating absorption, excretion, and deposition/resorption of phosphates in the body, maintaining a constant exchanging pool among various phosphates in the plasma. In the small intestine, active 1,25-$(OH)_2D_3$ promotes phosphate absorption, independent of its effect on calcium absorption (Peterlik and Wasserman, 1978; Kabakoff et al., 1982). In the bone, 1,25-$(OH)_2D_3$ enhances mobilization (resorption) of phosphate and calcium (Kowarski and Schachter, 1969). In the kidney, 1,25-$(OH)_2D_3$ promotes phosphate reabsorption, whereas PTH and calcitonin (Lang et al., 1981) exert the exact opposite roles. Homeostasis of phosphorus in ruminants is maintained primarily by salivary recycling (NRC, 2001). Although significant differences in liver, muscle, and plasma concentrations of 1,25-$(OH)_2D_3$ were found among three biological types of beef cattle (Montgomery et al., 2004), dietary phosphorus intake, ranging from 0.7 to 3 times the maintenance requirement, had no effect on plasma concentrations of 1,25-$(OH)_2D_3$ in aged dairy cows (Barton et al., 1987). In fish, phosphorus homeostasis is also mediated by absorption in the intestine, reabsorption in the kidney, and deposition in bones (Vielma and Lall, 1998). However, the exact molecular mechanisms of the regulation, including the role of vitamin D and the importance of an appropriate calcium:phosphorus ratio, are largely unclear (Lall, 2002).

Normal phosphorus nutrition and metabolism requires adequate levels of dietary calcium and an appropriate ratio of calcium:phosphorus (Littledike and Goff, 1987). Without adequate available calcium, phosphates cannot be deposited into the bones. However, excess dietary calcium forms insoluble complexes with phosphate or phytate in the intestine, rendering phosphorus unavailable for absorption (Lei et al., 1994; Liu et al., 2000). In addition, high levels of serum calcium inhibit the synthesis of 1,25-$(OH)_2D_3$, reducing phosphorus absorption in intestines (Lau et al., 1984). Therefore, it is difficult to produce or distinguish clinical signs of the primary phosphorus deficiency or toxicity, without confounding calcium or vitamin D nutrition. In many cases, phosphorus toxicity is produced by a relative excess of phosphorus in relation to low calcium. As high plasma phosphorus concentrations, associated with high dietary phosphorus intake, cause the lowering of plasma calcium, the parathyroid gland is subsequently stimulated to release more PTH to increase plasma calcium by accelerating bone resorption and renal phosphate excretion. Prolonged bone resorption leads to pronounced bone loss, so that the demineralized skeleton may be replaced by fibrous connective tissues (Bartter, 1964; NRC, 1980).

SOURCES AND BIOAVAILABILITY

Plant feeds, inorganic phosphate supplements, and bone, meat, poultry, and fish meals serve as the major sources of phosphorus for animals. Bioavailability of phosphorus from these sources is estimated by a variety of criteria. In some cases, the digestibility of phosphorus, as discussed above, is simply considered equivalent to bioavailability. Most times, phosphorus bioavailability is determined based on the effectiveness of a given source, relative to that of selected, highly available sodium or calcium phosphates (Cromwell, 1992), in improving performance, bone strength and integrity, and biochemical responses. Temperate or tropical forages contain 2.3–3.5 g P/kg (DM basis, Minson, 1990), and the phosphorus availability ranges from 64 to 86 percent to sheep (Field et al., 1984; Scott et al., 1995) and cattle (Martz et al., 1990). Plant protein feeds such as oilseed meals contain higher levels of phosphorus than those in cereals (5–12 vs. 2.7–4.3 g P/kg DM) (Reddy et al., 1982). Only 12–35 percent of the total phosphorus in most of these feeds is available to swine and poultry because of the high concentrations of phytate-phosphorus (Kornegay, 1996). In contrast, wheat, triticale, rye, and their byproducts have much higher phosphorus bioavailability (~50 percent) to nonruminant species due to their high intrinsic phytase activities (Pointillart, 1991; Han et al., 1997). The recently developed low-phytate corn has higher phosphorus availability to swine and poultry than normal corn (Spencer et al., 2000; Sands et al., 2001). Low-phytate soybean meal also has higher phosphorus bioavailability than normal soybean meal (Cromwell et al., 2000). The apparent digestibility of total ration phosphorus for dairy cows ranges from 30 to 60 percent (Knowlton et al., 2001; Valk et al., 2002; Wu et al., 2003; Weiss and Wyatt, 2004).

The phosphorus content in commonly used mineral salts for animal feeds ranges from 9 to 24 percent. Bone meal contains approximately 12.5 percent of phosphorus. More than 95 percent of phosphorus is available to swine and poultry from the following sources: hydrated dicalcium phosphate, monosodium phosphate, ammonium phosphate, fish meal, meat meal, meat and bone meal, monocalcium phosphate, potassium phosphate monobasic, monosodium phosphate, phosphoric acid, poultry by-product meal, tricalcium phosphate, and urea phosphate (Soares, 1995). In comparison, phosphorus in bone meal, blood meal, Curacao Island phosphate, defluorinated phosphates, and dried poultry waste is slightly less available (85–90 percent, Coffey et al., 1994).

However, phosphorus in metaphosphates and pyrophosphates is poorly available to nonruminants (Soares, 1995). Inorganic phosphorus is virtually completely (99 percent) available to fish, whereas the organic phosphorus is approximately 40 percent available (Roy and Lall, 2003).

There are several important issues related to the phosphorus supplementation. First, ground rock phosphate may be contaminated with high levels of fluoride (3–4 percent), resulting in fluoride toxicity. Second, the recent occurrence of bovine spongiform encephalopathy has resulted in restrictions against supplementing bone and meat meals of ruminant origin in diets. Third, inorganic phosphorus is a nonrenewable resource, and the readily available inorganic phosphorus deposit on Earth may be exhausted in 2080 at the current extraction rate (Forsberg et al., 2003). Last, and most important, high levels of manure phosphorus from the undigested phytate-phosphorus and/or excessive supplementation may cause environmental pollution. In many parts of the world, the phosphorus content of manure becomes the first factor limiting the manure application to arable land (Kornegay, 1996; Weiss and Wyatt, 2004; Koelsch, 2005).

Microbial phytases have been developed and supplemented into diets for swine, poultry, and fish to reduce phosphorus pollution of the environment from their waste (Lei and Porres, 2003). There is a dose-dependent effect of phytase on phytate-phosphorus bioavailability to both swine and poultry (Nelson et al., 1971; Simons et al., 1990, Cromwell et al., 1993), and 500 units of phytase activity per kilogram of feed may reduce dietary inorganic phosphorus supplementation by half and manure phosphorus concentration by 30–50 percent (Lei et al., 1993b; Augspurger et al., 2003). In addition, phytase can improve bioavailability of calcium, iron, and zinc by releasing them from phytate (Lei et al., 1993a,b; Stahl et al., 1999). However, degradation of phytate may release chelated lead (Pallauf and Rimbach, 1997; Zacharias et al., 1999), and render animals susceptible to oxidative stress mediated by high levels of dietary iron (Porres et al., 1999).

TOXICOSIS

Toxicosis from phosphorus is rather rare in food-producing animals. Although plant-based diets may meet nutrient requirements of phosphorus for ruminants (Wu et al., 2001; Erickson et al., 2002), concentrations and the bioavailabilities of phosphorus in those diets are too low to meet the needs for nonruminant species. Inorganic phosphorus supplements as feed ingredients are fairly expensive. Moreover, phosphate is readily excreted via urine, and thereby is well-tolerated (Underwood and Suttle, 1999). Thus, animals can tolerate a wide range of dietary phosphorus intakes if their diets are balanced with calcium. In many cases, the phosphorus toxicity is associated with metabolic disorders of calcium absorption and function, produced by a relative excess of phosphorus in relation to low calcium. Nevertheless, high levels of phosphorus may still be detrimental to animals even in the presence of adequate calcium (Laflamme and Jowsey, 1972; Carstairs et al., 1981; Matsuzaki et al., 1997).

Single Dose and Acute

Based on the review of 96 published and unpublished (by industry) studies in laboratory species (rats, mice, hamsters, rabbits, and guinea pigs), Weiner et al. (2001) concluded that all tested groups of inorganic phosphates, including phosphoric acid (Randall and Robinson, 1990), exhibited low acute oral toxicities. Among all the salts, tetrasodium pyrophosphate ($Na_4P_2O_7$) showed the lowest LD_{50} (1.38 g of compound/kg BW) and sodium trimetaphosphate $(NaPO_3)_3$ showed the highest LD_{50} (10.6 g of compound/kg) in rats.

Chronic

Urolithiasis (urinary calculi) can be produced by a relative excess of phosphorus in relation to calcium in ruminants. The malady is caused by the formation of stones or calculi in the kidney or bladder, resulting in obstruction of urine excretion, particularly in males. The continuous buildup of urine in the bladder eventually ruptures the bladder or urethra, followed by abdominal distension, depression, and death due to uremia. The incidence was approximately 70 percent in male lambs fed a high level of phosphorus (0.8 percent) and low calcium (0.44 percent), and also occurred in lambs fed lower levels of phosphorus (Table 23-1, Emerick and Embry, 1963, 1964). Different forms of sodium phosphates appeared to have equal ability to produce calculi, but elevating dietary calcium levels partially protected against the incidence in sheep (Bushman et al., 1965). Grazing sheep (45 kg BW) tolerated orthophosphoric acid and monosodium phosphate, administrated in water, up to 1.5 and 3.0 g of phosphorus per day for 70 days, respectively (McMeniman, 1973). However, all sheep died of receiving orthophosphoric acid at 3 g of phosphorus per day.

Nutritional secondary hyperparathyroidism can be induced in horses by low calcium, high phosphorus diets (Joyce et al., 1971). The disorder is also called fibrous osteodystrophy and is produced by an increased serum inorganic phosphorus and a decreased serum calcium concentration. As mentioned above, the low serum calcium stimulates the secretion of PTH, causing bone resorption and the replacement of the demineralized skeleton by fibrous connective tissue (Bartter, 1964). When horses are fed high levels of wheat bran that contains high phosphorus (1.15 percent) and low calcium (0.14 percent), their facial bones become enlarged by the invasion of the fibrous connective tissue in the area with significant calcium mobilization. Thus, it is sometimes called "bran disease" or "big head disease." A diet with a calcium:phosphorus ratio of 0.8:1.0 or lower may produce the symptom within 6 to 12 months (NRC, 1989). In this case, the high phosphorus intake

decreases calcium absorption, plasma calcium concentration, and renal calcium excretion, but increases both rates of calcium deposition and removal in bones (Schryver et al., 1971; Argenzio et al., 1974).

No signs of toxicosis were seen in Holstein-Friesian cows fed 0.69 percent dietary phosphorus (dry basis) for 14 weeks prepartum through 22 weeks of lactation (DeBoer et al., 1981). However, a similar level of dietary phosphorus (0.64 percent) reduced magnesium absorption in pregnant dairy heifers (Schonewille et al., 1994), compared with a lower level of phosphorus (0.22 percent). Primiparous cows fed 0.5 percent phosphorus (DM basis) showed depressed milk production during the second and third months of lactation compared to those fed 0.4 percent phosphorus (Carstairs et al., 1981). As high blood phosphorus inhibits the production of active vitamin D, high phosphorus intake (80 g/cow/d) during late pregnancy may increase the incidence of milk fever and hypocalcemia at parturition (Reinhardt and Conrad, 1980). However, feeding cows three times the maintenance requirement of phosphorus (0.69 percent phosphorus) for 28 days precalving showed an effect on the incidence of parturient paresis (Barton et al., 1987). Cows fed dietary phosphorus up to 0.67 percent (DM basis) showed no adverse response in milk production in long (2- to 3-year) experiments (Wu et al., 2000, 2001; Knowlton and Herbein, 2002) or under field conditions (Cerosaletti et al., 2004). Similarly, there was no difference in health or reproductive performance between cows fed 0.37 and 0.57 percent of phosphorus (dry basis) (Lopez et al., 2004a,b).

With appropriate dietary calcium:phosphorus ratios (1.3 to 2:1), pigs exhibited no adverse response in growth performance and/or bone traits to various dietary phosphorus levels up to 0.9 percent (Reinhart and Mahan, 1986; Combs et al., 1991b,c; Hall et al., 1991). However, dietary phosphorus in excess of 1 percent resulted in a quadratic decrease in feed use efficiency in growing pigs (Crenshaw, 1986). High dietary phosphorus, combined with very low calcium, also produced nutritional secondary hyperparathyroidism in pigs (Brown et al., 1966). Depressed egg production and eggshell quality was observed in laying hens fed 0.8–1.2 percent phosphorus (Harms et al., 1965; Charles and Jensen, 1975). The acid-base balance was disturbed by feeding high levels of acidogenic monobasic phosphate [$Ca(H_2PO_4)_2$] (Keshavarz, 1994). Egg production was reduced from 90 to 70 percent and feed intake was reduced from 105 to 78 g per hen per day when monobasic phosphate was supplemented at 1 percent phosphorus. In contrast, no adverse effect was produced by supplemental dibasic phosphate ($CaHPO_4$) at 2.0–2.4 percent phosphorus on performance or at 1.3 percent phosphorus on eggshell quality. Feeding broilers with 0.55–0.83 percent nonphytate-phosphorus (0.8–1.1 percent total phosphorus) rendered the birds susceptible to tibial dyschondroplasia, and the disorder could be alleviated by feeding high levels of calcium (1.5–1.7 percent) (Edwards and Veltmann, 1983).

Rapid bone loss and detached incisor teeth were seen in beagles fed a purified diet containing 1.2 percent phosphorus and 0.12 percent calcium (Krook et al., 1971). These disorders were associated with accelerated bone resorption mediated by PTH (Krook et al., 1971; Laflamme and Jowsey, 1972). When young adult dogs were fed three condensed sodium phosphates (poly-, tripoly-, and trimeta-phosphate) at 100 mg compound/kg per day for 30 days, no adverse effect was observed (Hodge, 1964). But, dogs fed higher levels of these phosphates (1–4 g/kg per day) for 5 months showed weight loss, alterations in eosinophil counts or in the proportion of neutrophils, increased heart weights, hypertrophy of the left ventricle, and tubular damage in the kidney. When dogs were fed 800 mg of dipotassium phosphate/kg per day for 14 or 38 weeks (Schneider et al., 1980a,b), the animals were vomiting, cachectic, and high in urine creatinine and blood urea nitrogen. There was also renal damage (disseminated tubular atrophy, focal scar tissue, and nephrocalcinosis).

Accelerated bone resorption was produced by 1.2 percent dietary phosphorus in adult rats fed a diet containing the same level of calcium (Anderson and Draper, 1972). Cumulative excretion of ^{45}Ca was increased, but urinary calcium excretion was decreased in rats fed 0.6 to 1.8 percent phosphorus compared to those fed 0.3 percent phosphorus (0.6 percent calcium) (Draper et al., 1972). Rats fed 1.8 percent or higher disodium phosphate, sodium tripolyphosphate, and tetrasodium pyrophosphate for 6 months or longer exhibit growth retardation, increased kidney weight, and renal calcification (Hahn, 1961). Nephrocalcionosis appeared in rats fed 1.2 percent phosphorus or high levels (Matsuzaki et al., 1997, 2001). These rats also had increased kidney weight and calcium and phosphorus concentrations, elevated urine volume, and high urine concentrations of albumin, N-acetyl-beta-d-glucosaminidase activity, and beta 2-microglobulin. Similar impacts of excess dietary phosphorus in relation to calcium have also been seen in other laboratory species (Table 23-1). When dietary phosphorus levels exceeded 1.2 percent, both Atlantic salmon reared in fresh water (Vielma and Lall, 1998) and juvenile haddock reared in seawater (Roy and Lall, 2003) showed depressed BW and feed use efficiency and increased mortality. The magnesium and zinc concentrations in the fish vertebrae ash were inversely correlated with dietary phosphorus levels (Vielma and Lall, 1998).

A number of inorganic phosphates have been tested in a series of standard systems, and no genotoxicity, mutagenicity, teratogenicity, or reproductive toxicity was observed at adequate or high levels (Weiner et al., 2001).

Factors Influencing Toxicity

Since high dietary phosphorus can often cause secondary reductions in Ca absorption, phosphorus toxicity may be prevented or alleviated by feeding higher levels of dietary cal-

cium. Excessive dietary magnesium promotes the formation of urinary calculi in sheep fed high phosphorus diets by enhancing the formation of magnesium phosphates that are integral to the growth of the phosphate calculi (Suttle and Hay, 1986). The acidogenic effects of monobasic phosphates on the performance or eggshell quality of laying hens can be ameliorated by alkaline salts of sodium or potassium bicarbonate (Keshavarz, 1994). Female rats are more prone to the high-phosphorus-induced nephrocalcinosis than male rats (Matsuzaki et al., 2002). Polyphosphate salts produce more severe nephrocalcinosis and kidney dysfunction than monophosphates in rats (Matsuzaki et al., 1999). Sodium aluminum phosphate, with a low solubility, has a lower toxicity than other phosphates (Weiner et al., 2001).

TISSUE LEVELS

Phosphorus comprises approximately 1 percent of BW. As 85 percent of body phosphorus is in bone, it has the highest phosphorus concentration of all tissues. On a dry, fat-free basis, different bones of various species contain 9–13 percent phosphorus that represents 11–20 percent of bone ash (Table 23-2). In most cases, bone ash, instead of bone phosphorus, is used to measure the effects of dietary supplemental phosphorus or phytase (Auspurger et al., 2003) because bone ash has a fairly constant concentration of phosphorus. However, deposits of phosphorus in bone, particularly in long bones, decrease with dietary phosphorus depletion (Underwood and Suttle, 1999; Wu et al., 2001), but remain fairly constant if dietary phosphorus levels are met or above the nutrient requirements (Draper et al., 1972).

Although 14–20 percent of the body phosphorus is in the soft tissues (Berner, 1997; Underwood and Suttle, 1999; McDowell, 2003), phosphorus concentrations in these tissues have been reported in only a few studies. Based on the limited data, the values range from 0.4 to 0.8 percent on a dry basis in several species (Table 23-2). As the main target organ of phosphorus toxicity, kidney can deposit high levels of phosphorus (up to 7.8 percent) and calcium (Matsuzaki et al., 1997). Effects of dietary phosphorus on plasma or serum inorganic phosphorus concentration have been assayed in many studies. The plasma inorganic phosphorus concentration, ranging from 20 to 100 mg/L, is slightly lower than that in serum, but much lower than that in the whole blood. Although the inorganic phosphorus in the blood represents only 0.3 percent of total body phosphorus, it is often sampled to assay for the body phosphorus status because it is very responsive to dietary phosphorus intake (De Boer et al., 1981; Reinhart and Mahan, 1986; Williams et al., 1991c; Erickson et al., 2002; Gentile et al., 2003; Lopez et al., 2004a,b). The phosphorus concentrations of milk from different species range from 0.6 to 1.6 g/L, but do not vary greatly with dietary phosphorus intakes within a given species (Table 23-2). In fish, both plasma and vertebrae phosphorus contents were increased as dietary phosphorus levels rose to approximately 1 percent (Roy and Lall, 2003; Vielma and Lall, 1998).

MAXIMUM TOLERABLE LEVELS

Assuming the presence of adequate levels of dietary calcium, the previous NRC (1980) committee suggested the following maximum tolerable levels of phosphorus (percent of diet): cattle, 1; sheep, 0.6; swine, 1.5; poultry, 1; laying hen, 0.8 percent; horse, 1; and rabbit, 1. Although there are insufficient new data, in particular from studies designed for testing maximum tolerance, to completely revise all these levels, several modifications and additions seem to be necessary. The maximum tolerable level of phosphorus for cattle is suggested to change from 1 to 0.7 percent (DM basis). This is because the 1 percent level stated by the previous committee was not specifically documented or justified and recent studies have shown that cattle performance or health were not affected by feeding dietary phosphorus up to 0.7 percent (DM basis, DeBoer et al., 1981; Knowlton and Herbein, 2002). As limited research has shown a decline in feed efficiency in pigs fed dietary phosphorus in excess of 1 percent (Crenshaw, 1986), it is appropriate and practically relevant to reduce the maximum tolerable level of phosphorus for swine from 1.5 to 1 percent. While the improved phytate-phosphorus use by supplemental phytases in diets for swine and poultry may shift the maximum tolerable levels of phosphorus downward, the release of phytate-bound calcium by phytase (Lei et al., 1993a) can help these animals cope with excess phosphorus. However, the increasing concern over manure phosphorus pollution of environment in the areas of intensive animal agriculture favors the lowering of dietary phosphorus levels. There is a limited amount of fish data to suggest the maximum tolerable level of phosphorus for this species as approximately 1 percent.

Based on the results from the studies presented in Table 23-1, rodents may be able to tolerate 0.6 percent phosphorus in diets. Weiner et al. (2001) have reviewed multiple subchronic (28–100 days) and chronic (21–104 weeks) toxicity studies of inorganic phosphates in laboratory animals, and concluded that kidney is the primary target organ of high phosphorus doses and that nephrocalcinosis and other renal disorders are due to the excessive phosphorus and calcium loads. A single subchronic and chronic NOEL (no-observed-effect level)/NOAEL(no-observed-adverse-effect level) has been suggested as 103 and 225 mg of compound/kg BW per day for all classes of inorganic phosphates, respectively (Weiner et al., 2001). Based on animal data, humans can tolerate up to 1 percent phosphorus in the diet or 70 mg/kg BW per day over a lifetime (Weiner et al., 2001).

FUTURE RESEARCH NEEDS

Recently, microbial phytases have been used in diets for nonruminants to improve their use of phytate-phosphorus

and to reduce their phosphorus excretion to the environment. Because of the release of phytate-phosphorus and the chelated calcium or other metals by these enzymes, the impact on the maximum tolerable phosphorus levels in their diets for swine and poultry should be examined.

SUMMARY

Direct toxic effects of phosphorus in food-producing animals, particularly in simple-stomached species, are more of a scientific issue than a practical concern. This is because (1) the amount of bioavailable phosphorus in plant-based feeds is low; (2) supplemental inorganic phosphorus is expensive; and (3) inorganic phosphates are intrinsically well tolerated and readily excreted via urine. Single large doses of phosphorus or short-time exposure to high levels of phosphorus produce only minor effects in the animals. However, prolonged exposure to high levels of dietary phosphorus does cause metabolic disorders such as urolithiasis in ruminants, nutritional secondary hyperparathyroidism in horses, and nephrocalcinosis in rats. Many of these phosphorus toxic effects are only observed when calcium intake is marginal or low. Most species, including fish, may tolerate 1 percent phosphorus in their diets, whereas the tolerable level is lower for sheep based on their susceptibility to urolithiasis.

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TABLE 23-1 Effects of Phosphorus Exposure in Animals

Animal	N[a]	Age/Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	10	Adult (12 kg)	1.05 g of P/d 2.8–3.1 g/d (1.10 g Ca/d)	Tablets of potassium phosphate	10 mo	Diet	Increased Ca retention Increased deposition of Ca in tissue, increased bone resorption	Laflamme and Jowsey, 1972
Dogs	10	Adult (10 kg)	1.2% (0.12% Ca)	K_2BPO_4 NaH_2PO_4 $CaHPO_4$	294 d	Diet	Loose incisor teeth, and severe bone loss	Krook et al., 1971
Dogs	1	Young adult	0 mg/d/kg 100 mg phosphate/d/kg BW	$Na_5P_3O_{10}$ $(NaPO_3)_3$ $(NaPO_3)_{10\text{-}15}$	30 d	Diet	No adverse effect	Hodge, 1964
Dogs, beagle	15		0 mg/d/kg 800 mg phosphate/d/kg	K_2HPO_4	14 or 38 wk	Diet	Vomited, cachectic, and elevated creatinine and blood urea nitrogen; disseminated tubular atrophy, focal scar tissue, and nephrocalcinosis	Schneider et al., 1980a,b
Mice	50	Adult	0.6% 1.2% (1.2% Ca)	$Ca(H_2PO_4)_2$	16 mo	Diet	Reduced bone breaking strength and ash, Ca, and P contents in femurs	Krishnarao and Draper, 1972
Rabbits, female	10		300–700 mg phosphate/kg	NaH_2PO_4	5–16 mo	Oral, water	Increased parathyroid weight	Fazekas, 1954
Rats	16	Adult (100g)	0.62% 1.20% (1.20% Ca)	$Ca(H_2PO_4)_2$	238 d	Diet	Accelerated bone resorption	Anderson and Draper, 1972
Rats	7	Adult (500 g, 8–14 m)	0.3% 0.6–1.8% (0.6% Ca)	$Ca(H_2PO_4)_2$ $Mg_3(PO_4)_2$ NaH_2PO_4	180 d	Diet	Increased cumulative excretion of ^{45}Ca, but decreased urinary Ca excretion	Draper et al., 1972
Rats	34–36/sex		0.5% phosphate 1.1–5.0% phosphate	Na_2HPO_4 $Na_3P_3O_{10}$ $Na_4P_2O_7$	>6 mo	Diet	Slight growth retardation, increased kidney weight, and renal calcification	Hahn, 1961

Rats	42	4 wk	0.5% 1.5% (0.5% Ca)	$K_5P_3O_{10}$	21d	Diet	 Nephrocalcinosis, increased kidney weight and Ca and P concentrations, increased urine albumin, N-acetyl-bet-d glucosaminidase activity, and beta 2-microglobulin	Matsuzaki et al., 1997
Rats	6	4 wk	0.32% 1.20% (0.53% Ca)	KH_3PO_4 $K_5P_3O_{10}$	21 d	Diet	 Nephrocalcinosis, more severe in those fed K $K_5P_3O_{10}$	Matsuzaki et al., 2001
Guinea pigs	15–22	1 wk (150–170 g)	0.5% 0.9% (0.8% Ca)	$CaHPO_4$ KH_2PO_4	150–600 d	Diet	 Reduced growth and survival time; deposits of Ca phosphate in tissues	Hogan et al., 1950
Guinea pigs	5–49	200 g	0.7% 0.9–1.7%	$CaHPO_4$ KH_2PO_4 NaH_2PO_4	84 d	Diet	 Slow weight gain, stiff joints, Ca phosphate deposits, and high mortality rate	House and Hogan, 1955
Chickens, hens	5	28 wk	0.68% 0.88% 1.08% 1.28–1.68% (3.3% Ca)	Defluorinated phosphate	280 d	Diet	 No adverse effect No adverse effect Reduced egg production	Harms et al., 1965
Chickens, hens	20–25	Laying hens	Monobasic phosphate: 150–600 meq/kg (1.0–2.4% P) Dibasic phosphate: 150–1,200 meq P/kg (0.79-2.40%P) (3.50% Ca)	$Ca(H_2PO_4)_2$ $CaHPO_4$	5–6 wk	Diet	Reduced egg production, feed intake, blood bicarbonates, egg specific gravity, or plasma calcium No effect on performance at 900-1,200 mEq/kg or eggshell quality up to 450 mEq/kg	Keshavarz, 1994
Chickens, broilers	50–100	2 wk	0.53% 0.61% 0.81–1.09% (0.63–1.67% Ca)	$CaHPO_4$	14–21 d	Diet	 Tibial dyschondroplasia (0.7% Ca) Tibial dyschondroplasia	Edwards and Veltmann, 1983
Swine	8	23 kg	0.4–1.2% P (0.4–1.2% Ca)	$CaHPO_4$	15 wk	Diet	Feed efficiency was reduced in pigs fed 1.1 and 1.2% P	Crenshaw, 1986

continued

TABLE 23-1 Continued

Animal	N[a]	Age/Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Horses	4	2 yr (150 kg)	0.2% 1.2% (0.4% Ca)	NaH_2PO_4	56 d	Diet	 Decreased Ca absorption or retention; increased bone Ca turnover, decreased plasma Ca	Schryver et al., 1971
Cows, Holstein	4–5	591 kg	0.34% 0.51% 0.67%	$CaHPO_4$ NaH_2PO_4	10 wk	Diet	 No adverse effect No adverse effect	Knowlton and Herbein, 2002
Sheep, lambs	16	27 kg	0.33% 0.62% 0.81% (0.44, 0.71, and 0.96% Ca) in a 3 x 3 factorial asr angement	Na_2HPO_4	131 d	Diet	 31% urinary calculi (0.4% Ca) 73% urinary calculi (0.4% Ca)	Emerick and Embry, 1963
Sheep, lambs	16	27 kg	0.35% 0.80% (0.51 and 0.90% Ca)	Na_2HPO_4	90 d	Diet	68 and 58% urinary calculi in animals fed 0.51 and 0.90% Ca, respectively	Emerick and Embry, 1964
Sheep, lambs	45	32 kg	0.25% 0.60% (0.31 and 0.58% Ca)	NaH_2PO_4 Na_2HPO_4 $Na_5P_3O_{10}$ $CaHPO_4$	84 d	Diet	 60–75% urinary calculi (no incidence in lambs fed $CaHPO_4$	Bushman et al., 1965
Sheep	5	Grazing	0 mg/d 500 mg/d 1,500 mg/d 3,000 mg/d	H_3PO_4 NaH_2PO_4	70 d	Oral (in water)	 No adverse effect of NaH_2PO_4 100% mortality produced by H_3PO_4 at 3,000 mg P/d	McMeniman, 1973
Fish, juvenile haddock	150	4.3 g	0.42–1.02% 1.22%	$Ca(H_2PO_4)_2$ H_2O	12 wk	Diet	No adverse effect Lower BW, specific growth rate, and feed use efficiency, but higher body lipids and protein contents, and urinary phosphates excretion	Roy and Lall, 2003
Fish, Atlantic salmon		15 g	0.4–1.1%; 1.3%	$Ca(H_2PO_4)_2 \cdot H_2O$	16 wk	Diet	Decreased BW, feed efficiency, and bone and plasma Mg and Zn, and increased mortality	Vielma and Lall, 1998

[a]Number of animals per treatment.
[b]Quantity of phosphorus dosed. SI conversion: 1 mg phosphorus equals 32.3 μmoles phosphorus.

TABLE 23-2 Phosphorus Concentrations in Fluids and Tissues of Animals

Animals	Quantity	Source	Plasma, mg/L	Serum, mg/L	Blood, mg/L	Milk, g/L	Bone	Muscle	Soft Tissues	Reference
Rats, 4-wk males on a 21-d study	0.5% P	$K_5P_3O_{10}$							1.5% (kidney)	Matsuzaki et al., 1997
	1.5% P (0.5% Ca)								7.8% (kidney) (dry basis)	
Rats, adult (100 g)	0.6% P	$Ca(H_2PO_4)_2$					12.8% P			Anderson and Draper, 1972
	1.2% P						12.9% P (dry, fat-free basis);			
Rats, adult (500g, 8–14 mo)	0.3% P	$Ca(H_2PO_4)_2$,	39				11.1%	0.69%		Draper et al., 1972
	0.6% P	$Mg_3(PO_4)_2$,	59				11.9%	0.81%		
	1.2% P	NaH_2PO_4								
	1.8% P		55				11.6%	0.71%		
			53				11.1% (dry fat-free, femurs)	0.71% (dry basis)		
	(0.6% Ca)									
Laying hens	0.55% P (3.50% Ca)	$Ca(H_2PO_4)_2 \cdot H_2O$	64.6							Keshavarz, 1994
Swine, starter (7 kg)	Basal (0.41 % P)		49.3							Gentile et al., 2003
	+ 1,250 units phytase/kg		99.5							
Swine, grower (20 kg)	0.40 P	$CaHPO_4$			61					Reinhart and Mahan, 1986
	0.60% P				79					
Swine, finisher (54 kg)	0.35% P	$CaHPO_4$			66					Reinhart and Mahan, 1986
	0.50% P				82					
Horses, 22 wk at the onset of the 30-wk study (182–369 kg)	low P (0.24–0.35%)	$CaHPO_4$ NaH_2PO_4			58–62					Cymbaluk and Christison, 1989
	normal P (0.68%)				61–62					
	high P (0.95–1.06%)				60–61					
Newborn calf	0.12% P			38.5	49.2	135.1				Williams et al., 1991c
	0.20% P			42.5	51.8	187.5				

continued

TABLE 23-2 Continued

Animals	Quantity	Source	Plasma, mg/L	Serum, mg/L	Blood, mg/L	Milk, g/L	Bone	Muscle	Soft Tissues	Reference
Holstein bull calves (46 kg) on a 16-wk trial	Basal	NaH_2PO_4			81.3					Agboola et al., 1988
	+ 5 g P/calf/d				90.1				1.3 mg P/g	
Weaned Angus heifers on a trial for 525–772 d	Basal (0.12% P)	NaH_2PO_4	36	45	105	0.88	123	0.44%	0.66 (liver)	Williams et al., 1991c
	0.20% P		42	47	99	1.0% (dry basis)	174 mg P/mL (fresh basis)	0.50%	0.66 (liver)	
Pregnant cows	35 g P (130 g Ca)	NaH_2PO_4 $NH_4H_2PO_4$	40							Reinhardt and Conrad, 1980
	70 g P (45 g Ca)		55							
Multiparous or uniparous cows	0.34%P	$NH_4H_2PO_4$		62.4						DeBoer et al., 1981
	0.51%P			68.6						
	0.69% P			71.8						
Multiparous Holstein cows	0.31% P	NaH_2PO_4		57			9.5%			Wu et al., 2001
	0.39% P			61			9.7%			
	0.47% P			65			9.9% (12th rib, dry basis)			
Holstein cows	0.34% P	NaH_2PO_4		39		0.89				Knowlton and Herbein, 2002
	0.51% P	$CaHPO_4$		45		0.90				
	0.67% P			57		0.89 (calculated average)				
Feedlot steers	0.24% P	$CaHPO_4$	50–56					0.20% (wet basis) or 0.68% (DM basis)		Montgomery et al., 2004
Goats, yearling Alpine does	Basal	$Ca(H_2PO_4)_2 \cdot H_2O$	57							Fredeen et al., 1988
	+ 0.5% P		86							
Lambs (25 kg) on a 105-d trial	0.3% P (0.4% Ca)	$CaHPO_4$					11.1%			Hutcheson et al., 1992
	0.6% P (0.8% Ca)						11.4% P (ash basis)			

Haddock, juvenile (4.3 g) for 12 wk	0.42%	$Ca(H_2PO_4)_2 \cdot H_2O$	12	6.8%	Roy and Lall, 2003
	0.62%		25	7.5%	
	0.82%		37	8.0%	
	1.02%		47	8.7%	
	1.22%		47	9.3% (DM basis, vertebra)	
Atlantic salmon, Parr (15 g) for 16 wk	0.40%	$Ca(H_2PO_4)_2 \cdot H_2O$	348	5.7%	Vielma and Lall, 1998
	0.51%		48	6.8%	
	0.61%		61	7.8%	
	0.73%		64	8.7%	
	0.83%		78	9.1%	
	0.93%		93	9.4%	
	1.13%		95	9.6%	
	1.33%		91	9.5% (DM basis, vertebrae)	

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Potassium

INTRODUCTION

Potassium (K) was first discovered in 1807 by Sir Humphrey Davy, who isolated it from potash (Peterson, 1997). Potassium ranks seventh in the order of abundance of elements in the Earth's crust with a concentration of 26,000 mg/kg by weight (McDowell, 2003). It is the third most abundant element found in the body of most animals. Potassium is a silvery, white metal in its pure state. Its atomic number is 19, and it is a member of the alkali group of metals, which also include lithium, sodium, rubidium, cesium, and francium. Potassium exists in three natural occurring isotopes with the mass numbers 39, 40, and 41 with the relative abundances of 93.1, 0.012, and 6.9 percent, respectively (Aikawa, 1983). The atomic weight of potassium is 39.098 and its specific gravity is 0.86. It melts at 63ºC and boils at around 760ºC.

Potassium is a very strong reducing metal and therefore is not found in its pure form in nature, but readily combines with other elements to form salts (McDowell, 2003). Potassium has a valence of +1 and joins to other elements or groups of elements through ionic bonds. The potassium compounds that have been used as supplements in diets to test the effects of potassium include potassium chloride (KCl), potassium bicarbonate ($KHCO_3$), potassium carbonate (K_2CO_3), potassium acetate (CH_3CO_2K), potassium citrate monohydrate ($K_3C_6H_5O_7{\cdot}H_2O$), potassium sulfate (K_2SO_4), tripotassium phosphate (K_3PO_4), potassium iodide (KI), and potassium gluconate ($C_6H_{11}O_7K$).

ESSENTIALITY

Potassium was determined to be an essential element in the diet of several animals in the early 19th century (McDowell, 2003). Nonruminant animals generally have a lower requirement for potassium than ruminants. The potassium requirement of various species has been published in several National Research Council (NRC) publications. The dietary potassium requirement of chickens ranges from 1,500 mg/kg for leghorn layers to 3,000 mg/kg for broilers from 0–8 weeks of age (NRC, 1994). The dietary potassium requirement of turkeys ranges from 7,000 mg/kg at 0–4 weeks to 4,000 mg/kg at 20–24 weeks (NRC, 1994b). The potassium requirement of swine is between 1,500 mg/kg to 3,000 mg/kg with younger animals requiring a higher dietary concentration than older animals (NRC, 1998). The dietary potassium requirement of sheep is 5,000–8,000 mg/kg of dry matter (NRC, 1985). Beef feedlot cattle have an estimated potassium requirement of 6,000 mg/kg of diet dry matter while the requirement for gestating beef cows is between 5,000 mg/kg and 7,000 mg/kg (NRC, 2000). Lactating dairy cows have the highest dietary potassium requirement at about 10,000 mg/kg of diet dry matter (NRC, 2001). Potassium is the mineral found in the highest concentration in milk (1,500 mg/L) and, therefore, lactating cattle require a higher potassium intake than nonlactating cattle (NRC, 2001). Weil et al. (1988) calculated the dietary potassium requirement for the growing dairy calf to be between 3,400 and 5,800 mg/kg of diet dry matter.

Research reviewed by McDowell (2003) showed that stress of animals tended to increase the requirement for potassium. Research in Florida found dairy cows subjected to heat stress improved feed intake and milk production as the amount of potassium in the diet was increased from 6,600 mg/kg to 10,800 mg/kg of diet dry matter (NRC, 2001). The NRC (2001) recommended a dietary concentration of 15,000 mg/kg dry matter for lactating dairy cows under thermal stress. Smith and Teeter (1987) found broilers subjected to 35ºC temperatures required dietary potassium of 15,000 to 20,000 mg/kg. Hutcheson et al. (1984) calculated that the amount of potassium required for transported calves was 247 mg/kg of body weight for the first two weeks after transport, approximately 20 percent more than what was recommended for calves that had not been transported. Juvenile tilapia require 2,000 to 3,000 mg/kg potassium in their diet (Shiau and Hsieh, 2001). Hills et al. (1982) found the dietary potas-

sium requirement for kittens increased from 3,000 to 5,000 mg/kg when protein content of the diet increased from 33 to 68 percent.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

The current methods used for analysis of potassium within body fluids include flame photometry, ion-selective electrodes, and atomic absorption spectrophotometry (Peterson, 1997). Electron probe microanalysis is used to determine potassium concentration within individual cells. Neutron activation analysis is used to measure the concentration of potassium within platelets (Glick, 1983). Erroneous results in the concentration of potassium within specimens normally result from improper preparation of the specimen for analysis rather than intrinsic effects within the analysis. A variety of blood-handling processes can result in invalid potassium results. Potassium concentration fluctuates diurnally; therefore, choice of sampling time should be considered. Cold temperature, mixing extent, and vigor can cause movement of potassium from within cells into the extracellular fluids, increasing potassium results. Other factors that can affect potassium results include choice of serum or plasma, length of storage time, skin-puncture and blood sampling technique, and metabolic state (Glick, 1983). Very high blood ammonia levels can sometimes interfere with potassium analysis if methods besides flame photometry are used (Glick, 1983). Total body potassium content can be estimated by using the natural isotopes by whole body counting or examining the distribution of administered radioactive isotope (for example, ^{45}K) (Peterson, 1997). However, total body potassium measurements are usually of little value, and it is the extracellular potassium concentration that is important in determining the potassium status of animals. Measurement of potassium status through blood, serum, or plasma analysis in animals is not a totally reliable indicator because of the discussion above and its regulation in the body.

REGULATION AND METABOLISM

Absorption and Metabolism

Most potassium is absorbed from the upper portion of the small intestine of nonruminant and ruminant animals through simple diffusion (McDowell, 2003). Potassium absorption also takes place in the rumen and omasum in ruminants. A small amount of potassium is absorbed in the lower part of the small intestine and in the large intestine in both nonruminants and ruminants. The true digestibility of potassium is 95 percent or greater in most feedstuffs (McDowell, 1992). A significant amount of the potassium absorbed in ruminants is from the saliva excretions, which are high in potassium, and in some cases potassium is used in saliva to replace sodium (McDowell, 1992). Disturbances of the intestinal tract, such as diarrhea, can reduce the absorption of potassium.

The role of potassium within the body includes acid-base regulation, osmotic pressure maintenance, nerve impulse transmission, muscle contraction, and carbon dioxide and oxygen transport (NRC, 2001). It is also involved in phosphorylation of creatine, pyruvate kinase activity, cellular uptake of amino acids and synthesis of protein, carbohydrate metabolism, and maintenance of normal cardiac and renal tissue, and as an activator or cofactor in many enzymatic reactions. Ninety-eight percent of the potassium within the body is located within the cells, with 2 percent of it located in the extracellular fluid. Potassium accounts for 75 percent of the total cations within body cells and sodium accounts for approximately 90 percent of the total cations in the extracellular fluid (McDowell, 1992).

The main excretory route for potassium is urine. The hormone aldosterone is indirectly responsible for the regulation of potassium through its effect on sodium reabsorption in the kidney. Fecal loss of potassium in dairy cattle is estimated at 2,200 mg of K/kg of dietary dry matter intake (NRC, 2001). Under heat stress conditions, ruminants and horses can lose significant amounts of potassium through sweat.

Metabolic Interactions

Potassium interacts with sodium and chlorine within the body to maintain acid-base balance and electrical and chemical concentration gradients (Kem and Trachewsky, 1983). The concentration of potassium within the cell is normally in the range of 100–160 mM (3,900–6,200 mg/L) while the concentration outside the cell is 3.5–5 mM (137–195 mg/L). In contrast, the sodium concentration inside the cell is 3–30 mM (69 to 690 mg/L) while concentration outside the cell is 130–145 mM (3,000 to 3,300 mg/L). The contrasting concentrations of potassium in cells and sodium outside cells create a chemical concentration gradient in which potassium would rapidly diffuse into the extracellular fluid while sodium would rapidly diffuse into the intracellular fluid if allowed to cross the cell membrane. These chemical gradients are maintained by energy generated within the cells (Kem and Trachewsky, 1983) through the use of a number of cellular pumps, including the primary transporters Na-K-ATPase and H-K-ATPase and the secondary transporters electroneutral 1Na:2Cl:1K cotransporter and potassium conductivity channels (Peterson, 1997).

The distribution of potassium within the body is regulated by a number of compounds including insulin, catecholamines, and aldosterone (Peterson, 1997). Insulin is released into the blood stream if potassium rises by as little as a few tenths of an mMol. Insulin then stimulates the uptake of potassium in the liver and skeletal muscles by increasing Na-K-ATPase pump activity. There is a rise in extracellular concentration of potassium with exercise. Exercise causes the release of catecholamines from the adre-

nal gland, which then trigger the rise of potassium in plasma through stimulation of the Na-K-ATPase pump that drives potassium back into cells. Cellular uptake of potassium is also accelerated by hyperpolarization. Aldosterone is necessary for normal rates of potassium excretion. Aldosterone influences potassium excretion by activating sodium channels that allow the entrance of sodium from the lumen of the nephron and the excretion of potassium (Peterson, 1997). The excretion of potassium always rises as the presence of the hormone aldosterone increases. The concentration of aldosterone increases when animals are under stress, therefore possibly explaining the increase in potassium requirements of animals raised and housed within stressful conditions.

SOURCES AND BIOAVAILABILITY

Environmental Exposure

The potassium level in forages ranges from slightly less than 10,000 mg/kg to more than 50,000 mg/kg of plant dry matter. Potassium is normally higher in forages than grains and plant protein feeds. The potassium level in forages can be quite variable and dependent on the species and variety, maturity, fertilization with potassium and nitrogen sources, crop management procedures, and environmental and soil conditions. Cool season grasses contain higher concentrations of potassium than warm season grasses; temperate legumes contain higher levels of potassium than tropical legumes (Underwood and Suttle, 1999). Mature pastures, winter pastures containing weathered forage, and hay that has been rained on can have levels of potassium below that required by ruminants (NRC, 2001; McDowell, 2003). The level of potassium in grains, plant protein feeds, and by-products feeds is generally less than the requirements of lactating dairy cows (NRC, 2001); however, it is generally adequate for meeting the potassium requirement of nonruminant animals that require less potassium in the diet than ruminants. The potassium concentration in grains ranges from 3,000 to 8,000 mg/kg, in vegetable proteins from 10,000 to 25,000 mg/kg, and in animal products from 3,000 to 20,000 mg/kg (McDowell, 2003). Bioavailability of potassium from most forages and grains is 85 percent or higher (Miller, 1995; NRC, 2001).

Supplementation Considerations

Potassium is readily provided in most feedstuffs and therefore its deficiency has not been recognized as a possible nutrition problem until recently. The amount of potassium in most diets for lactating dairy cows is normally greater than 15,000 mg/kg; therefore, no additional supplementation of potassium is needed (NRC, 2001). Supplementation of potassium can become necessary to meet nutritional requirements of lactating dairy cows and other ruminants as the percentage of energy concentrates and by-products increases in diets, especially as the recent trend in the industry is toward the increased feeding of concentrates and by-product feeds such as brewer's grains, distiller's dried grains, corn gluten meal, and cottonseed hulls (McDowell, 2003). Nonruminants generally do not require potassium supplementation, especially in high protein diets, as protein sources generally contain the highest concentration of potassium for feedstuffs classified as concentrates. The inorganic sources of supplemental potassium that are readily available for absorption in the gastrointestinal tract include KCl, $KHCO_3$, K_2CO_3, K_2SO_4, $C_2H_3KO_2$, and $K_3C_6H_5O_7 \cdot H_2O$ (NRC, 2001).

TOXICOSIS

Potassium toxicosis in healthy animals is rare. This is due to the body's ability to readily excrete potassium as well as regulate absorption. The major causes of hyperkalemia (a rise in the level of potassium to greater than 195 mg/L in the extracellular fluids) are excessive potassium intake (rarely the primary cause of hyperkalemia), reduced renal losses, and redistribution of potassium (Peterson, 1997). Renal losses of potassium can be reduced by acute renal failure, mineral corticoid deficiency, or potassium sparing diuretics. Hemolysis, necrosis, muscle injury, catecholamine antagonists, insulin deficiency, and abnormal skeletal muscle channels can lead to the redistribution of potassium causing hyperkalemia (Peterson, 1997). Hyperkalemia and hypokalemia are both associated with potentially fatal arrhythmias (Peterson, 1997). The responses of animals administered different levels of potassium are summarized in Table 24-1.

Low Levels

In a study involving 4-week-old Holstein and Jersey calves gaining 0.73 kg/day fed diets ranging in potassium from 5,500 to 13,200 mg/kg, Weil et al. (1988) found no toxic effects across the dietary range in potassium. In a second study, Weil et al. (1988) fed 6-week-old Holstein calves diets containing 3,400 or 5,800 mg/kg of the dry matter as potassium. Calves fed the 5,800 mg/kg potassium diet gained faster (0.74 kg/day versus 0.60 kg/day) and had a higher feed intake than calves fed the lower potassium diet.

Hutcheson et al. (1984) conducted several studies on the effects of potassium, added as KCl, in diets on calves for two weeks following shipping to feedlots. Concentrations of potassium in the diets tested ranged from 9,000 to 31,000 mg/kg. They concluded calf performance improved and blood packed cell volume increased as the potassium in the diet increased from 7,000 to 22,000 mg/kg. Mortality rate of the calves also decreased when potassium was included in the ration at the levels of 13,000 and 22,000 mg/kg. The level of sodium in the serum increased as the level of potassium in the diet increased (Hutcheson et al., 1984).

A set of experiments conducted by Teeter and Smith (1986) found no adverse effects when potassium, as KCl, was supplied in water with a corn-soybean base diet containing 7,300 mg/kg when fed to week-old chicken pullets for two weeks under near optimal environmental conditions. Potassium supplied in the water at levels from 500 to 1,500 mg/L had no effect on body weight gain or feed efficiency. Under heat stress conditions (35ºC, 70 percent relative humidity), offering the same water and dietary concentrations of potassium resulted in improved average daily gain in broilers receiving water containing 1,000 and 1,500 mg/L potassium, but blood pH and feed efficiency were not improved (Teeter and Smith, 1986). Another experiment found that weight gain was improved by providing 2,400 to 3,600 mg/kg potassium in water (Smith and Teeter, 1987). Supplementation of drinking water with KCl to heat-stressed broilers at levels up to 1,500 mg/kg KCl improved average daily gain to 27.4 g/day while supplementation with K_2CO_3 reduced average daily gain to 18.6 g/day, which was significantly below the average of 23.1 g/day without any potassium supplementation (Teeter and Smith, 1986). Water consumption was also reduced with the addition of K_2CO_3 under environmentally stressful conditions (Teeter and Smith, 1986).

A set of experiments conducted by Smith et al. (2000) tested the excreta moisture response in laying hens to the excess of several dietary minerals including potassium. The increase in the dietary concentration of potassium from 2,300 to 20,000 mg/kg caused a linear increase in water intake, water to feed ratio, and excreta moisture (11.95 ± 2.02 g/kg for each 1 g/kg increase in diet potassium content). The feed intake decreased linearly as the potassium concentration in the diet increased.

Two carcinogenesis studies in rats (Lina et al., 1994; Lina and Kuijpers, 2004) found base-forming potassium compounds such as $KHCO_3$ are strong promoters of urinary bladder carcinogenesis, while neutral potassium salts such as KCl are weak promoters. Lina et al. (1994) conducted an experiment in which rats were fed a control diet, or the control diet plus 7,800 or 15,600 mg/kg potassium as $KHCO_3$, or the control diet plus 15,600 mg/kg potassium as KCl. These diets were fed from 4 to 130 weeks to male and female rats. Both diets containing KCl and $KHCO_3$ increased urinary volume and potassium levels, while only $KHCO_3$ caused an increase in urinary pH. Potassium bicarbonate supplementation resulted in hyperplasia, papollomas, and transitional cell carcinomas of the urinary bladder compared to only a few neoplastic lesions in KCl fed rats. Potassium bicarbonate induces metabolic alkalosis as shown by an increase in the base excess in blood, urinary pH, and urinary net base excretion, whereas KCl had no effect on the metabolic acid-base balance (Lina and Kuijpers, 2004). High levels of both KCl and $KHCO_3$ increased levels of potassium in the serum and caused a decrease in growth rate. The chronic stimulation of the adrenal cortex by K+ in the diets containing KCl and $KHCO_3$ caused hypertrophy of the adrenal zona glomerulosa. From week 13 of supplementation onward, $KHCO_3$ caused the onset of oncocytic tubules while there was only a slight effect in those rats supplemented with KCl at 18 months. Lina and Kuijpers (2004) concluded that in the case of potassium supplementation, the different responses observed between KCl and $KHCO_3$ supplementation represented the physiological adaptation of the metabolic processes to base-forming salts.

High Levels

Studies using differing types and amounts of potassium have found that the palatability of various diets generally decreased as the percentage of potassium in the diet increased. Neathery et al. (1980) tested the palatability of four different sources of potassium with Holstein bull calves in 16 cafeteria-style feeding experiments. The feedstuffs contained potassium from different sources (KCl, K_2CO_3, $KHCO_3$, and $C_2H_3KO_2$) in the amounts of 20,000, 40,000, or 60,000 mg/kg. The most palatable potassium sources were $KHCO_3$ and $C_2H_3KO_2$, followed by KCl and with K_2CO_3 being the least palatable. When calves were fed only one source of potassium (KCl) at 20,000 mg/kg of the diet, there was no adverse effect on voluntary feed intake or growth. However, at 60,000 mg/kg of the diet, a decrease in voluntary feed intake and weight gain was observed.

A dietary concentration of 29,000 mg/kg potassium as supplied by $K_3C_6H_5O_7 \cdot H_2O$ caused reduced feed intake and weight gain when fed to rats for 17 days (Everts et al., 1996). Both a low level of potassium (1,000 mg/kg) and high level (29,000 mg/kg) of potassium in the diet from $K_3C_6H_5O_7 \cdot H_2O$ caused increased water intake and urinary excretion.

In a study with meadow voles, Mickelson and Christian (1991) found that as potassium concentration in the diet increased above 5,000 mg/kg, meadow voles selectively chose against consumption of a higher potassium diet. Potassium from a variety of sources was included in high potassium diets (390,000 mg/kg KCl, 360,000 mg/kg K_2SO_4, 80,000 mg/kg of K_2CO_3, $K_3C_5H_5O_7$, K_3PO_4, and 10,000 mg/kg KI) for feeding to voles. As concentration of potassium in the diet approached 35,000 mg/kg, voles chose to eat with increasing selectivity. There was no significant health or metabolic effects related to consumption of the high potassium diet except for a significant increase in water consumption.

Goff and Horst (1997) demonstrated that the feeding of potassium or sodium above dietary requirements in diets fed to dairy cows before parturition increased the incidence of milk fever in them. Jersey dairy cows entering their fourth or greater lactation were fed diets supplemented with $KHCO_3$ to achieve dietary potassium concentrations of 11,000, 21,000, or 31,000mg/kg. Incidence of milk fever increased as potassium in the diet increased; 2 of 20 cows, 10 of 20 cows, and 11 of 23 cows exhibited milk fever with diets containing 11,000, 21,000, or 31,000 mg/kg potassium, respectively. Lina et al. (2004) found that $KHCO_3$ is a strong

base-forming salt and that it has the effect of increasing urine and blood pH. This effect, plus a reduction in plasma hydroxyproline levels, was also found by Goff and Horst (1997) for diets containing $KHCO_3$ and $NaHCO_3$. Two different levels of calcium were also tested. It was concluded that dietary calcium concentration was not a major risk factor in the cause of milk fever, but the concentration of cations, especially potassium, could induce alkalosis in prepartum milk cows that reduces the dairy cow's ability to maintain calcium homeostasis (Goff and Horst, 1997). High potassium appears to induce milk fever or hypocalcaemia by reducing the sensitivity of bone and renal tissue to parathyroid hormone. In the kidney, the reduced sensitivity results in a decreased conversion of vitamin D_3 to 1,25-dihydroxyvitamin D_3, which is responsible for increasing absorption of calcium from the gut and increased mobilization of calcium from bone.

In ruminants, high potassium diets generally inhibit the absorption of magnesium from the gastrointestinal tract, particularly the rumen (Goff and Horst, 1997). Fisher et al. (1994) added K_2CO_3 to a base diet containing 16,000 mg/kg potassium to raise potassium levels to 31,000 and 46,000 mg/kg for feeding to dairy cows. The diet containing 46,000 mg/kg potassium caused a reduction in milk yield. As potassium level in the diet increased, plasma potassium levels increased and plasma magnesium levels decreased, indicating high levels of potassium interfere with the absorption of magnesium. The high dietary concentration of potassium also interfered with the utilization of calcium by reducing levels in urine and milk. Water intake and urine output increased as the level of potassium in the diet increased (Fisher et al., 1994). Ram et al. (1998) postulated that magnesium absorption from the rumen is regulated by transport mechanisms both sensitive and insensitive to potassium. They concluded from feeding diets to wethers of 10,000 and 36,000 mg/kg potassium containing $KHCO_3$ and 1,300, 2,500, and 3,700 mg/kg magnesium from magnesium oxide that 36,000 potassium in the diet increased the concentration of potassium in the rumen and reduced magnesium absorption by 0.32 g/day. Increasing magnesium in the diet at either high or low dietary potassium concentrations could offset the reduction in magnesium absorption (Ram et al., 1998). Leonhard-Marek and Martens (1996) used sheep rumen epithelium in vitro to show a decrease in mucosal-to-serosal magnesium flux (indicating decreased magnesium absorption) when luminal potassium concentrations were increased up to 80 mM. The effect of mucosal potassium on magnesium absorption was concentration-dependent with the depressing effect of potassium concentration showing a logarithmic behavior. From 5 to 80 mM mucosal potassium concentration, magnesium absorption decreased by a factor of almost 3; however, above 80 mM potassium concentration, no further decrease in magnesium absorption was observed. This research indicates the development of hypomagnesemia in ruminants feeding on lush, high-potassium pasture forages results from the potassium depolarizing rumen membranes and reducing the magnesium uptake into rumen epithelial cells. Jittakhot et al. (2004) fed diets containing 20,700, 48,000 or 75,500 mg of potassium per kg DM with either 40,600 or 69,100 mg of magnesium/day to 6 ruminally fistulated dry cows. Dietary potassium concentrations were achieved by feeding $KHCO_3$ and MgO was used as the magnesium source. At the highest magnesium supplementation level, magnesium absorption was significantly decreased as potassium levels increased (12,800, 8,900, and 4,800 mg/day). At the low magnesium supplementation, magnesium absorption was similar at 20,700 and 48,000 potassium levels (5,300 mg/day); however, it decreased to 800 mg/day at the 75,500 potassium level. It was concluded there was a negative linear correlation between rumen potassium level and absorption of magnesium as measured by urinary excretion.

Schonewille et al. (1999a) fed three grass diets containing either 26,000 mg/kg potassium, 43,000 mg/kg potassium, or 26,000 mg/kg plus $KHCO_3$ to achieve a similar potassium concentration to the 43,000 mg/kg diet to dry, nonpregnant cows. Both of the 43,000 mg/kg diets equally increased the concentration of potassium in the rumen before and after feeding and resulted in an apparent magnesium absorption from the rumen of about 2 percent compared to 10.8 percent for the 26,000 mg/kg potassium diet. Schonewille et al. (1999b) conducted a study testing the effects of $KHCO_3$, KCl, and K-citrate, supplemented to provide 41,000 mg/kg potassium in the diet, on magnesium absorption in wethers. Both $KHCO_3$ and K-citrate significantly reduced apparent magnesium absorption by 9.5 and 6.5 percent, respectively, while KCl tended to reduce apparent magnesium absorption by 5.5 percent. The consumption of $KHCO_3$ and K-citrate produced a significant transruminal potential difference while KCl did not. The authors concluded that the type of anion in potassium salts has an effect on magnesium absorption in addition to the effect of potassium concentration.

Neathery et al. (1979) drenched 6-month-old Holstein calves with 290 to 2,800 mg of potassium (using KCl) per kilogram of body weight. They found that the sodium content of the plasma generally increased about 1 hour after dosing. Respiration rates increased following dosing while carbon dioxide pressure, pH, and bicarbonate in the blood decreased. Clinical toxicosis signs, including excitability, muscle tremors of the legs, and excessive salivation, were observed with potassium doses greater than 580 mg K/kg BW. Some of the calves in the groups that received 1,730, 2,310, and 2,880 mg K/kg BW died. This study concluded that ruminants can safely consume large amounts of potassium in feeds if consumption is spread over time; however, when massive doses of potassium are given at one time, a breakdown in the homeostasis of potassium occurs resulting in death. Similar effects can be observed in horses with hyperkalemic periodic paralysis disease, a hereditary genetic defect, when high dietary potassium levels are fed, such as with the feeding of alfalfa hay (Reynolds et al., 1998).

Factors Influencing Toxicity

Potassium in and of itself is a relatively nontoxic element that is required in relatively large amounts to sustain life. However, disturbances in potassium metabolism can result in it being toxic. Most commonly, the physiological disorders that can lead to situations that have the potential to cause potassium toxicosis include impaired renal potassium excretion, acidosis, hypoaldosteronism, insulin deficiency, cellular injury, drugs, and genetics (Kaufman and Papper, 1983). In certain quarter horse families, the genetically inherited disease hyperkalemia periodic paralysis results in high levels of potassium in the blood, causing muscles to contract more readily than normal (Meyer et al., 1999). Feeding diets with less than 10,000 mg/kg potassium helps minimize the disease (Reynolds et al., 1998).

The form of potassium in diets can influence its toxicity. Inclusion of K-citrate and $KHCO_3$ in diets to raise potassium levels to 40,000 mg/kg decreased magnesium absorption, while the inclusion of KCl to achieve 40,000 mg/kg potassium in the diet only increased the potassium concentration in the rumen. These effects may be due to the basic salt properties of K-citrate and $KHCO_3$ compared to KCl, which is a neutral salt (Schonewille et al., 1999b). Raising magnesium levels in the diet can offset the effects of potassium on magnesium absorption. Teeter and Smith (1986) found that KCl exacerbated the toxic effects of NH_4Cl supplementation in chickens. Research reviewed in the NRC (1980) showed that animals can adapt to higher levels of potassium if the amounts supplied are increased incrementally. Sodium salts under some conditions can reduce the effects of high dietary potassium (NRC, 1980).

TISSUE LEVELS

Potassium is the third most abundant mineral in the body after calcium and phosphorus, representing approximately 0.3 percent of the body's dry matter (McDowell, 2003). Two-thirds of it is located in the skin and muscle. Studies summarized by the NRC (1980) showed that rats fed 50,000 mg/kg potassium diets had elevated amounts of potassium in the skeletal and heart muscles, kidney, and thymus; however, tissue levels of potassium were not significantly different than when 5,000 mg/kg potassium diets were fed (Meyer et al., 1950). A study by Drescher et al. (1958) found that there were no differences in the carcass or heart muscles for rats fed levels of potassium ranging from 0.60 to 15 mg K/kg BW. Thus, tissue levels of potassium are not very sensitive or indicative of dietary potassium levels.

MAXIMUM TOLERABLE LEVELS

The NRC (1980) set the maximum tolerable amount of potassium at 30,000 mg/kg for all ruminant and nonruminant species. A similar single maximum tolerable dietary level of potassium for all species under all conditions and fed all forms of potassium could not be established based on the research reviewed. In rats and other nonruminant animals, dietary concentrations above 10,000 mg/kg potassium can lead to some reduction in weight gain, feed intake, and increased urinary excretion. However, actual toxicity of potassium is dependent on the health and/or metabolic state of the animal and the potassium compound fed. Research reviewed by the NRC (1998) suggests that pigs can tolerate up to 10 times their requirement of potassium. Potassium salts like $KHCO_3$ that affect acid-base balance within the body are less tolerated than neutral potassium salts like KCl (Schonewille et al., 1999b). Dairy cows have a wide tolerance and requirement for potassium in diets. Cows close to parturition are at an increased risk for milk fever (hypocalcaemia) as dietary concentrations of potassium increase and cation balance of the diet becomes more positive (Goff and Horst, 1997). In contrast, lactating dairy cows, and particularly those under heat stress, have requirements for potassium above 15,000 mg/kg and can tolerate 30,000 mg/kg potassium diets. However, magnesium supplementation needs to increase in the diets of ruminants as dietary potassium levels increase above 15,000 mg/kg to decrease the probability of developing grass tetany (Schonewille et al., 1999a,b). Research suggests that ruminants can tolerate higher than 30,000 mg/kg potassium levels in their diet. In the spring, many grazing ruminants consume forages containing more than 40,000 to 50,000 mg/kg potassium. Conservatively safe maximum tolerable levels for nonruminants would be 10,000 mg/kg and for ruminants 20,000 mg/kg.

FUTURE RESEARCH NEEDS

The risk of potassium toxicity is low from current feedstuffs and water sources; therefore, tolerance levels are not well established and will need additional research if more accurate defined tolerance levels in healthy animals are desired. Future research is needed on the interaction of potassium fed in large excess of an animal's requirement on the metabolic and physiological functions of other minerals. Excess potassium feeding to dairy cows at parturition has been shown to result in hypocalemia but effects on other minerals (such as sodium and magnesium) and the complications resulting need additional research.

SUMMARY

Potassium is an essential nutrient that is found mainly in the cells of the animal body and is an important element in metabolic functions. The dietary needs for each species of animal vary, with ruminants generally requiring a higher level of potassium provided in the diet than nonruminants. Potassium is a fairly common element in most feedstuffs; therefore, while it is a nutritionally important element, its

essentiality has not been researched until relatively recently. Most diets provide adequate levels of this element and do not contain toxic levels of potassium. Potassium can be toxic if it is given in large enough doses, or if the body's potassium homeostatic system is compromised. Factors that influence its toxicity include the metabolic state of the animal, compound consumed, and interactions with other elements. The toxic effects of potassium include cardiac arrest, grass tetany through interference in magnesium absorption, and milk fever, although other metabolic pathways can cause both milk fever and grass tetany. The levels of potassium within the tissues are only slightly affected by the concentration of potassium within the diet.

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TABLE 24-1 Effects of Potassium Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Mice, meadow voles	14	≥ 20 g	5,000–35,000 mg/kg diet	Mineral mix consisting of 39% KCl, 36% K_2SO_4, 8% K_2CO_3, 8% K_3PO_4, 8% $K_3C_5H_5O_7$, 1% KI	7 d	Diet	Diet lower in % K was chosen between choice of 2 diets	Mickelson and Christian, 1991
Mice, meadow voles	10	≥ 20 g	5,000 mg/kg diet 15,000 mg/kg diet 25,000 mg/kg diet 35,000 mg/kg diet	Mineral mix consisting of 39% KCl, 36% K_2SO_4, 8% K_2CO_3, 8% K_3PO_4, 8% $K_3C_5H_5O_7$, 1% KI	27 d	Diet	No effects No effects No effects Increased water consumption	Mickelson and Christian, 1991
Rats, male	5	250 g	1,000 mg/kg diet 3,600 mg/kg diet 29,000 mg/kg diet	$K_3C_6H_5O_7 \cdot H_2O$	17 d	Diet	Increased total water intake and urine excretion No effects Reduced weight gain and feed intake; increased total water intake and urine excretion	Everts et al., 1996
Rats	10	5 wk	Control K% + 7,800 mg/kg diet Control K% + 15,600 mg/kg diet	$KHCO_3$ $KHCO_3$	4 wk	Diet	Linear increase in blood and urine pH Increased water intake; linear increase in blood and urine pH	Lina and Kuijpers, 2004
Rats	10	5 wk	Control K% + 7,800 mg/kg diet Control K% + 15,600 mg/kg diet Control K% + 15,600 mg/kg diet	$KHCO_3$ $KHCO_3$ KCl	13 wk	Diet	Linear increase in blood and urine pH Increased water intake; linear increase in blood and urine pH; increase in presence of oncocytic tubules Increased water intake	Lina and Kuijpers, 2004

Rats	15	5 wk	Control K% + 7,800 mg/kg diet	$KHCO_3$	18 mo	Diet	Linear increase in blood and urine pH; increase in simple urothelial hyperplasia	Lina and Kuijpers, 2004
			Control K% + 15,600 mg/kg diet	$KHCO_3$			Increased water intake; linear increase in blood and urine pH; increase in zona glomerulosa hypotrophy incidents, presence of oncocytic tubules and in simple urothelial hyperplasia	
			Control K% + 15,700 mg/kg diet	KCl			Increased water intake	
Rats	50	5 wk	Control K% + 7,800 mg/kg diet	$KHCO_3$	30 mo	Diet	Decreased body weight; linear increase in blood and urine pH	Lina and Kuijpers, 2004
			Control K% + 15,600 mg/kg diet	$KHCO_3$			Decreased body weight; increased water intake; linear increase in blood and urine pH; increase in zona glomerulosa hypotrophy incidents, the presence of oncocytic tubules and in simple urothelial hyperplasia	
			Control K% + 15,700 mg/kg diet	KCl			Decreased body weight; increased water intake; increase in zona glomerulosa hypotrophy incidents and in simple urothelial hyperplasia	
Rats	10 male, 10 female	5 wk	Control K% + 7,810 mg/kg diet	$KHCO_3$	4 wk	Diet	Increased urine pH; dose-related increase in urinary K excretion	Lina et al., 1994
			Control K% + 15,600 mg/kg diet	$KHCO_3$			Decreased body weight; increased urinary pH and excretion; dose-related increase in urinary K excretion	

continued

TABLE 24-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Rats	10 male, 10 female	5 wk	Control K% + 7,810 mg/kg diet	$KHCO_3$	13 wk	Diet	Increased urine pH; dose-related increase in urinary K excretion	Lina et al., 1994
			Control K% + 15,620 mg/kg diet	$KHCO_3$			Decreased body weight; increased urine pH; increased urine excretion; dose-related increase in urinary K excretion	
			Control K% + 15,700 mg/kg diet	KCl			Decreased body weight; increased urine excretion; dose-related increase in urinary K excretion	
Rats	15 male, 15 female	5 wk	Control K% + 7,810 mg/kg diet	$KHCO_3$	78 wk	Diet	Increased urine pH; dose-related increase in urinary K excretion; increase in simple and papillary epithelial hyperplasia in males	Lina et al., 1994
			Control K% + 15,620 mg/kg diet	$KHCO_3$			Decreased body weight; increased urinary pH and excretion; dose-related increase in urinary K excretion; increase in simple epithelial hyperplasia incidents and increase in papillary hyperplasia in females	
			Control K% + 15,700 mg/kg diet	KCl			Decreased body weight; increased urine excretion; increased urinary K excretion	

Rats	50 male, 50 female	5 wk	Control K% + 7,810 mg/kg diet	$KHCO_3$	130 wk	Diet	Increased urine pH; dose-related increase in urinary K excretion; increase in simple epithelial hyperplasia incidents	Lina et al., 1994
			Control K% + 15,620 mg/kg diet	$KHCO_3$			Decreased body weight; increased urinary pH and excretion; dose-related increase in urinary K excretion; increase in simple epithelial hyperplasia incidents; increase in papillary, nodular epithelial hyperplasia and transitional cell papilloma incidents in females	
			Control K% + 15,700 mg/kg diet	KCl			Decreased body weight; increased urine excretion; increase in urinary K excretion; epithelial proliferations in the bladder	
Chickens, laying hen	8	42 wk	5,000–20,000 mg/kg diet	Basal diet + KCl	16 d	Diet	Linear decrease in feed intake and increase in water intake as percent K increased; linear increase in weight of water excreted and moisture content of excreta	Smith et al., 2000
Chickens, pullets	10	4 wk	7,300 mg/kg diet, 500 to 1,500 mg/L in water	Basal diet + KCl in water	2 wk (24ºC, 55% RH)	Diet and water	No effects	Teeter and Smith, 1986
Chickens, pullets	10	4 wk	7,300 mg/kg diet, 500 to 1,500 mg/L in water	Basal diet + KCl in water	3 wk (35ºC, 70% RH)	Diet and water	No effects	Teeter and Smith, 1986
			7,300 mg/kg diet, 1,000 mg/L in water				No effects	
			7,300 mg/kg diet, 1,500 mg/L in water				Increased weight gain	
Chickens, pullets	10	4 wk	7,300 mg/kg diet, 1,500 mg/L in water	Basal diet + KCl in water	3 wk (35ºC, 70% RH)	Diet	Increased weight gain and water	Teeter and Smith, 1986

continued

TABLE 24-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Chickens, pullets	10	4 wk	7,300 mg/kg diet, 1,500 mg/L in water	Basal diet + K_2CO_3 in water	3 wk (35°C, 70% RH)	Diet and water	Reduced weight gain and water consumption	Teeter and Smith, 1986
Chickens, pullets	10	4 wk	7,300–14,300 mg/kg diet	Basal diet + KCl	3 wk (35°C and 70% RH)	Diet	No effects	Smith and Teeter, 1987
			15,300–19,300 mg/kg diet	Basal diet + KCl			Increased weight gain and feed efficiency	
Chickens, pullets	20	4 wk	17,300 mg/kg diet	Basal diet + KCl	23 d (26.6 to 36.7°C and 70% RH)	Diet	No effects	Smith and Teeter, 1987
			22,300 mg/kg diet				Improved weight gain	
			27,300 mg/kg diet				No effects	
Chickens, pullets	20	4 wk	7,300 mg/kg diet, 600 to 1,800 mg/L in water	Basal diet + KCl in water	22 d (26.6 to 36.7°C and 70% RH)	Diet and water	No effects Teeter, 1987	Smith and
			7,300 mg/kg diet, 2,400 to 3,600 mg/L in water				Increased weight gain; no signs of toxicosis at 0.36% K supplementation in water	
Cows	5	Mid-lactation	31,000 mg/kg diet	Basal diet + K_2CO_3	21 d	Diet	Decreased Ca content in milk; increased plasma K concentration	Fisher et al., 1994
			46,000 mg/kg diet				Decreased milk yield, Ca content in milk, and plasma Mg concentration; increased plasma K concentration	
Cows	20	4 lactations	11,700 mg/kg diet	Basal diet + $KHCO_3$	From 2 wk preparturition	Diet	10% milk fever cases	Goff and Horst, 1997
	20		21,600 mg/kg diet				50% milk fever cases; increased blood and urine pH; increased precalving feed intake; decreased postcalving feed intake	
	23		31,600 mg/kg diet				49% milk fever cases; increased blood and urine pH; increased precalving feed intake	
Cattle, feeder calves	25	Steers	14,000 mg/kg diet	Basal diet + KCl		Diet	Increased serum Na value	Hutcheson et al., 1984

Cattle, feeder calves	20	Steers	13,000 mg/kg diet	Basal diet + KCl	48 d	Diet	Decreased mortality rate; linear increase in packed cell volume	Hutcheson et al., 1984
			22,000 mg/kg diet				Decreased mortality rate; linear increase in packed cell volume	
			31,000 mg/kg				Linear increase in packed cell volume	
Cattle, calves	1	260 kg	290 mg/kg BW	KCl	Single dose	Drench	Increase in plasma K concentration; erratic and variably increasing respiratory and heart rates	Neathery et al., 1979
	2		580 mg/kg BW				Increase in plasma K concentration; erratic and variably increasing respiratory and heart rates; increase in packed cell volume	
	3		1,150 mg/kg BW				Increase in plasma K concentration; erratic and variably increasing respiratory and heart rates; increase in packed cell volume; excessive salivation; leg muscular tremors; excitability	
	10		1,730–2,880 mg/kg BW				Increase in plasma K concentration; erratic and variably increasing respiratory and heart rates; increase in packed cell volume; excessive salivation; leg muscular tremors; excitability; death	
Cattle	5	4.5 mo	27,700 mg/kg diet	Basal diet + KCl	3 wk	Diet	No effects	Neathery et al., 1980
			67,000 mg/kg diet				Reduced feed intake and average daily gain	
Cows	6	11.2 yr, 671 kg	4,260 mg/kg diet	Grass	4 wk	Diet	Decreased apparent absorption; urinary excretion and balance of Mg	Schonewille et al., 1999a
			4,310 mg/kg diet	Grass + $KHCO_3$			Increased Mg excretion in feces, decreased apparent absorption, urinary excretion and balance of Mg	

continued

TABLE 24-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Cattle	38 total blocked by breed and sex	3 wk	8,400 mg/kg diet 10,200 mg/kg diet 13,200 mg/kg diet	Basal diet + KCl	10 wk	Diet	No effects No effects No effects	Weil et al., 1988
Cattle	16 total blocked by sex	6 wk	5,800 mg/kg diet	KCl	8 wk	Diet	Increased dry matter intake and average daily gain	Weil et al., 1988
Goats, wether	8	2 yr	41,700 mg/kg diet	Control + $KHCO_3$	4 wk	Diet	Increased Mg excretion in feces; decreased urinary Mg excretion; decreased Mg absorption per day and as a percentage of intake; increased K concentration in rumen; increased ruminal pH	Schonewille et al., 1999b
			41,800 mg/kg diet	Control + KCl			Increased K concentration in rumen	
			40,400 mg/kg diet	Control + $K_3C_6H_5O_7 \cdot H_2O$			Increased Mg excretion in feces; decreased urinary Mg excretion; decreased Mg absorption per day and as a percentage of intake; increased K concentration in rumen	
Goats, wether	6	1 yr	36,000 mg/kg diet	$KHCO_3$, Mg diet	4 wk	Diet	Increased K concentration in rumen; decreased Mg absorption independent of Mg intake; rumen pH elevated 0.45 units	Ram et al., 1998.

[a]Number of animals per treatment.
[b]Quantity of potassium dosed. SI conversion: 1 mg potassium equals 25.6 µmoles potassium.

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Selenium

INTRODUCTION

Selenium (Se) has an atomic number of 34 and an atomic weight of 78.96. As a semimetal (metalloid) element, selenium has four natural oxidation states (–2, 0, +4, and +6) (NRC, 1983; Barceloux, 1999). In the –2 state, selenium exists as metallic selenide (Na_2Se), hydrogen selenide (H_2Se), or dimethyl selenide. A strong reducing agent and a relatively strong acid, hydrogen selenide is a very toxic gas at room temperature, and decomposes to form elemental selenium and water in air. Many naturally occurring minerals contain selenides of heavy metals (EHC 58, 1986). Methylated selenides are the primary detoxification compounds in the body; dimethyl selenides emit a strong garlic odor and can be detected in the breath of animals exposed to high levels of selenium. The 0 state of selenium is found in elemental selenium. At physiological pH, the primary form of selenium exists as Se^{-2} in selenocysteine. The elemental selenium (Se^0) has several allotropic forms including a red powder, red crystals, a dark brown moss, and a silver gray form. It boils at 684°C, and is very stable, highly insoluble, and unavailable to plants. The +4 state of selenium occurs as selenite in compounds such as sodium selenite (Na_2SeO_3), selenium dioxide (SeO_2), and selenious acid (H_2SeO_3). This state of selenium can be oxidized slowly to the +6 state in alkaline solution and in the presence of oxygen, or be readily reduced to elemental selenium by reagents such as ascorbic acid or sulfur dioxide. The weakly dibasic selenious acid can be formed from the reaction of selenium dioxide with water, and sometimes acts as an oxidizing instead of a reducing agent. Compounds containing the +6 state of selenium include sodium selenate (Na_2SeO_4), selenium trioxide (SeO_3), or selenic acid (H_2SeO_4). From these compounds, selenium is stable, soluble, and highly available to plants under alkaline conditions. Thus, the +6 state of selenium is potentially the form of this element most toxic to the environment.

Selenium and sulfur belong to the same group (VIa) in the Periodic Table, thus these elements share similar atomic sizes, bond energies, ionization potentials, and electron affinities (Tinggi, 2003). A large number of selenium analogues of organic sulfur compounds such as selenomethionine have been identified in plants, animals, and microorganisms. However, there are at least two major differences between selenium and sulfur (Tinggi, 2003). First, selenium tends to be reduced in biological systems in contrast to sulfur, which tends to be oxidized in those systems. Second, the acid strengths of selenium are greater than those of sulfur (e.g., pKa of H_2Se = 3.7; pKa of H_2S = 6.9). The strong acid strength of selenium enables the element, as selenol compounds (R-SeH), to be easily dissociated at physiological pH in catalytic reactions.

There are six stable isotopes of selenium (74, 76, 77, 78, 80, and 82) with natural abundances of 0.87, 9.02, 7.85, 23.52, 49.82, and 9.19 percent, respectively (EHC 58, 1986). The natural isotopic pattern is useful in determining selenium-containing fragments in mass spectrometry. Radioisotopes of selenium do not exist in nature, but can be produced by neutron activation or by radionuclear decay. The commonly used tracer in experiments is ^{75}Se with a half-life of 120 days. There are also two short half-life radioactive selenium isotopes (^{77m}Se and ^{81}Se with half-lives of 17.5 seconds and 18.6 minutes, respectively).

The Earth's crust contains approximately 0.09 mg of Se/kg, rendering the element the 69th in order of abundance (McDowell, 2003). It is found mainly in cretaceous rocks, volcanic materials, some seafloor deposits, and glacial drift in central Canada and North Dakota in the form of metallic selenides (NRC, 1980). In soil, selenium is present as basic selenite [$(Fe_{29}OH)SeO_3$], calcium selenate ($CaSeO_4$), elemental selenium, and organic compounds derived from plants (NRC, 1983). Weathering of selenium-containing rocks is the primary source of soil selenium, along with that from volcanic activity, dust, phosphate fertilizers, and water. The industrial applications of selenium are mainly for manufacturing rectifiers, xerographic copy machines,

photoelectric cells, glass, ceramics, rubber, pigments, and metal-plating solutions (NRC, 1980).

ESSENTIALITY

Although the biological significance of selenium was initially recognized with its toxicity to livestock, selenium deficiency is a more widespread practical problem. The nutritional essentiality of selenium was first established by its role in preventing diseases such as liver necrosis in rats, exudative diathesis in chicks, hepatosis dietetica in swine, white muscle disease in ruminants, and reproduction failure in various species (NRC, 1983). There are many clinical and subclinical signs of selenium deficiency in animals (McDowell, 2003). However, most of these clinical signs are not produced by selenium deficiency alone, but rather in combination with vitamin E deficiency (Oldfield, 2003). In some areas of China, selenium deficiency is associated with an endemic cardiomyopathy called Keshan disease in children and women of child-bearing age (Chen et al., 1980). Accelerated mutations of myocarditic coxsackievirus B3 from avirulent forms to the virulent forms in selenium deficiency might contribute to the occurrence of this disease (Beck et al., 1995). An osteoarthropathy in humans called Kashin-Beck disease (reported in China) is also considered a selenium-responsive disease (Ge and Yang, 1993).

Since the milestone discovery of selenium as an integral part of cellular glutathione peroxidase (Rotruck et al., 1973), significant progress has been made in understanding the molecular and biochemical functions of selenium. In mammals, selenium is an essential component of at least 12 enzymes: four glutathione peroxidases that use glutathione to break down hydroperoxides; three iodothyronine 5′-deiodinases that catalyze the deiodination of L-thyroxine to the biologically active thyroid hormone 3,3′5-triiodothyronine; three thioredoxin reductases that reduce oxidized proteins; a selenophosphate synthetase 2 that is involved in selenium activation of selenocysteine synthesis; and a methionine-R-sulfoxide reductase (selenoprotein R) (Brown and Arthur, 2001; Gladyshev et al., 2004). There are three characterized selenium-containing proteins: selenoprotein P that accounts for 60 percent of selenium in plasma, selenoprotein W that may be related to white muscle disease, and a 15 kDa selenoprotein that may be related to cancer (Brown and Arthur, 2001). In all these selenium-containing enzymes or proteins, selenium exists as the selenocysteine moiety that is encoded by UGA, normally a stop codon, and incorporated into the peptide by a mechanism called "co-translation," a process that involves four gene products, three reactants, and a unique mRNA sequence named selenocysteine insertion element (SECIS) in the 3′ untranslated region (Sunde, 1997). Without this sequence, the translation of selenium-containing proteins truncates at the UGA codon. Using this unique sequence and other signature characteristics of the known selenoproteins, another 11 new selenoproteins have been identified from the human genome. These are glutathione peroxidase 6 and selenoproteins H, I, K, M, N, O, R, S, T, and V (Gladyshev et al., 2004). Because cysteine replaces selenocysteine in the mouse and rat glutathione peroxidase 6, the rodent selenoproteome consists of 24 instead of the 25 selenoproteins suggested in humans. Overexpression or knockout of selected selenoprotein genes in mice has been applied to elucidate the metabolic functions of specific selenoproteins. This approach has led to exciting discoveries of new roles of glutathione peroxidase 1 (Cheng et al., 1998; Fu et al., 2001; McClung et al., 2004); glutathione peroxidase 2 (Esworthy et al., 2003; Chu et al., 2004); and selenoprotein P (Burk et al., 2003; Hill et al., 2003). The selenium requirements of various species including fish fall between 0.1 and 0.38 mg/kg of diet (NRC, 1993). Although the RDA for adult humans is 55–70 μg/day, supranutritional levels of selenium supplementation, in the form of selenium-enriched yeast, have been reported to reduce mortality due to lung, colon, and prostate cancers (Clark et al., 1996).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Although several methods can be used for selenium determination, the volatility and instability of certain forms of selenium and the non-homogeneity of sample materials are the common challenges for all analytical procedures (NRC, 1983). Thus, adequate precaution is required for sample collection, preparation, and storage to avoid loss of or contamination with selenium. Fluorometry is the most commonly used, and perhaps the most sensitive (detection limit to 2 μg Se/L) method for assay of total selenium in feeds, feces, water, urine, and tissues (Olson et al., 1975). Samples are wet-digested in mixtures of nitric, sulfuric, and perchloric acids, with or without additives such as hydrogen peroxide to destroy organic matter and free the selenium. A pre-digestion or concentration procedure may be added to remove excessive fat or to reduce the sample size. After the released selenious acids react with 2,3-diaminonaphthalene to form fluorescent piazselenol, it is extracted from an acid solution (pH 1–2) with either decahydronapthalene or cyclohexane and detected at an emission wavelength of 520 nm and an excitation wavelength of 390 or 366 nm. Although the fluorometric method has become an official assay for detecting selenium, it has certain limitations, and precautions must be taken when using this method (EHC 58, 1986). First, all traces of nitric acid in the digestion sample should be removed by heating it in a perchloric acid fume hood until perchloric acid fumes appear (about 15–20 min). Caution should be taken to avoid explosions. Secondly, the trimethylselenonium moiety in urine, some plants, and kidney samples may be resistant to wet digestion, requiring extended digestion time. Thirdly, animal samples should be digested in fresh forms to avoid losses of volatile selenium

compounds. Finally, the sensitivity of the fluorometric analysis may be improved by re-distilling cyclohexane and re-extracting 2,3-diaminonapthalene in 0.1 M hydrochloric acid.

Atomic absorption spectrometry can also be used for selenium analysis (NRC, 1983). If samples contain relatively high levels of selenium, flame atomization is useful, but atomization of selenium as hydrogen selenide is more sensitive and specific (EHC 58, 1986). It is possible to perform a direct analysis of selenium in certain samples without digestion by using an improved atomic absorption method based on Zeeman effect background (Cheng et al., 1999). Neutron activation analysis of selenium is very accurate, sensitive, and specific (EHC 58, 1986; Sunde, 1997). Thermal neutron activation is the most commonly used procedure for irradiating samples, producing ^{75}Se, ^{81}Se, and ^{77m}Se. Based on certain values, constants, or the irradiation and counting of selenium standards, the selenium content of the assayed sample can be determined by the detected radioactivity (van der Linden et al., 1974). In neutron activation analysis, sample destruction is not essential, but is preferred for the chemical separation of selenium. The limitation of neutron activation analysis of selenium is the unavailability of adequate equipment and the time required for the analysis. During recent years, speciation of selenium compounds in plant or animal samples using HPLC-ICP-MS (inductively coupled plasma-mass spectrometry) or HPLC-ESI (electronspray ionization)-MS has become a new focal point of selenium biology with limited success (Zayed et al., 1998; Kotrebai et al., 2000; Polatajko et al., 2004).

REGULATION AND METABOLISM

Absorption and Metabolism

For both ruminants and nonruminants, various forms of selenium are readily absorbed (up to 98 percent) in the small intestines, particularly the distal part for selenate (White et al., 1989; Vendeland et al., 1992). Selenocysteine and selenomethionine are absorbed via an active amino acid transport mechanism (Xia et al., 2000), whereas selenite is absorbed by simple diffusion, and selenate by a sodium-mediated carrier shared with sulfate (Wolffram et al., 1986, 1988; Barceloux, 1999). No homeostatic control of selenium absorption has been identified or presumably exists as dietary selenium levels or body selenium status has no apparent impact on its absorption efficiency (Vendeland et al., 1992; Windisch and Kirchgessner, 2000a, b). However, selenium absorption does vary, depending on its chemical form, the animal species, and a number of dietary factors. Organic selenium, such as selenomethionine or selenium-enriched wheat and yeast, has a greater absorption rate than inorganic selenium in many species (Heinz et al., 1996; Kim and Mahan, 2001a,b). Selenate is better absorbed than selenite (Vendeland et al., 1992), and both are better absorbed than elemental selenium (Barceloux, 1999). Due to the reduction of selenite and selentate, and the formation of insoluble particles in the rumen, cattle and sheep have lower absorption of selenium with greater variations than nonruminant species (Wright and Bell, 1966). High levels of dietary sulfur, lead, alfalfa hay, and cyanogenetic glycosides, along with high or low levels of dietary calcium, reduce selenium absorption in ruminants (Neathery et al., 1990; Ivancic and Weiss, 2001; Spears, 2003).

The absorbed selenium becomes associated with proteins in the plasma and carried to tissues. More than 95 percent of ^{75}Se disappeared from the blood after 6 hours following administration to calves (Neathery et al., 1990). In selenium adequacy, selenoprotein P represents >60 percent of plasma selenium in rats (Read et al., 1990). Increasing dietary selenium from 0.62 to 11.9 mg/kg resulted in a decrease of the portion of plasma selenium as selenoprotein P from 40–48 percent to 24–32 percent, resulting in more plasma selenium bound to plasma glutathione peroxidase and albumin (Hintze et al., 2002). Knockout of selenoprotein P interrupts the transport of selenium from liver to peripheral tissues such as testis (Hill et al., 2003). This implies that selenoprotein P is involved in the transport of selenium to tissues. A significant amount of selenium is associated with albumin in animals or humans with excessive selenium intake, especially the naturally occurring organic forms of selenium (Xia et al., 2000). In rhesus monkeys receiving 0.5 mg Se/L in drinking water, 68 percent of erythrocyte selenium was associated with erythrocyte glutathione peroxidase when sodium selenite was given, in comparison with 34 percent for selenomethionine (Butler et al., 1990). In selenium-adequate animals, kidney and liver have the highest selenium content, followed by spleen and pancreas (Kim and Mahan, 2001a,b; Lawler et al., 2004; Cristaldi et al., 2005). Muscle has moderate levels of selenium content, but accounts for the largest pool of body selenium because of its mass (Hintze et al., 2002). When organic selenium is fed to sheep and cattle at the same dietary level as inorganic selenium, it results in higher selenium concentrations in muscle, liver, kidney, and plasma in both species (Ullrey et al., 1977). Selenomethionine or selenium-enriched yeast is also more efficient in raising blood, milk, and tissue selenium in both humans and animals (Thompson et al., 1982; Knowles et al., 1999; Mahan et al., 1999; Mahan, 2000). The placenta or mammary gland transfer of selenium in cattle is quite effective (van Saun et al., 1989; Abdelrahman and Kincaid, 1995; Enjalbert et al., 1999), with a higher efficiency for the organic forms than the inorganic forms (Langlands et al., 1990; Zachara et al., 1993; Ortman and Pehrson, 1999). Approximately 6 to 20 percent of ingested dietary selenium goes into milk in lactating Holstein cows (Ivancic and Weiss, 2001).

Animals can excrete selenium via feces and exhalation, but urine is the primary excretion route and plays a quantitatively greater role in selenium homeostasis (Ellis et al., 1997; Windisch and Kirchgessner, 2000b; Ivancic and Weiss,

2001). Fecal selenium excretion is also an important route in animals fed high levels of dietary selenium (Ellis et al., 1997). Trimethylselenonium accounted for 2 percent of urinary selenium in rats fed 0.25 mg Se/kg (as selenite), but rose to 35–40 percent in rats supplemented with a high level of selenium in water (4 mg Se/L) (Janghorbani et al., 1990). The route, amount, and time course of selenium excretion are affected by the levels of selenium intake, the form of selenium, body selenium status, and dietary levels of copper, lead, mercury, and arsenic (NRC, 1983).

Metabolic Interactions, Regulations, and Mechanism of Toxicities

Metabolic pathways of various forms of selenium in the body are not fully understood, but it is clear that all forms of selenium are incorporated into selenium-dependent enzymes or proteins in a "co-translation" fashion. Using serine as the carbon source, selenite or selenate can be converted into selenocysteine (Sunde, 1997). The process requires the reduction of selenite to elemental selenium first and then to selenide (Ganther and Hsieh, 1974). Besides serving as a substrate of selenophosphate synthetase for the synthesis of selenocysteine, selenide can bind to selenium-binding proteins or be methylated sequentially into different end metabolites such as methaneselenol, dimethylselenide or trimethylselenonium (Hsieh and Ganther, 1977). Selenomethionine is readily metabolized into [Se]-adenosyl methionine (Markham et al., 1980) and subsequently into selenocysteine that may be degraded, catalyzed by a specific lyase, to release elemental selenium (Esaki et al., 1982). Because selenomethionine can be incorporated into proteins in place of methionine, expression of selenoenzymes and tissue selenium distribution in animals fed selenomethionine are affected by dietary levels of methionine (Waschuleswski and Sunde, 1988). Metabolism of methylselenocysteine or selenocystine resembles that of selenite instead of selenomethionine (Martin and Hurlbut, 1976; Deagen et al., 1987).

Expression of selenoenzyme mRNA and activity is largely regulated by dietary selenium level or body selenium status (Lei et al., 1997a, 1997b, 1998; Mahan, 2000; Kaur et al., 2003). Efforts have been made to model the flux of selenium among tissues (Patterson and Zech, 1992). Metabolic interactions of selenium with other nutrients or factors occur at different levels. As mentioned above, body selenium balance seems to be regulated primarily by excretion. Dietary sulfate and several heavy metals significantly increase selenium excretion and reduce selenium absorption or retention (Jensen, 1975; Donaldson and McGowan, 1989; Ivancic and Weiss, 2001; Hamilton, 2004). The strong antagonism between selenium and heavy metals such as mercury also occurs during the selenium passage across the placenta or mammary gland (Parizek et al., 1980).

At the metabolic functional level, selenium is closely associated with vitamins E and C, polyunsaturated fatty acids, iron, sulfur-containing amino acids, and iodine. It is very difficult, if not impossible, to produce clinical signs without a combined deprivation of both selenium and vitamin E (Underwood and Suttle, 1999). The general belief is that these two antioxidant nutrients scavenge free radicals at different sites and spare each other. However, the molecular mechanism of such interaction is unclear. High levels of dietary vitamin E (up to 100-fold the requirement) could not replace the role of selenium-dependent glutathione peroxidase in protecting mice against acute oxidative stress induced by pro-oxidants (Cheng et al., 1999). High levels of dietary polyunsaturated fatty acids or iron aggravate dietary selenium deficiency, whereas high levels of dietary methionine or vitamin C ameliorate selenium deficiency (NRC, 1983). As a component of three iodothyronine 5′-deiodinases, selenium cannot play its role in maintaining normal thyroid hormone without adequate iodine in the diet (Brown and Arthur, 2001).

Despite many decades of research, mechanisms of selenium toxicity still remain unclear. Three possibilities have been postulated (Raisbeck, 2000; Spallholz and Hoffman, 2002; Goldhaber, 2003). First, the possible substitution of selenium for sulfur, due to their similar chemical properties, in important biochemical reactions and structures such as disulfide bonds, may disrupt normal function and cell integrity. It has been suggested that replacing sulfur with selenium in keratin is related to the abnormality in hair and hoof produced by selenosis (Raisbeck, 2000). Secondly, the reaction between selenite and glutathione consumes or depletes cellular-free and protein-bound thiol levels, disturbing relevant enzyme activities (Vernie et al., 1978). Lastly, free radicals such as superoxide anions may be produced by the reactions of certain forms of selenium with tissue thiols, causing oxidative injuries to tissues (Hoffman, 2002; Kaur et al., 2003; Balogh et al., 2004). This oxidation hypothesis has received considerable attention (Barceloux, 1999). However, any single theory may not explain all modes of action due to the diverse chemical properties and metabolism of various selenium compounds.

SOURCES AND BIOAVAILABILITY

Soil selenium, via the growing of plant feeds and foods, serves as the primary source of selenium for the nutrition of humans and of grazing animals. However, the plant selenium content depends on the amount of water-soluble or available selenium, but not the total selenium in the soil (NRC, 1983). The former is determined by the parent materials, pH, aeration, humus, and total iron contents of the soil (Sun et al., 1985). Thus, selenium toxicosis, in particular to grazing animals, likely occurs in some areas of Colorado, South Dakota, North Dakota, northern Nebraska, Manitoba, Alberta, and Saskatuan that have arid or semi-arid climates, soils with pH levels above 7.0, and soils developed from shale (Davis et al., 2000a,b). In the high pH, well-aerated

soils, the predominant form of selenium is selenate, which is most soluble, mobile, and readily taken up by plants (Davis et al., 2000a, b). In contrast, Hawaiian and Puerto Rican soils contain selenium as high as 10–15 mg/kg, but do not produce toxic seleniferous vegetation because of the acidic pH (4.5–6.5) and the presence of ferric hydroxide (Lakin, 1972).

Most soils contain 0.1 to 2 mg of Se/kg. Both topsoil and subsoil should be sampled for selenium analysis because subsoil may have a higher pH and selenium content than topsoil. Soil selenium may be increased by applying manure from selenium-supplemented animals, selenium fertilizer, and selenium-bearing fly ash. Normal soil contains <2 mg of total Se/kg or <50 µg of water soluble Se/kg, and plants growing thereon contain <1 mg of Se/kg (Kappel et al., 1984). Seleniferous soils exceed all three of these selenium levels (Hintze et al., 2002; Lawler et al., 2004). Based on detailed and specific analysis of soil selenium distribution, U.S. soil is mapped into four types (Kubota and Allaway, 1972):

1. low—approximately 80 percent of all forages and grain contain <0.05 Se mg/kg;
2. variable—approximately 50 percent of the plants contain >0.1 Se mg/kg;
3. adequate—approximately 80 percent of all forages and grains contain >0.1 mg Se/kg; and
4. local areas where selenium accumulator plants contain >50 mg Se/kg.

The Great Lakes area and the Northeast, part of the West Coast, and Florida belong to the low selenium region. Pastures and forages from areas with selenium-responsive diseases contain as low as 0.02–0.05 mg Se/kg, but at least 0.1 mg Se/kg (dry basis) in many other areas (Underwood and Suttle, 1999). Cereal grains and other seeds contain 0.006 to 3 mg Se/kg, depending on soil selenium (NRC, 1983). Mean selenium concentrations in the feeds for cattle in Louisiana (Kappel et al., 1984) ranged from 0.057 to 0.295 mg/kg of dry feed, among which bahia grass, mixed ryegrass and oats, corn silage, and sorghum silage were <0.1 mg/kg whereas Bermuda grass, alfalfa hay, and concentrates were >0.1 mg/kg.

In seleniferous areas, accumulator plants such as *Astragalus racemosus* found in Wyoming and South Dakota, and annual legume *Neptunia amplexicaulis* found in Queensland, Australia, accumulate selenium at levels of 1,000–3,000 mg Se/kg, and sometimes even reach 4,000–15,000 mg Se/kg (Davis et al., 2000a,b). Soluble organic methylselenocysteine and selenocystathionine represent the predominant forms of selenium in these accumulator plants (Underwood and Suttle, 1999). A variety of plants can also absorb high levels of selenium from seleniferous soils up to 1,000 mg Se/kg. These plants are called indicators because they can be used to locate seleniferous soils. These plants are also called converters because it is believed that they absorb selenium from geological formations, such as virgin shales, and convert it into soluble compounds for the use by other plant vegetation. But, there is little direct evidence to support such a role by these plants (Raisbeck, 2000). Most ordinary plants grown on normal soil contain <3 mg Se/kg, and may reach 8–14 mg Se/kg when grown in seleniferous soils (NRC, 1983; Hintze et al., 2002; Lawler et al., 2004). The major form of selenium in seeds and forages is seleno-methionine (Allaway et al., 1967; Olson et al., 1970), including selenocystine, selenocysteine, and methylselenomethionine (NRC, 1983).

Supplementation of selenium-containing minerals is the most effective method to meet the selenium requirement of many species, in particular the nonruminants. The most commonly used sources in the United States are sodium selenite and sodium selenate. Other sources of selenium include calcium selenite (Mahan and Magee, 1991) and selenium dioxide. Various selenium-enriched yeast preparations are available and appear to have higher bioavailability than sodium selenite in raising tissue, egg, milk, and blood selenium levels, but lower bioavailability for repleting selenium-dependent glutathione peroxidase activity in pigs (Mahan, 2000). Bioavailability (digestibility) of selenium to Atlantic salmon follows the order from the highest to lowest: selenomethionine > selenite > selenocystine > fish meal (Bell and Cowey, 1989). The following approaches are used to enhance selenium intakes by grazing animals: (1) a free-choice selenium mineral supplement; (2) selenium fertilization of pasture; (3) injection of selenium; (4) a selenium drench; and (5) selenium ruminal bolus (McDowell, 2003). The selenium-fortified salt mixture has also been used for selenium delivery to the grazing animals. Wheat and hay grown on seleniferous soils have been tested for enriching tissue selenium in beef cattle (Hintz, et al., 2002; Lawler et al., 2004). Bioavailabilities of selenium in pet foods range from 27 to 53 percent, and a factor of 30 percent has been suggested for selenium in standard diets of dogs and cats (Wedekind et al., 1998). It is very important to mention that selenium is regulated by the federal government as a food additive. Sources of selenium and levels of supplementation for various species are covered by federal regulation (Title 21 Code of Federal Regulations, part 573.920). Sources of selenium that are not listed in this regulation are not permitted.

Selenium from normal surface or ground water (up to 1–3 µg/L) in many parts of the world contributes little or in negligible amounts to nutrition or toxicosis (NRC, 1983). Although some natural water may contain selenium up to 400 mg/L, public water supplies seldom exceed 10 µg Se/L (Barceloux, 1999). Irrigation of seleniferous soils or industrial contamination can substantially elevate water selenium to levels (>10 µg /L) toxic to fish and wildlife such as in the San Joaquin Valley in California (Hamilton, 2004). Water containing 10–25 µg Se/L may bear a garlicky odor, and

water containing 100–200 µg Se/L has an astringent taste (Pletnikova, 1970). Seawater contains approximately 0.04–0.12 µg Se/L (Barceloux, 1999).

TOXICOSIS

Selenosis or selenium poisoning occurs in three situations. First, grazing animals may suffer from subacute (blind staggers) or chronic (alkali disease) selenium toxicosis in seleniferous areas such as the Rocky Mountain and Great Plains regions of the western United States. Although the high selenium plants, including obligate and facultative selenium accumulators, have poor palatability and garlic-sulfur odor that do not attract animals to eat them, overgrazing and/or starvation may leave those animals no choice. High levels of selenium intake may also result from excessive soil ingestion (up to 15 percent of total DM intake) in periods of drought or on heavily stocked pasture in late autumn or winter (Rogers et al., 1990). Secondly, environmental contamination of agricultural drain water, sewage sludge, and industrial activities including fly ash from coal plants, oil refineries, and mining of phosphates and metal ores can cause selenium toxicosis in aquatic animals. Examples include fish kills and bird deformities at Belews Lake, NC (>10 µg Se/L); Martin Lake, TX; Kesterson Reservoir, CA (22 to 31 µg Se/L); and aquatic resources in southeastern Idaho and British Columbia (Barceloux, 1999; Hamilton, 2004). Because of its bioaccumulative nature in the food chain, selenium has become a primary concern among many elements studied under these conditions. Thirdly, selenium toxicosis can also be produced in various species by high levels of selenium supplementation under experimental conditions or poor management. In most cases, the selenium-intoxicated animals show depressed growth performance, elevated selenium concentrations in tissues, histopathology, clinical signs, or death.

Single Dose and Acute

Death may occur within a few hours or after several days, if animals consume a large quantity of accumulator plants such as *Astragalus racemosus,* which contains 100–9,000 mg of Se/kg (McDowell, 2003). These animals emit garlicky breath odor in exhaling dimethyl selenide and have an abnormal gait. After a short-distance walk, they show an initial characteristic stance with head lowered and ears dropped. The subsequent signs include vomiting, dyspnea, tetanic spasms, and labored respiration. Eventually they die of respiratory or circulatory failure (Raisbeck, 2000). Pathological changes include congestion of liver and kidney, endocarditis, myocarditis, and petechial haemorrages of the epicardium (NRC, 1983).

Grace (1994) suggested that the LD_{50} of sodium selenite for ruminants orally was 1.9–8.3 mg Se/kg BW. An oral dose of 9–20 mg of Se/kg BW may be lethal to calves (Puls, 1994). In sheep, the oral LD_{50} of sodium selenite is suggested as 10–15 mg Se/kg BW (Puls, 1994). Goats (6–8 months old) died of a single dose of 40 mg of sodium selenite/kg BW within 96 hours (Ahmed et al., 1990). For pigs, an oral dose of 4.2 mg of Se/kg of BW for 3 days is lethal (Puls, 1994). For poultry, the acute oral LD_{50} is 33 mg of Se/kg BW (Puls, 1994). A recent Polish study (Szeleszczuk et al., 2004) indicated that the lethal dose of selenium sodium selenite for one-day-old male chicks was 2 mg of Se/chick. The minimal lethal oral dose is 1.5–3 mg Se/kg BW for sodium selenite or sodium selenate in rabbits, rats, dogs, and cats (NRC, 1983; Puls, 1994). In a clinical case, a 3-year-old female Chihuahua dog was killed by a single IM injection of selenium at 2.5 mg/kg BW (Janke, 1989). The dog had congested capillaries in alveolar septae and abundant proteinaceous fluid in alveolar lumina. Liver and kidney from the dog had 12.9 and 12.1 mg Se/kg, respectively, in comparison with the normal concentrations of <3 mg Se/kg. The oral LD_{50} for sodium selenite ranges from 2.3 to 13 mg Se/kg BW in rabbits, guinea pigs, mice, and rats (EHC 58, 1986; Janke, 1989). The LD_{50} for methyl selenide, trimethylselenonium, or elemental selenium is 3- to 500-fold higher than that for selenite in these species (NRC, 1983). Hamilton and Buhl (1990) observed only increased surfacing behavior in Chinook salmon and Coho salmon exposed to seleno-DL-methionine up to 21.6 mg Se/L for 96 hours. Selenite was significantly more toxic to both species than selenate, as the estimated 96-hour LC_{50} was 13.4–17.0 mg Se/L for the former and 114–149 mg Se/L for the latter, respectively, in Chinook salmon in different waters.

Selenium injections are often administered to ruminants, especially neonatal ruminants, as a rapid means of selenium supplementation. The toxicity threshold for injected selenium is much lower than that for ingested selenium. Injectable selenium is usually composed of sodium selenate, sodium selenite, or barium selenate, commonly prepared to supply vitamin E as well, and generally administered at a rate of 0.06–0.1 mg Se /kg of BW at intervals of 1–6 months (Meads et al., 1980). Toxicoses are occasionally encountered following injection of selenium primarily because the concentration of selenium in the injectable preparation is underestimated or the preparation was given to supply vitamin E without considering the selenium content of the injection. The LD_{50} of intramuscular injection of selenite to 8- to 10-week-old lambs is 0.5 mg Se/kg BW (Caravaggi et al., 1970). Subcutaneous injection of selenite at a dose of 0.7 mg Se/kg BW killed 50 percent of 10- to 11-month-old wethers (Grace, 1994). The LD_{50} of subcutaneous selenate injection is 1 mg Se/kg BW in lambs and 1.9 mg Se/kg BW in adult cattle (Grace, 1994). For pigs, a dose of 1.2–2 mg injectable Se/kg BW is acutely toxic (Puls, 1994).

Subacute

"Blind staggers" is often considered a subacute selenosis in grazing animals. Sometimes it is also described as a

chronic selenosis produced by a relatively long exposure (weeks to months) to selenium-accumulator plants (Underwood and Suttle, 1999). The affected animals initially manifest signs of wandering, stumbling over objects, anorexia, and visual impairment, followed by increased severity of these signs plus development of weak front legs. Because the initial signs are not apparent, the toxicosis may not be noticed until the final stage when the animals show blindness, paralysis of the tongue and swallowing mechanism, rapid and labored respiration, salivation, and low body temperature. Once these clinical signs appear, animals usually collapse and die within a few hours. As the neuropathology of blind staggers cannot be reproduced by high levels of pure selenium alone, other factors including alkaloid poisoning, starvation, and polioencephalomacia might be responsible for or confounded with the disorder (O'Toole and Raisbeck, 1995).

Chronic

Ruminants and Horses

Alkali disease is a "classical" chronic selenosis in grazing cattle, horses, and sheep resulting from long exposure (>30 days) to seleniferous forages and grains containing 5–40 mg of Se/kg (NRC, 1983). This malady is usually associated with nonaccumulator plants instead of the obligate and facultative selenium accumulators, and can also be produced by overfeeding inorganic selenium or seleniferous feeds in these and other species (Kim and Mahan, 2001a; Kaur et al., 2003). The affected cattle, horses, and swine exhibit bilaterally symmetric alopecia and dystrophic hoof growth (Raisbeck, 2000). Other signs in cattle and horses include loss of appetite, unthriftiness, liver atrophy and cirrhosis, nephritis, myocardial necrosis, loss of vitality, loss of hair, and lameness (Table 25-1). Their hooves become elongated and slough off after prolonged exposure to high levels of selenium (O'Toole and Raisbeck, 1995). The lameness and pain associated with deformed hooves may make the animals reluctant to move for food or water, thereby dying of thirst and starvation (Underwood and Suttle, 1999). Because of the efficient transfer of selenium by the placental and mammary gland, calves and foals in seleniferous areas may be born with deformed hooves or show the deformity during the suckling period (Raisbeck, 2000).

Typical chronic selenosis, including hoof deformation and alopecia, was produced by feeding yearling steers 0.28 (as selenomethionine) or 0.8 (as selenite) mg Se/kg BW for 120 days (O'Toole and Raisbeck, 1995). Similar signs were also produced in calves fed sodium selenite at 0.25 mg Se/kg BW for 16 weeks (Kaur et al., 2003). These animals also exhibited elevated oxidative stress including lipid peroxidation of tissues. However, feeding steers seleniferous wheat or hay to supply 11.9 mg Se/kg of diet or 65 μg Se/kg BW for >100 days resulted in significantly increased tissue selenium concentrations, but no signs of toxicity (Hintze et al., 2002; Lawler et al., 2004). Adult Holstein cows tolerated sodium selenite at up to 87–118 μg Se/kg BW for 128 days (Ellis et al., 1997). Reduced growth performance and reduced blood packed cell volume were seen in 3-day-old calves fed 10, but not 5, mg Se/kg of diet (as sodium selenite) (Jenkins and Hidiroglou, 1986). There are no recent reports on experimentally produced selenium toxicosis in horses, but field cases of naturally occurring selenosis have been documented (Raisbeck et al., 1993; Witte and Will, 1993). The signs of toxicosis included hoof lesions and loss of mane and tail, without neurological disorders. Alfalfa samples from the affected areas contained up to 19–58 mg of Se/kg (Witte and Will, 1993).

Death and toxic selenium levels in tissues were observed after sheep grazed for 4 weeks on high selenium forage (<49 mg Se/kg DM) and drank high-selenium water (340–415 μg Se/L) (Fessler et al., 2003). However, no toxicosis was produced in sheep grazing on forages containing <13 mg Se/kg DM with normal water (<1.7 μg Se/L). Ewes endured selenium from sodium selenite (24 mg Se/kg diet) or *Astragalus* (29 mg Se/kg) incorporated into alfalfa pellets for 88 days, with only minor wool loss noted on the neck and sides of some ewes (Panter et al., 1995). Goats receiving repeated daily doses of 0.25, 0.5, or 1 mg of sodium selenite/kg BW for 225 days showed no clinical signs of toxicosis or histological changes (Ahmed et al., 1990). However, death was noted when the quantity of selenium was increased to 5 mg/kg.

Swine

Growth depression seems to be one of the most sensitive indicators of chronic selenosis in swine. Feeding pigs with sodium selenite at 0, 4, 8, 12, 16, and 20 mg Se/kg of diet had three effects: (1) a linear decrease in daily gain and feed intake; (2) a quadratic increase in hair selenium; and (3) a linear increase in blood selenium (Goehring et al., 1984a, b). External signs of selenosis such as hoof lesion and paralysis appeared in some pigs given 12 or 20 mg Se/kg. These results suggested that the toxic level of selenium in a corn-soybean meal diet for crossbred pigs was between 4 and 8 mg Se/kg of diet. In another study with growing pigs (12.5 kg BW) fed sodium selenite or calcium selenite for 35 days, growth and feed intake were reduced in pigs fed 10, but not 5, mg Se/kg compared with those fed 0.3 mg Se/kg (Mahan and Magee, 1991). Hair loss and separation of the hoof at the coronary band site occurred in pigs fed 10 mg Se/kg of diet as sodium selenite, but at 15 mg Se/kg as selenium-enriched yeast (Kim and Mahan, 2001a). Sodium selenite seemed to be more potent than selenium-enriched yeast in reducing growth performance, altering bile color, and elevating plasma glutamic oxalacetic transaminase activity. If the selenium intake is sufficiently high to severely reduce feed intake, pigs consistently develop neurological signs such as

bilateral malacia of grey matter in the spinal cord (Goehring et al., 1984b; Raisbeck, 2000). Neurological signs of paralysis developed in growing pigs (8–10 weeks old) fed selenium at 25 mg/kg diet for 6 weeks (Panter et al., 1996). However, the incident rate was 100 percent (5/5), 80 percent (4/5), and 40 percent (2/5) in pigs fed *Astragalus bisulcatus*, sodium selenite, and seleno-DL-methionine, respectively. Polioencephalomalacia also developed in pigs fed *Astragalus bisulcatus*. As the severity of the pathological changes was not directly related to tissue or blood selenium concentrations, other factors such as swainsonine (the toxic chemical in locoweed) might contribute to the responses in the *Astragalus bisulcatus*-treated group (Panter et al., 1996).

Poultry

Weight gain and feed efficiency in chicks were depressed when dietary selenium level was 5 mg/kg or higher (Jensen, 1975, 1986; Elzubeir and Davis, 1988; Donaldson and McGowan, 1989; Vanderkop and MacNeil, 1990). One study showed no adverse effect of 5 mg of Se/kg on growth performance of chicks (Jensen and Chang, 1976), but another study showed adverse effects of 1 and 3 mg of Se/kg (sodium selenite) on growth and tissue integrity of hybro-type chicks (Dafalla and Adam, 1986). The effect of sodium selenite seems to be more severe than that of selenomethionine (Lowry and Baker, 1989). Egg production and hatchability were decreased in emu hens during a 4-month selenium supplementation at 1.55 mg Se/kg DM (Kinder et al., 1995). Egg production was increased by 23 percent in the following years by decreasing selenium supplementation. When day-old ducklings were fed a wheat basal diet containing 30 mg Se/kg of diet as seleno-L- or DL-methionine, or selenized yeast, for two weeks, survival was 36, 100, and 88 percent, respectively (Heinz et al., 1996). When the basal diet was changed to a commercial duck feed, no mortality occurred with these sources. In another duckling study with three different sources of selenium at 10 mg Se/kg, no effect on health or survival of mallards was observed (Heinz and Hoffman, 1996).

Laboratory Animals

Feeding 4-week-old hamsters either sodium selenite or selenomethionine at 5.0 mg Se/kg of diet for 21 days produced no adverse effects (Julius et al., 1983). However, increasing dietary selenium level to 10 mg /kg or higher caused growth depression and (or) mortality. Similarly, rats given sodium selenite in drinking water at 4 mg Se/L for 1–2 years showed no changes in BW or survival compared with the controls (Jacobs and Forst, 1981a). In another 35-day experiment, rats given 64 mg Se/L of drinking water died by day 18. The survival was also decreased in rats given 8 and 16 mg Se/L. BW gain was decreased and serum alkaline phosphatase and glutamic oxaloacetic transaminase activities increased in the 16 and 64 mg Se/L groups. Reduced BW gain, decreased food and water intake, and increased dopamine metabolites in brain striatum resulted from giving young mice selenium in drinking water at 3 or 9 mg Se/L for 14 days as sodium selenite, but not as selenomethionine (Tsunoda et al., 2000). During a 50-week experiment, sodium selenite added to drinking water of rats at 8 mg Se/L caused a 50 percent reduction in BW gain, decreased white blood cell counts, and increased serum alkaline phosphatase and glutamic oxaloacetice transaminase activities (Jacobs and Forst, 1981b). However, those rats treated with 4 mg Se/L showed no adverse response compared with the controls (1 mg Se/L).

Fish

Because of the fish kills and bird deformities at Belews Lake, NC, Kesterson Reservoir, CA, and other sites, great progress on selenium toxicity in the aquatic species has been made during the last two decades. Hamilton (2004) has provided an excellent review on the background, progress, and current status of the field. Canadian researchers demonstrated that for rainbow trout, the minimal requirement of selenium was between 0.15 and 0.38 mg/kg of dry feed, the definite toxic level was 13 mg Se/kg diet, and the possible toxic level with prolonged exposure was 3 mg Se/kg. The massive disappearance of several fish species in Belews Lake, NC, was related to the rather high selenium level (5–10 μg/L) in the water. Studies on fish die-offs in Colorado and Wyoming indicated that the selenium toxicosis in fish was via the food chain. Based on mortality, growth depression, reproduction impairment, and migration, Hamilton (2004) listed the following adverse selenium levels for rainbow trout, Chinook salmon, fathead minnow, striped bass, bluegill, and razorback sucker: 2.4–70 mg Se/kg of dry feed (diets), and 47–472 μg/L (water). In most cases, reduced growth or survival occurred at dietary selenium levels close to 3 mg/kg or at whole-body selenium residues close to 4 mg/kg. The sources of selenium in these studies included sodium selenite, selenomethionine, and various types of fish. Selenomethionine seemed to be the most toxic form of selenium for fish.

Reproduction

Selenosis in poultry results in poor hatchability and deformed, rudimentary, or lack of legs, toes, wings, beaks, and eyes in the young (Latshaw et al., 2004). The chick embryo is very sensitive to selenium toxicity as shown by the 100 percent mortality of all embryos within 48 hours post administration of selenium (sodium selenite) at 0.02 mg per embryo (Szeleszczuk et al., 2004). Hatchability of eggs is affected by very low dietary selenium levels (Ort and Latshaw, 1978) that normally do not produce any effect in other species. A field study indicated that egg production and hatchability were de-

creased in hens during a 4-month supplementation of selenium at 1.55 mg Se/kg DM (Kinder et al., 1995). This level of dietary selenium also resulted in dead embryos and a high incidence of leg deformities in surviving chicks. These changes were partially reversed in the following years when selenium supplementation was decreased to 0.52 mg Se/kg. When adult male and female ducklings were given three sources of selenium at 10 mg Se/kg, hatching of fertile eggs was significantly lower for females fed seleno-DL-methionine (7.6 percent) or seleno-L-methionine (20 percent) than for controls (41.3 percent, 0.56 mg Se/kg) (Heinz and Hoffman, 1996). Both seleno-L-methionine and seleno-DL-methionine significantly decreased the number of 6-day-old ducklings produced per hen, and the former also decreased the survival percentage of young to 6 days old. Comparatively, the selenized yeast showed either no or much less effect on these measures, implying a lower toxicity. Recent studies on the adverse effects of high dietary selenium levels on reproduction of birds have been reviewed by Hamilton (2004).

The impacts of high selenium on reproductive performance in ruminants are far less clear than in avian species. Although there are field reports on decreased conception in cattle and sheep, no direct evidence has been produced experimentally (Raisbeck, 2000). BW gain, estrous cycle length and frequency, and lambing of yearling ewes were not affected by feeding a high level of sodium selenite (24 mg Se/kg of diet) or *Astragalus* (29 mg Se/kg) incorporated into alfalfa pellets for 88 days (Panter et al., 1995). When sows were fed a basal diet (0.13 mg Se/kg) supplemented with selenium (as sodium selenite) at 0, 2, 4, 8, or 16 mg/kg from the first estrous cycle through 9 weeks postpartum, conception rate, protein and fat content of colostrum or milk, number of piglets born, and piglet mortality were not affected by dietary selenium levels (Poulsen et al., 1989). Piglets born from sows on the two highest selenium levels tended to have lower birth weights than the controls, but the differences were not significant. Weaning weights of piglets (21 days) tended to be negatively influenced by selenium treatment ($P = 0.08$). At 9 weeks of age, piglet BWs and daily feed intakes (from 3 to 9 weeks) were lower in the two highest dose groups compared to the controls. A circular dark band was seen in the hoofs of some sows on high selenium supplementation. When gilts were fed 0.3, 3, 7, and 10 mg Se/kg of diet as sodium selenite or selenium-enriched yeast from 25 kg BW through the first parity, both sources of selenium were toxic at the two highest levels (Kim and Mahan, 2001b). It is intriguing that the organic selenium had more severe effects on reproductive performance of the gilts during gestation, whereas inorganic selenium was more detrimental to the nursing pigs during lactation.

Teratogenicity

Chick eggs and embryos are very susceptible to the teratogenic effect of selenium. Injecting sodium selenite into eggs up to 0.6 mg Se/kg resulted in 50 percent dead and 50 percent abnormal embryos (EHC 58, 1986). Methylselenic acid seems to be more potent in this regard than selenate, selenite, and selenomethionine. Breeding ring-necked pheasants receiving feed containing 9.3 mg of Se/kg had deformed beaks and abnormal eyes in 10 percent of chicks hatched and deformities in more than 50 percent of all embryos developed, including those that died in the shell (Latshaw et al., 2004). The eggs without embryonic development contained 2.05 mg of Se/kg. Likewise, teratogenesis is a biomarker of selenium toxicosis in fish and wild birds at the embryo-larval stage, and can be found in selenium-contaminated ecosystems such as in San Luis Drain, CA (Hamilton, 2004). Fish deformities include lordosis, kyphosis, scoliosis, and abnormal head, mouth, gill cover, and fin. Deformity was produced in mallard (adults or ducklings) by feeding 8–25 mg Se/kg of diet as selenite, selenomethionine, or selenized yeast (Heinz and Hoffman, 1996; Hamilton, 2004). Except for the deformed hooves, teratogenic effect of selenium in mammals is inconclusive. The relatively lower selenium accumulation in fetus than in eggs may be partially responsible for the stronger resistance of mammals to the teratogenic effect of selenium than that of birds (Barceloux, 1999).

Carcinogenicity and Mutagenicity

Although several earlier studies suggested that high levels of selenium promoted carcinogenesis, recent animal experiments and human epidemiological and intervention studies (Clark et al., 1996) have shown the opposite role of selenium. Thus, selenium salts are not listed as a suspected carcinogen by the International Agency for Research on Cancer or the U.S. National Toxicology Program (Barceloux, 1999).

Humans

Endemic human selenium toxicosis has been reported in seleniferous areas of China when whole blood selenium level was >1 mg/L and daily selenium intake was >900 mg (Yang and Zhou, 1994). The common symptoms of all patients included broken hair strands or nail damage. Chronic selenium toxicosis may also develop in individuals inhaling selenium fumes or ingesting overdoses of potent selenium tablets (Sunde, 1997). Symptoms include nausea and vomiting, nail thickening and ridging, hair loss, fatigue, irritability, abdominal cramps, watery diarrhea, paresthesis, dryness of hair, and garlicky breath (Barceloux, 1999).

Factors Influencing Toxicity

While the mechanism of selenium toxicity remains unclear, factors influencing its toxicity have been well studied. These factors include species, age, and physiological status

of the target animals, chemical form and nutritional nature of selenium, and dietary (water) conditions.

Species, Age, and Status of Animals

Poultry, fish, and aquatic birds seem to be more susceptible to the teratogenic effect of selenium than mammals (Barceloux, 1999; Hamilton, 2004). Adverse effects of high selenium intake on egg hatchability in poultry and reproduction in swine are evident (Kim and Mahan, 2001b). Sheep may be somewhat more resistant to toxicity of selenium from organic sources than are cattle and horses (Raisbeck, 2000). Susceptibility of different fish species to selenium toxicity varies greatly. For example, Coho salmon are more sensitive than Chinook salmon to inorganic selenium (Hamilton and Buhl, 1990). The elimination of fish species from contaminated aquatic communities is highly selective (Hamilton, 2004). Young animals are less tolerant of selenium than adult animals (Hamilton and Buhl, 1990; Raisbeck, 2000). Vitamin E-deficient animals are more susceptible to selenium toxicity than are vitamin E-adequate animals (NRC, 1983).

Chemical Form and Nutritional Nature of Selenium

As in the case of bioavailability, the relative toxicity of a given selenium compound is affected by its chemical form and solubility. The highly insoluble elemental selenium is much less toxic to many species than other more soluble forms such as selenite and selenate (NRC, 1983). It is generally true that organic forms of selenium are more effective in raising tissue selenium concentrations than the inorganic selenite or selenate (Kim and Mahan, 2001a; Hintze et al., 2002). However, the relative toxicity of selenium is not directly related to tissue selenium levels (Panter et al., 1996). Depression of growth performance was less by the same amount (15 mg/kg) of selenium as selenomethionine than as selenite in chicks (Lowry and Baker, 1989). Similarly, seleno-DL-methionine was less toxic to Chinook salmon and Coho salmon than selenite or selenate (Hamilton and Buhl, 1990). However, the opposite was true in other studies (O'Toole and Raisbeck, 1995; Hamilton, 2004). The same level of selenium from *Astragalus bisulcatus* produced more severe and disseminated lesions in pigs than that from sodium selenite or selenomethionine, suggesting that factors other than selenium might contribute to or modulate selenium toxicity (Panter et al., 1996). In mallard ducklings, both DL and L forms of selenomethionine were more toxic than selenized yeast (Heinz and Hoffman, 1996). Selenite is taken up more efficiently than selenate by aquatic plants, and is more toxic to aquatic invertebrates and fish (Hamilton and Buhl, 1990).

Dietary Conditions

Certain dietary conditions and factors can enhance or reduce selenium toxicity. In general, selenium is more toxic to animals if it is added in semi-purified diets than in practical diets (Heinz et al., 1996). Appetite and growth were impaired in swine fed 8 mg Se/kg of DM as selenite in a corn-soybean meal diet, but not in a wheat and oats diet (Goehring et al., 1984b). High levels of dietary protein and sulfur-containing amino acids (Hamilton, 2004) may help reduce selenium toxicity. Cyanogenic glycosides (Palmer et al., 1980) are considered the mediator of protection by linseed meal against chronic selenium toxicosis (Jensen and Chang, 1976). Dietary supplementation of sodium nitroprusside (Elzubeir and Davis, 1988) or monensin (Jensen, 1986; Vanderkop and MacNeil, 1990) has been shown to reduce selenium toxicosis in chicks. High levels of dietary sulfate and sulfur may protect against selenium toxicity as the former increases urinary excretion of selenate in rats (Halverson et al., 1962), and the latter decreases absorption of selenite in lactating cows (Ivancic and Weiss, 2001).

A number of trace elements including antimony, arsenic, bismuth, cadmium, copper, germanium, mercury, silver, and tungsten can affect selenium use (Rahim et al., 1986) and thus attenuate selenium toxicity in mammals, fish, and birds (Jensen, 1975; Donaldson and McGowan, 1989; Hamilton, 2004). The protection of arsenic compounds against selenium toxicosis has been shown in various species fed different forms of selenium (Levander, 1977; Lowry and Baker, 1989; Hamilton, 2004). A possible mechanism is the enhanced biliary excretion of selenium by arsenic. High levels of copper reduced selenium retention in tissues of fish, but had no biological effect (Hamilton, 2004). Long-term ingestion of moderate levels of copper might affect selenium distribution and retention in sheep (White et al., 1989). The antagonistic interaction between selenium and mercury (El-Begearmi et al., 1977; Urano et al., 1997; Hoffman and Heinz, 1998) is of great significance in fish for food safety and environmental protection. Although many have reported simultaneous bioaccumulation of these two elements, no evidence exists for a natural joint deposition of both in fishes, crustaceans, or mollusks (Hamilton, 2004). Mercury accumulation in fish carcass is reduced by relatively low levels of selenium addition, but may be increased by adding high levels of selenium (Klaverkamp et al., 1983). As combined selenium and mercury exposure is more toxic than separate exposures to aquatic life (Hoffman and Heinz, 1998), there is limited practical value of selenium and mercury antagonism. The consistent effects of other elements on the toxicity of different forms of selenium and the practical implications are unclear.

TISSUE LEVELS

Puls (1994) provided a comprehensive list of selenium concentrations in selected tissues and fluids in various species fed dietary selenium (mg/kg feed) at deficient (<0.10), marginally adequate (0.10–0.25), adequate (0.3–2), high (3–5), chronically toxic (>5), or acutely toxic levels (>20–80).

Data from the recent individual studies are summarized in Table 25–2. In most cases, kidney has the highest selenium concentration among all tissues assayed. The concentrations of selenium in kidney of steers fed normal to moderate dietary levels of selenium are about 2–2.5 mg/kg DM (Hintze et al., 2002; Lawler et al., 2004), whereas the concentrations of selenium in kidney of chicks and laying hens fed corn-soy or corn-torula yeast basal diets (0.03–0.10 mg Se/kg) range from 0.18 to 0.32 mg/kg (Latshaw, 1975; Osman and Latshaw, 1976). When fed selenium-adequate diets, liver:kidney selenium concentration ratios are approximately 1/3 in steers (Lawler et al., 2004), 1/10 in calves (Ammerman et al., 1980), 1/4 in pigs (Kim and Mahan, 2001a,b), and 1/2 in rats (Janhorbani et al., 1990). However, these two tissues seem to share similar selenium concentrations in chicks (Osman and Latshaw, 1976), laying hens (Latshaw, 1975), and hamsters (Julius et al., 1983). Spleen has slightly lower selenium concentrations than liver in both cattle (Lawler et al., 2004) and swine (Kim and Mahan, 2001a, b). Muscle has the lowest selenium concentrations among all assayed tissues across all species. Other soft tissues such as lung, heart, pancreas, and brain have selenium concentrations that fall between liver and muscle (Panter et al., 1996; Kim and Mahan, 2001a,b). All tissues can accumulate more selenium with increasing dietary selenium supplementation, and the elevation can reach 40- to 50-fold over the baseline or normal levels (Kim and Mahan, 2001a). In various species, organic selenium sources are more effective than inorganic selenium salts in raising tissue selenium levels (Lowry and Baker, 1989; Butler et al., 1990; Kim and Mahan, 2001a,b; Lawler et al., 2004), and selenomethionine seems to be most potent in this regard (Heinz and Hoffman, 1996; Panter et al, 1996).

Plasma selenium concentrations in various species range from 0.02 to 0.20 mg/L, and can be increased up to 3.2 mg/L in pigs fed selenized yeast at 20 mg Se/kg of diet (Stowe and Herdt, 1992; Kim and Mahan, 2001a). Red blood cells contain higher selenium concentrations than plasma (Salbe and Levander, 1990). Milk of U.S. cows contains 20–40 μg Se/L (Maus et al., 1980). A New Zealand study (Knowles et al., 1999) showed that milk produced by cows grazing on pasture identified as marginal to deficient in selenium (0.035 mg Se/kg of DM) contained 2.1 to 8.3 μg Se/L, varying with seasons. Treating cows with drenches of sodium selenite or selenized yeast at 2 or 4 mg Se/day for 133 days resulted in 1.5–5.5 times greater milk selenium concentrations (up to 25.3 μg Se/L). Approximately 71 percent of whole milk selenium was associated with casein, which from the selenium-treated cows contained as much as 1.4 mg Se/kg (Knowles et al., 1999). Sow milk contains approximately 40 mg Se/L, and a 10-fold increase over the baseline was produced by feeding gilts with selenized yeast at 10 mg Se/kg diet (Kim and Mahan, 2001b). Colostrum contains higher levels of selenium concentration than regular milk (Abdelrahman and Kincaid, 1995), and the level is also highly dependent on dietary selenium intake (Kim and Mahan, 2001b). Egg white contains approximately 0.4–0.5 mg Se/kg of DM, and can be increased to 2.47 mg Se/kg diet by feeding a moderately high level of organic selenium (0.42 mg Se/kg) (Latshaw, 1975; Jacobs et al., 1993). Concentrations of selenium in the eggs laid by mallards, shortly after placement on a diet that was 10 mg Se/kg as the DL or L form of selenomethionine, were elevated from 0.41 mg/kg in the control group (wet basis) to 9.1 mg/kg (Heinz and Hoffman, 1996). The elevation was greater ($P < 0.05$) than that produced by selenized yeast (22- vs. 16-fold).

Hair of cattle from areas of soil with normal amounts of selenium contains 1–4 mg Se/kg of DM, and may reach 10–30 mg Se/kg in seleniferous regions (Lawler et al., 2004). Gilts fed 0.3 mg Se/kg diet as selenite had a hair selenium concentration of 0.49 mg/kg (fresh basis), and reached 8–11 mg/kg in groups fed 7–10 mg Se/kg as selenized yeast (Kim and Mahan, 2001b). In swine, hooves had slightly lower selenium concentrations than hair, but were still as high as 29 mg/kg (fresh basis) in pigs fed 15 mg Se/kg as selenized yeast (Kim and Mahan, 2001a,b). Hair, hoof, and nail have been used for determining body selenium status, but caution should be given in sampling these tissues and interpreting the results because other factors such as dietary methionine affect the selenium content of these tissues (Salbe and Levander, 1990).

Through a homeostasis mechanism (via reduced intake or depuration), the juvenile fathead minnow reduced the whole body selenium concentration from the initial value of approximately 14 mg/kg to that close to the selenium concentrations in the food (5–11 mg of Se/kg) (Hamilton, 2004). Criteria based on tissue selenium are considered more reliable than selenium concentrations in water for predicting adverse effects of selenium in fish (Hamilton, 2004).

MAXIMUM TOLERABLE LEVELS

Selenium is considered rather toxic, and selenium poisoning can be a practical problem in grazing animals (Ullrey, 1992). The rather recent fish kills and bird deformities related to selenium bioaccumulation from agricultural irrigation and industrial contamination at several aquatic resources (Hamilton, 2004) have highlighted the immediate risk of selenium toxicity to the ecosystems. Meanwhile, the illustration of possible anti-cancer action by superanutritional levels of selenium in humans (Clark et al., 1996; Ip, 1998) has led to an interest in producing selenium-enriched meats, milk, and eggs by feeding animals with selenized grain, hay, or yeast (Lawler et al., 2004). Thus, establishing the accurate maximum allowable tolerable levels of selenium for various species has broad implications. The challenge is that these levels vary widely with the form and source of selenium, exposure duration, nature of diet, and end points of tolerance.

The maximum tolerable level of a mineral is the dietary level that, when fed for a defined period of time, will not

impair animal health and performance. A single level of 2 mg Se/kg of diet was suggested by the former NRC committee (NRC, 1980) as the maximum tolerable level for all species. This recommendation has been challenged (Underwood and Suttle, 1999; McDowell, 2003) as an underestimate of selenium tolerances by several species, especially for the ruminants under practical dietary conditions (Cristaldi et al., 2005). Based on the data presented in Table 25-1, various types of cattle showed no adverse response to 5–12 mg Se/kg of DM or 65–150 µg Se/kg of BW for up to 4 months. Sheep seem to be more tolerant of selenium than cattle as they displayed no toxic signs when fed 9–10 mg Se/kg DM (as selenite) (Echevarria et al., 1988; Cristaldi et al., 2005) or when grazing forages containing <13.0 mg Se/kg DM (Fessler et al., 2003). Only minor wool loss was observed in ewes fed 24–29 mg Se/kg DM as selenite or *Astragalus bisculcatus* (Panter et al., 1995). Therefore, it is reasonable to increase the maximum tolerable level of selenium for ruminants to 5 mg of Se/kg DM. This level seems to be appropriate for horses as well.

Compared with the selenium-adequate controls, pigs fed 3–5 mg Se/kg diet in several studies showed no inferior growth, reproduction, or health (Goehring et al., 1984a,b; Mahan and Magee, 1991; Kim and Mahan, 2001a,b; Poulsen et al., 1989). Because growth rate and feed intake were reduced by 5 mg Se/kg diet in one study with weanling pigs (Moxon and Mahan, 1981), and pigs are rather efficient in absorbing dietary selenium, it seems appropriate to set the maximum tolerable level of selenium for swine at 4 mg Se/kg of DM. Growth and appetite were reduced by adding selenite into drinking water of chicks at 4 mg of Se/L (equivalent to 7 mg Se/kg feed intake) (Cantor et al., 1984) or into feed above 5 mg of Se/kg (Jensen, 1975). As hatchability of eggs is very sensitive to selenium toxicity and 5 mg Se/kg DM appears to be the borderline for this effect (Ort and Latshaw, 1978) and also for the growth depression effect (Jensen and Chang, 1976; Jensen, 1986), the maximum tolerable level of selenium for poultry is set at 3 mg of Se/kg of feed.

Based on many laboratory and field studies (Hamilton, 2004), the selenium thresholds for adverse effects in fish and aquatic birds are 3–4 mg/kg DW in diet and 2–5 µg/L in water. Thus, a tentative maximum tolerable level of selenium is suggested as 2 mg of Se/kg of dry feed for fish and aquatic birds. Apparently, more research is needed for defined levels and appropriate safety factors for these species.

A study by Levander (1986) indicated that a dietary selenium concentration of 5 mg/kg was toxic to dogs. Although an upper limit of 2.0 mg of Se/kg of dog foods has been suggested by AAFCO (2001) for regulatory purposes, there are no data for this committee to set a maximum tolerable levels of selenium for dogs or cats (Wedekind et al., 2004).

A study in China (Yang and Zhou, 1994) indicates that 600 µg Se/day is considered the maximum individual safe selenium intake for humans. The current RDA is 55 µg/day for both men and women, and the tolerable upper intake level (UL) for adults is set at 400 µg/day by the Dietary Reference Intakes committee, Institute of Medicine (IOM, 2000). As eggs and muscle produced by animals fed diets supplemented with maximal tolerable levels of selenium, in particular with organic forms, may contain 0.7–2.5 mg Se/kg (Table 25-2), a consumption of 200 to 600 g of such products by a person per day could exceed the suggested UL. Thus, proper caution must be exercised whenever animal diets are supplemented with selenium.

FUTURE RESEARCH NEEDS

Maximum tolerable levels of selenium for given species in the future should be specifically defined with different forms of selenium, duration of exposure time, and nature of diet. Basic research on the impact of high levels of selenium on gene expression and cell signaling related to cell death and survival may help in understanding the molecular mechanisms of selenium toxicity. Effects of different dietary sources and levels of selenium on the expression and function of the selenoproteins other than glutathione peroxidase-1 should be studied in various farm animals. Impacts of feeding selenized feedstuffs to food animals on environmental accumulation, in particular on the possible toxicity to fish and aquatic birds, should be examined. Potential human nutrition and health benefits and risks of selenium-enriched animal products, generated from feeding animals with selenized plants or yeast, merit careful and long-term research.

SUMMARY

Selenium is a semimetal (metalloid) with four natural oxidation states (–2, 0, +4, and +6), and it shares similar chemical and biological properties with sulfur. Up to now, a total of 12 selenium-dependent enzymes and 13 selenoproteins have been identified in humans. The synthesis of seleno-enzymes or proteins is featured by the incorporation of selenium into the peptide as a selenocysteine moiety through a "co-translation" mechanism. Although the metabolic roles of many selenoproteins remain unknown, it is clear that the selenoenzymes are involved in antioxidation and thyroid hormone metabolism. Many sources of selenium are readily absorbed in the small intestine by animals and humans, and regulation of body selenium retention may occur via urinary excretion. In all species, selenium contents of tissues, blood, and milk (eggs) can be increased up to 50-fold of the normal baseline by dietary selenium supplementation. Organic selenium sources seem to be more effective than inorganic selenium salts, and selenomethionine may be more effective than selenized plants in raising tissue selenium concentrations.

Plant selenium contents depend on the amount of selenium in soil in which they grow, and vary greatly in different regions. Thus, both selenium deficiency and selenium toxi-

cosis are practical problems around the world. Selenium deficiency produces selenium-responsive diseases such as liver necrosis in rats, exudative diathesis in chicks, hepatosis dietetica in swine, white muscle disease in ruminants, and reproduction failure in various species. Selenium toxicosis may occur in animals grazing in seleniferous areas, in fish and birds that inhabit contaminated aquatic sites, and in animals overdosed with selenium under experimental condition or poor management. Sub-acute (blind staggers) and chronic (alkali disease) selenosis are more frequently seen than the acute toxicosis. Depression of growth performance is a very sensitive indicator of chronic selenium toxicosis across different species. Loss of hair and deformed hooves are also a feature of chronic selenosis in swine, cattle, and horses. Pigs consistently show neurological pathology under selenium toxicosis. Fish and birds including chicks and ducks are more susceptible to the teratogenic effect of selenium than mammals. Young animals are more sensitive to selenium toxicity than adult or old animals. Relative toxicity of different selenium supplements is largely related to their water solubility and nutrient bioavailability, and can be modulated by dietary factors such as protein, sulfate, vitamin E, and a number of trace elements including arsenic, copper, and mercury. Selenium is not listed as a suspected carcinogen. The maximum tolerable levels (mg/kg of diet or DM) of selenium are suggested as follows: ruminants, 5; swine, 4; poultry, 3; and fish and aquatic birds, 2.

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TABLE 25-1 Effects of Selenium Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Mice	5	6–7 wk BALB/c male (21–25 g BW)	0 1 3 9 mg/L	Sodium selenite Selenomethionine	14 d	Water	 No adverse effect Increased dopamine metabolites in striatum Reduced food and water consumption and BW gain	Tsunoda et al., 2000
Mice	7–10	4-wk, specific pathogen-free ICR male	0 2.4 4.8 and 7.1 mg Se/day	Seleno-DL-cystine	30, 60, or 90 d	Orally via a stomach tube, 6 d/wk	 No adverse effect Increased plasma aspartate aminotransterase and alanine aminotransterase activity	Hasegawa et al., 1994
Mice	Not listed	Study 1: Acute: 7 wk	Gavaged for 3 d with 0. 5 niL of Se at concentrations of 2,4,8,16 32 64 mg/L	Sodium selenite	10 d	Gavage	 No adverse effect One female mouse died after the second gavage All female mice in the 64 mg Se/L group died after the third gavage	Jacobs and Forst, 1981b
	10	Study 2: 7 or 18 wk	Se added in drinking water at 1, 4, 8, 16		46 d	Water	 All survived Increased serum alkaline phosphatase and glutamic oxaloacetic transaminase activities	

continued

TABLE 25-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Mice	50	Study 3: 6-wk female	64 mg/L Se added in drinking water at 1 or 4 8 mg/L		50 wk	Water	Reduction in survival and growth No adverse effect Reduced weight gain, and white blood cell counts and increased serum alkaline phosphatase and glutamic oxaloacetic transaminase activities	
Rats	10	Study 1: 5- or 12-wk Sprague-Dawley	Se added in drinking water at 1 or 4, 8 or 16 or 64 mg /L	Sodium selenite	35 d	Water	No adverse effect Decreased survival and weight gain 100% mortality by day 18	Jacobs and Forst, 1981a
	17–120	Study 2: 5- or 8-wk	Se added in drinking water at 0 4 mg/L		1–2 yr		Slightly greater age-dependent decline in hemoglobin and white blood cells	
Hamsters	10	4 wk	Study 1 0.25, 10, 20, 40 80 mg Se/kg of a casein based diet	Sodium selenite	21 d	Diet	Reduced BW gain and feed intake 30% mortality	Julius et al., 1983
				Sodium selenite or selenomethionine				
			Study 2 0.1, 5.0				No adverse effect	
			10 mg Se/kg of torula yeast-based diet				Reduced BW gain and feed efficiency	
			32				Reduction in survival and growth	

Chickens	30	Day-old Hubbard broilers or white leghorn chicks	Corn-soy basal +5 +10, 20 + 40, 80 mg Se/kg	Sodium selenite	2 wk	Diet	 Reduced BW gain Reduced growth performance 40–70% mortality	Jensen, 1975
Chickens	30	Single comb white leghorn cockerels	Corn-soy basal +5 +10, 20 mg Se/kg	Sodium selenite	2 wk	Diet	 No adverse effect Reduced growth performance	Jensen and Chang,1976
Chickens	40	Day-old male broiler chicks	Corn-soy basal (0.06 mg Se/kg) +0.1, 0.25, 1 + 5 mg Se/kg	Sodium selenite	3 wk	Diet	 No adverse effect Reduced growth performance	Jensen, 1986
Chickens	15	Male New Hampshire x Columbian chicks, 8 d post hatching	Corn-soybean meal basal diet 15 mg Se/kg of a corn-soybean meal diet	Sodium selenite or seleno-methionine	14 d	Diet	 Reduced weight gain and feed efficiency	Lowry and Baker, 1989
Ducklings	25	1-d (30-32 g)	0 15 30 mg Se/kg	Seleno-L-mehionine, seleno-DL methionine, selenized wheat, selenized yeast	14 d	Diet	 Reduced feed intake Reduced gain and feed intake, up to 44% mortality	Heinz et al., 1996
Ducklings	10–15 pairs	Adult males and females	0 10 mg Se/kg	Seleno-L-mehionine, seleno-DL-methionine, seleniferous yeast		Diet	 Decreased the percentage hatch of fertile eggs and number of 6-d-old ducklings produced per hen, decreased the survival percentage of young to 6-d-old	Heinz and Hoffman, 1996
Swine	16	Crossbred growing pigs	0 4 8 12, 16, and 20 mg Se/kg	Sodium selenite	35 d	Diet	 No adverse effect Reduced daily gain and feed intake Hoof lesion and paralysis	Goehring et al., 1984b

continued

TABLE 25-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Swine	15 (2 x 3 factorial with 2 sources of Se and 3 levels of Se)	12.5 kg growing pigs	0.3 5, 15 mg Se/kg	Sodium selenite and calcium selenite	35 d	Diet	 No effect on growth performance Reduced gain and feed intake	Mahan and Magee, 1991
Swine	5	8–10 wk	0.4 25 mg Se/kg	Seleno-DL-methionine, sodium selenate, *Astragalus* bisulcatus (300 mg Se/kg)	6 wk or until the pigs showed paralysis	Diet	 Neurological signs of paralysis, poor growth, symmetrical hair loss, dry scaling skin, and cracked, overgrown hooves	Panter et al., 1996
Swine	10 (a negative control + 2 x 4 factorial with 2 sources of Se and 4 levels of Se)	24.7-kg crossbred barrows	Basal (0.06-0.07 mg Se/kg), +5 +10, 15, or 20 mg/kg	Sodium selenite or Se-enriched yeast	12 wk	Diet	 No adverse effect Declined daily gain and feed intake, hair loss and separation of the hoof, and color change of bile	Kim and Mahan, 2001a
Swine	4–6 sows	Adults, 190–198 d	Basal sow diet (0.13 mg Se/kg) + 0 or 2 or 4 + 8 or 16 mg/kg	Sodium selenite	From 1st estrous through 9 wk postpartum	Diet	 No adverse effect Producing piglets with lower birth weights or weaning weights	Poulsen et al., 1989
Swine	11 (a 2 x 4 factorial with 2 sources of Se and 4 levels of Se)	25-kg gilts	Corn-soybean meal basal diet, + 0.3 or 3 + 7 or 10 mg Se/kg	Sodium seleniate or Se-enriched yeast	From 25 kg BW through one parity	Diet	 No adverse effect Lower gestation weights and lactation feed intakes; lower number of live pigs born and weaned, having pigs with hoof separation and alopecia	Kim and Mahan, 2001b

Cattle	9–12	Crossbred steers (351 kg BW)	Control (9.5 µg Se/kg BW) Se-treated groups (65µg Se/kg BW)	High-Se wheat high-Se hay and sodium selenate	120 d	Diet	 No effect on growth performance or carcass characteristics	Lawler et al., 2004
Cattle	4 (2 x 2 factorial: 2 dietary Se levels and 2 Se backgrounds)	374-kg Yearling steers from seleniferous or non-seleniferous areas	0.62 11.9 mg Se/kg of diet	Seleniferous or non-seleniferous wheat and hay	105 d	Diet	 No sign of toxicosis	Hintze et al., 2002
Cattle	2–5	Yearling steers, 10–12 mo	0.15 0.28 0.80 mg Se/kg BW	Sodium selenite and selenomethionine	120 d	Diet	 Hoof lesions in those fed selenomethionine Hoof lesions	O'Toole and Raisbeck, 1995
Cattle	6	Crossbred cow calves	0 0.25 mg/kg BW	Sodium selenite	16 wk	Diet	 Alopeica, cracking and enlargement of hooves, interdigital lesions, ring formation on the coronet region, and gangrene at tip of the tail	Kaur et al., 2003
Cattle	6	Adult Holstein (396–772 kg)	0 5.3–118 µg Se/kg BW	Sodium selenite	90–128 d		 No signs of toxicosis	Ellis et al., 1997
Cattle	8	3 d (42–45 kg)	0.2 1 3 5 10 mg Se/kg of diet in milk replacer (on DM basis)	Sodium selenite	42–54 d	Oral	 No adverse effect No adverse effect No adverse effect Reduced daily gain, feed efficiency, and blood packed cell volume	Jenkins and Hidiroglou, 1986

continued

TABLE 25-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Goats	2–10	6–8 mo	Single dose of	Sodium selenite	1 or 225 d	Oral as a drench		Ahmed et al., 1990
			160				Lethal	
			80				Lethal	
			or 40 mg of sodium selenite/kg BW;				Lethal	
			Repeated daily doses of					
			20.0				Death within 3 d	
			5.0				Death within 31 d	
			1.0				No sign of toxicity	
			0.5				No sign of toxicity	
			or 0.25 mg sodium selenite/kg BW, for 225 d				No sign of toxicity	
Sheep	5	50–80 kg yearling ewes	24 mg Se/kg	Sodium selenite	88 d	Diet	No adverse effect	Panter et al., 1995
			29 mg Se/kg	Astragalus bisculcatus			Minor wool loss	
			0.8 mg Se/kg	Alfalfa			No adverse effect.	
Sheep	24	Adult Columbia x Suffolk	Control forage: <0.32 mg Se/kg DM, drinking water containing < 1.7 µg Se/L	Se accumulated in forages due to phosphate mining or in normal areas	4-wk exposure followed by 2-wk depuration	Grazing (diet) and water	No signs of toxicity	Fessler et al., 2003
			Low Se forage: < 13.0 mg Se/kg DM, drinking water containing < 1.7 µg Se/L				No signs of toxicity	

		High Se forage: < 49.0 mg Se/kg DM, drinking water containing 340–415 µg Se/L (estimated intake 0.26 mg Se/kg BW/d)				One sheep died of Se toxicosis, and two others had Se concentrations in liver, kidney, and skeletal muscle indicative of high or toxic Se exposure	
Fish, fry of Chinook and Coho salmons	5–10	Various stages (0.5–2.6 g)	Various levels	Sodium selenite, sodium selenate, seleno-DL-methionine	24 or 96 h	Water	Selenite was more toxic than selenate. No mortality was caused by seleno-DL-methionine up to 21.6 mg Se/L, but this level produced pronounced surfacing behavior of at least 50% fish	Hamilton and Buhl, 1990

[a]Number of animals per treatment.
[b]Quantity of selenium dosed. SI conversion: 1 mg selenium equals 12.7 µmoles selenium.

TABLE 25-2 Selenium Concentrations in Fluids and Tissues of Animals

Animals	Quantity	Source	Whole Blood (B), Plasma (P), or Serum (S), mg/L	Milk, µg/L	Hoof or Nail, mg/kg
Female rhesus monkeys (6 kg, 3 year old) for 11- mo trial[a]	0.5 mg Se/L in water	Sodium selenite			
	0.5 mg Se/L in water	Selenomethionine			
Rats, adult Sprague-Dawley (268–332 g)	Basal diet (0.25 mg Se/kg)	Sodium selenite	0.56 (P)		
	Basal + water (4 mg/L)	Sodium selenite	0.68 (P)		
Rats, weanling male Sprague-Dawley rats for 8 week trial[a]	0.5 mg Se/kg diet	Sodium selenite	0.35 (P)		0.62[a]
	0.5 mg Se/kg diet	Selenomethionine	0.40 (P)		1.20[a]
	1.5 mg Se/kg diet	Sodium selenite	0.37 (P)		0.91[a]
	1.5 mg Se/kg diet	Selenomethionine	0.50 (P)		1.86[a]
	2.5 mg Se/kg diet	Sodium selenite	0.40 (P)		1.05[a]
	2.5 mg Se/kg diet	Selenomethionine	0.63 (P)		2.97[a]
Syrian hamsters, 4 wk old for 21-d trial[a]	0.25 mg Se/kg diet	Sodium selenite	0.17 (B)		
	10 mg Se/kg diet	Sodium selenite	0.49 (B)		
	20 mg Se/kg diet	Sodium selenite	0.78 (B)		
	40 mg Se/kg diet	Sodium selenite	1.11 (B)		
	80 mg Se/kg diet	Sodium selenite	2.35 (B)		
Male chicks, 8 d old for 14 d	Corn-soy basal diet				
	+ 15 mg Se/kg	Sodium selenite			
	+ 15 mg Se/kg	Selenomethionine			
Chicks, 4 wk old	Corn-torula yeast basal diet, 0.03 mg Se/kg				
	+ 0.06 mg Se/kg	Sodium selenite			
	+ 0.06 mg Se/kg	Selenomethionine			
	+ 0.06 mg Se/kg	Selenocysteine			
Laying hens	Corn-soy basal, 0.10 mg Se/kg				
	+ 0.32 mg Se/kg	Sodium selenite			
	Corn-soy basal, 0.42 mg Se/kg				
Turkeys, 14–20 wk old[a]	Practical diets, 0.13–0.20 mg Se/kg		0.19 (P)		
	+ 0.10 mg Se/kg	Sodium selenite	0.19 (P)		
	+ 0.20 mg Se/kg	Sodium selenite	0.20 (P)		
Young pigs, 8–10 wk old for 6-wk experiment[a]	Control, 0.4 mg Se/kg diet		0.3 (B)		
	25 mg Se/kg	Seleno-DL-methionine	5.6 (B)		
	25 mg Se/kg	*Astragalus bisulcatus*	2.0 (B)		
	25 mg Se/kg	Sodium selenate	2.4 (B)		
Growing crossbreed pigs (25 kg) for 12 wk[a]	Basal		0.11 (P)		0.41[a]
	5 mg Se/kg	Sodium selenite	0.66 (P)		1.26[a]
	10 mg Se/kg	Sodium selenite	1.10 (P)		4.89
	15 mg Se/kg	Sodium selenite	1.89 (P)		12.64[a]
	20 mg Se/kg	Sodium selenite	1.88 (P)		5.99[a]
	5 mg Se/kg	Selenized yeast	0.78 (P)		9.01[a]
	10 mg Se/kg	Selenized yeast	1.67 (P)		15.99[a]
	15 mg Se/kg	Selenized yeast	2.49 (P)		28.86[a]
	20 mg Se/kg	Selenized yeast	3.23 (P)		18.46[a]

Hair, mg/kg	Kidney, mg/kg	Liver, mg/kg	Muscle, mg/kg	Spleen, mg/kg	Reference
0.60[a]		0.24[a]	0.06[a]		Butler et al., 1990
4.20[a]		0.70[a]	0.68[a]		
	1.23	0.68	0.13		Janghorbani et al., 1990
	2.85	1.61	0.18		
0.38[a]		0.76[a]	0.09[a]		Salbe and Levander, 1990
1.23[a]		0.97[a]	0.38[a]		
1.41[a]		1.17[a]	0.12[a]		
3.01[a]		1.54[a]	0.91[a]		
2.14[a]		1.24[a]	0.15[a]		
4.60[a]		2.30[a]	1.45[a]		
	0.60[a]	0.50[a]	0.26[a]		Julius et al., 1983
	1.46[a]	1.56[a]	.024[a]		
	1.88[a]	5.36[a]	0.31[a]		
	3.18[a]	7.22[a]	0.46[a]		
	5.41[a]	10.10[a]	0.60[a]		
		0.7–2.0			Lowry and Baker, 1989
		14.0–19.0			
		53.2			
	0.18	0.15	0.05		Osman and Latshaw, 1976
	1.33	0.78	0.14		
	1.18	0.68	0.19		
	1.45	0.77	0.13		
	0.32	0.43	0.33		Latshaw, 1975
	0.74	0.82	0.42		
	2.47	1.92	1.18		
		0.66[a]	0.20[a]		Cantor and Scott, 1975
		0.66[a]	0.20[a]		
		0.66[a]	0.20[a]		
	7.2[a]	2.3[a]		2.1[a]	Panter et al., 1996
	118.3[a]	64.6[a]		75.9[a]	
	16.6[a]	12.4[a]		4.9[a]	
	20.1[a]	12.9[a]		7.1[a]	
	1.66[a]	0.40[a]	0.15[a]	0.24[a]	Kim and Mahan, 2001a
	3.11[a]	3.09[a]	0.33[a]	0.81[a]	
	6.67[a]	6.40[a]	0.28[a]	1.28[a]	
	8.78[a]	7.12[a]	0.32[a]	1.47[a]	
	8.57[a]	8.41[a]	0.32[a]	1.89[a]	
	5.30[a]	5.59[a]	3.38[a]	2.41[a]	
	9.71[a]	11.57[a]	5.93[a]	4.89[a]	
	13.77[a]	17.47[a]	10.31[a]	7.24[a]	
	16.29[a]	17.69[a]	7.65[a]	8.31[a]	

continued

TABLE 25-2 Continued

Animals	Quantity	Source	Whole Blood (B), Plasma (P), or Serum (S), mg/L	Milk, mg/L	Hoof or Nail, mg/kg
Gilts, from 25 kg to first parity[a]	0.3 mg Se/kg	Sodium selenite	0.19 (S)	40	0.27[a]
	3.0 mg Se/kg	Sodium selenite	0.38 (S)	170	0.50[a]
	7.0 mg Se/kg	Sodium selenite	0.65 (S)	180	1.42[a]
	10.0 mg Se/kg	Sodium selenite	0.82 (S)	760	2.00[a]
	0.3 mg Se/kg	Selenized yeast	0.20 (S)	90	0.84[a]
	3.0 mg Se/kg	Selenized yeast	0.50 (S)	650	3.61[a]
	7.0 mg Se/kg	Selenized yeast	1.37 (S)	1,610	16.17[a]
	10.0 mg Se/kg	Selenized yeast	1.74 (S)	4,140	12.27[a]
Crossbred steers (351 kg BW) for 120-d feeding	9.5 µg/kg BW				
	65.0 µg/kg BW	Sodium selenite			
	65.0 µg/kg BW	High Se hay			
	65.0 µg/kg BW	High Se wheat			
374-kg Yearling steers from seleniferous or non-seleniferous areas for 105-d feeding trial	Animals from seleniferos area				
	0.62 mg Se/kg diet	Control	0.2–0.5 (P)		
	11.9 mg Se/kg diet	High Se hay	0.6–0.7 (P)		
	Animals from non-seleniferos area				
	0.62 mg Se/kg diet	Control	~0.1 (P)		
	11.9 mg Se/kg diet	High Se wheat/hay	0.1–0.7 (P)		
Adult Holstein cows for 90–136 d (396–772 kg)	0 mg Se/cow/d		0.05 (S)		
	3 mg Se/cow/d (5.3 µg/kg BW)	Sodium selenite	0.06 (S)		
	20 mg Se/cow/d (34.5 µg/kg BW)	Sodium selenite	0.075 (S)		
	50–100 mg Se/cow/d (86.9 µg/kg BW)	Sodium selenite	0.10–0.25 (S)		
Holstein cows 60 d prepartum for 120 d	Control		0.11 (B)	40	
	+ 3 mg Se/cow/d	Sodium selenite	0.13 (B)	56 (colostrum)	
Holstein cows	0.1 mg Se/kg DM	Sodium selenite	0.16 (B)	18	
	0.1 mg Se/kg DM	Se-yeast	0.15 (B)	21	
Primiparous Swedish red and white cows	Basal (0.11 mg Se/kg DM)		0.10 (B)	14	
	+ 3 mg Se/cow/d (0.28 mg Se/kg DM)	Sodium selenite	0.14 (B)	16	
	+ 3 mg Se/cow/d (0.28 mg Se/kg DM)	Sodium selenate	0.14 (B)	16	
	+ 3 mg Se/cow/d (0.28 mg Se/kg DM)	Se-yeast	0.17 (B)	31	
Calves, 8 mo old[a]	Roughage and concentrate		0.021 (P)		
	+ 0.1 mg Se/kg	Sodium selenite	0.022 (P)		
	+ 0.2 mg Se/kg	Sodium selenite	0.024 (P)		
Holstein calves, 3 d old for 6- to 8-wk experiment	Basal, 0.2 mg Se/kg		0.08 (B)		
	1 mg Se/kg	Sodium selenite	0.10 (B)		
	3 mg Se/kg	Sodium selenite	0.19 (B)		
	5 mg Se/kg	Sodium selenite	0.26 (B)		
	10–40 mg Se/kg	Sodium selenite	0.24–2.08 (B)		
Yearling ewes (50–80 kg) for 88-d trial	Control, 0.8 mg Se/kg (in alfalfa)		0.45 (B)		
	24 mg Se/kg	Sodium selenite	2.4 (B)		
	29 mg Se/kg	*Astragalus bisculcatus*	1.3 (B)		
Lambs, 46 kg[a]	Complete roughage and grain, 0.06 mg Se/kg		0.032 (P)		
	+ 30 mg Se/kg in salts	Sodium selenite	0.078 (P)		
	Complete roughage and grain				
	+ 0.20 mg Se/kg	Sodium selenite	0.11 (P)		
	+ 0.20 mg Se/kg	Organic form of Se	0.16 (P)		
	+ 0.30 mg Se/kg	Sodium selenite	0.13 (P)		
	+ 0.30 mg Se/kg	Organic form of Se	0.16 (P)		

[a]Fresh (wet) tissue basis.

Hair, mg/kg	Kidney, mg/kg	Liver, mg/kg	Muscle, mg/kg	Spleen, mg/kg	Reference
0.49[a]	2.14[a]	0.58[a]	0.15[a]	0.34[a]	Kim and Mahan, 2001b
1.38[a]	2.56[a]	2.11[a]	0.17[a]	0.66[a]	
2.15[a]	3.99[a]	3.60[a]	0.24[a]	0.92[a]	
2.83[a]	4.42[a]	3.43[a]	0.23[a]	1.62[a]	
0.86[a]	2.49[a]	0.75[a]	0.28[a]	0.42[a]	
5.12[a]	4.99[a]	2.77[a]	1.69[a]	1.59[a]	
7.96[a]	8.69[a]	7.16[a]	4.14[a]	4.22[a]	
10.53[a]	9.29[a]	8.93[a]	5.33[a]	5.20[a]	
1.80	8.4	2.33	1.33	2.00	Lawler et al., 2004
4.00	10.05	9.91	1.55	2.60	
5.93	10.86	6.56	3.32	3.82	
10.54	12.89	10.79	4.41	5.16	
					Hintze et al., 2002
	2.12	0.97	1.20		
	3.58	4.69	2.09		
	2.45	0.89	0.40		
	4.73	5.94	1.56		
		~1.2			Ellis et al., 1997
		~1.4			
		~4.3			
		~6.5–15.0			
					Abdelrahman and Kincaid, 1995
					Fisher et al., 1995
					Ortman and Pehrson, 1999
	1.01[a]	0.09[a]	0.03[a]		Ammerman et al., 1980
	1.22[a]	0.11[a]	0.04[a]		
	1.29[a]	0.11[a]	0.04[a]		
	2.93	1.09	0.27		Jenkins and Hidiroglou, 1986
	3.08	3.43	0.32		
	3.42	4.74	0.034		
	4.02	9.90	0.59		
	5–32	28–188	0.6–1.1		
					Panter et al., 1995
	0.99[a]	0.19[a]	0.06[a]		Ullrey et al., 1977
	1.24[a]	0.27[a]	0.07[a]		
	1.26[a]	0.38[a]	0.09[a]		
	1.30[a]	0.62[a]	0.17[a]		
	1.22[a]	0.53[a]	0.11[a]		
	1.35[a]	0.66[a]	0.16[a]		

26

Silicon

INTRODUCTION

Silicon (Si) in its elemental form is a shiny metallic-looking substance that is hard and brittle with a crystalline structure similar to that of diamond. Silicon is the second most abundant element in the Earth's crust; 27.7 percent of the lithosphere is silicon. It occurs in nature mostly in the oxide and silicate form. Silicon dioxide (SiO_2) occurs mainly in the crystalline form as quartz or sand. Asbestos, feldspars, clays, and micas are examples of silicate minerals.

Silicon is prepared commercially by heating silica and carbon to 1,600°–1,800°C in an electric furnace (Hunter and Aberg, 1975). At this temperature carbon takes oxygen from silica to form carbon monoxide. Whereas silica is the term often used for naturally occurring substances composed mainly of silicon dioxide, silicone (organosiloxane) is the term used for synthetic polymers with a structure of alternating oxygen and silicon atoms (Hunter and Aberg, 1975).

Adding water to silicates liberates orthosilicic acid, which apparently is an important biological form of silicon. Orthosilicic acid ($Si(OH)_4$), also known as monosilicic acid or monomeric silica, polymerizes in neutral solutions at concentrations greater than 2 mmol/L. Some lower forms of life and plants may use this reaction to form polymeric silica, or phytolithic silica, for structure and growth. In higher animals and humans, monosilicic acid is thought to be a circulating form of silicon. Precipitated silica, or colloidal silicic acid (approximate formula of H_3SiO_3), is an insoluble form of silicon and occurs in nature as opal. The condensation of orthosilicic acid to form precipitated silica is a method for making silica gel, an adsorbent.

Silicon is tetravalent and has a strong affinity for oxygen. The chemistry of silicon is similar to that of carbon, its sister element (Wannagat, 1978). Silicon forms silicon-silicon, silicon-hydrogen, silicon-oxygen, silicon-nitrogen, and silicon-carbon bonds. Thus, organosilicon compounds are analogues of organocarbon compounds. However, the substitution of silicon for carbon, or vice versa, in organocompounds results in molecules with different properties because silicon is larger and less electronegative than carbon. A review of the aqueous chemistry of silicon shows that it is quite complex (Knight and Kinrade, 2001).

Elemental silicon is used as a processing aid in the manufacture of aluminum, aluminum alloys, iron and steel, and as the basic raw material to synthesize silicones (Corathers, 2002). The semiconductor industry, which manufactures chips for computers from high-purity silicon, accounts for only a small percentage of silicon use (Corathers, 2002). Silicon is a major component of ceramics, building materials, and glasses. Amorphous silica is used extensively in the food and pharmaceutical industries. It is used as an anti-caking agent for dry powders, a dispersion agent for dry powders in liquids to prevent clumping, an anti-settling or suspending agent, a stabilizer in oil-water emulsions, a thickening or thixotropic agent (viscosity control/dough modifier), a gelling agent, a flavor carrier, an extrusion aid, a clarification and separation aid, a general excipient (pharmaceuticals and cosmetics), and a support matrix for immobilization of enzymes (Villota and Hawkes, 1986). Colloidal silicic acid is used in beauty products, and in acne and gastritis medications.

ESSENTIALITY

Silicon is nutritionally essential for some lower forms of life (Carlisle, 1984). Silicon has a structural role in diatoms, radiolarians, and some sponges. It may be essential for some higher plants (e.g., rice). Diatoms, which are unicellular microscopic plants, have an absolute requirement for silicon as monomeric silicic acid for normal cell growth. The diatom, *Cylindrotheca fusiformis,* has five silicon transporter genes that tightly control silicon uptake and use in cell wall formation (Hildebrand et al., 1998). Findings from experiments comparing low intakes (<2.0 mg/kg diet) with physiological intakes (4.5–35 mg/kg diet) suggest that silicon has an essential role in collagen and glycosami-

noglycan formation or function, and thus influences bone formation, wound healing, and ectopic calcification in rats (Seaborn and Nielsen, 1993, 2002c). However, silicon is generally not accepted as an essential nutrient for higher animals, apparently because of the lack of a clearly defined specific biochemical function.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

The colorimetric determination of silicate using the ammonium molybdate reaction is quite sensitive but is prone to forming molybdenum blue (the final product of the colorimetric assay) in the presence of substances other than the yellow ammonium molybdate silicate complex (Schwarz, 1978). Thus, analytical techniques using inductively coupled plasma-atomic emission spectrometry (ICP-AES), direct current plasma atomic emission spectrometry (DCP-AES), or electrothermal atomic absorption spectrometry (ETAAS) have become methods generally used for the determination of silicon in biological and clinical samples (Klemens and Heumann, 2001). Recently, an inductively-coupled plasma-high resolution isotope dilution mass spectrometric (ICP-HRIDMS) method (Klemens and Heuman, 2001) was developed that may give more accurate determinations of silicon than in the past. Because the isotope-dilution step is performed during microwave-assisted sample decomposition in a closed system, loss of analyte after opening the digestion vessel has no effect on the analytical result (unlike other analytical methods).

Regardless of the instrumentation used, a major problem in silicon analysis is the high risk of contamination because of its ubiquity. This is probably the reason that more modern techniques and instrumentation indicate that silicon concentrations reported in the older biological and food composition literature often are too high. The use of clean room techniques to avoid dust, avoiding contact with glass, and use of water and reagents purified to remove silicon (Klemens and Heumann, 2001) are practices that help reduce contamination of samples to be analyzed. The digestion of biological samples to solubilize silicon is also a demanding task because of the low solubility of silica in acid media. Two methods that can be used are digesting with an acid mixture containing hydrofluoric acid or with an alkaline medium (Van Dyck et al., 1998; Hauptkorn et al., 2001). The hydrofluoric acid method is hazardous and care must be taken to prevent the etching of glassware (containing silicon). Currently, the most acceptable method is a nonoxidative alkaline digestion procedure using tetramethylammonium hydroxide and a high-pressure, microwave-assisted autoclave digestion system (Hauptkorn et al., 2001). Because of concerns about contamination and inadequate digestion, the validity of any silicon analysis can only be assured by the use of quality control procedures.

REGULATION AND METABOLISM

Absorption and Metabolism

A study with guinea pigs indicated that silicon is absorbed mainly as monomeric silicic acid (Sauer et al., 1959). In humans, monosilicic acid in foods and beverages is readily absorbed and excreted in urine (Baumann, 1960). Some of the absorbed monosilicic acid can come from food polymeric silica, which can be partly dissolved by fluids of the gastrointestinal tract. The mechanisms involved in the intestinal absorption and blood transport of silicon are unknown. Silicon is not bound to protein in plasma, where it is believed to exist almost entirely as undissociated monomeric silicic acid (Carlisle, 1984; Berlyne et al., 1986). Connective tissues, including aorta, bone, skin (and its appendages), tendon, and trachea, contain much of the silicon that is retained in the body (Carlisle, 1984; Adler et al., 1986), where it is thought to be present as a silanolate, an ether or ester derivative of silicic acid (Schwarz, 1974). Absorbed silicon is mainly eliminated via the urine, where it probably exists as orthosilicic acid and/or magnesium orthosilicate (Carlisle, 1984; Berlyne et al., 1986). The upper limits of urinary excretion apparently are set by the rate and extent of silicon absorption and not by the excretory ability of the kidney because peritoneal injection of silicon can elevate urinary excretion above the upper limit achieved by dietary intake (Sauer et al., 1959). The form of dietary silicon determines whether it is well absorbed. For example, humans absorbed only ~1 percent of a large dose of an aluminosilicate compound but absorbed >70 percent of a single dose of methylsilanetriol salicylate, a drug developed for the treatment of circulatory ischemia (Allain et al., 1983). The amount in the diet also affects silicon absorption. In rats, guinea pigs, cattle, and sheep, urinary excretion of silicon increases with an increasing intake of siliceous substances, but reaches a maximum that is not exceeded by increasing the intake (Bailey, 1981). For example, in sheep, the amount of silicon increased in urine as dietary silica increased from 0.10 to 2.84 percent, but reached a maximum of 250 mg of silica per day, which was less than 4 percent of the total intake (Jones and Handreck, 1965). Similar results were found in another study with sheep where only 0.8 percent of silica was absorbed from a diet supplying 20 grams per day (Bailey, 1981). These studies indicated that <1 percent of the silicon in diets that predispose to the formation of calculi in cattle and sheep is excreted in the urine. It has been suggested that the maximum urinary output of monosilicic acid is attained in ruminants when enough soluble silicon is ingested to saturate the reticulo-rumen fluid with monosilicic acid (Jones and Handreck, 1965).

Metabolic Interactions and Mechanisms of Toxicity

Ingested silicon has a relatively low order of toxicity. The most common pathological condition that may occur with a

high intake of silicon is urolithiasis (Bailey, 1981). A low output of water in urine relative to the output of orthosilicic acid, resulting in high urinary concentration or supersaturation of orthosilicic acid, apparently is a necessary condition for calculus formation. The formation of calculi is promoted by other urine properties, including a high pH (Schreier and Emerick, 1986; Emerick and Lu, 1987) and the presence of specific proteins (Bailey, 1981). In addition to urolithiasis, a high intake of silicon might interfere with the absorption or use of some essential nutrients, particularly zinc. An antagonism between zinc and silicon explains why dietary silicon decreases the zinc concentration in plasma and tissues of rats (Emerick and Kayongo-Male, 1990; Najda et al., 1992). In contrast to zinc, copper use or absorption apparently is enhanced by high dietary silicon in rats and turkeys (Emerick and Kayongo-Male, 1990; Najda et al., 1992; Kayongo-Male and Palmer, 1998). Also, relatively high dietary silicon (600 mg/kg) was protective against aluminum neurotoxicity characterized by depressed zinc and increased aluminum concentrations in brain that were apparently exacerbated by silicon deprivation (Carlisle and Curran, 1987; Carlisle et al., 1991).

SOURCES AND BIOAVAILABILITY

Forages and cereal grains high in fiber (e.g., oats and barley) are the major sources of silicon for animals (Bailey, 1981; Bowen and Peggs, 1984; Pennington, 1991). Silicon, present in plants as silica and soluble silicates, and in organic combinations, is bound to the cellulosic cell structure (Bailey, 1981). Hydrated silica known as opaline silica or silica gel is commonly deposited in plants in the form of particles called phytoliths. Each plant has a characteristic phytolith shape and these shapes vary enormously between plants (Carlisle, 1984). Soil type, plant species, transpiration rate, and nutrient supply affect the silica content of plants. Contamination of feeds, especially hay and pasture herbage, with soil elevates their content of silicon. The amount of silicon provided by the diet in areas where urolithiasis is a problem for ruminants can be extremely high (Bailey, 1981). The graminous species that constitute the main diet of afflicted animals contain high amounts of silica (up to 6 percent) DW. It has been estimated that yearling steers weighing 300 kg consuming native range hay or grass in the semi-arid Northern Great Plains of North America would consume about 500 g of silica per day. Sheep on common diets in Australia consume up to 20 g of silica per day and, when grazing on native grass, might consume as much as 40 g per day. Natural waters apparently are not a major source of silicon because they contain only 0.8–44 mg/L (Farmer, 1986). Other sources of silicon in animal feeds are grit added to some poultry diets, and sand added to some feeds as a "bulking" agent.

Early balance studies in animals indicated that almost all ingested silicon is unabsorbed. As indicated above; the low absorption probably was the result of intakes of silicon that exceeded the amount needed to achieve maximal absorption. Thus, these studies do not indicate the bioavailability of silicon when consumed in low or milligram quantities from various foods and feeds. A recent study with humans consuming diets providing about 30 mg of silicon per day indicated that a substantial amount of food silicon is absorbed. Jugdaohsingh et al. (2002) found that an average of 41 percent of dietary silicon was excreted in the urine (an indicator of absorption). Silicon in grains and grain products was readily absorbed, as indicated by a mean urinary excretion of 49 ± 34 percent of intake. In several of the grain products, silicon was as available as it was from fluids. For example, urinary silicon excretion was 41–86 percent from corn flakes, white rice, and brown rice, and 50–86 percent from mineral waters. Silicon in fruits and vegetables, except green beans and raisins, was readily absorbed, with mean urinary excretion 21 ± 29 percent of intake.

Additives in prepared feeds, foods, confections, and pharmaceuticals are another source of oral silicon. Amorphous silicates are considered safe additions to foods. Their use as anti-caking agents, for example, is permitted in amounts up to 2 percent by weight. A GRAS (Generally Recognized As Safe) committee concluded that silicates added to foods to enhance their physical properties are relatively inert and thus not bioavailable. Sodium zeolite A, a hydrated aluminosilicate that breaks down into monosilicic acid and aluminum in the gut (Benke and Osborn, 1979), has been used as a dietary supplement to increase chicken eggshell thickness (Roland, 1988), prevent parturient paresis in dairy cows (Thilsing-Hansen and Jorgensen, 2001), and decrease bone-related injuries in horses (Frey et al., 1992; Nielsen et al., 1993). The mean absorption of silicon from Zeolite A administered orally to dogs as a capsule, solution, or suspension was reported to be 2.33, 3.44, and 2.74 percent, respectively (Cefali et al., 1996).

TOXICOSIS

The toxic effect of high silica exposure through inhalation or injection, which can cause lung and renal damage, is not relevant to the mission of this document and thus will not be presented. Also, unlike the predecessor of this document (NRC, 1980), this review will not tabulate studies that show relatively high amounts of orally ingested silicon (generally between 250 and 500 mg/kg diet) as having no or beneficial effects. Examples of beneficial effects include prevention of aluminum toxicity to fish by silicic acid addition to water (Birchall et al., 1989) and improvement of growth, reduction of rachitic lesions, and enhancement of bone strength in calcium-deficient chicks by 0.75 percent and 1.5 percent dietary zeolite (Leach et al., 1990; Watkins and Southern, 1991). This contrasts with the exacerbation by 0.5–1.0 percent dietary zeolite of signs of phosphorus deficiency, including decreased feed efficiency, tibial ash, egg produc-

tion, and egg weight; zeolite did not affect these variables when dietary phosphorus was adequate (Roland, 1990; Moshtaghian et al., 1991). It is unclear whether the primary interaction is between phosphorus and silicon or phosphorus and aluminum. Although not a true toxicity action, a detrimental effect of silicon for ruminants is that it depresses DM digestibility of forages (Van Soest and Jones, 1968). Most silicon compounds are essentially nontoxic to humans when taken orally. Magnesium trisilicate, an over-the-counter antacid, has been used by humans for more than 40 years with only minimal apparent deleterious effects reported. Carlisle (1984) stated in a review that, as of 1964, only nine cases of urinary calculi containing silicon and associated with high and long-term use of magnesium silicate had been recorded.

Acute

The acute toxicity of silicon (as silica) is low. No significant acute toxicity or mortality has been reported in animals given doses of up to 3,000 mg/kg BW per day. An oral dose of 3 g of sand given to Leghorn cockerels did not affect true metabolizable energy of a laying hen diet or corn (Sibbald, 1980). Various handbooks have reported very high oral silicon LD_{50} values for rats and mice. For rats and mice, respectively, these include >22.5 and >15 g/kg as silicon dioxide (Hartley and Kidd, 1987), 1.1–1.6 and 1.1 g/kg (Gosselin et al., 1984; International Technical Information Institute, 1988) as sodium silicate, and 1.28 and 2.4 g/kg as sodium metasilicate (Clayton and Clayton, 1981–1982). The oral LD_{50} for amorphous hydrophobic silica (food additive) was reported to be >7.9 g/kg for rodents (Lewinson et al., 1994). The World Health Organization (1970) indicated that the probable lethal dose of oral silica or magnesium trisilicate for humans is over 15 g/kg BW, and for sodium silicate is between 0.5 and 5 g/kg BW. The LC_{50} for carp was reported as >10,000 mg/L/72 h (Hartley and Kidd, 1987).

Chronic

Table 26-1 summarizes the doses and effects of a chronic consumption of high amounts of silicon by various animals and humans. The table indicates that extremely high amounts of silicon are needed to have just relatively minor effects on growth. For animals, the most serious toxic effect of silicon is the formation of kidney stones in ruminants. Silicon urolithiasis is a concern in range animals in western Australia, western regions of Canada, and the arid northwestern United States where pasture plants contain high amounts of opaline silica. In other parts of the world, silicon toxicity is not a serious problem under practical farm or ranch conditions. Siliceous calculi have been found in the urinary tracts of at least 50 percent, and in some cases as many as 80 percent, of cattle in North American range herds (Bailey, 1981). Displacement of large calculi from the bladder to the urethra can obstruct the normal flow of urine and distend the abdomen to produce the condition known as "water belly." If treatment is not begun within a day or two, the obstruction is fatal because it causes the rupture of the bladder or urethra. Mortality as high as 5–10 percent has been found in sheep in areas of Australia where calculi most commonly occur, and up to 5 percent among range steers in problem areas of North America (Bailey, 1981).

An early study with female rats provided water containing 280 and 561 mg Si/L as sodium metasilicate for 2.5 years and found a decrease in young born and weaned (Smith et al., 1973). Amorphous silica apparently is nontoxic to reproduction. Rats, mice, rabbits, and hamsters fed 1,350, 1,340, 1,600, and 1,600 mg Si/kg BW per day, respectively, did not exhibit any developmental toxicosis or teratogenicity (Food and Drug Research Laboratories, 1973).

Factors Influencing Toxicity

Dietary fiber may affect silicon toxicity because in humans it significantly depressed silicon balance (Kelsay et al., 1979). Sex and age of animals apparently affect the response to high dietary silicon. Supplementing drinking water with sodium silicate to provide 280 mg Si/L depressed weight gain in female but increased gain in male rats (Smith et al., 1973), and 374 mg Si/L had a similar effect in sheep (Smith et al., 1972). In an experiment where rats aged 3–7 weeks at the start were fed a urolithic diet containing 2 percent tetraethylorthosilicate, the incidence of urolithiasis decreased 8.8 percent with each week increase in age (Emerick, 1986).

TISSUE LEVELS

Silicon is widely distributed in tissues. Highest concentrations are found in bone, nails, tendons, and vascular tissue. Table 26-2 shows representative silicon concentrations in some organs and fluids from various animals and humans. Many of these data were obtained by using older colorimetric analytical procedures; thus, the values given should be considered only approximate. Nonetheless, this table shows that no animal tissue or fluid used as a food will accumulate silicon to the extent that it is of toxicological concern for humans.

MAXIMUM TOLERABLE LEVELS

Because it is relatively nontoxic, establishing maximum tolerable levels for animals is difficult. The lack of developmental toxicosis or teratogenicity in several nonruminant animal species at intakes of 1,340–1,600 mg Si/kg BW per day as amorphous silicon dioxide (Food and Drug Research Laboratories, 1973) indicates that the tolerable level for this form of silicon is high. The maximum tolerable level for the more bioavailable silicates (e.g., sodium metasilicate) is less than that for silica, but still high based on findings of Smith

et al. (1973). They found that long-term (2.5 years) consumption of water containing 280 and 561 mg Si/L as sodium metasilicate moderately decreased reproductive performance of rats (less young born and weaned). Ruminants apparently tolerate higher intakes of silicates than nonruminants. No calculi were found in sheep fed diets containing up to 0.57 percent (5,700 mg/kg) silicon as sodium silicate (Emerick et al., 1959). Drinking water containing 374 mg Si/L as sodium silicate had equivocal effects on the growth of growing-finishing lambs (Smith et al., 1972). Prabowo and Spears (1992) found that supplementing a coastal Bermuda grass-based diet (containing 0.72 percent silica) with 1.5 percent silicic acid (4,350 mg Si/kg) only had minor effects on the metabolism of some minerals in lambs. The findings suggest that the added soluble forms of silicon were converted into insoluble forms in the gastrointestinal tract (Prabowo and Spears, 1992). Considering that diets containing up to 600 mg Si/kg as metasilicate were found beneficial to chicks and rats (Carlisle, 1984), and that 4,350 mg Si/kg diet as silicic acid had only minor effects in lambs, the maximum tolerable level of 2,000 mg Si/kg diet suggested for animals in 1980 (NRC, 1980) seems reasonable for soluble silicon compounds. Much higher tolerable levels are appropriate for insoluble forms of silicon (e.g., 50,000 mg/kg diet).

No acute oral silicon toxicity signs have been identified for humans. The occurrence of silica stones in people on long-term antacid therapy with magnesium trisilicate has been reported. Because of the inadequacy of available data, no maximum tolerable level for humans has been established in the United States. In the United Kingdom, a Safe Upper Level of 12 mg/kg BW per day has been suggested for humans based on an animal study performed by Takizawa et al. (1988). This value seems conservative, considering that Takizawa et al. (1988) found NOAELs of 50,000 mg/kg diet supplemental dietary silica, equivalent to 2,500 mg/kg BW per day for rats, and 7,500 mg/kg BW per day for mice. Thus, the amount of silicon in tissues of animals fed high silicon diets are not of concern for human health.

FUTURE RESEARCH NEEDS

There are no apparent pressing research needs in the area of silicon toxicity. However, the determination of mechanisms involved in silicon absorption and transport, mechanisms in and factors affecting silica urolith formation, and the silicon concentrations in various organs of domestic animals and humans, would further the understanding of the toxicological properties of various silicon compounds.

SUMMARY

Next to oxygen, silicon is the most abundant element in the Earth's crust. Silicon is tetravalent and has a strong affinity for oxygen, and thus occurs in nature mostly as the oxide or silicate. The chemistry of silicon is similar to carbon. Silicon is essential for some lower forms of life where it has a structural role. Silicon generally is not accepted as essential for higher animals and humans because it lacks a defined biochemical function. Analytical techniques that use ion-coupled plasma have become preferred for the analysis of silicon in biological materials. Ashing of samples is a critical step in the accurate analysis of silicon in low amounts because of contamination and low silica solubility concerns. Dietary silicon in low amounts is well absorbed (~50 percent) based on human findings. Based on studies mainly with ruminants, only a small amount of silicon (generally less than 4 percent) is absorbed when the diet contains high amounts of silicon as silica. Forages and cereal grains high in fiber are the major sources of silicon for animals. The harmful effects of an excessive intake of silicon in animals include a depression in roughage DM digestibility and formation of urinary calculi for ruminants, and depressed growth and abnormal reproduction for rats. Extremely high intakes of silicon are required to have minor effects on growth and reproduction. The maximum tolerable level for silicon as amorphous silicon dioxide is >1,300 mg/kg BW per day for animals. The maximum tolerable level for silicon as "soluble silica" (e.g., sodium metasilicate) of 2,000 mg/kg diet seem appropriate. Except for a few areas in the world where conditions are right for urolithiasis, silicon toxicity is not a major problem for domestic animals.

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TABLE 26-1 Effects of Silicon Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Dogs	24	Mature	5.2%	Silicic acid and talc	Up to 14 mo	Diet	Renal and cystic calculi; urinary blockage; bladder ulceration and perforation; death	McCullagh and Ehrhart, 1974
Mice	40	Weanling	50 g/kg	Silicon dioxide (syloid)	Up to 21 mo	Diet	Reduced growth	Takizawa et al., 1988
Rats	59	Mature	280 and 561 mg/kg	Sodium silicate	2.5 y	Water	Decreased young born and weaned	Smith et al., 1973
Rats	40	Weanling	25 and 50 g/kg	Silicon dioxide (syloid)	Up to 21 mo	Diet	Reduced liver weight	Takizawa et al., 1988
Rats	120 and 140	~50 g	2,700 mg/kg	Tetraethyl-orthosilicate	8 wk	Diet	Reduced weight; urolithiasis	Schreier and Emerick, 1986; Emerick and Lu, 1987
Rats	100	~200 g	0.05, 0.1, and 0.2%	Sodium metasilicate	18 wk	Water	Depressed antioxidant enzyme activity	Najda et al., 1994
Rats	120	53–55 g	2,700 mg/kg	Tetraethyl-orthosilicate	8 wk	Diet	Urolithiasis	Stewart et al., 1993
Rats	72	45 g	500 mg/kg	Tetraethyl-orthosilicate, sodium silicate, and sodium zeolite	8 wk	Diet	Depressed growth rate	Kayongo-Male and Jia, 1999
Turkeys	72	54 g	270 mg/kg	Tetraethyl-orthosilicate, sodium silicate, and sodium zeolite	4 wk	Diet	Depressed growth rate	Kayongo-Male and Jia, 1999
Cows	14	0–10 mo	3%	Prairie hay	10 mo	Diet	Calculi formation	Bailey, 1967
Sheep	160	Wether lambs	1%	Diet (hay)	91 d	Diet	Urolithiasis	Stewart et al., 1990
Sheep	18	32 kg	1,450 and 4,350 mg/kg	Silicic acid	19 d	Diet	Slightly depressed apparent absorption and retention of manganese and calcium	Prabowo and Spears, 1992

[a]Number of animals per treatment.
[b]Either mg/kg diet or % of total diet. SI Conversion: 1 mg silicon = 36.5 μmoles silicon.

TABLE 26-2 Silicon Concentrations in Fluids and Tissues of Animals (wet weight basis unless noted otherwise)

Animal	Silicon Intake	Milk	Plasma/ Serum	Skeletal Muscle	Liver	Heart	Kidney	Bone	Reference
Humans	~19 mg/d		38–516 µg/L						Van Dyck et al., 2000
Monkeys	Usual diet		1.9 mg/L	1.2 mg/kg	1.2 mg/kg	1.0 mg/kg	1.6 mg/kg	Shaft 23.1 Head 167.3 mg/kg	LeVier, 1975
Rats	~7 mg/d		0.2 mg/L	0.9 mg/kg	1.6 mg/kg	1.0 mg/kg	0.5 mg/kg	25.2 mg/kg	LeVier, 1975
Rats	Usual diet			~3.2 mg/kg	~2.0 mg/kg	~2.2 mg/kg	~3.8 mg/kg		Carlisle, 1984
Rats	5 and 270 mg/kg diet		0.51–0.69 and 0.59–0.83 mg/L						Emerick and Kayongo-Male, 1990
Rats	0.1–0.4 mg/g BW/d		~1.0–1.9 mg/L		11.2–14.3 mg/kg		25–31 mg/kg		Najda et al., 1992
Rats	2 and 10–35 mg/kg diet		0.25–0.28 0.30–0.32 mg/L		0.64–1.35 0.84–1.60 mg/kg DW			Femur 3.82 Vertebra 0.48–0.82 Femur 5.04 Vertebra 0.86–1.22 mg/kg DW	Seaborn and Nielsen, 2000a,b,c
Horses, mare	10.79 g/d and 44.29 g/d	224–460 and 468–896 µg/L	1,263–1,398 and 1,488–1,739 µg/L						Lang et al., 2001
Horses, foal	Mare's milk		1,146–1,406 and 1,337–1,887 µg/L						Lang et al., 2001
Sheep	Usual diet		0.40–1.03 mg/L						Hidiroglou et al., 1981

27

Sodium Chloride

INTRODUCTION

The Earth's oceans are, on average, 2.68 percent salt. Ocean-dwelling fish and other animals must constantly deal with excessively high salt environments. Salt is rare and difficult to find in most of the land areas of the world that support plant growth and therefore most terrestrial animals have had to develop mechanisms for strict conservation of salt, in particular the sodium ion of salt. Since the dawn of time, animals have instinctively forged trails to inland salt deposits, known as rock salt, to satisfy their need for salt. Salt was highly prized in ancient times as obtaining salt was one of the necessities of life. Though we take the availability of salt for granted, wars were fought over salt, and roads were built for the sole purpose of transporting it.

Salt is used for many commercial purposes as well, including water softening, de-icing our roadways, and meat preservation. Sir Humphrey Davy first separated salt into its constituent parts of sodium (Na) and chlorine (Cl) in 1807. The properties of chlorine and sodium place them among the most important of the basic raw materials that industry uses. Chlorine compounds of commercial importance include hydrochloric acid, chloroform, and sodium hypochlorite. Important sodium compounds include sodium carbonate (soda), sodium sulfate, sodium bicarbonate (baking soda), sodium phosphate, and sodium hydroxide.

ESSENTIALITY

Both the sodium and chloride elements of salt are essential nutrients for virtually all forms of life. Sodium and chloride are indispensable for maintenance of osmotic and acid-base balance. Sodium is the chief cation and chloride is the chief anion of the extracellular fluids. Blood is approximately 0.9 percent salt. Nerve and muscle resting membrane potentials are highly dependent on proper sodium and chloride concentrations. The chemistry of sodium in the body is almost entirely that of a monovalent cation—it rarely forms covalent bonds in the body with the exception of certain sodium compounds found in bone. Therefore sodium contributes greatly to the osmolarity of body fluids. Chloride is the ionized form of chlorine, a halogen, so it will readily accept an electron and its chemistry is entirely nonmetallic. When in solution, sodium ions attract a large number of water molecules, forming a water shell around each sodium ion. Potassium and chloride ions have a much smaller water shell around them. It is believed these water shields must be shed before these ions can cross the cell membrane. The size of the hydrated sodium ion, and the energy required to remove the water shell from sodium, prevent sodium from crossing cell membranes as easily as potassium and chloride do (Harper et al., 1997).

Salt may be the only mineral compound for which animals truly develop a craving. Salt deficiency is often accompanied by observations of animals with pica, or abnormal appetite, evidenced by their chewing or licking of wood, rocks, soil, urine, and bones. Eventually osmotic and acid-base disturbances cause a reduction in appetite. Animals fail to grow, develop rough hair coats, and lactation comes to a halt. Poultry will rapidly develop dehydration and become easily agitated. Long-term salt deficiency will cause death from dehydration. Most animals of economic importance to terrestrial agriculture require between 0.1–0.4 percent sodium and 0.3–0.5 percent chloride in their diet for optimal growth and productivity. Most plant origin feedstuffs are low in sodium and salt is added to most diets to meet the sodium requirements of the animal. For most species, the chloride requirement of the diet is adequately met by the combination of chloride from feedstuffs and from the salt added to meet the animal's sodium requirement.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Flame photometry, atomic absorption, and ion-sensitive electrodes can be used to measure sodium. Ion-sensitive

electrodes or electrometric methods based on formation of insoluble silver chloride during titration with silver nitrate are used to determine chloride content of body fluids and in diet components. The concentrations of chloride and sodium in biological fluids are commonly expressed as mEq/L, while feed analyses continue to express sodium and chloride content as a percentage of the feed.

Sodium content of body fluids and from feed sources is readily determined by atomic absorption spectrophotometry. For feed samples, the organic matrix can be destroyed by dry ashing in a muffle furnace. The remaining ash is dissolved in acid and the sodium determined by atomic absorption spectrophotometry. Cesium or potassium chloride is often added to the sample to control ionization within the sample. The presence of high concentrations of mineral acids following wet ashing of the sample can reduce the sodium signal.

Chloride level in biological fluids such as serum, plasma, and urine can be determined by a colorimetric method. Chloride in biological fluids is reacted with $Hg[SCN]_2$ at an acidic pH to produce a reddish-brown colored complex. The optical density of the color produced has a direct relationship with the chloride concentration in the solution.

Chloride ion concentration in body fluids and in dry-ashed feed samples dissolved in dilute nitric acid can also be measured with a chloride ion-selective electrode. The chloride ion-selective electrode is a pellet of silver chloride in direct contact with the sample solution. Because silver chloride has extremely low solubility in water, the silver chloride pellet never reaches chemical equilibrium with the sample water. Instead, a small amount of chloride ion dissolves into the sample. The resulting relative surplus of silver ions at the surface of the pellet creates a measurable electrical potential that varies with the concentration of chloride ions in the sample. This potential is measured with an external reference electrode, and then scaled to chloride ion concentration. Ion-selective electrodes are sensitive only to the ionized form of chloride. Un-ionized forms of chloride (for instance, insoluble salts or organic compounds), will not be detected. All chloride sensors suffer interferences from other ions, working best when the concentrations of bromide, iodide, cyanide, silver, and sulfide ions are much lower than the chloride ion concentration.

REGULATION AND METABOLISM

Between 85 and 95 percent of consumed sodium and chloride within diets is absorbed across the wall of the gastrointestinal tract. Absorption of sodium and chloride occurs primarily in the upper small intestine but can occur throughout the intestinal tract. Large amounts of sodium and chloride enter the gastrointestinal tract in salivary, pancreatic, gastric, bile, and intestinal epithelial cell secretions. Sodium must be secreted in large amounts in the upper small intestine to create the concentration gradient that will power facilitated transport of diet-derived glucose and amino acids across the intestinal mucosa. Chloride is secreted into the lumen of the stomach to create a low pH vital to proteolysis. Low pH also provides protection from ingested bacteria. These sodium and chloride ions are quickly reabsorbed by the intestinal tract distal to their point of secretion. When they are not, an osmotic diarrhea will ensue. Diarrhea can rapidly deplete extracellular stores of these minerals with life-threatening consequences to tissue perfusion and acid-base balance.

Most terrestrial animals evolved in an environment with a scarce supply of sodium. Therefore an intricate mechanism designed to be extremely adept at conservation of sodium developed. Key to this process is the very efficient absorption of sodium from the intestinal tract. When sodium is absorbed in excess of needs it is readily excreted by the kidneys, if water is available. Excretion of sodium in urine, salivary, and intestinal secretions is controlled by the complex interplay of several hormones. The principal hormone, aldosterone, is secreted by the adrenal glands in response to a decline in plasma sodium concentration or systemic blood pressure. Aldosterone causes increased renal conservation of sodium while increasing renal potassium excretion. Atrial natriuretic peptide secreted by the cardiac atrial cells in response to hypertension increases renal excretion of sodium. Vasopressin, secreted by the posterior pituitary in response to excessively high blood osmolarity, will stimulate renal reabsorption of water to lower plasma sodium concentration. Several gastrointestinal hormones, such as gastrin, gastric inhibitory peptide, secretin, and vasoactive intestinal peptide can affect the rate of secretion of chloride and sodium into the lumen of the gut. Chloride metabolism is intimately tied to sodium metabolism, as factors that stimulate sodium excretion generally tend to increase chloride excretion. However, in order to produce a low pH in the lumen of the stomach, chloride secretion into the lumen is enhanced while sodium absorption from the lumen is increased.

Aquatic animals have different challenges than terrestrial animals. Nearly all species of animal, whether vertebrate or invertebrate, will maintain their extracellular and intracellular osmotic concentrations between 250 and 400 mOsm. Why this particular level provided an evolutionary advantage remains conjecture, but one theory contends that when extracellular fluids exceed 400 mOsm the cell must use amino acids and urea to help maintain intracellular osmotic pressures (Steele et al., 2004). This represents an inefficient use of amino acids and may also lead to problems with disposition of nitrogen.

Ocean-dwelling crustaceans, fish, and higher vertebrates will typically be in an environment that is 1,000 mOsm, with salt contributing the bulk of osmotic particles to the solution. Sodium chloride represents about 77 percent of the total dissolved solids in seawater, with calcium, magnesium, and carbonate comprising the largest proportion of the remaining solute. Because their gills and skin (consider the Asiatic crab-eating frog) are not totally impervious to water, marine

animals are constantly trying to retain water within their bodies since the high salt water environment they reside in draws water away from their body. Unfortunately, their only option for obtaining water is to drink seawater. To survive they must have a mechanism to excrete the salts in seawater and retain the water to osmoregulate. Kidneys cannot make urine that is more concentrated (has higher osmolarity) than the extracellular fluids, so renal excretion of salt will not be adequate. Therefore these species must use other organs specialized in excretion of sodium and chloride. For most ocean-dwelling fish this organ is the gill, which is equipped with high numbers of sodium-potassium ATP-ase pumps capable of removing sodium from the blood and pumping it against its concentration gradient back into the sea. Chloride appears to follow the sodium by moving down the electrical gradient created by the movement of the sodium. Though birds have skin that is less impervious to water loss than the gills of fish or the skin of amphibians, they also lose water, which must be replenished. Seabirds have special glands located in the region of the ethmoid turbinates of the skull that extract salt from seawater, allowing them to live on the oceans of the world. Myriad other adaptations exist in nature to allow osmoregulation when living in the ocean (Schmidt-Nielsen, 1974).

Vertebrates living within fresh water face the opposite problem. Since their bodies have greater osmolarity than the water bathing them, water is constantly trying to enter their body. They do not drink water, as more water than they can use is absorbed across their gills and/or skin. In addition to avoiding excessive water gain they also must try to avoid salt loss from their body as salt will tend to diffuse from the extracellular fluids to the fresh water. These species excrete water via their kidneys, which form a very dilute urine. Though salt is not in great supply in their environment, freshwater fish adapt to constant salt loss by utilizing extremely powerful methods to absorb any existing sodium and chloride from the water across their gills. Amphibians use their skin in a similar fashion. Freshwater species are often able to absorb sodium from water that is as low as 6–7 μM sodium, while their own extracellular fluids are approximately 100 mM sodium, a 10,000-fold difference in concentration (Stobbart, 1965). Freshwater fish find osmoregulation easier in hard water than in pure water—it is easier to maintain extracellular fluids at 300 mOsm in water that is 5–10 mOsm than when the water is 1–2 mOsm!

Unfortunately, some other cations and anions that are considered toxic, such as mercury, can also be absorbed by the mechanisms designed to help the freshwater inhabitant absorb sodium and chloride to osmoregulate. Just as they are very efficient at pulling sodium and chloride from the water they may also inadvertently concentrate contaminants that might be within that fresh water. In many cases higher salt in the fresh water can allow the salt to out-compete minerals such as mercury, cadmium, chromium, and zinc for binding sites during absorption, and reduce the uptake of these toxins from the water (Hall and Anderson, 1995).

In all species, when sodium or chloride is added separately to the diet (not as sodium chloride) the acid-base status of the animal is likely to change. This aspect of sodium and chloride metabolism—which is often the factor determining the toxic level of these individual minerals—is described in detail in the section on Minerals and Acid-Base Balance.

SOURCES AND BIOAVAILABILITY

Almost one-third of the irrigated land on Earth is not suitable for growing crops because it is contaminated with high levels of salt. High soil salinity causes both hyperionic and hyperosmotic stress effects on plants, and the consequence of these on the ability of the plant to take up and retain water can be plant demise (Hasegawa et al., 2000). As a result, terrestrial plants, by and large, do not contain enough sodium to meet the dietary sodium requirement of animals. Most grasses and legumes will contain less than 0.05 percent sodium. In coastal areas, winds can carry sea salt a short distance inland and this salt is sometimes deposited in relatively high concentrations on the outside of plants grown in these areas, producing forages much higher in sodium and chloride. However, internally these plants remain low in sodium.

Chloride content of plants is much more variable and generally reflects soil chloride content and fertilization practices. Alfalfa, grasses, corn, wheat, and other common crop plants often range from 0.2–1.2 percent chloride. One variable that commonly affects plant chloride content is whether potash was used to supply potassium to the crop. The potassium requirements of crops are generally described on the basis of potash, K_2O_5, equivalents. However, more than 90 percent of the potash sold in the United States is actually muriate of potash, better known as potassium chloride, originating from huge deposits in Saskatchewan. Crops that are heavily fertilized with "potash" are likely to be relatively high in chloride.

Animal-derived dietary components such as meat meal, blood meal, and fish meal are very high in sodium content. Blood meal is about 0.4 percent sodium and Menhaden fish meal can be 0.8 percent sodium. Carnivorous animals consuming diets high in animal-derived products do not generally require salt supplementation.

All salt available in the United States for addition to diets of animals is at least 98 percent NaCl and must comply with the National Research Council's Food Chemicals Codex Sodium Chloride Monograph (NRC, 1996a). It specifies that salt may contain up to 2 percent impurities. Included in the 2 percent, salt may commonly contain up to 13 mg/kg sodium ferrocyanide or up to 25 mg/kg of green ferric ammonium citrate to prevent caking in table salt. In addition potassium iodide is often added at levels of 0.006 to 0.010 percent to prevent iodine deficiency disorders (iodized salt). Dextrose, when added (typically at about 0.04 percent), acts as a stabilizer for potassium iodide in salt. Otherwise the potassium

iodide tends to dissociate into "free" iodine, which vaporizes and may be lost from the salt. "Halite" is a lower grade of rock salt sold for de-icing driveways and for nonfood uses. It isn't pure enough to meet the standards of "food grade." Impurities often color it gray or even brown.

Sodium salts commonly used in the feed industry include sodium phosphate, sodium carbonate and bicarbonate, sodium sulfate, and sodium selenite. In all the above salts, the toxic dose of the salt is dictated by the anion of the salt rather than the sodium of the salt, unless the sodium is causing an uncompensated metabolic alkalosis. Chloride is a common anion found in combination with many of the cationic macro and trace minerals, such as calcium chloride, manganese chloride, and magnesium chloride. Because the chloride anion is generally better absorbed than the cation in these salts, ingestion in excessively high amounts would be expected to cause acidification of the extracellular fluids, which can be life threatening.

TOXICOSIS

Sodium toxicity and chloride toxicity can occur separately, primarily because of the effect these ions can have on acid-base physiology (see chapter on Minerals and Acid-Base Balance). However, when fed as sodium chloride, the effects on acid-base physiology are negligible. The remainder of this chapter will only consider the effects of sodium chloride toxicity to the animal when fed in this form.

Single Dose Toxicity

A summary of the studies demonstrating the effects of a single dose of salt on health of animals is presented in Table 27-1. Salt administered directly into the mouth of animals, such as the dog and cat, can irritate the stomach, which will cause emesis (vomiting) within 10 minutes. Although better methods for inducing emesis exist, such as administration of Syrup of Ipecac or 3 percent hydrogen peroxide, veterinarians sometimes prescribe emergency administration of salt to dogs suspected of ingesting toxic substances to help rid them of the toxin. The quantity of salt that induces emesis in dogs is 1–3 teaspoons (5–20 g), administered in the back of the throat to ensure swallowing (Davis, 1980). Beyond emesis, life-threatening hypernatremia (high blood sodium) can develop in animals given this bolus dose of salt, especially if water is not offered immediately after vomition, or if vomition fails to occur. Gastric bleeding can also be observed in some dogs. Horses and rats cannot be induced to vomit—they lack the striated muscle in their esophagus necessary for retrograde flow of stomach contents.

In rats, the LD_{50} for salt is reported to be 3.75 g salt/kg BW. Note that this dose of salt given to the rat could not be lost through vomition. Hypernatremia was the cause of death (Boyd and Shanas, 1963). There are no data on the LD_{50} of a single oral dose of salt for larger animals.

The voluntary ingestion of salt, when offered as salt alone, is unlikely to ever be great enough to cause toxicity. However when mixed with other palatable foods it is possible to ingest a toxic amount of salt. In one case report, an Airedale terrier developed continuous seizure activity after ingesting a salt-flour mixture used as sculpting clay for small figurines. Serum concentrations of sodium (211 mEq/L; normal = 145–158 mEq/L) and chloride (180 mEq/L; normal = 105–122 mEq/L) were greatly elevated. Brain sodium level (108 mEq/L; normal = <80 mEq/L) was believed responsible for swelling in the brain, which caused the convulsive seizures (Khanna et al., 1997). In another report, a pot-bellied pig ingested enough potato chips to develop hypernatremia, causing ataxia and apparent blindness (Holbrook and Barton, 1994).

Rapid establishment of ataxia and death occurred in chickens given 4 g salt/kg BW administered into the crop (Blaxland, 1946). A single cow was given two oral drenches of 454 g of salt mixed with one pint of water within a 24-hour period—which would approximate 2 g salt/kg BW. The cow developed diarrhea, ataxia, and knuckling over at the fetlocks but eventually recovered (Jones, 1930).

Access to water can greatly affect the toxicity of salt. Pigs given 2.5 g salt/kg BW as an oral drench, and not permitted access to water, developed ataxia and tremors, and died within 2 days. The same amount of salt given to pigs with access to water had no observable effects (Todd et al., 1964).

Hard-working horses lose great amounts of salt and water in their sweat. Rehydration therapy of 0.9 percent (9,000 mg/kg) physiological saline solutions can be offered to the horse, and horses will voluntarily consume up to 12 L of this fluid. They must have access to fresh water later. Long term, horses cannot tolerate water that supplies more than 10 times their daily salt requirement (Nyman et al., 1996; Butudom et al., 2004).

Freshwater fish can withstand rather dramatic increases in saline content of their water for short periods of time. Aquarium enthusiasts take advantage of this fact to remove parasites from the gills of fish—the parasites find the salinity of the water lethal but the fish can recover when placed back into fresh water. Most freshwater fish will tolerate water that is 3 percent salt (slightly more salt than seawater) for up to 10 minutes (Swann and Fitzgerald, 1993). Lower salinity can be tolerated longer.

Acute Toxicity

Salt intoxication occurs in two phases. During the first phase, ingested sodium is rapidly absorbed into the blood, causing a rapid rise in blood sodium and chloride concentrations, especially if water is restricted and prevents renal excretion of sodium. As plasma sodium rises above 160 mEq/L there is a strong tendency for water to move from the cerebrospinal fluid into the plasma. If severe enough, intracellular water will be drawn from the cells in the brain. This re-

sults in cellular dehydration and brain shrinkage. As the brain shrinks away from the calvaria the blood supply to brain cells can be disrupted, causing hemorrhage and thrombosis (Finberg et al., 1959; Harber et al., 1996). If cell dehydration is severe enough convulsions and death follow. If this first episode is not severe enough to kill the animal, a second phase of salt intoxication may occur over the next few days. In order to avoid dehydration during hypernatremia, brain cells will increase the osmolarity of their intracellular spaces. This is, in part, accomplished by cell uptake of sodium, chloride, and potassium from the cerebrospinal fluid. However, of greater importance is the accumulation of organic osmoles such as taurine, glutamate, glutamine, and phosphocreatine within the intracellular fluids of brain cells. If the hypernatremia is rapidly corrected, as might occur if water is suddenly offered to an animal that had limited access to water for a prolonged period, the osmolarity of the extracellular fluids would fall below that of the brain cell intracellular fluids. Water would tend to move into the cells, causing brain cells to swell. This occurs because the brain cells cannot remove the organic osmoles from the intracellular spaces quickly. Brain cells become hyperosmotic compared to the extracellular fluids. Since the bones encasing the brain cannot expand to accommodate the edematous cells, pressure builds on the brain cells and causes necrosis of brain cells. This can cause convulsions and death (Hogan et al., 1969). In veterinary medicine it is more common for animals to be observed in this second phase of salt intoxication. This syndrome is occasionally referred to as "water intoxication," as the rapid restoration of normal blood osmolarity by water ingestion is often the factor precipitating clinical symptoms. Correction of severe salt toxicity or water deprivation should therefore occur over a period of 2–3 days so that the brain's adaptive mechanisms to prevent cellular dehydration and cerebral edema are not overwhelmed (Angelos and Van Metre, 1999). A summary of the studies demonstrating the effects of acute oral exposure to salt of animals is presented in Table 27-2.

Suckling calves fed a milk replacer containing 2.6 percent salt exhibited nervous symptoms and pathological increases in blood sodium and chloride concentrations. These animals had no access to water other than that used to reconstitute the milk replacer (Pearson and Kallfelz, 1982). It was suggested that a high salt whey had mistakenly been used in the milk replacer, since whey produced during the manufacture of certain types of cheese can be as much as 8 percent salt.

Lactating dairy cows provided with 4 percent salt in a grain mix, fed for 14 days at the rate of 1 kg/2 kg milk produced, exhibited no adverse effects, and the composition of the milk was not changed (Demott et al., 1968). These cows were fed hay and silage ad libitum so the estimated total salt content of the diet was approximately 0.8–1 percent. These cows also had full access to water. Pigs can tolerate 3 percent salt in their diet, even if water intake is restricted, but not eliminated. However, 5.3 percent salt in the diet cannot be tolerated if water is not provided ad libitum (Done et al., 1959).

Sudden introduction of water and/or salt to animals deprived of these substances can also cause toxicosis. Six of a group of 100 feeder lambs that had been deprived of sodium chloride, then more recently deprived of water, developed water deprivation—sodium chloride intoxication soon after water and a mineral supplement containing sodium chloride were reintroduced. The clinical signs included somnolence, intense thirst, and generalized muscle fasciculations. Serum chemical analyses revealed profound hypernatremia and hyperchloremia. Postmortem examination of the dead lambs revealed microscopic evidence of cerebral edema and cerebrocorticonecrosis (Scarratt et al., 1985). Trueman and Clague (1978) reported high mortality in steers fed a salt supplement and then put into paddocks without water for 30 hours.

The poultry industry generally avoids addition of more salt to a diet than the birds need because the birds drink more water to permit salt excretion. The additional water lost in the urine causes the litter in the house to become wet, which greatly decreases air quality within the house. The study of Smith et al. (2000) demonstrates that for every 0.25 percent increase in salt content of the diet there will be an additional 9 g water excreted per g of feces. Typical poultry rations are less than 0.5 percent salt. Within 1–2 days of being fed a diet that was 1.85 percent salt, a commercial flock of 5–11-day-old turkey poults experienced 4 percent mortality preceded by respiratory distress and ascites in affected birds (Swayne et al., 1986). A commercial flock of growing turkeys fed a diet with 8 percent salt exhibited diarrhea within two days and a 6.7 percent mortality rate over the five days the birds were fed the diet (Wages et al., 1995).

Scrivner (1946) found that 1 percent sodium chloride in the feed for turkey poults was without effect, whereas 1 percent salt in the drinking water resulted in 100 percent mortality characterized by edema and ascites within 48 hours. Some birds died rapidly, before they could develop ascites. At 2 percent salt in the feed, half the poults developed edema and ascites.

About 150 migrating waterfowl died and another 250 became weak and lethargic from suspected salt poisoning after resting in White Lake, a highly saline lake in North Dakota. Frigid temperatures made fresh water in other lakes unavailable, forcing the birds to ingest the saline waters. Sick birds recovered when removed from the salt water and released into freshwater marshes. Brain sodium levels were higher in dead geese submitted for necropsy than in controls (Windingstad et al., 1987). Another interesting cause of death, which may be termed a salt toxicity, occurs in waterfowl resting on highly saline ponds. If the temperature drops dramatically the salt begins to precipitate and crystallize within the feathers of the sleeping birds floating in the water. The weight of the salt crystals prevents the birds from flying

and they drown or are stranded, which forces them to drink these highly saline waters (Wobeser and Howard, 1987; Gordus et al., 2002).

Chronic Salt Toxicity

A summary of the studies demonstrating the effects following long-term exposure to salt via diet or water on health of animals is presented in Table 27-3. The daily salt requirement for mature beef cattle is less than 30 g/head/day. Voluntary salt intake often exceeds minimum needs. However there are limits to the amount of salt that cattle will eat voluntarily and salt can be used to restrict the consumption of highly palatable or expensive supplemental feeds by animals at pasture where daily feeding is impractical. In such instances, daily voluntary intake of salt will average 1.5 g salt/kg BW for most classes of cattle. In some studies, cows reach their voluntary salt intake limit at 1 g/kg BW while in other studies, salt intake will not be affected until the cow has consumed 2 g salt/kg BW (Riggs et al., 1953; Rich et al., 1976; Schauer et al., 2004). When salt is added to a supplement to limit intake, adult beef cows can consume about 500 g salt/day over long periods of time without adverse effects provided they have plenty of drinking water. Excessive addition of salt to such supplements will cause excessive feed restriction and cause reduced performance in the cattle.

In ponies, supplemental grain intake could be restricted by inclusion of salt at 16 percent of the grain supplement. Feeding supplemental grain that was 8 percent or less salt was not sufficient to keep the ponies from eating all the grain put before them in one day (Parker, 1984). Ponies weighing 200 kg were fed 5 percent salt diets at 1.5 percent of BW/day for four weeks without deleterious effect. Since their total salt intake was 175 g/day, the ponies tolerated 0.88 g salt/kg BW when expressed on a BW basis.

In animals with access to water, the tolerable concentration of salt in the diet is very high. When total dietary salt fed to lambs or ewes exceeded 7.6 percent, there was a small reduction in weight gain of lambs and in lambs produced per ewe (Meyer and Weir, 1954; Jackson et al., 1971). Lambs weighing 27 kg were fed 5.85 percent salt rations for nearly four months with no deleterious effects (Jackson et al., 1971). Assuming these lambs were consuming 2 percent of their BW in DM/day, these lambs were safely ingesting 1.8 g salt/kg BW. Beef steers weighing 370 kg were fed a 9.33 percent salt diet with no adverse effects (Meyer et al., 1955). Assuming these steers were consuming 2 percent of their BW in DM/day, these steers were safely ingesting 1.86 g salt/kg BW. However, newly weaned calves suffered a reduction in feed intake and growth rate when the diet was 7 percent salt. Those calves fed a 4.75 percent salt diet grew normally (Leibholz et al., 1980). Assuming these calves weighed 70 kg and were consuming 2.5 percent of their BW in DM/day, these calves were safely ingesting 1.2 g salt/kg BW.

Paver et al. (1953) fed chicks up to 3.52 percent salt diets with no observable toxic effects. However, day-old turkey poults may be more susceptible to salt toxicity than day-old broiler chicks. Day-old turkey poults fed 0.7, 1.2, and 1.7 percent salt diets for 14 days had similar feed intake and rates of growth. Though poults fed 2.7 percent salt diets grew normally, many died due to lung congestion and myocardial abnormalities within 7 days of the initiation of the study (Morrison et al., 1975). Roberts (1957) was able to feed mature turkeys up to 4 percent salt diets with no adverse effects. In the Roberts study it was noted that water intake increased dramatically in birds given the high salt diet, allowing increased renal excretion of salt. In the study of Morrison et al., it was noted that the poults failed to increase water intake with increasing salt. This difference in behavior likely played a role in the increased mortality among young poults given the higher salt diets. Young fowl are generally considered more susceptible to salt toxicosis than older birds because their kidneys are too immature to excrete sodium and chloride rapidly when placed on a high salt diet (Mohanty and West, 1969).

Zentek and Meyer (1995) found that dogs fed a diet containing approximately 2.9 percent sodium (7.4 percent salt) reduced their intake of the diet. The diet induced vomiting in at least one dog. Boemke et al. (1990) found that potassium excretion and potassium balance were negatively impacted in dogs fed diets that were greater than 2 percent sodium (5 percent salt). Based on potassium balance alone their data suggest the maximum tolerable salt level in the diet is 3.75 percent. Cats can tolerate very high levels of salt in the diet when water is provided. Burger (1979) found that cats preferred lower salt diets but would consume diets that were 3.8 percent salt without ill effects. Kittens grow well and suffer no ill effects when fed diets that are 2.5 percent salt, though they prefer to eat lower salt diets (Yu et al., 1997).

The tolerance for salt in the drinking water is much lower than the tolerance for salt in the diet. It is useful to keep in mind that the ocean's waters are about 2.68 percent salt, and blood is about 0.9 percent salt. Growing sheep can tolerate 1 percent salt in the drinking water for an extended period of time with no observable adverse effects. However, when salt content of drinking water exceeds 1.5 percent there is a reduction in weight gain, and at 2 percent salt in the water, overt signs of weakness and marked weight reduction occur (Pierce, 1957). Pregnant sheep forced to drink water that was 1.3 percent salt had an increased rate of stillborn lambs and elevated blood chloride levels (Potter and McIntosh, 1974). Growing cattle exhibited reduced growth when drinking water was 1.25 percent salt (Weeth and Haverland, 1961).

Lactating dairy cows forced to drink 0.25 percent salt water (1,000 mg/L sodium and 1,500 mg/L chloride) produced less milk, but did not exhibit hypernatremia or a reduction in feed intake (Jaster et al., 1978). Brackish water containing just 287 mg/L sodium and 580 mg/L chloride in conjunction with other minerals was responsible for reduced

water consumption and reduced milk production in lactating dairy cows (Solomon et al., 1995). The lower tolerance for brackish water suggests the salt may not have been the only factor contributing to the decreased performance of cows drinking brackish water.

Young birds can be adversely affected by drinking water that is as low as 0.4 percent salt (Krista, 1961). Older birds are somewhat more tolerant. Egg production of laying hens is not affected when water is 0.7 percent salt, but 1 percent salt in the water greatly reduces egg production (Krista, 1961).

Prepartum diets high in sodium and/or potassium have been implicated as causes of udder edema in cattle. Studies by Hemken et al. (1969), Conway et al. (1977), and Jones et al. (1984) demonstrated an increased incidence or severity of udder edema with sodium chloride supplementation. In the experiments of Hemken et al. (1969), restriction of sodium chloride and water intake reduced the severity and incidence of udder edema in pregnant heifers. A lower incidence and severity of udder edema were found when diets contained no supplemental salts of sodium or potassium (Randall et al., 1974). In a controlled study, heifers were fed one of four diets: (1) no added sodium or potassium, (2) 227 g/day sodium chloride added, (3) 227 g/day potassium chloride added, or (4) 227 g/day sodium chloride and 227 g/day potassium chloride added. These diets were fed during the last 40 days before expected parturition. Addition of sodium or potassium chloride increased the severity of udder edema. The combination of adding both sodium and potassium chloride increased the severity numerically but not statistically (Randall et al., 1974).

Nestor et al. (1988) reported the findings of a 2×2 factorial experiment in which prepartum rations contained two levels of supplemental potassium bicarbonate (0 versus 272 g/head per day) and two levels of supplemental sodium chloride (23 versus 136 g/head per day). The severity of udder edema was greater when pregnant heifers were fed additional potassium bicarbonate or sodium chloride separately, but the combination of feeding both supplemental sodium chloride and potassium bicarbonate did not increase udder edema scores. The failure to get an additive effect of sodium and potassium is difficult to understand. However it is possible that feeding just one of the minerals, either sodium or potassium alone, upsets the aldosterone balance of the body more than when they are fed in combination. If this system is not functioning properly, excessive retention of sodium or potassium could occur, which may be contributing to the edema of the udder and ventral abdomen. Perhaps the ratio of these two minerals in a diet is critical to the function of the renin-angiotensin-aldosterone endocrine system.

Factors Influencing Toxicity

The major determining factor in salt intoxication is the availability of drinking water. Acute salt toxicity is essentially eliminated if animals can use water to rid their bodies of unwanted salt to regulate blood osmolarity.

TISSUE LEVELS

Normal blood sodium concentration is between 130 and 150 mEq/L for most species (Table 27-4). Normal blood chloride concentration is 95–110 mEq/L. Hypernatremia, with the possibility of clinical signs affecting the nervous system, can be observed when mammalian blood sodium concentration exceeds 160 mEq/L (Senturk and Huseyin, 2004). Plasma sodium concentrations above 225 mEq/L and plasma chloride concentrations above 125 mEq/L are lethal in mammals and birds. Cerebrospinal fluid sodium is normally 130–142 mEq/L in cattle and pigs. During salt toxicity cerebrospinal fluid sodium concentration is generally above 160 mEq/L (Puls, 1994). Brain tissue levels of sodium and chloride do not always correlate well with salt intoxication especially if clinical symptoms are occurring during the rehydration phase of the intoxication (Wells et al., 1984). High dietary salt concentration has little effect on tissue salt content, unless access to fresh water is restricted. Drinking water that is high in salt can cause hypernatremia and increased salt content of the brain.

MAXIMUM TOLERABLE LEVELS

In the absence of water, or if salt is in the only available drinking water, there is an increased risk of salt toxicity. However, it is beyond the scope of this report to set a maximum tolerable level that considers all these possible circumstances. The maximum tolerable level for salt in the diet of the species listed below is calculated with the assumption that water is freely available to the animals. The maximum tolerable level is based on the level of salt that can be safely fed without causing either a reduction in feed intake that affects production, or induction of clinical signs of salt intoxication such as diarrhea or nervous system dysfunction. Sodium absorbed independent of chloride, from sources such as sodium bicarbonate, will cause morbidity due to induction of metabolic alkalosis at levels well below those that will affect bodily fluid osmolarity. Similarly, chloride absorbed independent of sodium, from sources such as calcium chloride, risks induction of severe metabolic acidosis. These situations are described in the chapter on Minerals and Acid-Base Balance, and will not be discussed further here.

In ruminants and horses, about 1 g salt/kg BW can be consumed without adversely affecting feed intake (Riggs et al., 1953; Rich et al., 1976; Schryver et al., 1987; Schauer et al., 2004). Beyond these levels feed intake and productivity could be observed to decline in some animals, though there is considerable variation in the capacity for voluntary salt intake between individual animals. Assuming 1 g salt/kg BW is the maximum total load of salt the body can adapt to without adversely affecting diet intake, it is possible to predict

the maximal concentration of salt that can be in a diet without adversely affecting intake and therefore growth or performance of the animal. A 410-kg beef steer in a feedlot might be expected to consume 9 kg DM/day (2.2 percent of BW) each day (NRC, 1996b). Its voluntary salt intake would be about 410 g. Any diet containing more than 410 g salt/9 kg DM or 4.55 percent salt would be expected to limit intake of the steer. For a 650-kg lactating dairy cow consuming 3.3 percent of her BW each day, a similar calculation predicts that the cow could tolerate up to 3 percent salt in her diet without adversely affecting feed intake. A 450-kg horse consuming 1.5 percent of its BW in DM could tolerate a 6.6 percent salt diet.

There is only one applicable study in poultry, the study by Morrison et al. (1975) in turkey poults, and it suggests the tolerance for dietary salt of poultry may be higher than in mammals. If we assume that at 2 weeks of age the turkey poults weighed about 270 g and consumed about 25 g diet/day, the 1.7 percent salt diets that were safely fed to the poults supplied 1.57 g salt/kg BW. Although there are concerns to the industry in terms of wet droppings when birds are fed high salt diets, the maximum tolerable amount of salt that can be fed to poultry without affecting feed intake is 1.5 g/kg BW.

No long-term toxicity studies on the effects of high salt diets have been conducted in pigs. However, based on short-term studies by Done et al. (1959), adverse effects of salt were only seen with >5 percent salt diets and only when access to water was restricted. This study also suggested pigs readily tolerate 3 percent salt in the diet. Assuming these pigs were eating 3 percent of their BW in DM/day these pigs were safely consuming 0.9 g salt/kg BW. It is likely that 5 percent salt diets, providing about 1.5 g salt/kg BW, would be tolerated if water were freely accessible, but data from long-term studies on high salt diets do not exist. The maximum tolerable salt load for pigs is therefore conservatively set at 1.0 g salt/kg BW.

Cats can safely consume diets that are 3.8 percent salt but they prefer lower salt diets. Since the studies of Burger (1979) involved adult cats, an assumption that these were 4-kg cats consuming about 1.6 percent of their BW in DM/day can be made. This is a maximal tolerable limit of just 0.6 g salt/kg BW. Kittens safely consumed a 2.5 percent salt diet (Yu et al., 1997). The kittens can be assumed to have weighed approximately 800 g and consumed about 5.5 percent of their BW in DM/day. Therefore they were safely consuming about 1.37 g salt/kg BW. Unfortunately data do not exist to allow a more precise estimate in cats. The study of Boemke et al. (1990), which used potassium balance as a criteria for safety, suggests the maximum tolerable dietary salt level for dogs is 3.75 percent. Assuming these adult dogs weighed 15 kg and were consuming 1.7 percent of their BW in DM/day, dogs can safely consume 0.64 g salt/kg BW.

While it is expected that feed intake would be reduced once the maximum salt load is reached, there are instances where animals will consume toxic amounts of salt. Usually this is associated with the rapid ingestion of a newly offered high salt diet. In these cases, clinical illness can be observed in animals fed very high salt diets, even when adequate water is supplied. At what dietary salt level this occurs is difficult to define. In young growing birds, diets that are 2.7 percent salt lead to rapid mortality (Morrison et al., 1975). At this dietary salt level it is estimated that the birds are consuming 2.5 g salt/kg BW. In suckling calves fed 2.6 percent salt milk replacer, toxicity and mortality were observed (Pearson and Kallfelz, 1982). The salt load on these calves can be estimated to have been 3.1 g salt/kg BW. The studies by Meyer et al. (1955) suggested reduced lambing rates in 32-kg ewes fed a 13.1 percent salt diet. Assuming the ewes consumed 2 percent of their BW in diet DM/day, the salt load that caused this toxicity in the sheep was approximately 2.6 g salt/kg BW. For all species it is likely that animals consuming more than 2.5 g salt/kg BW will suffer adverse effects that include increased mortality.

It is beyond the scope of this report to determine the concentration of salt in the water at which each freshwater and saltwater fish species will no longer be able to regulate its internal osmolarity. Some fish can, with time, learn to osmoregulate in both fresh and sea water during various times of their lives. In general, freshwater fish will not tolerate water that is more than 1.5 g sodium chloride/L (Arenzon et al., 2003), and ocean-dwelling fish will not survive when sodium chloride in the water exceeds 3 percent, though some fish are adapted to survive in inland lakes and seas with a somewhat greater salt content (Wang et al., 2003). The Dead Sea is so-called because no fish or even brine shrimp will survive in its waters, which are 300 g/L salt, about ten times the salinity of the oceans.

FUTURE RESEARCH NEEDS

There seems to be little research that precisely defines the effects of salt on feed intake in cats and dogs. Also in these pet species, there is little reported work on the effect of salt in the diet on dogs and cats with hypertrophic cardiomyopathies.

An area of great concern, but one that has few data, concerns the role of salt in the pathogenesis of udder edema, a common affliction of dairy cows. Graded levels of salt below those previously reported to induce udder edema in the studies of Hemken et al. (1969) and Nestor et al. (1988) need to be added to dry cow diets and tested for their effect on udder edema. This could allow definition of a maximum tolerable level of salt in diets intended for dry dairy cows, a level above which udder edema is likely.

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TABLE 27-1 Single Oral Dose Salt Toxicity

Animal (N)	Age	Quantity	Route	Effect(s)	Reference
Dogs	Any	5–20 g	Over back of throat	Vomition in 10–15 min Gastric irritation Hypernatremia if emesis does not occur and water is not consumed within a short time	Hardy, 1989
Rats		3.75 g/kg BW	Oral gavage	Hypernatremia, convulsion, death in 50%	Boyd and Shanas, 1963
Chickens (9)	60–90 d	4 g/kg BW	Instilled into crop	Diarrhea, death	Blaxland, 1946
Swine (4)	14 kg	2.5 g/kg BW	Drench—allowed access to water	No adverse effects	Todd et al., 1964
	14 kg	2.5 g/kg BW	Drench—not allowed access to water	Ataxia, tremors, convulsions, death within 2 d	
Cattle (1)	Adult	2 g/kg BW	Drenched twice with 1 lb salt in 1 pt water within 24 hr	Diarrhea, knuckling over at fetlocks, muscle spasms	Jones, 1930

TABLE 27-2 Acute Salt Toxicity (Exposure ≤ 2 Weeks)

Animal (N)	Age/Weight	Quantity	Route	Duration	Effect(s)	Reference
Turkeys (20,000)	13 wk toms	8.04%	Diet	5 d	Polydipsia, diarrhea within 2 d. 6.7% mortality over 5 d. Post mortem findings included hepatic congestion, fluid filled intestines, cerebral necrosis.	Wages et al., 1995
Turkeys (commercial flock)	5–11 d	1.85%	Diet	1–2 d	4% mortality. Sudden deaths. Rapid onset of respiratory distress and ascites in affected birds. Myocardial damage.	Swayne et al., 1986
Swine (6)	30 kg	6%	Diet—restricted access to water	5 d	Rapid onset of nervous signs including ataxia, weakness, blindness in one pig	Bohstedt and Grummer, 1954
Swine (3)	Adult	3%	Diet—water restricted	11 d	No adverse effects	Done et al., 1959
Swine (7)	Adult	5.3%	Diet—water restricted	10 d	Muscle tremors, incoordination, convulsions, death, brain lesions typical of meningoencephalitis	Done et al., 1959
Cattle (2)	Lactating	1, 2, or 4% salt in grain mix. Total diet salt content estimated to be 0.8–1.0%.	Fed 1 kg grain/2 kg milk produced	14 d	No adverse effects. No significant changes in milk composition or freezing point	Demott et al., 1968
Cattle (~100)	Suckling veal calves	2.6% salt milk replacer on DM basis	Milk replacer fed with no access to other water. High sodium whey used in milk replacer.	Several d	Hyperesthesia, nystagmus, muscle twitching, intermittent convulsions, hypernatremia (200 mEq/L), and hyperchloremia	Pearson and Kallfelz, 1982

TABLE 27-3 Chronic Salt Toxicity (Exposure >2 Weeks)

Animal (N)	Age	Quantity	Route	Duration	Effect(s)	Reference
Chicks (64)	1 d	4,000 mg salt/L	Drinking water	28 d	Watery feces, but no detrimental effects	Krista et al., 1961
Chicks (64)	1 d	7,000 mg salt/L	Drinking water	28 d	High mortality; decreased growth, watery feces, decreased activity	Krista et al., 1961
Laying hens (30)	Pullets	7,000 mg salt/L	Drinking water	12–16 wk	No effect on egg production; wet feces	Krista et al., 1961
Laying hens (30)	Pullets	10,000 mg salt/L	Drinking water	12–16 wk	Reduced egg production. Wet feces	Krista et al., 1961
Turkeys (49)	1-d poults	4,000 mg salt/L	Drinking water	28 d	Increased mortality and reduced weight gain in surviving chicks	Krista et al., 1961
Ducks (7)	1 d	4,000 mg salt/L	Drinking water	21 d	No adverse effects	Krista et al., 1961
Ducks (7)	1 d	7,000 mg salt/L	Drinking water	21 d	Reduced growth rate	Krista et al., 1961
Ducks (7)	1 d	4,000 mg salt/L	Drinking water	21 d	High mortality; great reduction in weight in survivors	Krista et al., 1961
Turkeys	1-d poults	1.7% salt	Diet	14 d	No adverse effects	Morrison et al., 1975
Turkeys	1-d poults	2.7% salt	Diet	14 d	Increased mortality during first few d of life; ascites not noted; lung congestion and myocardial distension	Morrison et al., 1975
Horse (3)	200-kg ponies	5% salt	Diet fed at 1.5% BW	28 d	No adverse effects	Schryver et al., 1987
Cattle (12)	Lactating dairy	0.25% (2,500 mg/L)	Water	4 wk	Reduction in milk production, observed no hypernatremia, observed no change in feed intake, cows drank more of the saline water	Jaster et al., 1978
Cattle (41)	Lactating dairy	287 mg/L sodium 580 mg/L chloride	Brackish drinking water	Full lactation	Reduced water intake and reduced milk production	Solomon et al., 1995
Cattle (6)	180–220 kg	1.00% 1.20% 1.25% and 1.50% 1.75%	Water Water Water Water	30 d 30 d 30 d 30 d	No adverse effects Increased water consumption Reduced growth rate Anorexia, weight loss, reduced water intake	Weeth and Haverland, 1961
Cattle (14)	370-kg steers	9.33%	Diet	84 d	No adverse effects	Meyer et al., 1955
Cattle (9)	Pregnant dairy heifers	227 g sodium chloride added to diet	Diet	40 d	Increased udder edema	Randall et al., 1974
Cattle (11)	Pregnant dairy heifers	136 g sodium chloride in the diet	Diet	40 d	Increased udder edema	Nestor et al., 1988

continued

TABLE 27-3 Continued

Animal (N)	Age	Quantity	Route	Duration	Effect(s)	Reference
Cattle (10)	3-wk calves	0.75, 2.75 and 4.75% salt	Diet	8 wk	Normal feed intake and growth	Leibholz et al., 1980
Cattle (10)	3-wk calves	7% salt	Diet	8 wk	Decreased feed intake and growth	Leibholz et al., 1980
Sheep (10)	Ewes 32 kg	4.8 and 9.1% salt	Diet	253 d	No adverse effects	Meyer et al., 1954
		13.1%	Diet	253 d	Greater weight loss during lactation; fewer lambs raised/ewe	
Sheep (5)	27-kg lambs	1.78, 3.31, 4.83, 5.85%	Diet	119 d	No adverse effects observed	Jackson et al., 1971
Sheep (5)	27-kg lambs	7.63%	Diet	119 d	Small reduction in weight gain	Jackson et al., 1971
Sheep (6)	55 kg	1.0%	Water	460 d	No adverse effects	Pierce, 1957
		1.5%	Water	460 d	Decreased feed intake and weight loss	
		2.0%	Water	460 d	Pronounced weight loss; increased blood sodium and chloride concentrations; diarrhea, generalized weakness	
Sheep (8)	Pregnant and lactating ewes	1.3%	Water	>80 d	Stillborn lambs, increased mortality; increased blood chloride in ewes	Potter and McIntosh, 1974

TABLE 27-4 Sodium and Chloride Concentrations in Fluids and Tissues of Animals

Animal	Element	Serum[a]	Muscle[b]	Liver[b]	Brain[b]	Milk[b] or Eggs[b]
Poultry	Sodium chloride	122–163 110–128	600–800 950–1,000	1,800–2,400 1,900	1,600–1,750	290 (yolk) 460 (albumen) 290 (albumen)
Pigs	Sodium chloride	140–150 98–106	600–800	1,750 2,840	1,850–2,030 1,200–2,500	340–400 900–1,020
Cattle	Sodium chloride	135–150 95–110	700–900	900–1,800 1,050	800–1,400	300–600 1,050–1,150
Sheep	Sodium chloride	140–157 95–103	650–750	2,000–4,000	1,100–1,250	
Fish	Sodium chloride	146–155 134–139	1,100–1,500			

[a]Reported as mM/L.
[b]Reported as mg/kg fresh tissue weight for all tissues.

SOURCES: Compiled from data of Barlow et al., 1948; Todd et al., 1964; and Puls, 1994.

28

Sulfur

INTRODUCTION

Sulfur (S) is one of the few elements known and described in detail in the ancient world and was referred to as Sulpur in Latin. In Genesis liquid sulfur (which is elemental sulfur with a temperature >132°C) was referred to as "brimstone" and was associated with the fires of hell, probably because elemental sulfur occurs in a yellow, relatively pure state in the vicinity of volcanoes and hot springs. It is also widely distributed in nature as iron pyrites, galena, sphalerite, cinnabar, stibnite, gypsum, epsom salts, celestite, barite, and other such substances. Sulfur has an atomic number of 16 and an atomic weight of 32.06.

Sulfur is a pale yellow, odorless, brittle solid, which is insoluble in water but soluble in carbon disulfide. Organic compounds containing sulfur, such as methionine, are very important to normal life. Calcium sulfate, ammonium sulfate, carbon disulfide, sulfur dioxide, and hydrogen sulfide are but a few of the many important inorganic compounds of sulfur.

The sulfur atom in organic and inorganic compounds can be present in at least five oxidation states: –2 (sulfide, organic thiols such as cysteine and glutathione, and most other organosulfur compounds); 0 (elemental sulfur); +2 (sulfenic acid); +4 (inorganic sulfite, and sulfinic acids such as cysteinesulfinic acid and hypotaurine); and +6 (inorganic sulfate, and sulfonic acids such as taurine and cysteic acid). Sulfur atoms in sulfur-containing amino acids are divalent (–2) and thus in the most reduced state, and the sulfur atom of sulfate is in the fully oxidized hexavalent (+6) state.

Until 2000, sulfur was commercially recovered from wells sunk into the salt domes along the Gulf Coast of the United States. Using the Frasch process, heated water was forced into the wells to melt the sulfur, which was then brought to the surface. Sulfur also occurs in natural gas and petroleum as hydrogen sulfide and other forms. These were once emitted as a waste during coal washing or during combustion, but are now recovered and used. Bituminous coal used in the coke-making processes is about 0.6 to 2.6 percent sulfur, depending on the grade of coal purchased. In the past, burning of coal and other fossil fuels released large amounts of sulfur dioxide into the atmosphere, which was converted to sulfuric acid when it contacted water and caused acidification of lakes and rivers downwind of the emissions. Today, much of the sulfur is removed from coal before it is burned and scrubbers within the smokestacks allow recovery of a good deal of the remaining sulfur, which has greatly reduced sulfurous emissions, though they still remain a concern. Much of the sulfur harvested is used to produce sulfuric acid, which in turn, is used in chemical reactions to produce a wide variety of products, including phosphate fertilizer and white paper. Sulfuric acid production is the major end use for sulfur, and consumption of sulfuric acid has been regarded as one of the best indexes of a nation's industrial development. More sulfuric acid is produced in the United States every year than any other chemical. Sulfur compounds are also used in the production of gunpowder and fungicides, the vulcanization of rubber, and the manufacture of synthetic fibers such as rayon. Sulfur in the form of sulfites is commonly added to foods as a preservative.

In 2003, approximately 8.8 million tons of sulfur were produced in the United States, with 45 percent of that coming from the petroleum refineries of Texas and Louisiana. Still the United States imported nearly 3 million tons of sulfur to meet domestic industrial needs for sulfur—mostly as sulfuric acid. Agricultural chemicals, primarily for fertilizer production, accounted for 70 percent of all sulfur used in the United States in 2003 (USGS, 2004).

ESSENTIALITY

The body is approximately 0.15 percent sulfur by weight. The sulfur is primarily incorporated into a number of organic molecules vital to life, making sulfur an essential nutrient. Sulfur-containing compounds include several sulfur-containing amino acids and their metabolites (methionine, cysteine, cystine, homocysteine, taurine, cystathionine, and

cysteic acid), thiamine, biotin, lipoic acid, co-enzyme-A, glutathione, chondroitin sulfate and cartilage mucopolysaccharides, fibrinogen, heparin, ergothionine, and certain forms of estrogens. These compounds are found in nearly all tissues of the body. Many of these sulfur-containing compounds required by the body can be synthesized from methionine. Unfortunately, no vertebrate is capable of producing methionine from inorganic sulfur in the diet. Therefore, organic sulfur in the form of methionine is an essential nutrient in the diet of all vertebrate animals. Two other essential sulfur-containing compounds cannot be produced from methionine and must also be supplied in the diet of vertebrates (or produced by microbes in the forestomachs). These are thiamine and biotin, which are classified as water soluble B vitamins. Cystine can also be converted to many of the sulfur-containing compounds required by the body and can spare methionine for other purposes if it is present in the diet, but because cystine can be made from methionine it is not considered essential. There is evidence that some inorganic sulfur in the form of sulfates can be absorbed and incorporated into organic compounds such as taurine and cartilage mucopolysaccharides. As much as 15 percent of the dietary requirement for cystine can be replaced by inorganic sulfate present in the diet (Sasse and Baker, 1974; Soares, 1974). Examined in its entirety, the contribution of inorganic sulfur sources to the essential functions of sulfur in the body are small. Diets that contain just 0.05 percent sulfur will saturate the ability of the body to use dietary inorganic sulfur for production of some of the organic sulfur-containing compounds (Anderson et al., 1975). However, no amount of inorganic sulfate added to the diet can replace methionine, thiamine, or biotin.

Some important species differences exist with respect to the essentiality of this element. For instance cats (the entire family Felidae) cannot synthesize taurine from methionine, so taurine becomes an essential nutrient in feline diets. Because vertebrate tissues cannot produce methionine, thiamine, and biotin from inorganic sulfur in the diet, there is no dietary requirement for inorganic sulfur in nonruminant species. Bacteria within the rumen can synthesize methionine, thiamine, and biotin in quantities that are high enough to support much of the need for methionine of the host ruminant. For most ruminants, sulfur must be between 0.18 and 0.24 percent of the diet to allow the microbes of the foregut to produce sufficient sulfur-containing compounds to support bacterial growth for good rumen function and to provide sulfur-containing compounds to meet the demands of the host ruminant. In these species, methionine, thiamine, and biotin do not ordinarily need to be added to the diet.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Various methods exist for determination of sulfur content of feedstuffs, forages, and water samples. Turbidometric tests for sulfur have been used for a long time. Samples of water, diet, or feedstuffs are analyzed by wet ashing the sample in nitric acid and mixing the sample with barium chloride to form barium sulfite precipitates. The resulting turbidity of the solution can be measured by spectrophotometry at 440 nm and the turbidity increases with sulfur content of the sample. The method suffers some variability problems as the barium sulfate crystallization process does not always form uniform crystals.

Sulfur content of feeds can also be determined by combusting the dry samples of feed or forage in an oxygen-rich atmosphere at 1350°C. Any sulfur-containing compounds break down, freeing sulfur, which is then oxidized to form sulfur dioxide (SO_2). The SO_2 gas produced during combustion of the sample flows through an infrared detection cell that measures the concentration of SO_2. Though SO_2 is measured, it is most common to convert that determination back into percentage of sulfur when reporting analyses of diet or forage samples.

In some cases, such as water samples, a sulfide ion-specific electrode can be used to measure sulfide ions in aqueous solutions quickly and accurately. The sulfur atoms of the sulfur compounds in water (sulfide and sulfates) are first converted to inorganic sulfide by acid treatment so they will be detected by the electrode. The presence of silver in the water could interfere with the determination but because of the extreme insolubility of silver sulfide, the two ions are virtually never present in solution together (Ubuka, 2002).

REGULATION AND METABOLISM

Organic sulfur-containing compounds are absorbed across the intestinal wall by specific transport processes. The sulfur-containing amino acids are incorporated into proteins or they are used to produce other organic sulfur-containing compounds. Eventually, all these organic sulfur-containing compounds will be catabolized to inorganic sulfate. Inorganic sulfur compounds that might be in the diet such as sulfides, sulfites, and sulfates are generally converted to sulfides or sulfates prior to absorption. Sulfate is a nonmetabolizable strong anion and can add acidity to the fluids of the body (see chapter on Minerals and Acid-Base Balance). In ruminants, most dietary sulfur, whether ingested as amino acids or inorganic sulfur, is reduced to sulfide within the rumen by certain types of bacteria that use sulfur as an electron acceptor. Sulfide can be incorporated into microbial protein by certain types of bacteria or the sulfide can be absorbed into the portal circulation where it is quickly and efficiently (in most cases) oxidized to sulfate in the liver.

Sulfates fed to animals are relatively well absorbed. Ruminants can absorb an estimated 77–87 percent of sulfur from sodium sulfate or calcium sulfate (gypsum) (Bouchard and Conrad, 1973). From studies of the effects of sulfur on acid-base status it would appear that, on a molar basis, sulfate is absorbed at about 30 percent the efficiency of

chloride (Goff, et al. 2004). Inorganic sulfur in the form of elemental sulfur is poorly available for absorption by animals. It is about 35–65 percent as available for absorption as sulfate (Henry and Ammerman, 1995) and is relatively insoluble in water. It is likely that some small proportion is converted to sulfide, which would have deleterious effects if not rapidly converted to sulfate by the liver.

Sulfur is taken up by tissue cells mainly in the divalent state as sulfur-containing amino acids. These are then metabolized via the quadrivalent state (sulfinic acids), and finally oxidized to the hexavalent form (sulfates). The great majority of dietary sulfates entering the body or produced in the body during catabolism of organic sulfur-containing compounds are eventually excreted in the urine (Harrington and Lemann, 1970; Magee et al., 2004). Inorganic sulfur, absorbed across the intestinal tract, adds to the strong anion sulfate in the blood and affects the acid-base balance of the body as described previously.

SOURCES AND BIOAVAILABILITY

The primary sources of sulfur in the diet of most species are the sulfur-containing amino acids. Plants can use inorganic sulfur in the soil to produce methionine, thiamine, and biotin and serve as an important source of sulfur-containing compounds for vertebrates. Plants high in protein, such as alfalfa, are about 0.3 percent sulfur, while relatively low protein corn silage is about 0.14 percent. Meat and poultry products (especially feather meal) are much higher in protein and also higher in sulfur-containing amino acids. Rendered meat meal is about 0.51 percent sulfur and fish meal can be as high as 1.16 percent sulfur (NRC, 2001). Corn gluten feed and corn steep liquor (by-products of the refinement of corn for ethanol), corn syrup, corn gluten, corn oil, and cornstarch have gained popularity as livestock feeds. They tend to be high in sulfur content (0.45 percent to 0.85 percent sulfur) and due to their low prices they may comprise a large proportion of the diet of some ruminants.

Typical diets for cattle and small ruminants that are adequate in protein or that will provide adequate microbial-derived protein contain about 0.20 percent sulfur. For horses, diets that are adequate in protein contain about 0.15 percent sulfur. For swine, the typical corn-soybean meal diet providing adequate protein for growth will be about 0.20 percent sulfur. Dogs and cats are often fed diets that are formulated with meat and tend to be higher in protein. These diets are often as high as 0.50 percent sulfur depending on the amount of meat in the diet.

Calcium sulfate (gypsum) and magnesium sulfate (epsom salts) are used as calcium and magnesium sources in animal diets. Potassium sulfate is used in ruminant diets as a source of potassium for electrolyte balance and sulfur for rumen bacteria. Ammonium sulfate is used in some diets as an acidifying agent. The cationic trace minerals zinc, copper, manganese, and iron are often added to diets as the sulfate salts—primarily because the sulfate salts are soluble in water and therefore, often are among the most bioavailable of the inorganic forms of these trace minerals. Many of the mineral supplements used to meet the phosphorus requirements of animals contain a significant amount of sulfur. For instance, dibasic ammonium phosphate is about 2.16 percent sulfur and dicalcium phosphate is approximately 1.14 percent sulfur.

There are six sulfiting agents (sulfur dioxide, sodium and potassium metabisulfite, sodium and potassium bisulfite, and sodium sulfite) currently being used to help prevent spoilage and discoloration in foods, and these are used almost exclusively in processed food products. These agents have been banned by the Food and Drug Administration for use on fresh fruits and vegetables that are meant to be consumed raw and on fresh meat and poultry products. Sulfites are still used to help prevent black spot in shrimp and its use in shrimp is supposed to be labeled if the sulfite residue is 10 ppm or more. Certain humans are sensitive to sulfites and have asthmatic attacks or headaches following its ingestion. It is unknown if these symptoms occur in animals. If one considers that the major threat of sulfite residues for humans comes from red wines, it is unlikely to be a major problem for animals.

TOXICOSIS

The toxicity of sulfur is highly dependent on the form of the sulfur ingested. Elemental sulfur is generally very unreactive and very innocuous in nonruminant species. It is also less soluble in rumen fluid than other oxidized forms of sulfur and tends to be less toxic in ruminants as well, though rumen bacteria are capable of further reducing elemental sulfur to more toxic compounds, such as hydrogen sulfide.

Hydrogen sulfide (H_2S) is an extremely toxic sulfur-containing compound. Very low amounts of this gas can prove rapidly fatal. Inhaled H_2S is a particular concern for swine confinement operations where the pigs are kept on slatted floors above a manure pit where H_2S may be produced during anaerobic digestion of the manure. Death occurs shortly after exposure of pigs to 470 ppm H_2S in the atmosphere. Levels as low as 15–20 ppm H_2S inhaled over a period of two months can cause fibrosis of pulmonary tissues (O'Donoghue and Graessner, 1962). Production of H_2S by bacteria within the rumen can be fatal to ruminants and will be discussed in depth in the section on ruminant polioencephalomalacia.

There are many sulfur-containing compounds that can be injurious to animals when ingested over the short or long term. Plants of the Brassica family (kale, cabbage, broccoli) are included as a major part of the ration of ruminants in some parts of the world. These plants contain isothiocyanates that can interfere with thyoperoxidase activity in the thyroid gland, blocking thyroxine production and thus leading to hypothyroidism and goiter. Kale poisoning, or a severe

haemolytic anaemia, was discovered in cattle in Europe in the 1930s, but its link with the hydrolytic product of S-methyl cysteine sulfoxide was only shown years later (Smith, 1980). The effects of other bioactive sulfur-containing compounds present in plants are reviewed by Stoewsand (1995) and will not be considered further in this review. Humans have figured out how to use sulfur-containing compounds for biological warfare. For example, sulfur mustard killed thousands of men and horses during the First World War and was employed by Iraq against Iran from 1984–1987. It causes severe local blistering burns if it touches the skin or is inhaled (Rice, 2003).

Sulfur-containing organic compounds are commonly found among the myriad compounds causing toxicosis in animals maintained on pastures within oil fields. Cattle will voluntarily ingest petroleum and chemicals used in the exploration, production, and transportation of crude petroleum (Coppock et al., 1996). Sulfur-containing gases in oil fields are irritating to the mucosa of the eye and respiratory tract.

One other sulfur compound deserves some mention. Dimethyl sulfoxide (DMSO) is widely used in horses and other animals as a topical anti-inflammatory (sometimes illegally). It is capable of crossing the skin and entering the tissues under the skin. It is also a very good solvent and can solubilize substances that are not readily soluble in water. In and of itself DMSO is not very toxic, even when ingested orally. Because DMSO is a good solvent and is capable of crossing the skin, it is sometimes used to drag other drugs across the skin directly into the inflamed joint or other area of the body for localized treatment. It is the combining of DMSO with other substances that makes topical application of DMSO potentially dangerous (Brayton, 1986). As an example, combining DMSO with a topical counter-inflammatory joint treatment containing mercury caused systemic mercury poisoning in a horse (Schuh et al., 1988).

The 1980 NRC publication *Mineral Tolerance of Domestic Animals* presented an exhaustive review of the toxicities of inhaled, intravenous, and subcutaneous doses of various organic and inorganic sulfur compounds. This review will only focus on toxicities caused by oral ingestion of inorganic sulfur compounds and the sulfur-containing amino acids (Table 28-1). Oral ingestion of sulfate salts such as calcium sulfate or magnesium sulfate can also affect acid-base balance of the body. This aspect of sulfur toxicity is described in the chapter entitled "Minerals and Acid-Base Balance."

Single Dose

There are few data on the toxicity of a single dose of sulfur compounds in domestic species. At one time elemental sulfur, often referred to as "flowers of sulfur," was used extensively in veterinary and human medicine as a purgative and was thought to have a variety of curative properties. Ales (1907) described a situation where 300 g of a flowers of sulfur gruel had been administered to five horses to treat "collar gall"—i.e., sores where the yoke or the harness collar rubbed against the neck of the horse. Within three hours the horses exhibited violent abdominal pain and colic, had a fetid diarrhea, and collapsed. One died and the others recovered over a period of days.

In a similar case, 20 yearling heifers were given about 250 g elemental sulfur mixed with a small amount of corn grain as part of a "treatment" for ringworm. All animals developed a severe watery diarrhea and the "rotten egg" smell characteristic of hydrogen sulfide was noted around the cattle. Several of the heifers died within the first day with more than half of the animals dying over the next three days (Julian and Harrison, 1975).

Toxicity of Sulfur

It is commonly noted in studies with nonruminant species that switching animals to a high sulfur diet or water source has a cathartic effect, though wetter feces do not seem to affect animal performance in and of themselves. There are no reports of acute toxicosis (poor performance observed with less than 10 days of consumption) for sulfur in nonruminant species. In ruminants, the data implicating high sulfur diets as the cause of death from polioencephalomalacia does not warrant distinguishing between acute and chronic effects of high sulfur exposure. Ruminants and nonruminants will be considered separately as the etiology of toxicosis is very different in the two groups of animals.

Nonruminants

Pigs are able to consume diets that are up to 0.42 percent sulfur (supplemented with potassium sulfate) for 4 months with no deleterious effects (Dale et al., 1973). Diets using higher dietary levels of sulfur have not been reported. Piglets and sows can tolerate drinking water that is 3,000 mg sulfate/L (1,000 mg sulfur/L) with no deleterious effects on growth or reproduction. These levels of sulfate in the water do have a cathartic effect and increase the water content of the feces, but this does not affect the health of the pig (Paterson et al., 1979).

Leach et al. (1960) found that increasing dietary sulfur from 0.41 to 1.20 percent decreased growth rate of chicks dramatically. When using calcium sulfate (gypsum) to increase the dietary sulfur, these results might be explained away as being due to induction of a metabolic acidosis in the chicks (see chapter on Minerals and Acid-Base Balance). However, similar results were observed when a mixture of sodium and potassium sulfate, which would not be expected to acidify the chick's blood, was added to the diet, suggesting an effect of sulfur on growth independent of effects on acid-base balance. Egg production decreased in laying hens given drinking water that was 10,000 mg sulfate/L (3,333 mg S/L) when magnesium sulfate was used as the source of sulfate, but not if sodium sulfate was used to supply the

sulfate. Egg production was markedly reduced when hens were given 12,000 mg sulfate/L drinking water with sodium sulfate as the source of sulfate (Krista et al., 1961). Adams et al. (1975) titrated the effects of drinking water with 250, 1,000, 4,000, and 16,000 mg sulfate/L on laying hen performance. They used either magnesium sulfate or sodium sulfate as the source of sulfate. Feed consumption was significantly depressed and egg production was decreased in birds given water 4,000 mg sulfate/L, regardless of sulfate source. However, nearly 15 percent of birds given 4,000 mg sulfate/L water using magnesium sulfate as the source of sulfate died, while sodium sulfate did not kill the birds. All birds given 16,000 mg sulfate/L water died.

The use of sulfites to preserve meat meant for pet food is not without problems (and is not permitted by many countries). The addition of sulfur dioxide to meat can rapidly inactivate thiamine within the rest of the diet. A cat with allergic dermatitis was fed a diet of fresh meat preserved with sulfites and a multivitamin supplement for 38 days to exclude food allergy as a cause of its dermatopathy. The cat died as a result of acute thiamine deficiency (Steel, 1997). Dogs and cats consuming meat preserved with sodium metabisulfite containing from 400 to 1,480 mg sulfur dioxide/kg developed ataxia, depression, and in some cases convulsions over a period of several months (Studdert and Labuc, 1991). Although sulfur dioxide is highly regulated in meat meant for human consumption, some meat intended for pet foods is still preserved with it, usually by soaking it in a brine of sodium metabisulfite.

Little is known of sulfur toxicity in fish. Sulfur dioxide emitted from smokestacks, and its deposition as sulfuric acid rain, has impaired surface water quality in the Adirondack and Catskill regions of New York by lowering pH levels, decreasing acid-neutralizing capacity, and increasing aluminum concentrations—all of which are thought to contribute to reduced diversity and abundance of aquatic species in lakes and streams. There are also linkages between acidic deposition and fish mercury contamination and eutrophication of estuaries (Driscoll et al., 2003).

Methionine and Cysteine Toxicity

Methionine is approximately 21.5 percent sulfur and cysteine is about 26.5 percent sulfur. The addition of 5 g methionine/kg diet to a basal ration that was 0.35 percent methionine and 0.35 percent cysteine (bringing total diet sulfur to about 0.17 percent) was fed to growing chicks with no deleterious effects. However, increasing the basal diet methionine to 1.35 percent caused a 40 percent decrease in growth of the chicks (Katz and Baker, 1975). The added methionine raised total sulfur in the diet to just 0.38 percent—a dietary sulfur level Leach et al. (1960) used as a level for the control diet in their studies, suggesting the toxicity of methionine is independent of the sulfur the methionine brings to the diet.

Cats given DL-methionine (1 g/kg BW/day) developed severe hemolytic anemia with marked increase of methemoglobin (MetHb) concentration and Heinz-body formation 6–10 days after treatment began (Maede et al., 1987).

Ruminants and Polioencephalomalacia

Ruminants comprise the principal species likely to develop sulfur toxicosis, which most commonly presents as rapidly developing central nervous system symptoms such as ataxia, blindness, and seizures, often followed by death. These adverse effects of sulfur in the diet or water are quite different from the adverse effects of sulfur in nonruminants, in which sulfate entering the small and large intestine causes an osmotic diarrhea as the most significant observable clinical finding. Brain lesions are most commonly described as polioencephalomalacia—swelling of the cerebrum, evidenced by flattened gyri and shallow sulci. The cerebral cortex may be thinned. The distribution is symmetrical. Histological section may reveal a pale layer near the junction of the gray and white matter. The affected areas of the cortex will fluoresce under ultraviolet light.

Rumen microbes generally convert a good proportion of the dietary sulfur to sulfide, which can be absorbed and converted to sulfate by the liver or go on to form hydrogen sulfide or sulfur dioxide (which are gases) within the rumen. Dougherty et al. (1965) discovered that during the eructation process ruminants normally inhale rumen gases into the lung. They further demonstrated that large amounts of hydrogen sulfide generated in the rumen could be absorbed across the lungs during eructation and would cause symptoms of central nervous system disruption in the sheep. When they placed high amounts of hydrogen sulfide into the rumen of a sheep with a blocked trachea so that rumen gases could not be drawn into the lungs, there were no clinical signs observed, providing direct evidence that inhalation of the rumen gases was a required factor contributing to the appearance of clinical symptoms. The hydrogen sulfide gas does not cross the rumen wall and enter the blood. Hydrogen sulfide is a potent inhibitor of cytochrome C oxidase, vital to cellular respiration (Beauchamp et al., 1984), and since the brain has a very high energy requirement it is logical that sulfur intoxication would be associated with symptoms of central nervous system failure and brain lesions.

Hydrogen sulfide can build up in the gas cap above the rumen fluid, with H_2S concentration in these gases being many fold higher than concentrations of H_2S in the rumen fluid (Gould et al., 1997; Loneragan et al., 1998). Rumen gas H_2S concentrations peaked about 1–3 weeks after cattle were placed on a higher sulfur diet (0.37 percent sulfur from sodium sulfate) and this corresponded to the time when clinical symptoms appeared in the cattle. After this peak, hydrogen sulfide levels in rumen gas decreased and no further clinical cases of polioencephalomalacia developed, which

must reflect some kind of adaptation of rumen microbes to the higher-sulfur diets (Gould et al., 1997).

Other workers have demonstrated that sulfur dioxide can cleave thiamine. Cleavage of thiamine is suggested as a cause of thiamine deficiency in the ruminant, leading to cerebrocortical necrosis (Edwin and Jackman, 1982). The lesions of polioencephalomalacia commonly described during sulfur intoxication of ruminants are similar, but not identical, to the cerebrocortical necrosis associated with thiamine deficiency (Jeffrey et al., 1994). Further, most cases of polioencephalomalacia attributed to sulfur intoxication are not accompanied by decreased blood thiamine concentrations (Gould, 2000).

The SO_2 generated in the rumen will also react with water in the rumen to form sulfuric acid, which is thought to be responsible for hemorrhagic lesions in the wall of the rumen above the rumen fluid level and abdominal pain in animals dying of acute sulfur intoxication (Julian and Harrison, 1975; Kandylis, 1984).

Most reports of polioencephalomalacia are made in beef cattle or lambs on high concentrate diets. Gould et al. (1991) reported that 5 of 9 growing steers fed a diet that was 0.36 percent sulfur developed polioencephalomalacia. Low et al. (1996) reported that 21 of 70 growing lambs fed a 0.43 percent sulfur high concentrate diet developed symptoms or lesions of polioencephalomalacia. Zinn et al. (1997) found that increasing diet sulfur from 0.20 to 0.25 percent using ammonium sulfate as the sulfur source caused a reduction in feed intake and average daily gain and reduced carcass quality of feedlot steers. It is not known if the ammonium added to the diet, which was primarily composed of steam flaked corn, caused this response or if the sulfate was truly responsible for this response. Ammonium added to diets of neutral or alkaline pH is sometimes converted to ammonia, which is volatile and irritating to cows and as a consequence reduces intake of the diet. Rumsey et al. (1978) used elemental sulfur as a sulfur source and reported no problems in feedlot steers fed a 0.42 percent sulfur diet, but steers fed a 0.98 percent sulfur diet went completely off feed. Slyter et al. (1988) fed purified diets that were up to 1.72 percent sulfur from elemental sulfur to newly weaned calves for up to 50 days with no deleterious effects. Dairy cows are rarely reported to suffer from polioencephalomalacia, despite the fact that addition of sulfate (in the form of anionic salts) to bring diet sulfur to 0.5 percent is commonly practiced as a means of controlling milk fever in cows in late gestation (Block, 1984; Oetzel et al., 1988; Gaynor et al., 1989).

Water coming from wells can often contain high concentrations of sulfur, usually in the form of sulfate. Water quality reports typically express sulfur content of water in units of sulfate/L. Concentrations as high as 5,000 mg sulfate/L are not uncommon (NRC, 1977). Embry et al. (1959) failed to observe any adverse effects in cattle drinking water that was 7,000 mg sodium sulfate/L (4,760 mg sulfate/L) but observed toxicosis in cattle drinking 10,000 mg sodium sulfate/L (6,800 mg sulfate/L). A series of studies (Weeth and Hunter, 1971; Weeth and Capps, 1972) examined the effect of drinking water with added sodium sulfate on feed intake and water intake of growing beef heifers fed predominantly hay rations, culminating in the conclusion that 2,500 mg sulfate/L (or 834 mg sulfur/L) represents the maximal safe concentration of sulfate in drinking water (Digesti and Weeth, 1976). However the study of Loneragan et al. (2001) demonstrated that significant decreases in BW and carcass yield occurred when drinking water for feedlot steers was 1,219 mg sulfate/L but not when water was 582 mg sulfate/L.

Miscellaneous Considerations

High dietary sulfur can reduce the availability of trace minerals to ruminants, probably through formation of insoluble sulfides and other complexes with the trace minerals within the rumen. The copper-sulfur-molybdenum interaction in the rumen can form copper tetrathiomolybdate, which renders the copper unavailable to the animal. Increasing diet sulfur from 0.2 to 0.4 percent can cause a 50 percent reduction in copper absorbed from the diet (Suttle, 1991). Similarly, increasing diet sulfur content reduced true digestibility of dietary selenium from 50.5, when no sulfate was added to the diet, to 46.0, and to 42.3 percent as diet sulfur was increased by 0.2 and 0.4 percent, respectively. The high dietary sulfur level placed these cows in negative selenium balance (Ivancic and Weiss, 2001).

Sodium metabisulfite and sodium sulfite are also used to preserve silage, especially wilted grass or alfalfa silage intended for ruminants. When properly conducted, the majority of sulfur dioxide produced from the sulfite is converted to sulfuric acid, bringing the pH down to preserve the ensiled material. Cows receiving 9–10 g sulfur per day, as sulfur dioxide incorporated into their diet, exhibited no deleterious effects (Weigand et al., 1972). However, adding 15 g sulfur from sodium sulfite decreased rumen production of acetate, which was associated with a reduction in milk fat synthesis (Alhassan et al., 1969).

Methionine Toxicity

Methionine imbalance can also be toxic to ruminants. Abe et al. (2000) examined the occurrence of methionine imbalance and toxicity using thirty 70- and 100-kg bull calves. The animals had been trained to maintain reflex closure of the reticular groove after weaning at 5 weeks of age. Calves received a corn-soybean meal diet. Postruminal administration of 6 g of DL-methionine each day increased ADG, feed intake, gain/feed, and nitrogen retention compared with a control group receiving nitrogen-free supplement. Administration of 12 g of DL-methionine per day did not improve these variables. Addition of 18 and 24 g methionine per day resulted in BW loss and decreased gain/feed and nitrogen utilization efficiency. In a study by Satter et al. (1975), the

toxicity of DL-methionine and methionine hydroxy analog infused into the rumen or abomasum was gauged by relative feed consumption. A continuous intraruminal infusion of at least 3 days' duration of DL-methionine equivalent to about 2.5 percent or more of dietary DM intake was required to reach a toxic amount. This was approximately four times the amount necessary when it was infused into the abomasum. Methionine hydroxy analog equivalent to about 1 percent or more of dietary DM intake was toxic when infused into either the rumen or abomasum. This is in large excess of the amount of methionine analog typically added to diets.

Factors Affecting Toxicity

Nonruminants are relatively secure from the acute mortality from sulfur toxicosis observed in ruminants. Generally, nonruminants respond to excessive dietary sulfur by reducing feed intake. Within ruminants there is great disparity in dietary levels at which toxicosis is observed. Several factors are likely involved. Feedlot animals are often fed diets that are 90 percent concentrate and very low in fiber. In contrast, dairy cows are rarely fed diets that are more than 60 percent concentrate. Readily fermentable carbohydrate in the diet appears to increase the activity of sulfate, reducing bacteria and causing more H_2S to be produced. High grain diets also reduce rumen pH, which can also increase the toxicity of dietary sulfur. H_2S trapped in solution within the rumen fluid becomes less soluble as the pH decreases, causing the amount of H_2S in the gas cap to increase (Gould, 2000).

Diets that are 0.4 percent sulfur can induce polioencephalomalacia in feedlot cattle fed on high concentrate, low fiber diets. Yet dairy cattle have commonly been fed 0.5 percent sulfur diets with no reports of polioencephalomalacia. The data suggest that the tolerance for dietary sulfur is dependent on dietary concentrate content. Spears and Lloyd (2005) found that 0.46 percent sulfur diets were well tolerated in corn silage-based diets, but caused a dramatic reduction in feed intake and gain when a 0.46 percent sulfur high concentrate diet, typical of feedlot diets, was administered. Water sulfate tolerance seems to also be dependent on diet. Loneragan et al. (2001) demonstrated a dramatic reduction in growth and carcass yield when water sulfate exceeded 600 mg/L. These steers were fed a finishing ration that was a high-concentrate diet. In the study of Digesti and Weeth (1976), heifers fed a grass hay diet ad libitum could drink water that was 2,500 mg sulfate/ L with no health or growth problems.

The mineral content of the diet may also play a role in the susceptibility to polioencephalomalacia from dietary sulfur. Gould et al. (1991) demonstrated that 0.36 percent sulfur feedlot diets could result in polioencephalomalcia. In this study it may be important to note that the basal ration barely met the animals' requirement for copper. Copper and some other trace minerals have a high affinity for sulfides. Though this generally renders the trace mineral unavailable, it may provide some small degree of protection from polioencephalomalacia by reducing conversion of sulfide into neurotoxic hydrogen sulfide within the rumen.

When water is an important source of dietary sulfur, the risk of polioencephalomalacia may be increased during hot weather as animals drink more water.

TISSUE LEVELS

Measurement of ruminal gas sulfide concentration is a relatively sensitive indicator of the predilection of cattle to develop polioencephalomalacia. Rumen gas sulfide is normally less than 500 mg/L but typically rises above 3,000 mg/L in cows developing polioencephalomalacia (Gould et al., 1997; Loneragan et al., 1998). Blood sulfur in cattle is between 1.5 and 1.8 mg/ml. The concentration of sulfur in soft tissues of the body is generally between 1,000 and 2,000 mg/kg WW and does not change appreciably in response to diet (Table 28-2). Tissue sulfur content consists of sulfur contained within methionine and cysteine of tissue proteins and sulfur present as sulfate within tissues.

MAXIMUM TOLERABLE LEVELS

The maximum tolerable level of sulfur in diets of nonruminant and ruminant animals will be considered separately. For nonruminant (simple-stomached) animals the criteria for determining the maximum tolerable level of sulfur is the highest dietary level that can be safely fed without affecting health or performance. When the maximum tolerable level of dietary sulfur is exceeded, the typical reaction of these animals is a reduction in feed intake, which may or may not be accompanied by osmotic diarrhea. It is difficult to find data to justify a maximum tolerable level for dietary or water sulfur concentration in nonruminant species. For swine the maximum tolerable dietary sulfur level is 0.4 percent. Swine can tolerate water that is 3,000 mg sulfate/L (1,000 mg sulfur/L). Dogs and cats fed diets consisting primarily of meat will generally consume diets that are 0.5 percent sulfur with no ill effects. Dogs and cats fed diets composed of large amounts of fish may be receiving diets that are even higher in sulfur. For example, menhaden fish meal is 1.16 percent sulfur (NRC, 2001). No reported studies examined the effects of high dietary sulfur in these species. Therefore since cats and dogs typically consume diets that are 0.5 percent sulfur, the maximum tolerable level of sulfur in the diet of these species will be set at 0.6 percent.

Data on poultry only exist for chickens but it is reasonable to assume most birds will respond similarly to the chicken. Chicks can safely tolerate 0.4 percent sulfur diets. Their tolerance may be higher but in the only existing study, the next highest dose of sulfur tested was 1.2 percent and this depressed growth (Leach et al., 1960). Though data of Krista et al. (1961) suggest chickens may tolerate as much as 10,000 mg sulfate in drinking water, the data of Adams et al.

(1975) do not justify setting this level as the maximal tolerable level. Because Adams et al. (1975) demonstrated reduced performance when water contained 4,000 mg sulfate/L, it seems prudent to set the maximum tolerable level for sulfate in drinking water of birds to 1,000 mg/L (333 mg sulfur/L), based on the upper level tested in Adams' study (1975).

Cattle fed diets typical of beef animals in the finishing phase have a lower tolerance for sulfur, based on avoidance of polioencephalomalacia or reduced weight gain, than do cattle fed higher forage diets. Therefore two maximum tolerable levels for sulfur are applicable—depending on the diet the animal is being fed. Cattle and sheep fed diets with less than 15 percent forage are at risk of polioencephalomalacia when diet sulfur is as low as 0.35 percent. Therefore the maximum tolerable level of sulfur in diets that are more than 85 percent concentrate is 0.30 percent. Drinking water for cattle, and probably other ruminants, fed these high concentrate diets should contain less than 600 mg sulfate/L (200 mg sulfur/L). The maximum tolerable dietary sulfur level based on avoidance of polioencephalomalacia for cattle and other ruminants fed diets with at least 40 percent forage is 0.50 percent sulfur. Cattle and other ruminants on these higher forage diets can safely drink water that is 2,500 mg sulfate/L (834 mg sulfur/L). Data on dietary sulfur interactions with copper and selenium availability in ruminants would dictate that sulfur content of cattle diets be limited to the requirement of the animal, which is 0.2 percent dietary sulfur for dairy and 0.15 percent in beef cattle and other ruminants. Though this may be an important factor affecting trace mineral absorption, the data do not warrant using this criterion when determining the maximum tolerable dietary sulfur level.

FUTURE RESEARCH NEEDS

The dearth of experimental evidence available does not allow a very precise definition of the maximum tolerable dietary sulfur level for many species. For instance, there are no published studies on the effects of dietary or water sulfur content on the health and performance of horses. Also, the maximum tolerable sulfur levels for nonruminant species described in this chapter are often well below the levels of dietary sulfur known to produce deleterious effects. However, no studies that have examined these intermediate exposures to dietary sulfur exist.

Further work may elucidate the role diet has on the high susceptibility of feedlot beef animals to sulfur in their diets and drinking water. Since rumen bacterial population shifts are implicated in the greater susceptibility to sulfur toxicity of ruminants on high concentrate diets, identification of the rumen microbes responsible for hydrogen sulfide generation in the rumen may suggest methods for protecting the cattle from polioencephalomalacia. For example, a practical unanswered question concerns the effect monensin might have on sulfur toxicity in cattle.

SUMMARY

Excessive dietary sulfur is rarely a practical concern in nonruminant species. Ingredients used to formulate rations for these species would not be expected to pose a risk of the animal manifesting sulfur toxicosis. Even in cattle fed feedlot finishing diets, where death from polioencephalomalacia can occur with 0.35 percent sulfur diets, it is uncommon for feedstuffs to cause dietary sulfur to exceed 0.25 percent. However, in many sections of the country, the water that animals are forced to drink can be very high in sulfate. From a practical standpoint water represents the greatest source of exposure of animals to sulfur. When cattle are fed high concentrate diets, water sulfur concentrations in excess of 200 mg/L can be associated with reduced performance. In contrast, finishing swine on similar diets can tolerate water that is 1,000 mg sulfur/L.

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TABLE 28-1 Effects of Sulfur Exposure in Animals

Toxicity from a Single Dose of Sulfur per os

Animal (N)	Age	Quantity	Source	Effect(s)	Reference
Horses (5)	Adult	300 g	Elemental S in an oral drench	Violent colic, fetid diarrhea	Ales, 1907
Horses (4)	Adult	0.27 g sulfur/kg BWT	Magnesium sulfate solution administered via intragastric tube	Loose stools, but well tolerated	Freeman et al., 1992
Cattle (20)	Yearling	250 g	Elemental S in an oral drench	Severe watery diarrhea, abdominal pain, rotten egg smell to rumen, 60% death within 3 d	Julian and Harrison, 1975

Effects of Sulfur Exposure from Diet or Water Sources

Animal (N)	Age	Quantity	Source	Duration	Effect(s)	Reference
Cats (200)	All ages	420–1,480 mg SO_2/kg	Sodium metabisulfite	Months	Ataxia, depression, seizures in about 10%	Studdert and Labuc, 1991
Dogs (65)	All ages	420 mg SO_2/kg	Sodium metabisulfite	Months	Ataxia, depression, cerebrocortical necrosis of the brain consistent with thiamine deficiency	Studdert and Labuc, 1991
Chicks (24)	8 d	0.35% methionine (0.17% sulfur)	Basal chick diet with 0.35% cystine	6 d	Good growth ingredients	Katz and Baker, 1975
Chicks (24)	8 d	1.35% methionine 0.35% cystine (0.38% sulfur)	Basal chick diet with added methionine	6 d	40% reduction in growth	Katz and Baker, 1975
Chicks (17)	Growing	0.41% S 1.20% S 1.20% S	Basal diet Basal diet+ $CaSO_4$ Basal diet + Na_2SO_4 and K_2SO_4	28 d 28 d 28 d	Normal growth Depressed growth Depressed growth	Leach et al., 1960
Laying hens (30)	Pullets	200 mg sulfate/L drinking water 10,000 mg sulfate/L drinking water 10,000 mg sulfate/L drinking water 12,000 mg sulfate/L drinking water	Tap water Added $MgSO_4$ to drinking water Added Na_2SO_4 to drinking water Added Na_2SO_4 to drinking water	12–16 wk 12–16 wk 12–16 wk 12–16 wk	Control Slight depression in egg production, wetter feces No deleterious effects, wetter feces Marked depression in egg production	Krista et al., 1961

continued

TABLE 28-1 Continued

Effects of Sulfur Exposure from Diet or Water Sources

Animal (N)	Age	Quantity	Source	Duration	Effect(s)	Reference
Laying hens (12)	Pullets	250 mg sulfate/L	Added $MgSO_4$ to drinking water	3–4 wk	No deleterious effects	Adams et al., 1975
		250 mg sulfate/L	Added Na_2SO_4 to drinking water	3–4 wk	No deleterious effects	
		1,000 mg sulfate/L	Added $MgSO_4$, to drinking water	3–4 wk	No deleterious effects	
		1,000 mg sulfate/L	Added Na_2SO_4 to drinking water	3–4 wk	No deleterious effects	
		4,000 mg sulfate/L	Added $MgSO_4$ to drinking water	3–4 wk	Depressed feed intake and egg production 15% mortality	
		4,000 mg sulfate/L	Added Na_2SO_4 to drinking water	3–4 wk	Depressed feed intake and egg production No increased deaths	
		16,000 mg sulfate/L	Added $MgSO_4$ to drinking water	3–4 wk	All died within 12 d	
		16,000 mg sulfate/L	Added Na_2SO_4 to drinking water	3–4 wk	All died within 12 d	
Pigs (18)	5–6 wk	Basal (0, 15% S) diet + 0.22% S added	Potassium sulfate	20 d	No effect of the added sulfate which raised total diet S to approximately 0.37%	Dale et al., 1973
Pigs (12)	5–6 wk	Basal (0.15% S) diet + 0.27%S	Sodium sulfate	130 d	No deleterious effects of the added sulfate, which raised total diet S to 0.42%	Dale et al., 1973
Pigs (18)	3–4 wk	1,836 mg sulfate/L drinking water	Added $MgSO_4$ and Na_2SO_4 to water that was also high in saline	42 d	No deleterious effects on weight gain Scours observed first weeks when water sulfate was increased	Anderson and Stothers, 1978
Sows (19)	Gestation through lactation	320; 1,820; and 3,320 mg sulfate/L water	Sodium sulfate added to drinking water	3 mo	No deleterious effects on sow litter size or growth of piglets	Paterson et al., 1979
Pigs (18)	4 wk	320 mg sulfate/L water	Control tap water	28 d	Control rate of growth	Paterson et al., 1979
		3,000 mg sulfate/L water	Added Na_2SO_4 to drinking water	28 d	No significant effect on growth, feces not as firm	
		3,000 mg sulfate/L water	Added $MgSO_4$ and Na_2SO_4 to Water	28 d	No significant effect on growth, feces not as firm	

Cattle (9)	115–180 kg	0.36% S diet	Sodium sulfate in a 17% starch, 17% dextrose, low fiber diet Minimal Cu, Zn, and Mo level	25 d	5 of 9 calves developed polioencephalomalacia within 21 d No significant decrease in brain or tissue thiamine concentrations Increased rumen sulfide concentrations when compared to control calves fed long stem hay diet	Gould et al., 1991
Cattle (4)	Weaned calves	0.34, 0.94, 1.72% S	Elemental S in diet that was 27% starch, 40% sugar, and 20% corn cobs	39–50 d	No differences in weight gain or feed intake	Slyter et al., 1988
Cattle (4)	120- to 160-kg steers	0.37% S	Sodium sulfate added to diet high in starch and sugar and low in fiber	17 d	3 of 4 steers developed polioencephalomalacia	Gould et al., 1997
Cattle (36)	384-kg heifers	0.15% S 0.20% S 0.25% S	Basal diet = low fiber finishing ration Basal + $(NH_4)_2SO_4$ Basal + $(NH_4)_2SO_4$	76 d 76 d 76 d	Good growth Good growth Decreased average daily gain and decreased longissimus muscle area	Zinn et al., 1997
Cattle	Feedlot	0.14% S 0.42% S 0.98% S	Elemental S added to diet that was a 90% concentrate finishing diet		Control = good growth No deleterious effect Severe depression in feed intake within 2 wk necessitating removal from trial	Rumsey et al., 1978
Cattle (40/gp)	Feedlot steers	0.12, 0.3, and 0.46% S	Ammonium sulfate added to corn silage diet	84 d	No effect on growth, DM intake or gain/feed	Spears and Lloyd, 2005
Cattle (40/gp)	Feedlot steers	0.13, 0.31, and 0.46%	Ammonium sulfate added to a high concentrate diet		Significant reduction in gain and DM intake at 0.46% S Some reduction in DM intake at 0.31%S	Spears and Lloyd, 2005
Cattle (48)	304-kg steers	136; 291; 582, 1,219; and 2,360 mg sulfate/L	Sodium sulfate added to drinking water Steers on high concentrate, 0.16% S, ration	16 wk	Linear decrease in BW, average daily gain, and carcass yield with increasing sulfate in water	Loneragan et al., 2001

continued

TABLE 28-1 Continued

Animal (N)	Age	Quantity	Source	Duration	Effect(s)	Reference
Cattle (9)	256-kg growing heifers	3,493 mg sulfate/L	Sodium sulfate in drinking water	30 d	35% decrease in water consumption 30% decrease in feed consumption	Weeth and Hunter, 1971
Cattle (12)	Growing heifers	1,250 mg sulfate/L	Sodium sulfate in drinking water	90 d	No deleterious effects	Weeth and Capps, 1972
		2,500 mg sulfate/L	Sodium sulfate in drinking water	90 d	No deleterious effects	
Cattle (9)	Growing	2,814 mg sulfate/L	Sodium sulfate in drinking water	30 d	12% decrease in feed intake	
Cattle	Adult	4,760 mg sulfate/L	Sodium sulfate in drinking water	Chronic	No deleterious effects	Embry et al., 1959
		6,800 mg sulfate/L	Sodium sulfate in drinking water	Chronic	Reduced feed intake, poor performance	
Lambs (71)	20 kg	0.43% S	Concentrate diet	60 d	21 of 71 exhibited polioencephalomalacia, symptoms (cortical blindness, depression, head-pressing) between 15 and 32 d of switch to 0.43% S diet	Low et al., 1996

SI conversion: 1 mg sulfur equals 31.2 µmoles sulfur.

TABLE 28-2 Sulfur Concentrations in Tissues of Sheep

	Plasma	Kidney	Liver	Muscle	Heart
Sulfur content (mg S/kg fresh weight)	27–36	1,600–1,800	2,600–2,800	1,200–1,400	1,600–1,800

NOTE: Other species are expected to be similar. Tissue sulfur content is not significantly increased by addition of sulfur to the diet.

SOURCE: Compiled from data of Puls (1994) and NRC (2001).

29

Tin

INTRODUCTION

Tin (Sn) has an atomic number of 50 and appears in group 4A of the periodic table at the boundary between metals and nonmetals. It exists naturally in both the divalent (stannous or tin 2+) and tetravalent (stannic or tin 4+) oxidation states. Industrially important forms of inorganic tin include stannous chloride, stannic oxide, and stannous fluoride, and industrially important compounds containing organic tin include dimethyltin, dibutyltin, tributyltin, dioctyltin, triphenyltin, and trichlohexyltin families (ATSDR, 2003).

Tin is a relatively scarce element; its abundance in the Earth's crust is about 2 mg/kg. The major sources of tin in 2002 were mines in China, Peru, and Indonesia, and recycling of scrap tin (Carlin, 2002). No tin has been mined in the United States since 1993.

Tin is a soft, malleable metal that is used as a protective coating for iron and steel cans and containers (21 percent of total use). Tin is also a component of various alloys, including pewter, brass, the solders for joining pipes or electrical/electronic circuits, and bearing alloys. Its use in electrical solders, construction, and transportation constitutes 24, 11, and 14 percent of total use, respectively (Carlin, 2002). Organotin compounds are used in various pesticides and as stabilizers of plastics (Kumpulainen and Koivistoinen, 1977). Tin is used as an anticaries agent in dentifrices and as a reducing agent of ^{99m}technetium for nuclear medicine (Schäfer and Femfert, 1984).

ESSENTIALITY

Schwarz et al. (1970) reported that low levels of tin (0.5–2 mg/kg diet of tin) supplied as stannic sulfate promoted growth in suboptimally growing rats fed purified amino acid-based diets and housed in plastic isolator systems. These observations have not been confirmed and experts doubt that tin is essential because of other limitations in the diets that Schwarz fed to rats (Schroeder et al., 1964; Mertz, 1986).

Limited data suggest that tin has cariostatic properties. Rats fed diets supplemented with 15 to 75 mg/kg diet of tin were found to develop fewer caries in some studies (McDonald and Stookey, 1973; Stookey et al., 1974). Tin fluoride was found to have more antiplaque properties against *Streptococcus mutans* than other fluoride compounds (Ferretti et al., 1982; Leverett et al., 1984).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Prior to the mid-1980s the preferred methods for analyses of inorganic tin were spectrophotometric methods that required separation of the tin by distillation, precipitation, or extraction (Horwitz, 1979; Greger and Baier, 1981). These analyses were more sensitive than standard atomic absorption spectrometry. However, ATSDR (2003) reported that the methods most commonly used for analyses of tin in biological and environmental materials in 2003 involved inductively coupled plasma atomic emission analysis and flame or furnace atomic absorption. The samples are generally digested in acid and sometimes extracted with a resin.

Both gas chromatography and high performance liquid chromatography are used to separate and identify organotin compounds (ATSDR, 2003). However, the methods for determination of specific tin compounds are less well developed than for total tin. No ongoing studies involving analytical techniques of tin or tin compounds were identified in a search of Federal Research in Progress in 2003 (ATSDR, 2003).

REGULATION AND METABOLISM

Absorption and Metabolism

Generally rats (Hiles, 1974; Fritsch et al., 1977; Greger and Johnson, 1981; Johnson and Greger, 1985) and humans (Calloway and McMullen, 1966; Tipton et al., 1969;

Johnson and Greger, 1982) fed 100 mg/kg diet of tin as stannous chloride excreted more than 90 percent of the tin in the feces. However, human subjects fed very low levels (0.11 mg/day of tin) of inorganic tin often lost only 50 percent of their tin intake in feces (Johnson and Greger, 1982). Sullivan et al. (1984) found that neonatal rats absorbed tin more than adult rats when both were gavaged with solutions containing ^{113}Sn (0.9 percent versus 0.01 percent of dose, respectively).

Some of the tin, especially tin 2+ lost in the feces, may be of endogenous origin. Hiles (1974) noted that 12.1 percent of a single intravenous dose of ^{113}Sn 2+ appeared in the feces but only 3.1 percent of a single intravenous (IV) dose of ^{113}Sn 4+ appeared in the feces. Moreover, when ^{113}Sn 2+ was injected into rats with their bile ducts cannulated, 11.5 percent of the dose was collected in the bile; virtually none of the ^{113}Sn from an IV dose of ^{113}Sn 4+ was collected in the bile.

Little tin is excreted in the urine of rats (Hiles, 1974; Fritsch et al., 1977) or humans (Tipton et al., 1969; Johnson and Greger, 1982; Perry and Perry, 1959). Urinary losses of tin reflect large differences in tin intake. Eight human subjects excreted four times more tin (122 versus 29 µg/day of tin) when fed 50 mg as stannous chloride rather than 0.11 mg tin daily (Johnson and Greger, 1982).

Absorption of some organotin compounds (e.g., triethyltin and trimethyltin) is high (Kimbrough, 1976). For example, the lethal dose of triethyltin is the same whether administered intraperitoneally (IP) or orally. The lethal oral dose of trimethyltin tin is about twice the lethal ip dose (Kimbrough, 1976). The absorption of dimethyltin is also much more rapid than of inorganic tin (Noland et al., 1983). Little has been reported on the excretion of organotin compounds, which may have two phases. Initially after injection, ^{113}Sn as bis-tributyltin oxide is rapidly lost from the bodies of mice. After ten days, the tin from the tributyltin is lost at a rate parallel to that of inorganic tin (Brown et al., 1977).

SOURCES AND BIOAVAILABILITY

Most fresh foods naturally contain less than 1 mg/kg of tin (Schroeder et al., 1964; Greger, 1988). Thus the amount of tin in the diets of livestock is small but humans and pets may be exposed to dietary tin from other sources.

Food additives are a minor source of dietary tin for humans (NRC, 1979; Greger, 1988). Stannous chloride is a GRAS-approved coloring agent, preservative, and sequestrant. A variety of organotin compounds have been used as ascaricides and fungicides (Schäfer and Femfert, 1984). Dibutyltin compounds have been used as anthelmintics for poultry (Barnes and Stoner, 1959). Alkyl tin compounds have been widely used as stabilizers and catalysts in plastics that come in contact with food (Sherlock and Smart, 1984) but the migration of tin from these compounds into foods is thought to be small (Kumpulainen and Koivistoinen, 1977).

The major source of dietary tin for humans (and probably for some pets) is canned foods (Schroeder et al., 1964; Greger and Baier, 1981; Sherlock and Smart, 1984; Blunden and Wallace, 2003). Foods packed in cans that were totally coated with lacquer generally contain <4 mg/kg food of tin (Greger and Baier, 1981). Light-colored foods (e.g., pineapple, grapefruit and orange juices, applesauce, tomato paste), which are often packed in unlacquered cans to prevent discoloration (Sistrunk and Gascoigne, 1983), contained 40–150 mg/kg food of tin when the cans were first opened (Greger and Baier, 1981; Sherlock and Smart, 1984). However, the amount of tin in canned foods is affected by storage conditions. Canned foods accumulated more tin when stored for several months, especially if ambient temperatures were high (Calloway and McMullen, 1966; Nagy et al., 1980) or if the foods had high nitrate levels or had a low pH (Davis et al., 1980; Sherlock and Smart, 1984). The biggest accumulation of tin in food occurred when food was stored in opened, unlacquered cans. Several acidic foods (grapefruit sections, crushed pineapple, and tomato sauce) stored in opened, unlacquered cans in the refrigerator for 1 week accumulated more than 250 mg/kg food of tin (Greger and Baier, 1981).

Public water supplies in the United States were reported to contain 1.1 to 2.2 µg/L of tin in 1977, and seawater was reported to contain 0.2 to 0.3 µg/L of tin (NRC, 1977). Waste from industrial uses of inorganic tin compounds (in dyeing fabrics, weighting silks, tinning vessels, and producing lacquers, nail polishes, and varnishes) and agricultural uses of organotin compounds as pesticides contributed tin to public water supplies. Similarly, stannous fluoride is used in many dentifrices and consequently reaches municipal sewers (NRC, 1977). Little of this tin is believed to remain in the water supply because many tin salts are insoluble in water (NRC, 1977). However, Hallas et al. (1982) noted that mixed inoculums of microorganisms from Chesapeake Bay sediments transformed inorganic tin to organotin compounds, such as dimethyltin and trimethyltin.

Bioavailability

Rats absorbed a single dose of 20 mg of tin 2+ more efficiently than of tin 4+ (2.85 versus 0.65 percent) but the anion components (fluoride, ascorbate, or pyrophosphate) of dietary tin did not affect absorption (Hiles, 1974). Fritsch et al. (1977) observed that changes (in terms of sucrose, ascorbic acid, potassium nitrate, albumen, oil, or ethanol) in diet did not affect the absorption of ^{113}Sn, but Kojima et al. (1978) noted that organic acids increased the absorption of dietary tin. Johnson and Greger (1985) observed that a 3-fold increase in dietary zinc levels (e.g., from ≈15 to ≈52 mg/kg diet zinc as zinc sulfate) increased fecal losses of tin when rats were fed 100–200 mg/kg diet of tin.

The various organotin compounds differ in bioavailability. Mammals appear to absorb trimethyltin and triethyltin much more than other organotin compounds

(Kimbrough, 1976; Mushak et al., 1982). The bioavailability of organotin compounds in aqueous solutions is greatest at neutral and slightly alkaline pH and is reduced in the presence of dissolved organic carbon (Rudel, 2003).

TOXICOSIS

Inorganic Tin

There are a few key signs of toxicosis in animals and humans, injected, fed once, or chronically fed inorganic tin. Common mechanisms underlie these signs and symptoms. Hence the toxicosis of inorganic tin is organized by types of symptoms or signs (Table 29-1).

Gastrointestinal Symptoms

There are only a few reports on the toxic effects in humans of single large doses of inorganic tin (Warburton et al., 1962; Benoy et al., 1971; Barker and Runte, 1972). The symptoms included nausea, abdominal cramping, diarrhea, and vomiting and generally developed after individuals consumed canned juices or acidic beverages prepared in tinned vessels. These beverages contained 500 to 2,000 mg/L of tin.

Other animals may be less sensitive to inorganic tin than humans. Benoy et al. (1971) found that 20 to 30 percent of cats but none of the dogs dosed with juices containing 1,370 mg/L of tin vomited. No signs of toxicosis were observed in cats or rats dosed with citric acid solutions containing up to 1,200 mg/L of tin, or in dogs and cats fed foods that contained 400–470 mg/kg food of tin. Mushak et al. (1982) observed no toxic effects in rat neonates orally dosed with 500 mg/kg BW of tin.

Chronic feeding of inorganic tin also affects the gut. Rats fed a diet containing 0.8 percent tin chloride (about 300 mg/kg BW/day of tin) for 13 weeks experienced pancreatic atrophy (Dreef van der Meulen et al., 1974). The gastrointestinal tracts of rats fed high levels of tin (≈ 2,000 mg/kg diet of tin) for 28 days hypertrophied (Johnson and Greger, 1984). Increased cell turnover in the small intestines was noted in rats fed 900 mg/kg of tin (Janssen et al., 1985).

Growth and Zinc Utilization

The effects of inorganic tin on growth are dependent on the dose and form of the tin fed. Growth of rats was usually depressed when dietary tin levels were elevated above 500 mg/kg diet of tin (deGroot, 1973; de Groot et al., 1973; Dreef van der Meulen et al., 1974; Johnson and Greger, 1984). The ingestion of soluble tin compounds (e.g., stannous chloride, stannous sulfate, and stannous oxalate) affected growth more than insoluble tin compounds (e.g., stannous oxide, stannous oleate, and stannous sulfide) (de Groot et al., 1973). However, the addition of 5 mg/L of tin as stannous chloride to the water provided to mice for 540 days did not affect their growth (Schroeder and Balassa, 1967).

Feed intakes of rats were sometimes depressed when animals were fed >500 mg/kg diet of tin (Johnson and Greger, 1984; Rader, 1991). Pekelharing et al. (1994) reported a linear inverse response of feed intake to dietary tin levels from 10–200 mg/kg diet of tin.

It is well established that zinc deficiency will depress food intake and growth of animals (Mertz, 1986). The effect of tin on growth was partially due to the interaction between tin and zinc. Rats fed 500 mg/kg diet of tin and ≈15mg/kg diet of zinc as zinc sulfate for 23 days have suppressed levels of zinc in bones and soft tissues (Greger and Johnson, 1981; Johnson and Greger, 1984). Among rats fed 100–200 mg/kg diet of tin, tibia zinc levels were depressed after 21 days (Johnson and Greger, 1984) and tibia, kidney, and plasma zinc concentrations were depressed after 28 days (Rader, 1991; Pekelharing et al., 1994).

At least part of this effect was due to the effect of dietary tin on apparent absorption of zinc. Johnson et al. (1982) found that human subjects lost an additional 2 mg zinc daily in feces when fed 50 mg tin versus 0.1 mg tin daily; this resulted in significantly poorer overall retention of zinc by these subjects. Valberg et al. (1984) confirmed these results and found that a dose of 36 mg inorganic tin depressed the absorption by humans of ^{65}Zn from 4 mg of zinc as zinc chloride or from a turkey test meal. The mechanism by which tin affects zinc absorption appears to be dose dependent. Johnson and Greger (1984) found that when rats were fed high levels (≈ 2,000 mg/kg diet of tin and ≈15 mg/kg diet of zinc as zinc sulfate), their gastrointestinal tracts were hypertrophied and endogenous losses of zinc in the feces were significantly increased. Janssen et al. (1985) observed increased cell turnover in the small intestines of rats fed 900 mg/kg diet of tin. However, Johnson and Greger (1984) observed that when moderate levels of tin (200–500 mg/kg diet of tin and [a]15 mg/kg diet of zinc as zinc sulfate) were fed, endogenous losses of zinc in feces were constant but the true absorption of zinc tended to be depressed.

Hematological Status and Iron, Copper, and Selenium Utilization

The ingestion of high levels of tin (3,000 mg/kg diet of tin) as stannous chloride induced anemia in rats (deGroot, 1973; deGroot et al., 1973). Others observed reduced hematocrits among rats fed 500 mg/kg diet of tin as compared to pair-fed controls (Janssen et al., 1985) and rats fed about 300 mg/kg BW/day of tin (Dreef van der Meulen et al., 1974). Moderate intakes of tin (100 mg/kg diet of tin) induced anemia in rats only if the rats were also fed low levels of copper (Riecks and Rader, 1990; Rader, 1990; Rader et al. 1991).

The effects of dietary tin on other measures of iron status were not consistent among studies despite similar protocols (i.e., stannous chloride was used as the source of dietary tin

in purified diets; the feeding periods lasted 21–28 days; and growing rats were used). The ingestion of moderate levels of tin (200 mg/kg diet of tin) depressed plasma iron concentrations in rats fed 35 mg/kg diet of iron (Beynen et al., 1992) but not serum iron concentrations in rats fed 35 or 250 mg/kg diet of iron (deGroot, 1973); depressed transferrin saturation, but did not increase total iron binding capacity of serum (Beynen et al., 1992); depressed plasma and spleen iron concentrations (Beynen et al., 1992), but not liver, kidney, or tibia concentrations of iron in rats fed ≈37 mg/kg diet of iron (Greger and Johnson, 1981; Johnson and Greger, 1985).

It is doubtful that the anemia induced by ingestion of tin was caused primarily by tin suppressing iron absorption. The injection of tin (>1 mg/kg BW of Sn^{+2}) into in situ rat intestinal segments reduced the absorption of ^{59}Fe (<0.3 mg/kg BW of iron) (Schäfer and Forth, 1983). However, others could not demonstrate that tin affected iron absorption of rats fed ≈36 mg/kg diet of iron and 100 mg/kg of tin (Rader et al., 1991) or humans fed daily ≈19 mg of iron and ≈ 50 mg of tin (Johnson, et al., 1982). In contrast, the effect of dietary tin on tissue concentrations of copper and copper-containing proteins appeared to be dose dependent. The plasma copper levels of rats fed high levels of tin (500 mg/kg diet of tin) were depressed to less than 20 percent of the levels found in control animals. Kidney and liver levels of copper were also severely depressed in these animals (Greger and Johnson, 1981; Johnson and Greger, 1985). Ingestion of 200 mg/kg diet of tin usually depressed copper concentrations in soft tissues among rats fed adequate levels of essential elements (≈5 mg/kg diet of copper, ≈37 mg/kg diet of iron, and ≈16 mg/kg of zinc) (Greger and Johnson, 1981; Johnson and Greger, 1985). Ingestion of only 100 mg/kg diet of tin reduced serum ceruloplasmin levels and sometimes liver copper concentrations in rats fed adequate levels of copper (≈5 mg/kg diet of copper, ≈36 mg/kg diet of iron, and ≈32 mg/kg diet of zinc) but consistently reduced liver copper concentrations among rats fed marginally adequate levels of copper (0.5 mg/kg diet of copper) (Riecks and Rader, 1990; Rader, 1991; Rader et al., 1991). Ingestion of 100 mg/kg diet of tin was also associated with depression of serum superoxide dismutase (a copper-containing enzyme) activity especially among rats fed marginally adequate levels of copper (Riecks and Rader, 1990; Rader, 1991; Rader et al., 1991). Anemia is a common symptom of copper deficiency. Moreover, deGroot (1973) demonstrated that the addition of copper to diets of rats eliminated the anemia induced by feeding 150 mg/kg diet of tin.

Johnson et al. (1982) found that the addition of 50 mg tin daily (equivalent to 100 mg/kg dry diet of tin) to the diets of humans for 20 days had no effect on the apparent absorption of copper or on plasma copper or ceruloplasmin concentrations. This may reflect adequate copper intake by the subject. Yu and Beynen (1995) reported that oral tin depressed true absorption of copper but that decreases in biliary excretion of copper compensated so that apparent absorption appeared unchanged. Accordingly, animals with marginal copper status might not be able to compensate when tin depresses the true absorption of copper.

In theory, tin may also induce anemia by affecting heme synthesizing and catabolizing enzymes. Injection of tin into mice (Chiba et al., 1985) and rabbits (Zareba et al., 1986) depressed erythrocyte δ-amino levulinic acid dehydratase (δ-ALAD is a heme synthesis enzyme) activity. Injections of tin induced hemeoxygenase (an enzyme involved in heme catabolism) activity in the kidneys and livers of rats (Kappas and Maines, 1976; Dwivedi et al, 1985). However, only the ingestion of very high doses of tin (2,000 not 200 mg/kg diet of tin) depressed the activity of blood δ-ALAD in rats (Johnson and Greger, 1985).

The effect of injected tin (as stannous chloride) on δ-ALAD could be counteracted by also injecting zinc into rabbits (Zareba et al., 1986) or sodium selenite into mice (Chiba et al., 1985). Interestingly, dietary interactions have been demonstrated not only for tin and zinc (as already discussed) but also for tin and selenium. Hill and Matrone (1970) showed that high dietary level of tin depressed the apparent absorption of selenium from chick intestinal segments. Greger et al. (1982) demonstrated that human subjects fed 50 versus 0.11 mg/day of tin apparently absorbed significantly less selenium.

Bone and Calcium Utilization

Yamaguchi et al. (1982b) observed that the hydroxyproline content of the femoral epiphyses was decreased in rats given tin orally (1 mg/kg BW of tin twice a day for 28 days). Ogoshi et al. (1981) observed that the compressive strength of femurs of young rats given tin (300 mg/L of tin) in their drinking water was significantly decreased. Japanese researchers have observed that oral exposure (3 mg/kg BW of tin twice daily for 90 days) to tin depressed the activity of acid, and sometimes alkaline, phosphatases in serum, duodenum, and bone (Yamaguchi et al., 1980, 1981).

It is doubtful that these effects of tin on bone were primarily modulated through effects on calcium metabolism. The Japanese workers have found that oral exposure to levels of tin as low as 50 mg/kg diet of tin depressed calcium concentrations in bone and serum but elevated calcium levels in kidneys of growing rats (Yamamoto et al., 1976; Yamaguchi et al., 1980, 1981). Johnson and Greger (1985) also observed that moderate levels of inorganic tin (100 mg/kg diet of tin) depressed the calcium content (but not concentration) of tibias but observed no changes in plasma calcium level of weanling rats fed recommended levels (≈5,000 mg/ kg diet of calcium). Oral exposure to tin (30 mg/kg BW by gavage) was found to increase biliary volume and calcium content in rats (Yamaguchi and Yamamoto, 1978) but had no effect on fecal or urinary calcium losses in rats fed 11,000 mg/kg diet of calcium (Yamaguchi et al., 1982a) or humans fed ≈800 mg calcium daily (Johnson and Greger, 1982).

Organic Tin Compounds

In general, organotin compounds are more toxic than inorganic tin (ATSDR, 2003). However, the toxicity of various organotin compounds varies greatly.

Relative Effects of Oral Doses of Various Organotin Compounds in Mammals

The LD_{50} of triphenyltin acetate, triphenyltin hydroxide, and tricyclohexyltin hydroxide given orally to rats was 360–540 mg/kg BW of tin whereas the LD_{50} of these compounds when injected (IP) into rats was 11.9–13 mg/kg BW of tin (Kimbrough, 1976). In contrast, the LD_{50}'s of trimethyltin and triethyltin administered orally was 10–30 mg/kg of tin and administered IP was 7–16 mg/kg of tin (Dyer et al., 1982b).

Mushak et al. (1982) observed that trimethyltin, triethyltin, and tri-n-butyltin were more toxic than other triorganotin and diorganotin compounds. Oral doses of trimethyltin (0.66 mg/kg BW of tin), triethyltin (1.3 mg/kg BW of tin), tri-n-propyltin (4.2 mg/kg BW of tin), and tri-n-butyltin (1.0 mg/kg BW of tin) killed at least some of the neonatal rats dosed. Similar oral doses of tin as tricyclohexyltin, triphenyltin, diethyltin, and dimethyltin produced no fatalities and did not affect weight gain of the pups.

The effect of organotin compounds in these studies on feed intake was important. For example, rat pups orally dosed with tri-n-butyltin (1 mg/kg BW) appeared to die of starvation (Mushak et al., 1982). Rats injected with triethyltin (1.5 mg/kg BW of tin) reduced feed intake abruptly (DeHaven et al., 1982). MacPhail (1982) demonstrated that trimethyltin and triethyltin induced flavor aversions in rats.

Some of the differences in toxicity of various organotin compounds reflected the greater gut absorption of trimethyltin and triethyltin than other organotin compounds (Kimbrough, 1976). The speed of elimination of organotin compounds and hence the retained tissue levels of tin may also be important. For example, higher concentrations of tin were found in the tissues of rats during the first 24 hours after injections of 6 mg/kg of triethyltin than after similar doses of trimethyltin, but the rate of loss of triethyltin from the tissues was also more rapid (Cook et al., 1984). However, tissue levels of tin did not predict the ultimate toxicity of organotin compounds. Mushak et al. (1982) demonstrated that although pups orally dosed with a variety of organotin compounds (1–30 mg/kg BW) accumulated tin in their brains, kidneys, and livers, only rat pups dosed with trimethyltin and triethyltin showed any neurotoxic effects.

Rats appear to be less sensitive to organotin compounds than rabbits, guinea pigs, sheep (Kimbrough, 1976), and mice (Wenger et al., 1982). Dyer et al. (1982b) found larger rats to be more sensitive than smaller rats.

The comparative toxicity of various organotin compounds to fish was generally similar to the comparative toxicity of the organotin compounds to mammals. de Vries et al. (1991) exposed rainbow trout during the early life stages (110 days) to organotin compounds. The diorganotin compounds (dibutyltin and diphenyltin) were three orders of magnitude less toxic than triorganotin compounds (tributyltin, triphenyltin, and tricyclohexyltin). Tricyclohexyltin was most toxic; no fish survived exposure to 3 nm for 1 week. The NOEL for dibutyltin and diphenyltin was 160 nM (40 and 60 µg/kg water) and for tributyltin and triphenyltin was 0.12 nM (40 and 50 ng/kg water).

Not only is tributyltin more toxic to fish than dibutyltin, it also has accumulated more in fish tissues. Farmed fish in the area of Naples, Italy, were more apt (85 percent of fish sampled) to accumulate tributyltin (2–260 µg/kg) than dibutyltin (10 percent of fish sampled and levels of 1–26 µg/kg) (Amodio-Cocchieri et al., 2000).

Trimethyltin Toxicosis

Rats injected with trimethyltin developed spontaneous seizures, tail mutilation, vocalization, and hyperactivity; cell loss was largely confined to the inferior pyramidal cells (Dyer et al., 1982b). Similarly, rats dosed orally once with trimethyltin (7 mg/kg of tin) developed hyperactivity and lost pyramidal cells (Rupert et al., 1982; Dyer et al., 1982a). Like in the rat, hyperactivity was a symptom of toxicity in mice but extensive necrosis was observed in the granule cells, not the pyramidal neurons, of the hippocampus (Chang et al., 1982).

Triethyltin Toxicosis

Orally administering triethyltin compounds for 14–27 days produced an edema in the myelin of the central nervous system and depressed food and water intake of rats (Kimbrough, 1976; Squibb et al., 1980; Mushak et al., 1982). Rats fed 20 mg/kg diet of tin as triethyltin for about 2 weeks developed paralysis in their hind limbs; symptoms regressed when exposure ceased (Magee et al., 1957). Rat pups dosed orally with 1, but not 0.3, mg/kg of tin as triethyltin exhibited tremors and microscopic changes in myelin (Mushak et al., 1982). Feeding rats a diet low (<0.4 percent of total energy) in a-linolenic acid made them more sensitive to IP injections of triethyltin (Bourre et al., 1989).

The effects of triethyltin toxicity have been observed in humans (Barnes and Stoner, 1959; ATSDR, 2003). In 1954 in France, Stalinon (a pharmaceutical containing diethyltin diodine and linoleic acid) was sold for treatment of Staphylococcal skin infections, osteomyelitis, anthrax, and acne. About 100 people using the product died. Other symptoms included headache, vomiting, abdominal pain, visual disturbances, rapid weight loss, and paralysis. Recovery after discontinuing use of the product was slow and often incomplete. The product was contaminated with triethyltin and the

toxic dose was estimated to be 70 mg of triethyltin in 8 days. However, other organotin compounds were found in the product.

Tri-n-butyltin and Dibutyltin Toxicosis

Single large oral doses of tributyltin (500 mg/kg BW), but not ingestion of a diet with 2.5 mg/kg/day of tributyltin oxide for 106 weeks, produced hemorrhages in the digestive tracts of mice (ATSDR, 2003). Rats dosed with 1 mg/kg BW/day of tin as tributyltin acetate for 24 days developed fibrosis in the portal triad region of the liver with inflammation of the bile ducts (Mushak et al., 1982).

Several groups of investigators observed that single oral doses of dibutyltin to rats (50 mg/kg) and hamsters (30 mg/kg) and chronic ingestion of dibutyltin chloride (23, but not 7.7, mg/kg BW/day) by rats for 2 weeks inflamed the wall of the bile duct (ATSDR, 2003). If the inflammation of the bile duct caused perforation of the duct, fibrosis in the pancreas and liver occurred among animals in which the pancreatic and bile ducts are combined (e.g., rats, mice, hamsters) but not in animals with separate ducts (i.e., rabbits and guinea pigs) (Kimbrough, 1976; ATSDR, 2003).

Additional Side Effects of Diorganotin Compounds

Teratological effects, especially of the skeleton, have been observed when pregnant rats are fed di-n-butyltin acetate (>10 mg/kg/day), but not n-butyltin trichloride (even at doses of 400 mg/kg/day), for 10 days during gestation (Noda et al., 1992).

Gastric intubation of rats and chicks for 10–14 days with the dichloride salts of dimethyltin, dibutyltin, and dioctyltin resulted in depression of the thymus weights of young rats and depression of the weight of the bursa Fabricii in chickens (Renhof et al., 1980). Trout exposed to >800 M dibutyltin and >0.6 nM tributyltin for 133 days did not exhibit atrophy of the thymus but were less resistant to an intraperitoneal challenge with a secondary pathogenic bacterium (deVries et al., 1991).

TISSUE LEVELS

The Agency for Toxic Substances and Disease Registry (2003) reported that no studies were found concerning the distribution of tin in tissues of humans after oral administration of inorganic or organic tin. The overall apparent retention of tin by human subjects in balance studies (whether the tin was naturally present, a contaminant from cans, or as stannous chloride) was low (i.e., not significantly different than equilibrium) (Callaway and McMullen, 1966; Tipton et al., 1969; Johnson and Greger, 1982b).

However, tin has been found in at least trace amounts in most mammalian tissue (Schroeder et al., 1964; Schäfer and Femfert, 1984). Generally, all soft tissues contained <1 mg/kg of tin but bone contained 0.5–8.0 mg/kg of tin (ATSDR, 2003). (All tissue tin concentrations are reported in this report on a wet weight basis unless noted otherwise.)

Rats fed diets (21–28 days) supplemented with inorganic tin (100–2,000 mg/kg diet of tin as stannous chloride) (Johnson and Greger, 1985) or orally dosed (twice a day for 90 days) with a solution of inorganic tin (0.3–3.0 mg/kg BW/day of tin as stannous chloride) (Yamaguchi et al., 1980) accumulated tin in their tibias, kidneys, and livers generally in proportion to their exposure to tin. The average tin concentrations in kidneys and livers of control rats in these studies ranged from 0.14–0.52 mg/kg wet tissue of tin and of rats orally dosed with tin ranged from 0.24–8.5 mg/kg wet tissue of tin; the average tin concentrations in bone of control rats ranged from 0.3–2.1 mg/kg wet bone of tin and of rats orally dosed with tin ranged from 4.3–45.7 mg/kg wet bone of tin (Table 29-2).

Ingested organic tin compounds (i.e., trimethyltin, triethyltin, tributyltin, and tripropryltin) accumulated more in the liver than in other soft tissues and even the levels in the liver were less than 0.6 μg/g wet tissue among neonatal rats that developed clinical signs (Mushak et al., 1982).

No data are available on the accumulation of tin in tissues of livestock fed controlled levels of tin. However, farmed fish in the area of Naples, Italy, were more apt (85 percent of fish sampled) to accumulate tributyltin (2–260 μg/kg) than dibutyltin (10 percent of fish sampled and levels of 1–26 μg/kg) (Amodio-Cocchieri et al., 2000).

MAXIMUM TOLERABLE LEVELS

Acute responses to inorganic tin have been observed when humans consumed a single dose of beverages containing 0.5–2.0 mg/L of tin (Warburton et al., 1962; Benoy et al., 1971; Barker and Runte, 1972). Chronic symptoms (i.e., decreased zinc absorption) were observed in humans fed 50 mg/day of tin for 20 days (Johnson et al., 1982). These subjects probably consumed about 0.5 kg dry weight of diet per day or about 100 mg/kg dry diet of tin.

The Agency for Toxic Substances and Disease Registry (2003) suggested a NOAEL of 32 mg/kg/day of tin for chronic oral exposure to inorganic tin based on limited work with rats. If the rats weighed 200 g and consumed 15 g diet per day, the toxic signs occurred when about 400 mg/kg diet of tin was consumed. These calculations did not consider data cited in this document. Several studies cited observed adverse effects (lowered status and impaired absorption of zinc and copper and anemia) in rats fed 100–200 mg/kg diet of tin (deGroot, 1973; Greger and Johnson, 1981; Johnson and Greger, 1984; Riecks and Radar, 1990; Radar et al., 1991; Pekelharing et al., 1994; Yu and Beynen, 1995).

Since the estimates in rats and humans are similar and no data are available for livestock, it is suggested that livestock should not be fed chronically more than 100 mg inorganic tin/kg diet.

Based on limited data primarily in rats, the Agency for Toxic Substances and Disease Registry (2003) suggested the following LOAELs for oral exposure to various organotin compounds for humans: dibutyltin, 3.8 mg/kg/day; tributyltin, 1 mg/kg/day; triethyltin, 0.66 mg/kg/day; and trimethyltin, 1 mg/kg/day. If a 200-g rat consumed 15 kg diet per day, chronic toxic effects would be expected to occur when diets contained more than 50 mg/kg diet of dibutyltin, more than 13 mg/kg diet of tributyltin or trimethyltin, or 9 mg/kg diet of triethyltin. These guidelines also appear logical for livestock.

FUTURE RESEARCH NEEDS

Future research on inorganic tin should focus on the interactions of inorganic tin with essential minerals because that data will yield the most sensitive indications of adverse effects of ingested inorganic tin. Almost no research could be found on the toxicity of tin compounds to livestock. A few studies with a variety of species would make application of data collected with rats to livestock possible.

Chronic studies on the metabolism (excretion and breakdown of compounds in the gut and liver) of organotin compounds are needed. Without these studies, interpretations of studies in which rats were injected with organotin compounds are often questionable. More information is also needed on interconversions of inorganic tin and organotin compounds by microorganisms in landfill leaches and lake and estuary sediments. The latter information is particularly important to those concerned with the toxicity of organotin compounds to fish.

SUMMARY

Tin is not considered to be an essential element. Exposure of livestock to high levels of inorganic tin is unlikely. If it occurred, the animals, which were in marginal nutritional status in regard to zinc or copper, would be most sensitive to chronic high doses of inorganic tin.

Organotin compounds are many times more toxic than inorganic tin. The symptoms of organotin compound toxicosis are also different than those of inorganic tin.

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TABLE 29-1 Effects of Tin Exposure in Animals

Animal	N[a]	Quantity[b]	Source	Duration	Route	Effect(s)	References
Humans	8	≈ 50 mg Sn/d (0.66 mg Sn/kg BW)	Stannous chloride	20 d	Diet	Depressed Zn absorption and urinary Zh	Johnson et al., 1982
Humans	8	≈ 50 mg Sn/d	Stannous chloride	20 d	Diet	Depressed Se absorption	Greger et al., 1982
Rats	10	300–10,000 mg Sn/kg diet	Stannous chloride and orthophosphate, sulfate, oxalate, tartrate, sulfide, oxide salts of tin	4 and 13 wk	Diet	Growth retardation, anemia esp. for those fed stannous chloride. No effect of tin sulfide, oleate, or oxides	DeGroot et. al., 1973
Rats	10	150 and 5,300 mg Sn/kg diet	Stannous chloride	4 and 6 wk	Diet	Growth depression and anemia. Anemia prevented by additional dietary copper	DeGroot, 1973
Rats	10	Gradual increase to 8,000 mg Sn/kg diet	Stannous chloride	13 wk	Diet	Growth retardation, anemia, pathology in pancreas and small intestine	Dreef van der Meulen et al., 1974
Rats	10	1–3 mg/kg BW	Triethyltin bromide	2 wk	Oral	Edema of CNS white matter tracts that reduced when dosing stopped	Squibb et al., 1980
Rats	6	0.3, 1.0, 3.0 mg/kg BW	Stannous chloride	90 d	Oral	Highest levels depressed enzyme activity in serum, liver, bone	Yamaguchi et al., 1980
Rats	7	206 mg Sn/kg diet	Stannous chloride	21 d	Diet	Depressed bone Zn, kidney Zn and Cu, zinc absorption	Greger and Johnson, 1981
Rats	3 litters of 8 pups each	0.07–10 mg Sn/kg BW	Trimethyltin, triethyltin, tri-n-propyltin, tri-n-butyltin, tricycloheyyltin, triphenyltin, diethyltin, dimethyltin	< 28 d	Oral	Highest doses of trimethyl triethyltin, tri-n-propyltin, tri-n-butyltin, triphenyltin caused death; growth retardation common. Trimethyltin damaged hippocampus; triethyltin caused myelin edema; tri-n-butyltin caused liver damage	Mushak et al., 1982 Johnson et al., 1982 Greger et al., 1982
Rats	5	1.0 mg Sn/kg BW	Stannous chloride	28 d	Oral	Decreased myaroxiproline content of femoral diaphysis	Yamaguchi et al., 1982b
Rats	7	100, 200, 500, 2,000 mg Sn/kg diet	Stannous chloride	27 d	Diet	Depressed Zn in plasma, tibia, liver, and kidney; highest Sn levels caused GI hypertrophy and increased gut endogenous loss of Zn	Johnson and Greger, 1984
Rats	7	100, 200, 500, 2,000 mg Sn/kg diet	Stannous chloride	27 d	Diet	Depressed Cu in plasma, liver, and kidneys; highest level depressed enzyme activity and bone Ca	Johnson and Greger, 1985

continued

TABLE 29-1 Continued

Animal	N[a]	Quantity[b]	Source	Duration	Route	Effect(s)	References
Rats	10	100 mg Sn/kg diet	Stannous chloride	4 wk	Diet	Depressed Cu status; enzyme changes. Marginal Cu status intensified tin effect	Riecks and Rader, 1991
Rats	10	100 mg Sn/kg diet	Stannous chloride	4 wk	Diet	Depressed Cu status; anemia	Rader et al., 1991
Rats	12–16	50–400 mg/kg 1.75–15 mg/kg	n-butyltin trichloride di-n-butyltin diacetate	10 d	Oral	Dibutyltin caused resorption of fetus and skeletal teratology	Noda et al., 1992
Rats	7	10–200 mg Sn/kg	Stannous chloride	28 d	Diet	Depressed Cu and Zn status; anemia	Pekelharing et al., 1994
Rats	12	100 mg Sn/kg diet	Stannous chloride	28 d	Diet	Decreased bihary Cu and decreased true absorption of Cu	Yu and Beynen, 1995
Chickens	15	100 and 500 mg/kg BW	Dimethyltin, dibutyltin, di-octyltin chlorides	10–14 d	Oral	Decrease in bursa fabricii in chickens	Renhof et al., 1980
Fish, rainbow trout		0.12–0.15 nM	Trimethyltin, tributyltin, triphenyltin, tricyclohexyltin	110 d	In water	Tricyclohexyltin, tributyltin, triphenyltin caused death. Ditributyltin caused atrophy of thymus	deVries et al., 1991
Fish, rainbow trout	50–100 fry						deVries et al., 1991

[a]Number of animals per treatment
[b]Quantity of tin dosed. SI conversion: 1 mg tin equals 8.4 μmoles tin.

TABLE 29-2 Tin Concentrations in Fluids and Tissues of Animals[a]

Animal	Quantity	Source	Duration	Route	Tissues	References
Humans	Unknown	Unknown	Lifetime	Diet	Soft: < 1; bone: 0.5–8.0	ATSDR, 2003
Rats	0.3 mg/kg BW; 2 times/d 1.0 mg/kg BW; 2 times/d 3.0 mg/kg BW	$SnCl_2$ $SnCl_2$ $SnCl_2$	90 d 90 d 90 d	Gavage Gavage Gavage	Soft: < 0.5; bone: ≈4 Soft: < 0.5; bone: ≈7 Soft: < 0.5; bone: ≈22	Yamagucchi et al., 1980
Rats	100 mg/kg diet 200 mg/kg diet 500 mg/kg diet 2,000 mg/kg diet	$SnCl_2$ $SnCl_2$ $SnCl_2$ $SnCl_2$	27 d 23–27 d 23 d 21 d	Diet Diet Diet Diet	Soft: < 1; bone: ≈6 Soft: ≈1.5; bone: ≈10 Soft: ≈3.5; bone: ≈20 Soft: ≈8.5; bone: ≈46	Johnson and Greger, 1985
Rats	0.66 mg/kg BW 0.44 mg/kg BW 1.0 mg/kg BW 1.2 mg/kg BW	Trimethyltin Triethyltin Tributyltin Tripropyltin	2–29 d 2–29 d 2–29 d 2–29 d	Gavage Gavage Gavage Gavage	Liver: 0.4 Liver: 0.5 Liver: 0.6 Liver: 0.6	Mushak et al., 1982
Farmed fish	Unknown	Unknown	Lifetime	Water around Naples	Tributyltin: < 0.3 mg/kg dibutyltin: < 0.03 mg/kg	Amodio-Cocchieri et al., 2000

[a]Values are mg/kg wet weight of soft tissues (liver, kidney, etc.) and bone of tin.

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Vanadium

INTRODUCTION

Vanadium (V), atomic number 23, is a bright white (NRC, 1980) or a soft silvery gray metal in the pure state (WHO, 2001). Vanadium was named after Vanadis, the Norse goddess of beauty, youth, and luster, in 1830 by Nils Sefstrom, due to the striking colors of vanadium-containing crystals and salts (Nechay et al., 1986). The two natural isotopes for vanadium are V^{50} and V^{51} of which V^{51} occurs at a 99.76 percent frequency in the environment (Stoecker and Hopkins, 1984). Metallic vanadium, a corrosion-resistant metal, does not occur in a pure form in nature, but exists in the oxidative states –1 to +5 with +3, +4, and +5 being the most common. The pentavalent state (+5) is the most common form of the element found in the environment with +3 and +4 states being quickly oxidized in the presence of water to +5. The most stable forms of vanadium are the quadrivalent salts (Barceloux, 1999). Vanadium pentoxide, a red-yellow or green crystalline powder, is the most commonly available form of vanadium (WHO, 2001). The compounds containing vanadium that are generally used in the toxicology studies are ammonia metavanadate (NH_4VO_3); sodium metavanadate ($NaVO_3$); sodium orthovanadate (Na_3VO_4); calcium orthovanadate ($Ca_3(VO_3)_2$); vanadyl sulphate ($VOSO_4$); vanadium pentoxide (V_2O_5); vanadyl sulphate pentahydrate ($VOSO_4 \cdot 5H_2O$); and bis(maltolato)oxovanadium (BMOV).

Vanadium is present in more than 50 naturally occurring minerals, and in oil and coal. The most important minerals containing vanadium used for industrial purposes are patronite (contains 10 percent vanadium and iron oxides), carnotite ($K_2O \cdot 2U_2O_3 \cdot V_2O_5 \cdot 3H_2O$), roscoelite ($2K_2O \cdot 2Al_2O_3(Mg, Fe)O \cdot 3V_2O_5 \cdot 10SiO_2 \cdot 4H_2O$), and vanadinite ($9PbO \cdot 3V_2O_5 \cdot PCl_2$) (Budavari et al., 1989). Vanadium is mined in South Africa, Russia, the United States, Finland, and China. Vanadium pentoxide is contained in a vanadium slag produced during the smelting of iron ore (WHO, 2001). Vanadium pentoxide may also be present in amounts as high as 10–15 percent in the dust, soot, boiler scale, and fly ash that accumulates from the burning of vanadium-rich oils, certain oils that are found in Venezuela and Mexico (Leonard and Gerber, 1994). Vanadium is used in relatively small portions in some steels: cutting steel, high-strength steel, and wear-resistant iron (NRC, 1980). Vanadium reviews have been provided by the NRC, 1980; Jandhyala and Hom, 1983; Stoecker and Hopkins, 1984; Lagerkvist et al., 1986; WHO, 1988; Barceloux, 1999; and WHO, 2001.

ESSENTIALITY

Vanadium is an essential element in various enzymes in algae, bacteria, fungi, and lichens (Nielsen, 2000). The enzymes include haloperoxidases, which catalyze the oxidation of halide ions by hydrogen peroxide to facilitate the formation of a carbon-halide bond. Some bacteria require vanadium for the enzymatic reaction of reducing nitrogen gas to ammonia.

There are conflicting reports as to the essentiality of vanadium for animals. The National Research Council (NRC, 1980) concluded that in the species tested, vanadium is essential for normal growth and proper physiological function. However, Nielsen (1995) concluded vanadium research conducted between 1971 and 1974 (NRC, 1980) was inconclusive as the amount of vanadium in control treatments was higher than the amount of vanadium available in normal diets, and the form of vanadium used in the studies had a very high availability. This led to difficulty in determining if the results from these studies were deficiency signs or if the level of vanadium used in the controls actually had significant pharmacological actions (Nielsen, 1985; French and Jones, 1993; Nielsen, 1995). The composition of the diet also has been found to affect the response of rats to vanadium and many of the early deficiency studies on vanadium fed diets that were not balanced for nutrients (Nielsen, 1985).

Reviews by Nielsen (1995, 2000) found circumstantial evidence that vanadium was an essential nutrient for rats and goats. Anke et al. (1986, 1989) conducted a set of experi-

ments on goats to determine vanadium essentiality. Goats that received less than 10 ng V/g of diet had higher incidents of abortion, produced less milk in the first 56 days of lactation, and had a lower conception rate on first insemination than goats fed the 0.5 or 2 μg V/g diet. Approximately 40 percent of the kids from the vanadium-deficient goats died within 7 to 91 days of life (Anke et al,, 1989). Only 8 percent of the kids from the goats that received 2 μg V/g diets died during the same time. The vanadium-deficient goats had higher serum creatine and β-lipoprotein, and lower serum glucose levels (Anke et al., 1986). They also had skeletal deformations in the forelegs and a thickening of the forefoot tarsal joints (Anke et al., 1986). Uthus and Nielsen (1990) found vanadium-deprived rats had a higher thyroid weight and thyroid-to-body weight ratio than controls fed 1 μg V/g diet. Other deficiency signs of vanadium in rats were decreased erythrocyte glucose-6-phosphate hydrogenase, cecal total carbonic anhydrase, and an altered response to high and low dietary iodide (Nielsen, 1996). However, because a defined biochemical function has not been identified in higher animals, vanadium is currently not considered an essential element.

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Concerns on the reliability of tests to analyze vanadium have been raised (Lagerkvist et al., 1986). The low amount of vanadium in the environment and especially in animal and plant tissues makes it difficult to measure amounts accurately using older methods of analysis. Contamination of samples from vanadium present in air and other sources such as stainless steel and cutting steels can alter the amounts in a sample. Seiler (1995) reviewed the methods available for analyzing the vanadium content in biological materials. In the past, spectrophotometric methods have been used to determine low concentrations of vanadium, but the risk of erroneous results due to the laborious sample preparation and the lack of sensitivity of the test is high. The most common method for vanadium analysis in biological materials is atomic absorption spectrometry (Seiler, 1995), but it only has a detection limit down to 0.2 ng/ml, whereas neutron activation analysis (NAA) can be used to detect lower levels (Lagerkvist et al., 1986). The NAA method can detect vanadium below 0.2 ng/ml and has been used to detect the erroneous results of methods used for vanadium analysis in earlier studies.

REGULATION AND METABOLISM

Absorption and Metabolism

French and Jones (1993) and Nielsen (1995) have provided reviews on the regulation and metabolism of vanadium. Studies cited in NRC (1980) indicate vanadium is absorbed from the digestive tract at an efficiency of 1 percent or less. Conklin et al. (1982) reported the uptake of 40 μg of vanadium given orally to rats as radioactive V_2O_5 was 2.6 percent. Roshchin et al. (1980) found similar results to Conklin et al. (1982), but other studies have reported less than 5 percent absorption (French and Jones, 1993) to more than 10 percent absorption of ingested vanadium (Bogden et al., 1982; Wiegmann et al., 1982). Studies cited by French and Jones (1993) suggest that the length of the fasting period and composition of the experimental diet may have resulted in higher dietary efficiencies. Thompson et al. (2002) tested the bioavailability of three vanadium-containing compounds—ammonium metavanadate, vanadyl sulfate, and bis(maltolato)oxyvanadium—that differ in chelation and oxidation states in male rats weighing 180–210 g. They found neither oxidation state nor complexation alone were adequate predictors of relative absorption or tissue vanadium uptake. In general for nonruminants, most ingested vanadium is converted to vanadyl (VO^{2+}) in the stomach; however, vanadate (HVO_4^{2-}) escaping conversion is absorbed three to five times more effectively from the gastrointestinal tract then vanadyl (Nielsen, 1995). The excretion of absorbed vanadium is fairly rapid with 40–60 percent of a given dose eliminated in one to three days after exposure (Barceloux, 1999). Absorbed vanadium is excreted mainly by the kidneys (NRC, 1980; Nielsen, 1995) with a minor amount excreted in the feces. Skin is only a minor route of exposure for vanadium (Barceloux, 1999).

Metabolic Interactions

Vanadium's high number of oxidative states makes it a very multifunctional element in the body and likely contributes to its ability to have effects at relatively low levels. In mammals, vanadium can function as a growth factor, a mitogen, and an anti-diabetic agent (Davison et al., 1997). Vanadium compounds have been shown to mimic the biological actions of insulin (Dai et al., 1995; Tsiani and Fantus, 1997). Vanadyl readily binds to nucleic acids, amino acids, phosphates, phospholipids, glutathione, oxalate, citrate, and lactate excreta (Nechay, 1984). Vanadium inhibits a large number of enzyme systems in vitro including ATPases, phosphates, and phosphoric transfer enzymes. The vanadate ion is one of the most potent inhibitors of the Na^+/K^+ ATPase pump (Jandhyala and Hom, 1983; Barceloux, 1999). However, specific activation or inhibition of an enzyme by vanadium has not been found (Nielsen, 1995).

SOURCES AND BIOAVAILABILITY

Environmental Exposure

Vanadium pentoxide is the most important vanadium-containing compound used in industry. High amounts of vanadium can be present in the air around industrial

complexes that use vanadium pentoxide. The WHO (2001) reported air levels of vanadium range from 0.001 to 1,460 ng/m^3. The concentration of vanadium in the ocean crust is approximately 250 mg/kg, and 160 mg/kg in the continental crust (Leonard and Gerber, 1994). Rain in North America and Europe has been found to contain 1.1–46 µg/L of vanadium (Galloway et al., 1982). Both fresh water and sea water contain less than 3 µg/L (Hamada, 1998). Vanadium concentrations in marine organisms are quite variable with planktonic organisms estimated to be 1 mg/kg DM and fish .08–3 mg/kg DM (WHO, 2001). The average concentration of vanadium in soils worldwide is approximately 100 mg/kg (WHO, 2001). The average concentration in the United States was found to be 60 mg/kg and ranged between < 7 mg/kg to 500 mg/kg (WHO, 2001).

Food is the major source of exposure to vanadium for the general population. Vanadium is usually present in food in the form of vanadyl (French and Jones, 1993) and at concentrations of less then 1 ng V/g (Barceloux, 1999). Foods that are relatively high in vanadium (0.05–2 µg/g) include black pepper, mushrooms, parsley, dill seed, shellfish, and some prepared foods (Nielsen, 1991). Dairy products, meat, seafood, and whole grains have vanadium concentrations in the 5–30 ng/g range (McDowell, 2003). The estimated dietary intake of vanadium by humans is 10–60 µg/day (Barceloux, 1999).

A concern in animal feed is the concentration of vanadium in rock phosphates used as phosphorus sources in diets. The NRC (1980) reported that some rock phosphates may contain up to 6,000 mg/kg vanadium. Concentrations of vanadium in phosphorus sources vary by purity, with monocalcium phosphates ranging from 46–796 mg/kg, dicalcium phosphates ranging from 36–185 mg/kg, and thermochemically produced defluorinated phosphates ranging from 20–164 mg/kg (Sullivan et al., 1994). Grazing animals can be exposed to higher levels of vanadium through unavoidable ingestion of soil (NRC, 1980; McDowell, 2003).

Supplementation Considerations

Nielsen (2000) reported that vanadium has become a component in a large number of pills and other dietary supplements to enhance strength and ward off diabetes. This trend is caused by the discovery that vanadium mimics the action of insulin in animal models (Poucheret et al., 1998). However, Domingo's (2000) review on diabetes studies with rats found that there are many complications associated with supplementing vanadium for this purpose, and toxic effects from long-term buildup of vanadium in the tissues is of great concern.

TOXICOSIS

Reviews on vanadium toxicity have been provided by the NRC (1980), Leonard and Gerber (1994), and Domingo (1994, 1996, 2000). The major clinical signs and effects of vanadium toxicosis are a reduction in weight gain of growing animals, weight loss in adult animals, and death. In rats, toxic levels of vanadium ingestion led to reductions in reproduction, reduced fluid intake, diarrhea, and changes in behavior and learning patterns. In laying hens, elevated vanadium intake has led to reduced egg production, feed intake, feed conversion efficiency, and albumin quality as measured by Haugh units. Vanadium appears to inhibit enzymes and damage cells through lysis. Vanadate has been found to activate cardiac adenylate cyclase and inhibits the (Na, K)-ATPase pathway and many other phosphohydrolase enzymes through its ability to mimic phosphate in a transitional state (Kustin, 1998).

High Quantities and Acute

Llobet and Domingo (1984) reported the LD_{50} (14-day) for rats and mice given oral administration of sodium metavanadate ($NaVO_3$) and vanadyl sulphate pentahydrate ($VOSO_4 \cdot 5H_2O$). For rats, the LD_{50} was 98 mg/kg^{-1} BW for $NaVO_3$ and 448 mg/kg^{-1} BW for $VOSO_4 \cdot 5H_2O$. In mice, the LD_{50} (14-day) was 74.6 mg/kg^{-1} BW for $NaVO_3$ and 467.2 mg/kg^{-1} BW for $VOSO_4 \cdot 5H_2O$. The toxicity of vanadium increases as the valence increases and for vanadyl (V^{+4}) the LD_{50} (14-days) was determined to be 90.3 and 94.2 mg/kg BW for mice and rats, respectively. The LD_{50} (14-day) for vanadate (V^{+5}) was determined to be 40 and 31.2 mg/kg^{-1} BW in mice and rats, respectively. Toxicity of orally ingested vanadium is dependent on the valence of the vanadium element with higher valences being more toxic and having a lower LD_{50} than lower valances.

Hansard et al. (1982) determined the toxicity of ammonium metavanadate, calcium orthovanadate, and calcium pyrovanadate given by capsule to 41-kg BW wethers. An initial dose equivalent to 100 mg of elemental vanadium of each compound was given. Quantity was increased by 50 mg per two-day intervals to determine the amount of vanadium necessary to decrease feed intake to 75 percent of control-fed wethers. The initial decline in feed intake was observed at vanadium intakes of 400–500 mg/head/day or 9.6–12 mg/kg BW. The decline in feed intake was accompanied by diarrhea. All three compounds were similar in toxicity as they brought about the decline in feed intake at approximately the same time. Tissue analysis of the sheep showed extensive mucosal hemorrhage of the small intestine and petechial subcapsular hemorrhage of the kidneys for all compounds dosed. Acute toxicosis was determined by giving 3 sheep a dose of 40 mg of vanadium as NH_4VO_3/kg BW. The toxic levels killed two sheep within 80 hours and elevated the vanadium content in kidney, spleen, bone, muscle, liver, and lung tissues of the others.

NRC (1980) reported that a dose of 20 mg V/kg BW given orally to calves as ammonium vanadate resulted in diarrhea, dehydration, emaciation, and prostration that lasted for 3 days. The gross pathological changes were hemorrhagic

inflammation of the intestinal tract, ruminal ulcers, diffuse hemorrhage around the kidney and heart, and congestion of the liver and lungs.

The WHO (2001) has summarized the toxicity (LC_{50}) of vanadium compounds for fresh- and saltwater fish. The LC_{50} (96 hour) for freshwater trout and salmon ranged from 6–24 mg of vanadium per liter of water. Toxicity of vanadium appears to increase with increasing water hardness and as pH increases from 5.5 to 8.8 (Stendahl and Sprague, 1982).

Low Quantities and Chronic

Toxicosis via ingestion of vanadium through food is very uncommon in humans. The most common animal models used in vanadium toxicology literature are chickens and rats. The ingestion of vanadium can alter the function of the rumen. An in vitro study found the addition of 7 mg/kg of vanadium as sodium ortho- or metavanadate to the rumen fluid of lambs reduced DM digestibility (NRC, 1980).

A number of studies on the toxicity of vanadium in the diet and water are summarized in Table 30-1. A decrease in the albumin quality of eggs from laying hens was apparent when contaminated phosphorus sources provided as little as 4.6 mg/kg of vanadium in the total diet (Sell et al., 1982). Reductions in albumin quality were proportional to the concentration of vanadium from NH_4VO_3 in the diet. At 27.5 mg/kg vanadium in diets of laying hens, albumin quality and egg production were significantly lower at the end of the first 4 weeks of the study than lower dietary treatments, and chickens did not recover as fast from this high dosage after vanadium was reduced in the diet (Sell et al., 1982). Growing chicks fed diets containing 50 mg/kg of vanadium as calcium orthovanadate gained less weight (205 vs. 237 g) during the first 28 days of life and had lower liver, gizzard, spleen, and bursa weights, but not as a percent of BW, compared to chicks fed a control starter diet (Kubena et al., 1985). Fifty-week-old laying hens fed 40 mg/kg of added vanadium in their diet for 7 weeks had decreased albumin quality (18.7 Haugh unit decrease), lowered egg weights (by 3.8 g), lowered egg production (by 28.8 percent per day), and a loss in BW of 149 g (Ousterhout and Berg, 1981). The total feed intake for hens fed a 40 mg/kg added vanadium diet was 880 g less than the control hens over the seven-week treatment period.

Paternain et al. (1990) found that administrating vanadyl sulphate pentahydrate by gavage to pregnant mice at quantities of 75 and 150 mg/kg^{-1} BW on days 6–15 of gestation resulted in a reduction in maternal weight that was dose related, and lower liver and kidney weights than control mice. However, administration of vanadium at either dosage by gavage did not affect feed intake.

Hilton and Bettger (1988) reported that rainbow trout refused diets containing 493 mg V/kg of diet. Concentrations as low as 10.2 mg of sodium orthovanadate/kg of diet reduced weight gain during a 12-week feeding period.

Factors Influencing Toxicity

Domingo (1996) reviewed the influence of vanadium on reproduction and development in rats and mice and found the effects of vanadium toxicosis were dependent on many factors, including the valence of the vanadium compound, the chemical form of the vanadium compound, the period of dosing, the length of exposure, and the size of the dose. The toxicity of vanadium compounds tends to increase as the valence increases (Nechay et al., 1986). Vanadium toxicity is fairly low when it is ingested, moderate when it is inhaled, and high when it is injected (Nechay et al., 1986).

Various studies have tested the ability of different compounds to reduce the toxicity of vanadium. Benabdeljelil and Jensen (1990) conducted a series of experiments testing the effectiveness of ascorbic acid and chromium in countering the negative effects of dietary vanadium on interior egg quality. Ascorbic acid fed at 100–5,000 mg/kg of diet effectively protected hens against decreases in interior egg quality at 10 mg/kg of diet feeding of vanadium, but did not prevent a reduction in total egg weight. Chromium at 10 or 50 mg/kg in diets containing 10 mg/kg vanadium did not prevent a decrease in albumin quality. This also was true when chromium was added at 30 or 150 mg/kg to diets containing 30 mg/kg added vanadium. A study by Miles et al. (1997) found that the antioxidants vitamin E, ascorbic acid, and beta-carotene could be used in diets containing 10 mg/kg vanadium to partially restore interior egg quality. Ousterhout and Berg (1981) found that the inclusion of ascorbic acid at 0.4–0.5 percent of the diet effectively protected the hen from reductions in albumin quality, BW, and egg production from vanadium levels of up to 40 mg/kg. However, ascorbic acid didn't counteract vanadium's reduction in egg weight. Replacing soybean meal with 20 percent cottonseed meal had the same effect as the ascorbic acid. Ousterhout and Berg (1981) found that adding ethylenediamine tetraacetic acid at levels four to eight times the molar concentration of vanadium and varying the protein percentage in the diet using soybean meal had no consistent effect in countering the effects of vanadium. Replacing the grain with sucrose and replacing the soybean meal with herring fish meal were found to intensify the negative affects of vanadium on performance. Studies by Kubena et al. (1985, 1986) found a toxicity-enhancing synergism between orchratoxin A and vanadium in male chicks. Diets containing as little as 2.5 mg/kg orchratoxin A and 12.5 mg of vanadium resulted in lower weight gain than when orchratoxin A was fed alone.

The chelating agent, Tiron (sodium 4, 5-dihydroxybenzamine-1,3-disulfonate), when administered at 235–470 mg/kg BW to rats that have received 16 mg/kg BW othorvanadate orally per day for 6 weeks, was shown to be an effective antidote in vanadium-loaded rats (Sanchez et al., 1999). Vanadium-loaded rats exhibited altered behavior of avoidance to stimuli in an open field, but reverted back to normal behavior with Tiron injections. Yamaguchi et al.

(1989) found that injections of zinc sulfate (15.3 µmol of Z/100 g BW) when administered simultaneously with 20 µmol of V/100 g BW in weanling rats prevented the toxic effects of vanadium on bone metabolism. However, Zaporowska and Wasilewski (1992) found that including zinc (in drinking water) with the dose of vanadium caused the rats to have a more pronounced reduction of feed intake than the vanadium itself.

TISSUE LEVELS

The distribution of vanadium varies throughout the different tissues in the body (Table 30-2 and Table 30-3); most vanadium accumulates in the organ tissues. The highest concentrations are in the liver, kidney, and bone (French and Jones, 1993). Feeding 9.6–12 mg/kg BW of vanadium from ammonium metavanadate, calcium pyrovanadate, or calcium orthovanadate to wethers increased vanadium levels in muscle, lung, liver, and kidney tissue by 10-, 13-, 8- and 33-fold, respectively, over control-fed wethers with tissue level increases similar across all three sources of vanadium (Hansard et al., 1982). In an acute experiment with three sheep dosed with vanadium at 40 mg/kg BW, vanadium levels in kidney tissue were increased 69-fold at the time of death (Hansard et al., 1982). Vanadium concentration in the kidney tissue was the highest of all tissues tested, averaging 25.83 mg/kg on a DM basis. The lowest tissue vanadium concentration was in muscle, averaging 0.63 mg/kg DM.

Paternain et al. (1990) found vanadyl sulphate pentahydrate administered orally at doses of 37.5, 75, and 150 mg/kg BW/day to pregnant mice on days 6–15 of gestation linearly increased concentrations of vanadium in the liver, kidney, spleen, whole fetus, and placenta as dosage level increased. Sanchez et al. (1998) administered metavanadate orally to rats at 4.1, 8.2, and 16.4 mg/kg of BW/day for 8 weeks. Tissues levels of vanadium increased as the amount of vanadium dosed increased. Lowest tissue concentration of vanadium was in the brain, followed by muscle, liver, kidneys, bone, and spleen—except at the 16.4 mg/kg dose where bone became the tissue with the highest vanadium concentration. Tissue concentrations ranged from 0.029 µg V/g tissue WW in brain at the 4.1 mg/kg dose to 2.852 µg V/g tissue WW in bone at the 16.4 mg/kg dose. Other tissue contents are summarized in Tables 30-2 and 30-3.

MAXIMUM TOLERABLE LEVELS

NRC (1980) suggested the maximum tolerable dietary levels for vanadium: 50 mg/kg for cattle and 10 mg/kg for poultry. More recent research indicates poultry can tolerate up to 25 mg/kg of vanadium in diets and possibly up to 50 mg/kg (depending on the source of vanadium) without significant decreases in weight gain or health effects (Table 30-1). However, egg albumin quality declines at levels as low as 4.6 mg/kg of vanadium in diets and egg weights decrease proportionally as the amount of vanadium in diets increase. Thus, for concerns about egg quality, the previous 10 mg/kg of diet maximum tolerable level appears appropriate, but slightly high for maximum egg quality. In mice, the maximum tolerable levels are not clearly definable with adverse effects on weight gain and reproduction occurring between 6 and 17 mg/kg BW intake of vanadium per day. Rats change their learning behavior at intakes of vanadium as low as 1.7 mg/kg BW/day, but intakes above 7 mg/kg BW appear necessary to observe decreased weight gain. At intakes of 10 mg V/kg BW, sheep have reduced feed intake and develop diarrhea.

FUTURE RESEARCH NEEDS

The effect of vanadium per se and vanadium sources on toxicosis and on physiological changes in rats and poultry has been researched. Information on health, alterations in production, and even toxicity of vanadium in many domestic animal species, particularly ruminants, and fish is unavailable. Additional research on the interaction of antioxidants, mycotoxins, and overall nutrient content of the diet and vanadium toxicity is needed.

SUMMARY

Vanadium has recently been found to be an essential element in rats and goats. Vanadium is present in the environment in low but adequate levels. There is a low risk of animals suffering from a deficiency in vanadium; however, vanadium toxicity can occur and in certain species at fairly low intake levels. In vitro, vanadium has been shown to inhibit (Na, K)-ATPase and influence the function of other enzyme systems. Vanadium administered to sheep at an ever-increasing rate caused a strong drop in feed intake at the 400–500 mg/day level. When administered a 40 mg/kg dose of vanadium as sodium orthovanadate, sheep died within 80 hours. Signs of toxicosis of vanadium in laying hens are usually a reduction in albumin quality at dietary vanadium levels as low as 5 mg/kg of diet; as levels increase, egg production decreases, growth rate stops, and laying hens can atrophy. Levels of 100 mg/kg of vanadium in laying hens' diets can cause death. Low levels of vanadium have been shown to modify behavior in rats and mice. The largest concern for vanadium in feedstuffs is the concentration of vanadium in phosphorus mineral sources, as vanadium concentrations in other feedstuffs are low.

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TABLE 30-1 Effects of Vanadium Exposure in Animals

Animal	N	Age or Weight	Quantity Vanadium	Source	Duration	Route	Effect(s)	Reference
Mice	24	28–30 g	8.36 mg/kg BW/d	$NaVO_3$	64 d	Drinking water	No adverse effect	Llobet et al., 1993
			16.71 mg/kg BW/d				No adverse effect	
			25.07 mg/kg BW/d				Reduced male fertility, testicular and/or epididymis sperm counts	
			33.42 mg/kg BW/d				Reduced male fertility, BW gain, epididymis weight, testicular and/or epididymis sperm counts	
Mice	16	25–30 g	7.55 mg/kg BW/d	$VOSO_4 \cdot 5H_2O$	d 6–15 of gestation	Water, gavage	Decrease in BW gain, fetal BW and fetal body length; significantly lower gravid uterine weight; increase in number of reabsorptions per litter	Paternain et al., 1990
	20		15.10 mg/kg BW/d		d 6–15 of gestation	Water, gavage	Decrease in BW gain, fetal BW, and fetal body length; significantly lower gravid uterine, liver, and kidney weights; increase in number of reabsorptions per litter and number of stunted fetuses	
	20		30.19 mg/kg BW/d					
Rats	8	180–210 g	9.68 mg/kg BW/d	NH_4VO_3	12 wk	Drinking water	Significant decrease in daily fluid intake, no significant change in haematological indexes or erythrocyte osmotic fragility	Dai et al., 1995
	8		7.64 mg/kg BW/d	$VOSO_4$			Significant decrease in daily fluid intake, no significant change in haematological indexes or erythrocyte osmotic fragility	
	8		9.17 mg/kg BW/d	bis(maltolato) oxovanadium (BMOV)			Significant decrease in daily fluid intake, no significant change in haematological indexes or erythrocyte osmotic fragility	
Rats		270–320 g	10.6 mg/kg BW/d	$VOSO_4$	52 wk	Drinking water	Decrease in rate of weight gain	Dai et al., 1994
			16.9 mg/kg BW/d		1, 51 wk	Drinking water	Decrease in rate of weight gain more significant than 10.6 $mg \cdot kg^{-1}$ dose	
			28.1 mg/kg BW/d		1, 1, 50 wk	Drinking water	Decrease in rate of weight gain more significantly than 16.9 $mg \cdot kg^{-1}$ dose	
Rats	3	150–250 g	2.76 mg/kg BW/d	Na_3VO_4	77 d	Drinking water	No adverse effects	Roman et al, 1981
Rats	12	200–220 g, male	1.71 mg/kg BW/d	$NaVO_3$	8 wk	Water, gavage	Some changes in activity and learning	Sanchez et al., 1998
	12	200–220 g, male	3.42 mg/kg BW/d				Some changes in activity and learning	
	12	200-220 g, male	6.84 mg/kg BW/d				Decrease in BW, some changes in activity and learning	

continued

TABLE 30-1 Continued

Animal	N	Age or Weight	Quantity Vanadium	Source	Duration	Route	Effect(s)	Reference
Rats		60–65 g (3 wk)	0.509 mg/kg BW/d	V_2O_5	3 d	Orally administer, dissolved in water and NaOH solution	Increase in alkaline phosphatase activity; increase in DNA content in wet bone	Yamaguchi et al., 1989
			1.018 mg/kg BW/d				Increase in alkaline phosphatase activity; increase in DNA content in wet bone	
			5.09 mg/kg BW/d				Increase in alkaline phosphatase activity; increase in DNA content in wet bone	
			7.635 mg/kg BW/d				Increase in calcium concentration in serum; increase in alkaline phosphatase activity	
			10.18 mg/kg BW/d	V_2O_5			Increase in calcium and phosphorus concentration in serum; increase in alkaline phosphatase activity; death	
Rats	13 males 14 females	2 mo	19.7 mg/kg BW males 25.8 mg/kg BW females	NH_4VO_3	4 wk	Drinking water	Diarrhea; death; decrease in average weight gain and fluid intake; decrease in erythrocyte and haemoglobin levels; polychromatophilic erythroblasts	Zaporowska and Wasilewski, 1992
Rats	11 males	2 mo	19.7 mg/kg BW	NH_4VO_3	4 wk	Drinking water	Decrease in average weight gain, fluid intake and feed intake; decrease in erythrocyte and haemoglobin levels	Zaporowska and Wasilewski, 1991
Rats	10	80.8–102.4 g	2.09 mg/kg in water	$NaVO_3$	3 mo	Drinking water	No adverse effects	Domingo et al., 1985
	10		4.18 mg/kg in water				No adverse effects	
	10		20.89 mg/kg in water				Plasma concentration of urea and uric acid increased	
Rats	7	210–230 g	69.2 mg/kg in water	Na_3VO_4	d 10–20 of gestation	Drinking water	322 ± 190 (SD) ppb V in blood; significant decrease in water consumption and number of fetuses per pregnancy	Ganguli et al., 1994
Rats	30 males 60 females	180–200 g	87.1 mg/kg in water	NH_4VO_3	Males 70 d, females 61 d	Drinking water	Reduced fertility with males affected more severely than females; reduced reproductive performance	Morgan and El-Tawil, 2003

Rats		Pregnant females, fetuses, and pups	300 mg/L vanadium sulfate, 5 g/L NaCl in water	$VOSO_4$	last 3 d of pregnancy and first 25 d of resulting pups' life	Drinking water	Retardation of body growth, reduction in survival rate at weaning, male rats had a significant reduction in the number of rearing postures	Poggioli et al., 2001
Chickens	20	43-wk laying hens	10 mg/kg – diet	NH_4VO_3	28 d	Diet	Decreased albumin quality	Benabdeljelil and Jensen, 1990
	20		30 mg/kg – diet				Decreased albumin quality and rate of egg laying	
	20		100 mg/kg – diet				Decreased albumin quality, rate of egg laying, BW, and feed intake	
Chickens	35	32-wk laying hens	20 mg/kg – diet	NH_4VO_3	56 d	Diet	Decreased albumin quality and fertile hatch percentages	Bressman et al., 2002
			40 mg/kg – diet				Decreased albumin quality, fertile hatch percentages, feed intake, egg production and feed conversion; increased embryonic mortality	
			60 mg/kg – diet				Decreased albumin quality, fertile hatch percentages, feed intake, egg production and feed conversion; increased embryonic mortality	
Chickens	35	52-wk laying hens	12.8 mg/kg – diet	NH_4VO_3	42 d	Diet	Reduced interior egg quality by 24 d	Davis et al., 2002
	35		22.8 mg/kg – diet		42 d	Diet	Reduced interior egg quality by 72 hr	
	35	52-wk laying hens	32.8 mg/kg – diet			Diet	Reduced feed conversion; reduced interior egg quality by 72 hr; decline in egg production	
Chickens	20	37-wk laying hens	22 mg/kg – diet	NH_4VO_3	28 d	Diet	Decreased albumin quality, albumin height, and inner thin albumin	Eyal and Moran, 1984
	20		32 mg/kg – diet			Diet	Reduced weight gain; decreased albumin quality, albumin height, and inner thin albumin; proportion of albumin decreased; reduced magnum weight and carcass fat	
Chickens	20	1 d	12.5 mg/kg – diet	$Ca_3(VO_4)_2$	28 d	Diet	No adverse effect	Kubena et al., 1986
			25 mg/kg – diet				No adverse effect	
Chickens	30	1 d	50 mg/kg – diet	$Ca_3(VO_4)_2$	28 d	Diet	Reduced growth	Kubena et al., 1985

continued

TABLE 30-1 Continued

Animal	N	Age or Weight	Quantity Vanadium	Source	Duration	Route	Effect(s)	Reference
Chickens	18	29-wk laying hens	12.5 mg/kg – diet	$Ca_3(VO_4)_2$	28 d	Diet	No adverse effect	Kubena and Phillips, 1983
	18		25 mg/kg – diet				No adverse effect	
	18		50 mg/kg – diet				Reduced weight gain; decreased egg production	
	18		100 mg/kg – diet				BW loss; decreased egg production; death	
Chickens	20	50-wk laying hens	40 mg/kg added vanadium – diet	NH_4VO_3	7 wk	Diet	Decreased albumin quality, egg-laying rate, BW, egg weight, and feed intake	Ousterhout and Berg, 1981
Chickens	40	25-wk laying hens	4.6 mg/kg – diet	Dicalcium phosphate	29, 44, 66 or 80 wk	Diet	Decreased albumin quality	Sell et al., 1986
			7.4 mg/kg – diet				Decreased albumin quality	
Chickens	5	41-wk laying hens	10.2 mg/kg – diet	Dicalcium phosphate (Dical)	4 wk		Initial reduction in albumin quality, albumin quality recovered by 28 days	Sell et al., 1982
			27.5 mg/kg – diet	Dical + NH_4VO_3			Reduction in albumin quality; decreased egg production and feed consumption	
			30.4 mg/kg – diet	Dical + NH_4VO_3			Reduction in albumin quality; decreased egg production and feed consumption	
Chickens	4	49-wk laying hens	9.9 mg/kg – diet	Dicalcium phosphate	6 wk	Diet	Reduction in albumin quality	Sell et al., 1982
Chickens	4	49-wk laying hens	4.0 mg/kg added for 4 wk and then 0 mg/kg added for 6 wk – diet	Dicalcium phosphate	4 wk		No reduction in albumin quality	Sell et al., 1982
			6.0 mg/kg added – diet		10 wk		Reduction in albumin quality	
			7.9 mg/kg added – diet		10 wk		Reduction in albumin quality	
Chickens	3 pens of 11–15	Broiler breeders	50 mg/kg – diet	$NaVO_3$		Diet	Bleaching effect on brown egg shells	Sutly et al., 2001
			100 mg/kg – diet				Bleaching effect on brown egg shells	
Chickens	5	25-wk laying hens	18.6 mg/kg for 1–14 d, 34.4 mg/kg 15–28 d – diet	NH_4VO_3	28 d	Diet	Decreased albumin quality, egg production and BW	Toussant and Latshaw, 1994

Sheep	3	41 kg	10.0–11.2 mg/kg BW	NH_4VO_3	18 d	Capsule	Reduced feed intake; diarrhea; extensive mucosal hemorrhage in the small intestine; diffuse subcapsular hemorrhage of the kidney; death	Hansard et al., 1982
	3	41 kg	9.6–12.0 mg/kg BW	$Ca_2V_2O_7$	18 d	Capsule	Reduced feed intake; diarrhea; extensive mucosal hemorrhage in the small intestine; diffuse subcapsular hemorrhage of the kidney; death	
	3	41 kg	9.7 mg/kg BW				Reduced feed intake; diarrhea; extensive mucosal hemorrhage in the small intestine; diffuse subcapsular hemorrhage of the kidney; death	
	3		40 mg/kg BW				Diarrhea, death, toxic hepatitis, fatty degeneration	

SI conversion: 1 mg vanadium equals 19.6 µmoles vanadium.

TABLE 30-2 Vanadium Concentrations in Selected Tissues of Animals

Species	Age	Quantity	Compounds	Bone	Liver	Kidney	Muscle	Spleen	Lung	Reference
Mice	Pregnant mice	37.5 mg/kg BW	$VOSO_4 \cdot 5H_2O$		0.484 ± 0.147 mg/kg WW	0.493 ± 0.140 mg/kg WW		0.707 ± 0.318 mg/kg WW		Paternain et al., 1990
		75 mg/kg BW			0.850 ± 0.265 mg/kg WW	0.769 ± 0.317 mg/kg WW		1.340 ± 0.540 mg/kg WW		
		150 mg/kg BW			1.589 ± 0.796 mg/kg WW	1.912 ± 1.087 mg/kg WW		2.369 ± ±1.308 mg/kg WW		
Rats	200–220 g, male	1.71 mg/kg BW	$NaVO_3$	0.544 ± 0.312 mg/kg WW	0.083 ± 0.041 mg/kg WW	0.414 ± 0.150 mg/kg WW	0.036 ± 0.014 mg/kg WW	0.920 ± 0.388 mg/kg WW		Sanchez et al., 1998
		3.42 mg/kg BW		0.753 ± 0.354 mg/kg WW	0.189 ± 0.0046 mg/kg WW	0.534 ± 0.158 mg/kg WW	0.132 ± 0.042 mg/kg WW	1.102 ± 0.512 mg/kg WW		
		6.84 mg/kg BW		2.852 ± 1.101 mg/kg WW	0.391 ± 0.071 mg/kg WW	1.381 ± 0.455 mg/kg WW	0.201 ± 0.033 mg/kg WW	2.529 ± 0.331 mg/kg WW		
Rats	170–200 g	5 mg/kg diet	$NaVO_3$		0.103 ± 28 mg/kg WW					Bogden et al., 1982
		25 mg/kg diet			0.554 ± 115 mg/kg WW					
Rats	80.8–102.4 g	2.09 mg/kg water	$NaVO_3$							Domingo et al., 1985
		4.18 mg/kg water				0.58 ± 0.09 mg/kg WW		0.48 ± 0.06 mg/kg WW		
		20.89 mg/kg water			0.30 ± 0.05 mg/kg WW	2.92 ± 1.34 mg/kg WW		1.19 ± 0.13 mg/kg WW	0.24 0.04 mg/kg WW	

Chickens	Chicks 1–28 d	50 mg/kg diet	$Ca_3(VO_4)_2$		0.307 mg/kg WW	0.298 mg/kg WW	0.192 mg/kg WW			Kubena et al., 1985
Sheep, Exp 1	41 kg	10–11.2 mg/kg BW	NH_4VO_3		3.51 ± 0.56 mg/kg DW	22.74 ± 5.32 mg/kg DW	0.83 ± 0.07 mg/kg DW		1.42 ± 0.18 mg/kg DW	Hansard et al., 1982
		9.6–12.0 mg/kg BW	$Ca_2V_2O_7$		2.83 ± 0.23 mg/kg DW	20.24 ± 5.02 mg/kg DW	0.75 ± 0.12 mg/kg DW		1.48 ± 0.26 mg/kg DW	
		9.7 mg/kg BW	$Ca_3(VO_4)_2$		3.68 ± 0.42 mg/kg DW	24.37 ± 6.47 mg/kg DW	0.97 ± 0.09 mg/kg DW		2.6 ± 0.22 mg/kg DW	
Sheep, Exp 2	45.4 kg	40 mg/kg BW	NH_4VO_3	4.54 mg/kg DW	7.92 mg/kg DW	38.3 mg/kg DW	0.97 mg/kg DW	3.61 mg/kg DW		Hansard et al., 1982
	40.8 kg	40 mg/kg BW	NH_4VO_4	2.11 mg/kg DW	2.61 mg/kg DW	20.4 mg/kg DW	0.53 mg/kg DW	1.82 mg/kg DW	4.22 mg/kg DW	
	54.4 kg	40 mg/kg BW	NH_4VO_5	1.23 mg/kg DW	2.04 mg/kg DW	18.8 mg/kg DW	0.4 mg/kg DW	0.89 mg/kg DW	2.31 mg/kg DW	

TABLE 30-3 Vanadium Concentrations in Other Fluids and Tissues of Animals

Species	Weight	Dose	Compound	Heart	Brain	Kidney Cortex	Kidney Medulla	Blood	Plasma	Reference
Rats	170–200 g	5 mg/kg diet 25 mg/kg diet	$NaVO_3$		0.0034 ± 2.2 mg/kg WW 0.016.0 ± 5.1 mg/kg WW	0.628 ± 338 mg/kg WW 1.812 ± 495 mg/kg WW	0.203 ± 77 mg/kg WW 0.663 ± 266 mg/kg WW	0.029 ± 14 mg/kg WW 0.110 ± 26 mg/kg WW	0.046 ± 13 mg/kg WW 0.210 ± 69 mg/kg WW	Bogden et al., 1982
Rats	200–250 g	10.6 ± 0.7 mg/kg d	$VOSO_4$						0.18 ± 0.05 mg/L	Dai et al., 1994
Rats	200–250 g	16.9 ± 1.3 mg/kg d 28.1 ± 2.9 mg/kg d							0.31 ± 0.05 mg/L 0.46 ± 0.11 mg/L	Dai et al., 1994
Rats	80.8–102.4 g	2.09 mg/kg water 4.18 mg/kg water 20.89 mg/kg water	$NaVO_3$	0.30 mg/kg WW						Domingo et al., 1985
Rats	200–220 g, male	1.71mg/kg BW 3.42 mg/kg BW 6.84 mg/kg BW	$NaVO_3$	0.029 ± 0.010 mg/kg WW 0.092 ± 0.021 mg/kg WW 0.111 ± 0.023 mg/kg WW						Sanchez et al., 1998
Sheep	45.4 kg	40 mg/kg BW	NH_4VO_3	1.14 mg/kg DW						Hansard et al., 1982
	40.8 kg	40 mg/kg BW	NH_4VO_4	0.82 mg/kg DW						
	54.4 kg	40 mg/kg BW	NH_4VO_5	0.56 mg/kg DW						

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Zinc

INTRODUCTION

Zinc (Zn) has an atomic number of 30 and appears in group 12 of the Periodic Table. In biological systems, zinc is virtually always in the divalent state. Zinc typically forms complexes with a coordination number of 4 and with a tetrahedral disposition of ligands around the metal. Zinc is a strong Lewis acid, meaning that it is an electron acceptor. It does not exhibit redox chemistry. Zinc readily forms complexes with amino acids, peptides, proteins, and nucleotides. It has a particular affinity for thiol and hydroxy groups and for amine electron donors.

Zinc is a bluish-grey element. A large proportion of all zinc is used to galvanize metals such as iron to prevent corrosion. The oxide (ZnO) is used in the manufacture of paints, rubber products, cosmetics, pharmaceuticals, floor coverings, plastics, printing inks, soap, textiles, electrical equipment, and other products. It is also used in ointments. The sulfide (ZnS) is used in making luminous dials, x-ray and TV screens, paints, and fluorescent lights. In 2001, more than 9 million tons of zinc were produced. Approximately 47 percent was used for galvanizing, 19 percent in brass and bronze, 14 percent in zinc-based alloys, 9 percent in chemicals, and the remaining 11 percent in other uses.

ESSENTIALITY

Zinc essentiality in plants was established in 1869 (Raulin, 1869), in experimental animals in 1934 (Todd et al., 1934), in swine in 1955 (Tucker and Salmon, 1955), and in humans in 1961 (Prasad et al., 1961). Additional zinc is needed for growth and lactation. The efficiency of zinc absorption increases during pregnancy and lactation in rats (Davies and Williams, 1977). Milk of most animals generally contains 3–5 mg/L of zinc (McDowell, 2003). The use of a factorial approach to estimate the zinc requirements of sheep and cattle during growth, pregnancy, and lactation is discussed by Underwood and Suttle (1999). Milk zinc concentrations in humans are not influenced by maternal zinc status or dietary zinc intakes (Krebs et al., 1985).

The biochemical basis for essentiality is traced to the discovery of zinc as a requirement for activity of carbonic anhydrase in 1940 (Kielin and Mann, 1940) and zinc finger protein domains in 1985 (Vallee et al., 1991). Since discovery of zinc deficiency in humans, interest in the biochemical and clinical aspects of zinc nutrition has increased exponentially. Approximately 300 enzymes are associated with zinc (Cousins and King, 2004). Because of its stability and coordination flexibility, zinc is able to carry out many diverse biological functions in protein, nucleic acid, carbohydrate, and lipid metabolism (Vallee and Falchuk, 1993). Zinc is required for DNA replication and transcription and is a cofactor for many zinc-dependent gene regulatory proteins. Zinc deficiency arrests growth and development and produces system dysfunction (Cousins and King, 2004). The biological functions of zinc can be divided into three categories: catalytic, structural, and regulatory (Cousins, 1996).

The clinical signs of zinc deficiency include reduced growth, feed intake, and feed efficiency; listlessness; reduced testicular growth; parakeratotic lesions that are most severe on the legs, neck, head, and around the nostrils; failure of wounds to heal, and alopecia (Ott et al., 1965). Thymus atrophy and impaired immune function have also been observed in zinc-deficient animals (Perryman et al., 1989).

DIFFICULTIES IN ANALYSIS AND EVALUATION

Analytical procedures for zinc include atomic absorption spectrophotometry and inductively-coupled plasma emission spectrophotometry (Sunderman, 1973). Zinc reference standards are available from the National Institute of Standards and Technology. Before analysis, any organic material associated with the zinc must be destroyed. A microwave digestion system using nitric acid in a closed system with high temperature and pressure is the method of choice. Alternatives include wet ashing with sulfuric acid or nitric acid or

dry ashing in a muffle furnace. When ashing temperatures exceed 500°C, losses occur via volatilization. Extreme care must be taken to prevent contamination of samples; these measures include the use of ultra-pure reagents, deionized water, acid-washed glassware, and zinc-free laboratory supplies. Blanks should be included in all analyses.

Of the zinc radioisotopes, only ^{65}Zn (half-life, 245 days) has been widely used in research. Stable isotopes of zinc and corresponding natural abundances are ^{64}Zn, 49 percent; ^{66}Zn, 29 percent; ^{67}Zn, 4 percent; ^{68}Zn, 19 percent; and ^{70}Zn, 1 percent. These have been effectively used in human studies of zinc metabolism (Turnlund and Keyes, 1990).

REGULATION AND METABOLISM

Absorption

Zinc is absorbed all along the intestinal tract, but the highest rates of absorption occur in the jejunum of humans and nonruminant animals. In ruminants, there is also absorption from the rumen (Georgievskii et al., 1979). Zinc uptake by the small intestine occurs via two processes: (1) a nonmediated (nonsaturable) process that is not affected by dietary zinc intake and (2) a mediated (saturable) process that is stimulated by zinc depletion (Solomons and Cousins, 1984; Cousins, 1996). The nonsaturable process does not require energy and may reflect paracellular zinc uptake or diffusion of zinc into the cell (Cousins, 1996). The net result of these two processes is that fractional zinc absorption is inversely related to dietary levels of zinc. Numerous dietary factors affect the apparent absorption of zinc. These factors include feed source, phytate, amino acids, and the presence or absence of other divalent cations such as iron, calcium, and copper (Hambidge et al., 1986; Lonnerdal, 1989; Abdel-Mageed and Oehme, 1990a). In general, high intakes of iron, calcium, and phytate reduce the availability of zinc for absorption, whereas certain amino acids (i.e., histidine, cysteine) enhance its absorption.

Thus, the efficiency of zinc absorption in animals can vary widely from as little as 15 percent to more than 60 percent (McDowell, 2003). Under usual conditions, about one-third of the dietary zinc consumed by humans is absorbed (King and Keen, 1999). Studies in experimental animals suggest that zinc transporter proteins assist in regulating zinc absorption and whole body homeostasis (Harris, 2002). Uptake and retention of dietary zinc is greater in growing than in mature organisms (Weigand and Kirchgessner, 1979).

Transport

Albumin appears to be the major portal carrier for newly absorbed zinc (Cousins, 1989). Changes in the systemic level of albumin may alter zinc absorption. Total plasma zinc is bound primarily to albumin (70 percent) or α-2 macroglobulin (20–30 percent). There is a large molar excess of albumin compared with zinc, which assures that an adequate transport system exists. Other plasma proteins that bind zinc include transferrin, histidine-rich glycoprotein, and perhaps metallothionein. Plasma zinc concentrations respond markedly to external stimuli, including fluctuations in zinc intake, fasting, and various acute stresses, such as infection (King and Keen, 1999). Most reductions in plasma zinc levels are believed to reflect increased hepatic zinc uptake, perhaps resulting from hormonal control.

Excretion

The primary route of zinc excretion is in the feces (Miller, 1970). Fecal zinc represents unabsorbed dietary zinc as well as zinc that is secreted into the gut from the body and is not subsequently absorbed (i.e., endogenous fecal zinc). The endogenous fecal losses are a mix of pancreatic and intestinal secretions (King et al., 2000). Meals stimulate endogenous zinc secretion, and over half of zinc in the intestinal lumen postprandially comes from endogenous secretions (Matseshe et al., 1980). Both total and endogenous fecal excretion of a tracer dose of ^{65}Zn and of stable dietary zinc were significantly reduced in calves and goats fed low zinc diets (Hambidge et al., 1986).

Urinary and integumental zinc losses comprise less than 20 percent of the total losses under normal conditions (Underwood and Suttle, 1999; King et al., 2000; McDowell, 2003). Urinary losses rise with trauma, muscle catabolism, and the administration of chelating agents such as EDTA (Hambidge et al., 1986). Urinary zinc levels do not respond to changes in zinc intake unless the diet is virtually free of zinc (King and Keen, 1999). Urinary excretion of zinc by sheep and calves is generally <1 mg per day with little effect due to zinc supply in the diet.

Regulation

The results of tracer studies and isolated cells suggest that the zinc-binding protein, metallothionein (MT), is involved in the regulation of zinc metabolism. It appears that MT is inducible by dietary zinc via the metal response element (MRE) and MTF-1 mechanism of transcriptional regulation (Cousins, 1996). An increase in cellular MT is associated with increased zinc binding within the cells. Metallothionein may act as a Zn^{+2} buffer, controlling the free Zn^{+2} level or helping to control an intracellular zinc pool that is responsive to both hormones and diet. Zinc transporters that regulate influx or efflux further allow cells to adapt to differences in zinc intake independent of MT. Cytokines, primarily interleukins 1 and 6, influence zinc metabolism (Cousins, 1996). Acute infection where proinflammatory cytokines are released leads to secretion of cytokines that increases zinc uptake into liver, bone marrow, and thymus and reduces the amount going to bone, skin, and intestine.

Metabolic Interactions

Interactions with other divalent cations in the intestinal lumen may also influence zinc bioavailability. High individual doses of iron (25, 50, or 75 mg) in a water solution inhibited the absorption of zinc from a 25-mg zinc dose (Solomons and Jacob, 1981), but the interaction is less pronounced when intakes are closer to "physiological levels" (Lonnerdal, 2000). Nevertheless, there are reports of iron supplementation reducing zinc absorption in individuals with increased iron and zinc needs (e.g., pregnant and lactating women, patients with ileostomies; O'Brien et al., 2000; Chung et al., 2002; Troost et al., 2003). The interaction between calcium intake at high supplementation levels and zinc absorption has not been resolved (Wood and Zheng, 1997). Modest increases in copper intake do not interfere with zinc absorption (August et al., 1989). High levels of tin and cadmium inhibit zinc absorption, but the extent to which lower, physiological levels affect absorption in humans is unknown. Although most of these interactions have been described in studies of humans, it is assumed the same interactions occur in animals.

Mechanisms of Toxicity

Excessive accumulation of zinc within the cells is thought to disrupt the function of essential biological molecules, such as protein, enzymes, and DNA. This leads to the clinical signs of chronic toxicosis. Alterations in the protein can disrupt function and lead to toxic consequences. Also, excessive amounts of zinc in the gastrointestinal tract can lead to reduced copper absorption from the diet, leading to a systemic copper deficiency (Sandstead, 1995).

Acute excessive intakes of zinc can be a local irritant to tissues and membranes causing gastrointestinal distress, with signs including nausea, vomiting, abdominal cramps, and diarrhea (Abdel-Mageed and Oehme, 1990a). High luminal zinc concentrations may also damage the brush border membrane of the small intestine and allow zinc to enter the cell and bind nonspecifically to cellular proteins and other ligands (ATSDR, 2003).

Zinc is relatively nontoxic to birds and mammals. Rats, pigs, poultry, sheep, cattle, and humans exhibit considerable tolerance to high intakes of zinc. Nevertheless, zinc toxicosis has occurred in a number of species. Exposure to excessive zinc is primarily by ingestion. Other possible pathways for zinc exposure are water and air. Sources of exposure include drinking water, feed, and polluted air. Initial signs of zinc toxicosis in animals usually consist of reduced feed intake, growth rate, and other measures of performance or signs of secondary deficiencies of other minerals, such as copper. High levels of zinc also affect rumen metabolism, probably via a toxic effect on ruminal microorganisms (Ott et al., 1966b).

SOURCES AND BIOAVAILABILITY

Sources

Diet

Pasture herbage zinc content ranges from 17 to 60 mg/kg dry weight with most values falling between 20 and 30 mg/kg. Industrial pollution increases the zinc content of grass from 5- to 50-fold (Mills and Dalgarno, 1972). The zinc concentration in plants usually falls with advancing maturity, and leguminous plants invariably carry higher zinc levels than grasses grown and sampled under the same conditions (Hambidge et al., 1986). Heavy dressings with lime and to a lesser extent with superphosphate can greatly reduce pasture zinc levels. Cereal grains typically contain 20–30 mg/kg zinc, whereas soybean, peanut, and linseed meal contain 50–70 mg/kg. Fishmeal, whale meal, and meat meal may contain 90–100 mg/kg zinc (Hambidge et al., 1986). When animal diets need to be supplemented to provide adequate zinc, the usual form is either zinc sulfate or zinc oxide. Other forms such as zinc acetate, zinc carbonate, zinc citrate, zinc chloride, and zinc picolinate and also zinc-amino acid complexes and zinc proteinates are occasionally used (Baker and Ammerman, 1995). When the purpose is to provide pharmacological levels of zinc to enhance growth of chicks and young pigs, zinc oxide is usually added.

Water

The standard for zinc level in drinking water is 5 mg/L (NRC, 1978). EPA also recommends that drinking water should contain no more than 5 mg/L of zinc because of taste (ATSDR, 2003). This concentration is almost never reached in surface water, municipal drinking water supplies, or in drinking water collected from the home tap. Industrial pollution, such as that derived from dumping plating baths or mining operations, can produce very high concentrations of zinc. Streams tend to become purified by precipitation of zinc with clay sediments or hydrous iron and manganese oxides. A concentration of 25 mg/L zinc was recommended as a safe upper limit in drinking water for livestock and poultry (NRC, 1978). Storage of food and water in galvanized containers can contaminate the contents with large amounts of zinc, particularly under acidic conditions. Other potential sources of excess zinc include pesticides, fungicides, and industrial pollution.

Bioavailability

The bioavailability of zinc is the fraction of zinc intake that is retained and used for physiological functions. Zinc absorption is determined by three factors: the animal's zinc status, the total zinc content of the diet, and the availability

of soluble zinc from the diet's food components (Lonnerdal, 2000). If the animal's zinc status is discounted, zinc absorption is largely determined by its solubility in the intestinal lumen, which in turn is affected by the chemical form of zinc and the presence of specific inhibitors and enhancers of zinc absorption. A comprehensive report on the bioavailability of zinc for animals was published by Baker and Ammerman (1995).

In general, zinc is absorbed more efficiently from aqueous sources in the absence of food and from animal products. Phytate (myoinositol hexaphosphate), which is present in plant products, especially cereals and legumes, irreversibly binds zinc in the intestinal lumen and accounts for the lower efficiency of absorption from plant foods. The negative effect on absorption is exerted by the inositol hexaphosphates and pentaphosphates (Lonnerdal, 2000). Phytates with less phosphate have little to no effect on zinc absorption. Fiber is often implied as having a negative effect on zinc absorption, but this is usually because most high fiber foods are also high phytate foods (Lonnerdal, 2000).

The binding of zinc to low molecular weight ligands or chelators that can be absorbed also has a positive effect on zinc absorption because the solubility of zinc is increased. Certain chelators (e.g., EDTA), amino acids (histidine or methionine), and organic acids (citrate) have been used to enhance zinc absorption. These have been shown to have mixed benefit. Recently, plant breeding or genetic engineering strategies that either reduce the content of inhibitors (e.g., phytate) or increase the expression of compounds that enhance zinc absorption (e.g., amino acids) have been considered to improve the bioavailability of zinc from plant foods (Lonnerdal, 2003).

In recent years, bioavailability has become more important in livestock diets as producers attempt to reduce the amount of zinc excreted in feces. In pigs, when retention was used as an indication of absorption, zinc proteinate, zinc polysaccharide, and zinc methionine were reported to be superior to inorganic forms (oxide and sulfate) (Kessler et al., 1996; Rupic et al., 1997). However, Wedekind et al. (1994) reported that neither zinc methionine nor zinc lysine were as bioavailable to pigs as zinc sulfate. Cheng et al. (1998) found that zinc lysine did not improve zinc absorption compared with zinc sulfate, but the addition of lysine, beyond that contained in the zinc source, reduced hepatic zinc. Using multiple linear regression slope ratios of bone zinc in the chick, Cao et al. (2000b) reported that the absorption of zinc-proteinate, zinc-amino acid chelate, and zinc-polysaccharide were 83–139 percent of zinc sulfate (set at 100 percent). With this same technique, Sandoval et al. (1997a) found zinc oxide was 74 percent as available as the sulfate form. Edwards and Baker (1999) reported that various zinc oxide sources fed to chicks were 93 to 39 percent absorbed relative to zinc sulfate. Cao et al. (2000b) reported that slope ratios of liver, kidney, and pancreas zinc concentrations and liver MT in lambs were 130, 113, and 110 percent for a zinc proteinate, a zinc amino acid chelate, and a zinc methionine, respectively, compared with 100 percent for zinc sulfate.

TOXICOSIS

Exposure by Feed

Acute

Acute, high-dose oral exposure to zinc compounds generally results in gastrointestinal distress with clinical signs of nausea, vomiting, abdominal cramps, and diarrhea; exposure levels resulting in these effects in several different species range from 2 to 8 mg Zn/kg/day (ATSDR, 2003). The irritant effect of zinc sulfate led to its former use as an emetic. Common sources of zinc contamination include galvanized coating on iron and steel (cages and nails, metal nuts from transport cages, and fencing), automotive parts, batteries, fungicides, and topical medications. The less soluble salts of zinc such as the oxide and stearate are commonly found in protective ointments and cosmetics and do not usually cause acute zinc toxicosis.

Acute zinc toxicosis has been reported in dogs and humans (Murphy, 1970; Hornfeldt and Koepke, 1984). It is characterized by an intravascular hemolytic anemia, gastrointestinal upset from direct irritation, and, potentially, multiorgan failure. There have been reports of dogs that have ingested large amounts of zinc from pennies, metal nuts from the dog's kennel, and other foreign metal objects (Hornfeldt and Koepke, 1984; Caldwell, 1994; Gandini et al., 2002; Mikszewski et al., 2003). Pennies minted after 1982 are made predominantly of zinc (96–98 percent) with a copper (2.5 percent) coating. Clinical signs include anorexia, gastrointestinal distress, hemolytic anemia, acute pancreatitis, hepatomegaly, and renal disease (Gandini et al., 2002; Mikszewski et al., 2003).

Acute zinc toxicosis also occurred in sheep given 3 g of zinc in 20 ml of a solution of zinc sulfate using a drenching gun in a study of the use of zinc to prevent lupinosis (Allen et al., 1986). Two pellets, each containing 2.5 g of zinc, were given immediately after the drench. Nineteen of the 230 sheep died within 3 days of the treatment. Necropsies showed severe fibrosing pancreatitis, abdominal lesions, and mild kidney changes. A reduction in feed intake was noted in those animals that survived. Previous studies have shown that sheep tolerate a single administration of 3 g of zinc when given by ruminal intubation. But, when given with a drenching gun, zinc is likely to cause intoxication because the concentrated solution of zinc stimulates irritation and closure of the esophageal groove and severe lesions of the abomasum (Smith et al., 1979). The liver, kidney, and bone seem to be able to accumulate zinc to tolerable concentrations before other organs, such as the pancreas, go into failure.

Chronic

Zinc toxicity is difficult to relate to the physical and chemical properties of this element because it is not believed to be carcinogenic, mutagenic, or teratogenic, and it does not have a classical genetically caused storage disease. As Vallee and Falchuk (1993) stated, "Zinc is the only pre-, post-, and transitional element that has proven to be essentially nontoxic." A major effect of chronically excessive zinc intake is insufficient tissue copper caused by inducing metallothionein and increasing copper-bound metallothionein in intestinal cells and decreasing copper absorption. This causes a copper deficiency anemia (ATSDR, 2003). Other effects of chronic zinc toxicosis include reductions in immune function (decrease in lymphocyte stimulation by phytohemagglutinin) and high-density lipoprotein (HDL) cholesterol (Cousins, 1996). A summary of experiments on chronic zinc toxicity is presented in Table 31-1. High dietary zinc intake in chickens produced a pause in egg production and molting via precipitous decreases in food intake (McCormick and Cunningham, 1984). Feeding zinc to chickens at 500 mg/kg diet produced dysfunction of pancreatic acinar cells and exocrine status (Lu et al., 1990). It is unclear why the pancreas seems to be so sensitive to chronically high intakes of zinc, but studies suggest that pancreatic accumulation is related to the metallothionein content of that tissue (Oh et al., 1979).

A natural occurrence of zinc toxicosis in male Holstein veal calves provides useful information on the metabolic consequences of chronic zinc toxicosis in growing ruminants (Graham et al., 1987). Due to an error in manufacturing, an additional amount of zinc sulfate was added to the milk replacer. The mean concentration of milk replacer totaled about 700 mg/kg rather than 150 mg/kg; total zinc intake averaged 1.5 to 2 g per day. The high-zinc milk replacer was fed for 35 days. Clinical signs appeared 23 days after feeding the high-zinc milk replacer. Of the 85 calves examined, 75 percent had pneumonia, 73 percent had ocular signs, 54 percent had diarrhea, 40 percent were anorectic, 18 percent were bloated, 9 percent had cardiac arrhythmias, 3.5 percent had convulsions, and 3.5 percent were polydipsic/polyphagic. Nineteen percent of the calves died. Necropsy data showed that tissue manganese, copper, and iron levels were normal (Graham et al., 1988). However, infarcts in liver, kidney, and heart tissue were observed. Marked atrophy and necrosis of pancreatic acinar tissue were observed. Calves with a cumulative intake of more than 45 g of zinc for 35 days were 60 times more likely to die than calves exposed to less than 45 g. These data suggest that the zinc intake of preruminants should be less than 500 mg/kg diet. A subsequent study showed that only 700 or 1,000 mg/kg of dietary zinc fed to preruminant calves altered weight gain, food intake, and feed efficiency (Jenkins and Hidiroglou, 1991).

The effect of chronically high intakes of zinc on copper metabolism has been studied extensively in chicks, ruminants, and experimental animals. Rama and Planas (1981) fed 80 or 5,000 mg/kg zinc with adequate copper and iron to one-day-old chicks. The concentrations of iron and copper were decreased in the plasma and liver. An intramuscular injection of iron corrected these depressed concentrations, whereas an oral iron supplement only partially returned hepatic iron to normal. However, when copper was injected, all copper parameters returned to normal including ceruloplasmin, but iron was not corrected. The authors concluded that the interference of zinc was at the intestinal level. Administration of diets containing 2,000 or 4,000 mg/kg zinc dramatically increased liver, pancreas, and bone zinc levels while reducing both gain and gain/feed markedly (Southern and Baker, 1983). Excess dietary zinc also reduced liver copper deposition.

Sheep are a unique livestock species because they are extremely sensitive to copper intake and have low hepatic copper concentrations at birth compared with their concentrations at 30 and 60 days of age (Saylor and Leach, 1980). This pattern is in contrast to other mammals where copper concentrations are highest in the liver at birth. Saylor and Leach (1980) found that when sheep were fed 543 mg/kg zinc, ceruloplasmin activity, plasma copper concentration, and hematocrits were reduced, but hepatic zinc and copper were not changed. Dosing sheep with 1 g Zn/10 mL of solution directly into the rumen three times per week for 2–14 weeks induced renal lesions and elevated plasma creatinine concentrations (Allen and Masters, 1985). Excess dietary zinc, 750 mg/kg diet, induced severe copper deficiency in pregnant ewes and caused a high incidence of abortions and stillbirths (Campbell and Mills, 1979). Supplemental copper failed to prevent the adverse effects of high zinc on weight gain, feed consumption, efficiency of feed use, and lamb viability. The authors speculated that a depressed feed intake of the ewes given the high-zinc diets caused the high mortality rates of the lambs.

L'Abbe and Fischer (1984) reported that 120–240 mg/kg zinc decreased hepatic superoxide dismutase and cardiac cytochrome *c* oxidase activities in the rat, suggesting that copper deficiency could result from zinc concentrations that were only four times that recommended.

Hill et al. (1983) reported that when sows were fed 5,000 mg/kg zinc for two parities, copper was reduced and iron was increased in the livers of their offspring at birth. However, the same group (Carlson et al., 1999) showed that when newly weaned pigs were fed 3,000 mg/kg zinc for 4 weeks, copper and iron in the liver were not altered compared with pigs fed 150 mg/kg zinc. If the pigs were weaned at 11 days of age, copper concentration in the kidney increased; if pigs were weaned at 24 days of age copper concentration was not changed. These data suggest that the severity of zinc excess can be reduced by dietary provision of adequate iron. Barone et al. (1998) found that fetal rats from dams fed 1,000 mg/kg zinc had higher hepatic zinc and copper and higher plasma iron than fetuses from dams fed 32 mg/kg zinc. However, plasma copper and hepatic iron were not different.

An interaction between zinc and copper has also been observed in humans. Administration of zinc as a therapeutic agent to sickle cell anemia patients caused hypocupremia (Prasad et al., 1978) that could be reversed by copper supplementation. Similarly, self-supplementation with zinc for "prostate trouble" resulted in sideroblastic anemia that was reversed in 80 days when supplements were discontinued (Patterson et al., 1985).

The mechanism underlying the interaction between zinc and copper probably involves metallothionein. Both zinc and cadmium induce MT (Tacnet et al., 1991). Using gel filtration analysis, Evans et al. (1970) reported that both cadmium and zinc displaced copper from sulfhydryl binding sites on MT, and, therefore, antagonized copper metabolism.

Exposure by Water

The mean concentrations of zinc in ambient water and drinking water range from 0.02 to 0.05 mg/L and from 0.01 to 0.1 mg/L, respectively. The concentration of zinc in drinking water can often be higher than the concentration in the raw water from which the drinking water was obtained because zinc may leach from transmission and distribution pipes. The concentration of zinc in standing water from galvanized household water pipes was 1.3 mg/L (ATSDR, 2003).

The bioavailability of zinc given in water is very high. Consequently, zinc has been added to the drinking water of ruminants to improve their zinc intake (Smith, 1980). The addition of 0.25, 0.5, and 1.0 g Zn/L reduced water consumption by 8, 35, and 54 percent compared to controls over a 9-week period. The effect was greatest in the early weeks of the experiment. Moderate pancreatic damage was recorded in the animals in the high zinc treatment group. It appeared that the unpalatable nature of high aqueous zinc solutions was insufficient to prevent toxicosis.

Factors Influencing Toxicity

The types and severity of adverse effects are related to zinc exposure level and duration; animal age, sex, species, and nutritional status; and composition of the diet. In general, young animals and reproducing females are more vulnerable than adult, nonreproducing animals. Young animals may be more vulnerable than older animals because they tend to have a higher efficiency of zinc absorption. Exposure to high levels of zinc in the diet prior to and/or during gestation has been associated with increased fetal resorptions, reduced fetal weights, altered tissue concentrations of fetal iron and copper, and reduced growth in the offspring (ATSDR, 2003). Administration of 200 mg Zn/kg/day to dams throughout gestation reduced growth and tissue levels of iron and copper in fetal rats (ATSDR, 2003), but no changes were seen when the diet provided 100 mg Zn/kg/day, suggesting that the placenta was able to act as an effective barrier to zinc at the lower dietary level.

Ruminants seem to be more susceptible to zinc toxicity compared to rats, pigs, and poultry (Abdel-Mageed and Oehme, 1990a). This may be due to the adverse effects of the high zinc intake on ruminal microorganisms. At high levels of zinc intake in lambs there was a reduction in the volatile fatty acid concentration and acetic acid to propionic acid ratio in the rumen (Ott et al., 1966a). Cellulose digestion by ruminal bacteria in vitro was reduced by zinc concentrations in the medium of 10–20 μg/mL (Martinez and Church, 1970).

The chemical form of administered zinc influences its toxic effects. Sheep given a single intraruminal dose (120, 240, or 480 mg Zn/kg BW) or a thrice-weekly dose of 240 mg Zn/kg BW for 4 weeks showed a divergent response to the chemical form of zinc administered (Smith and Embling, 1984). Sheep receiving the EDTA-zinc had an increase in urinary zinc but only a transient elevation in plasma zinc, whereas zinc sulfate caused a marked, sustained increase in plasma zinc. The effect of supplemental zinc on mortality varies with the form of zinc given. Six of the seven animals receiving multiple doses of zinc sulfate died, whereas none of the animals receiving zinc oxide or zinc EDTA died. Major pancreatic injury occurred in sheep dosed with sulfate or oxide, but only mild changes were observed in sheep dosed with zinc EDTA. Diarrhea was mild and transitory in the EDTA-dosed sheep, but more severe and persistent in those dosed with sulfate. These divergent effects of the chemical forms on zinc toxicity presumably are related to zinc bioavailability from the different forms. Zinc sulfate is considered to be highly available and readily absorbed. Zinc oxide is less soluble and available for absorption. The EDTA probably acts as a chelator and facilitates the excretion of excessive zinc levels in tissues. Diethylenetriaminepentaacetic acid (DTPA) and cyclohexanediaminetetraacetic acid (CDTA) are also effective against zinc intoxication (Abdel-Mageed and Oehme, 1990b).

The presence or absence of other cations in the diet can also influence zinc toxicity. The interaction between zinc and copper is described earlier in this chapter. Elevation of dietary calcium from 0.7 to 1.1 percent was effective in protecting against the toxic effects of 4,000 mg Zn/kg diet in young pigs (Abdel-Mageed and Oehme, 1990b).

TISSUE LEVELS

The zinc content of the bodies of adult animals (milligrams per kilogram of crude defatted tissue) ranges from 25 for swine to 50 for rabbits (Georgievskii et al., 1979). In newborn animals, the zinc contents are somewhat lower: from 10 for swine to 30 for cattle (mg per kg of crude defatted tissue). Tissue distribution of zinc, however, varies considerably, with some tissues (e.g., the prostate) having a very high concentration. Approximately 85 percent of the total

body zinc is in skeletal muscle and bone. About 95 percent of body zinc is intracellular, with 40 percent of the cellular zinc found in the nucleus. A variable amount of cytosolic zinc may reside in vesicles and may serve as a cellular zinc reserve. The amount of "free" Zn^{2+} in cells appears to be very low (O'Halloran, 1993; Outten and O'Halloran, 2001). Zinc contents of various tissues, averaged from several different species fed diets without excess zinc, are as follows (Georgievskii et al., 1979): blood (mg/L) 0.6–5; liver (mg/kg fresh) 40–65; hide (mg/kg fresh) 3–6; and skeletal muscle (mg/kg fresh) 5–9.

Zinc concentrations of selected fluids and tissues are presented in Table 31-2. These data indicate that excess intakes cause zinc to be deposited in the liver, pancreas, kidney, and bone. There is little or no accumulation in milk from dairy cows and skeletal muscle of various species (Table 31-2) or spleen of sheep (Rosa et al., 1986; Henry et al., 1997).

MAXIMUM TOLERABLE LEVELS

A summary of data on the maximum tolerable level of zinc intake for animals published since 1980 is given in Table 31-1. Cat diets providing up to 230 mg Zn/kg BW/day as zinc oxide for several months did not produce any adverse effects (Drinker et al., 1927). When 600 mg Zn/kg of diet was fed to adult cats for six weeks, plasma zinc concentration rose to 120 µg/dL compared to 90 µg/dL in cats fed 100 mg Zn/kg (Sterman et al., 1986). No clinical abnormalities were reported, suggesting that the safe upper limit of dietary zinc for adult cats is 600 mg/kg, at least for short periods of time. Dogs fed diets that provided up to 80 mg Zn/kg BW/day in the form of zinc oxide for several months suffered no ill effects (Drinker et al., 1927).

Based on data published before 1980, the maximum tolerable level of zinc for poultry was set at 1,000 mg/kg diet (NRC, 1980). Although earlier studies indicated that this level of zinc was tolerated without decreased growth or feed efficiency in diets adequate in all nutrients, recent studies indicate that this level can result in negative effects. Lesions in the pancreas and gizzard of chicks fed 1,000 mg/kg were reported by Dewar et al. (1983). When diets were marginally deficient in iron, 1,000 mg/kg also resulted in depressed growth (Blalock and Hill, 1988). Mild histological changes in the thyroid were observed in chicks fed 200 mg Zn/kg diet, and this level decreases plasma levels of thyroxin in laying hens (Kaya et al., 2001; 2002); however, functional or pathological consequences of these changes have not been demonstrated. Zinc caused decreased growth and signs of pancreatic pathology when supplemented at 500 mg/kg to purified diets (Lu and Combs, 1988; Lu et al., 1990). More recently, several studies (Sandoval et al., 1997a, 1998, 1999; Cao, 2000b) have reported reductions in feed intake and weight gain when zinc exceeded 500 mg/kg diet, especially when the source was zinc sulfate. Taken together, evidence supports lowering the maximum tolerable level for poultry to 500 mg/kg diet.

The maximum tolerable level of zinc for swine is difficult to establish. Several studies reviewed in the 1980 report (NRC, 1980) demonstrated that pigs tolerated zinc at levels of 1,000 mg/kg of diet for many weeks, but 2,000 mg/kg diet resulted in signs of toxicosis. The current maximum allowable zinc content in pig diets in Europe is 250 mg/kg, but this is based on environmental concerns. Recently, numerous experiments have demonstrated that supplementing weaned pig diets with 1,500–3,000 mg/kg zinc as zinc oxide stimulates their growth (Hill et al., 2000; Mavromichalis et al., 2000; Carlson et al., 2004; Davis et al., 2004). Most of these experiments have been for only 3 or 4 weeks, but a few have lasted 5 or 6 weeks. Furthermore, most studies have used zinc oxide, which is less bioavailable to pigs than zinc sulfate (Wedekind et al., 1994). Because of this, the maximum tolerable level is left unchanged as 1,000 mg/kg of diet. Despite their prohibition in the EU, growth promotion levels of zinc are used for short periods in many other countries. Although not considered in determining the maximum tolerable level, the potential negative environmental impact of excessive zinc intakes is an important consideration (Jondreville et al., 2003).

The maximum tolerable concentration of zinc for cattle was previously set at 500 mg/kg (NRC, 1980); recent research supports this recommendation. Young calves fed milk replacer tolerated 500 mg Zn/kg of diet for 5 weeks without adverse effects; but 700 mg/kg of diet reduced gain, feed intake, and feed efficiency (Jenkins and Hidiroglou, 1991). In one experiment, dairy cattle were fed a diet with a zinc content of 1,000 mg/kg for 16 weeks with no adverse effects.

For sheep, the previous maximum tolerable level was set at 300 mg/kg of diet, and there is insufficient new evidence to justify a change. In one experiment (Henry et al., 1997), levels as high as 2,100 mg/kg were fed for as long as 30 days without reducing feed intake, but tissues were not examined for histological lesions.

Because there is little accumulation of zinc in skeletal muscle or milk, excess intake of zinc by animals has little consequence for human health. Very high intakes of zinc do cause some increase in zinc content of kidney and liver (Table 31-2) but not heart (Henry et al., 1997).

FUTURE RESEARCH NEEDS

Maximum tolerable levels for many species, including horses, dogs, and cats, are not well established, and these species merit further research. Pharmacological levels of zinc intake have been shown to be effective in promoting growth in livestock. Further studies are needed to determine the optimal levels, sources, and duration of feeding of pharmacological levels to achieve the optimum benefit and the least environmental impact.

SUMMARY

Zinc is an essential nutrient for all animal species and is required for a wide variety of metabolic functions. Different compounds of zinc differ widely in bioavailability and probably in toxicity. Although animals are able to tolerate much higher levels of zinc than of many other minerals, signs of toxicosis generally develop when dietary concentrations exceed 1,000 mg/kg. In addition to reduced feed intake and growth rate, signs of toxicosis often involve damage to the pancreas. Swine are more tolerant than most other species of high zinc levels. Indeed, levels as high as 3,000 mg/kg of diet for periods of 3 or 4 weeks promote growth and feed efficiency of young pigs.

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TABLE 31-1 Effects of Zinc Exposure in Animals

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Broiler chicks	30	1 d	0.5 g/kg 1 g/kg 2 g/kg 4 g/kg	Zn acetate	4 wk	Diet	No effect on BW, feed intake, or feed conversion efficiency	Oh et al., 1979
Broiler chicks	30	1 d	1 g/kg 2 g/kg 4 g/kg 8 g/kg 16 g/kg	Zn acetate	5 wk	Diet	Levels > 4 g/kg reduced BW, feed intake, and feed conversion efficiency and increased mortality	Oh et al., 1979
Broiler chicks	42	1 d	1 g/kg 2 g/kg 4 g/kg 6 g/kg	ZnO	28 d or 42 d	Diet	Lesions of gizzard and pancreas At 2, 4, and 6 g/kg levels, decreased weight gain, increased tissue Zn, lesions of gizzard and pancreas	Dewar et al., 1983
Broiler chicks	15	7 d	2 g/kg 4 g/kg	$ZnCO_3$	3 wk	Diet	At 2 g/kg, no adverse effects on weight gain, feed conversion efficiency, hemoglobin, or hematocrit; at 4 g/kg, reductions in growth traits, hemoglobin, hematocrit, and accumulations of Zn in liver, pancreas, and bone	Southern and Baker, 1983
Broiler chicks	15	7 d	2 g/kg 4 g/kg	$ZnCO_3$	3 wk	Diet	At 2 g/kg, small reductions in weight gain and feed conversion efficiency; at 4 g/kg, large reductions in weight gain and feed conversion efficiency	Bafundo et al., 1984
Broiler chicks	10	1 d	1 g/kg 2 g/kg 5 g/kg	ZnO	14 d	Diet	At all levels, decreased BW, increased kidney and liver Zn, and decreased kidney and liver Cu	Blalock and Hill, 1988
Broiler chicks	20	1 d	2 g/kg 4 g/kg	ZnO	21 d	Diet	Increased kidneyand liver Zn	Blalock and Hill, 1988
Broiler chicks	30	1 d	0.5 g/kg 1 g/kg 2 g/kg	ZnO	20 d	Diet	No adverse effect at 0.5 or 1 g/kg; increased pancreas Zn at 2 g/kg	Lu and Combs, 1988
Broiler chicks	100	1 d	5 g/kg	ZnO	28 d	Diet	Decreased BW, increased serum Zn, reduced serum concentrations of thyroid hormones, growth hormone,and cholesterol	Dean et al., 1991
Broiler chicks	18–21	1 d	300–1,200 mg/kg	$ZnSO_4$ $ZnCO_3$ ZnO Zn Metal	21 d	Diet	Feed intakes were affected by source; for $ZnSO_4$, concentrations of 600 mg/kg or greater reduced feed intake; little or no effect from the other sources	Sandoval et al., 1997a
Broiler chicks	12	1 d	500 mg/kg 1,000 mg/kg 1,500 mg/kg	$ZnSO_4 \cdot 7H_2O$	21 d	Diet	Concentrations of 1,000 and 1,500 mg/kg reduced both feed intake and weight gain	Sandoval et al., 1998

continued

TABLE 31-1 Continued

Animal	N[a]	Age or Weight	Quantity[b]	Source	Duration	Route	Effect(s)	Reference
Broiler chicks	6	14 d	1,000 mg/kg 1,000 mg/kg 1, 000 mg/kg	$ZnSO_4$ Zn acetate $ZnCl_2$	7 d	Diet	No effects on feed intake or weight gain in the first experiment, but reductions in weight gain in the second	Sandoval et al., 1999
Broiler chicks	42	1 d	200 mg/kg 400 mg/kg	$ZnSO_4 \cdot 7H_2O$	21 d	Diet	No effect on feed intake or BW	Cao et al., 2000a
Broiler chicks	18	1 d	200 mg/kg 400 mg/kg 600 mg/kg	$ZnSO_4 \cdot 7H_2O$	3 wk	Diet	Small, but statistically significant, reductions in feed intake and weight gain	Cao et al., 2000b
Broiler chicks	48	1 d	200 mg/kg 400 mg/kg	$ZnSO_4 \cdot 7H_2O$	3 wk	Diet	No effect on feed intake or weight gain	Cao et al., 2000b
Broiler chicks	20	1 d	125 mg/L 500 mg/L 1,000 mg/L	$ZnSO_4 \cdot 7H_2O$	60 d	Water	Reductions in feed intake, BW, feed efficiency, and thyroid hormone levels at the 500 and 1,000 mg/L levels	Dönmez et al., 2001
Broiler chicks	15	1 d	125 mg/L 500 mg/L 1,000 mg/L	$ZnSO_4 \cdot 7H_2O$	60 d	Water	No adverse effect on erythrocytes or leukocytes; small reduction in hemoglobin at 1,000 mg/L	Dönmez et al., 2002
Laying hens	62	36–52 wk	20 g/kg	ZnO	5 d	Diet	Reduced egg production; reduced Cu, K, and Zn in eggs	Palafox and Ho-A, 1980
Laying hens	11	18 mo	10 g/kg 20 g/kg	ZnO	28 d	Diet	At both levels, decreased feed intake and lesions of gizzard and pancreas	Dewar et al., 1983
Laying hens	60	60–66 wk	20 g/kg	ZnO	4 d	Diet	Reduced feed intake; reduced BW; reduced ovary and oviduct weight; no change in liver, kidney and pancreas weights; increased tissue concentrations of Zn especially in pancreas	Williams et al., 1989
Laying hens	13	56 wk	25 mg/kg 50 mg/kg 100 mg/kg 200 mg/kg	ZnO	12 wk	Diet	Additions of >50 mg/kg led to reductions in levels of thyroid hormones	Kaya et al., 2001
Laying hens	13	56 wk	200 mg/kg	ZnO	12 wk	Diet	No accumulation of Zn in pancreas, liver, gizzard, or thyroid. Small increase in thyroid Fe at 200 mg/kg	Kaya et al., 2002
Calves	26	NP[c]	706 µg Zn/g of milk replacer	NP[c]	28 d	Diet	Pneumonia, polyuria, pica, and weight loss	Graham et al., 1988
Calves	7	3 d	40 200 500 700 1,000 µg Zn/g DM of milk replacer	Zho	5 wk	Diet	No adverse effects No adverse effects No adverse effects Reductions in feed intake, weight gains, and gain/feed Reductions in feed intake, weight gains, and gain/feed	Jenkins and Hidiroglou, 1991

Dairy cows	5	Yearling	0.25 g/L 0.50 g/L 1.00 g/L	$ZnSO_4 \cdot 7H_2O$	9 wk	Water	Progressive reductions in water intake, mild pancreatic lesions in first two groups, moderate pancreatic lesions in highest group	Smith, 1980
Dairy cows	10	3 primiparous and 7 multiparous	1 g/kg 2 g/kg	$ZnSO_4 \cdot H_2O$	16 wk	Diet	No adverse effects Reductions in feed intake and milk yield	Miller et al., 1989
Beef cattle	12	93–156 kg	0.28 g/L 0.56 g/L 1.12 g/L	$ZnSO_4 \cdot 7H_2O$	8 wk	Water	No adverse effects due to Zn supplementation; in fact there was a positive weight gain response to the supplemental Zn in all three treatments	Wright et al., 1978
Sheep	2	NP[c]	2 g/kg	ZnO	49 or 72 d	Diet	Severely reduced feed intake	Allen et al., 1983
Sheep	6–9	NP[c]	731 mg/kg 1.41 g/kg	ZnO	49	Diet	Pancreatic lesions in both groups	Ellis et al., 1984
Sheep	12	64 kg	700 mg/kg 1,400 mg/kg 2,100 mg/kg	$ZnSO_4 \cdot 7H20$	Up to 30 d	Diet	No effect on feed intake at any level	Henry et al., 1997
Fish, carp	NP[c]	NP[c]	NP[c]	$ZnSO_4 \cdot 7H_2O$	NP[c]	Diet	A summary of five different studies indicated that growth and survival was reduced at concentrations >294 g/kg of dry diet	Clearwater et al., 2002
Fish, Atlantic salmon	30	8.5 g	0.32 mg/L 0.42 mg/L 0.56 mg/L 0.75 mg/L	$ZnSO_4 \cdot 7H_2O$	61 d	Water	Initial reductions in feed intake, but adaptation to the highest level by the end of the experiment	Farmer et al., 1979
Fish, Atlantic salmon	30	8.5 g	0.115 mg/L 0.240 mg/L 0.560 mg/L	$ZnSO_4 \cdot 7H_2O$	80 d	Water	No adverse effects on growth rate	Farmer et al., 1979
Fish, brown trout	10	8–10 g	0.10 to 6.40 mg/L	$ZnSO_4 \cdot 7H_2O$	96 hr	Water	The median lethal concentration (LC_{50}) values ranged from < 0. 14 mg/L in alkaline soft water to 3.20 mg/L in acidic hard water	Everall et al., 1989
Fish, rainbow trout	10	50 g	0.11 to 4.26 mg/L	$ZnSO_4 \cdot 7H_2O$	72 hr	Water	The median lethal concentration (LC_{50}) was 2 mg/L	Lovegrove and Eddy, 1982
Fish, rainbow trout	10	4–7 g	NP[c]	$ZnCl_2$	96–120 hr	Water	The median lethal concentration (LC_{50}) is pH dependent and at pH 7 was 4.46 and 0.17 mg/1 at high and low hardness, respectively	Bradley and Sprague, 1985
Fish, rainbow trout	NP[c]	NP[c]	NP[c]	Several different sources	NP[c]	Diet		Clearwater et al., 2002

[a]Number of animals per treatment group.
[b]Quantity of zinc dosed. SI conversion: 1 mg Zn equals 15.3 μmoles zinc.
[c]NP = not provided.

TABLE 31-2 Zinc Concentrations in Fluids and Tissues of Animals (mg/kg or Mg/L)[a]

Animal	Quantity	Source	Duration	Route	Muscle	Kidney	Liver	Pancreas	Bone	Egg	Milk	Serum	Reference
Broiler chicks	1 g/kg 2 g/kg 4 g/kg	ZnO	4 wk	Diet			117[b] 271[b] 845[b]						Dewar et al., 1983
Broiler chicks	2 g/kg 4 g/kg	$ZnCO_3$	3 wk	Diet			379[b] 1,144[b]	1,775[b] 8,201[b]	1,826[c] 4,308[c]				Southern and Baker, 1983
Broiler chicks	2 g/kg 4 g/kg	$ZnCO_3$	3 wk	Diet			272[b] 1,108[b]						Bafundo et al., 1984
Broiler chicks	1 g/kg 2 g/kg 5 g/kg	ZnO	2 wk	Diet		68 104 413	112 361 935						Blalock and Hill, 1988
Broiler chicks	5 g/kg	ZnO	4 wk	Diet								3	Dean et al., 1991
Broiler chicks	500 1,000 1,500 mg/kg	$ZnSO_4 \cdot 7H_2O$	21 d	Diet		96[b] 99[b] 113[b]	68[b] 71[b] 100[b]		647[c] 849[c] 1,005[c]			2.20 2.45 2.76	Sandoval et al., 1998
Broiler chicks	200 mg/kg 400 mg/kg 600 mg/kg	$ZnSO_4 \cdot 7H_2O$	3 wk	Diet					455[c] 480[c] 534[c]				Cao et al., 2000b
Broiler chicks	200 mg/kg 400 mg/kg	$ZnSO_4 \cdot 7H_2O$	3 wk	Diet					380[c] 416[c]				Cao et al., 2000b
Laying hens	20 g/kg	ZnO	5 d	Diet						54			Palafox and Ho-A, 1980
Laying hens	20 g/kg	ZnO	45 d	Diet		251[b]		1,678[b]		189[b]			Williams et al., 1989
Laying hens	25 mg/kg 50 mg/kg 100 mg/kg 200 mg/kg	ZnO	12 wk	Diet			57 66 56 66	56 65 53 59					Kaya et al., 2002
Pigs	25 mg/kg 50 mg/kg 75 mg/kg 100 mg/kg 125 mg/kg 150 mg/kg	$ZnSO_4$		Diet								0.25 0.50 0.90 0.90 0.90	Jondreville et al., 2003[d]
Calves	706 µg Zn/g of milk replacer	NP[e]	28 d	Diet	22	236	345						Graham et al., 1988

Calves	40 200 500 700 1,000 µg Zn/g DM of milk replacer	ZnO	5 wk	Diet	59[b] 62[b] 72[b] 82[b] 94[b]	138[b] 634[b] 1,210[b] 1,779[b] 2,498[b]	677[b] 1,625[b] 3,138[b] 3,821[b] 3,647[b]				1.5 2.5 5.6 6.1 16.6	Jenkins and Hidiroglou, 1991
Dairy cows	0.25 g/L 0.50 g/L 1.00 g/L	$ZnSO_4 \cdot 7H_2O$	9 wk	Water		171[b] 318[b] 790[b]	258[b] 464[b] 1,004[b]	356[b] 549[b] 1,834[b]			1.74 2.18 2.22	Smith, 1980
Dairy cows	1 g/kg 2 g/kg	$ZnSO_4 \cdot H_2O$	16 wk	Diet						5.5 6.5	2.2 6.5	Miller et al., 1989
Sheep	46 mg/kg 543 mg/kg	$ZnCO_3$	60 d	Diet	24 26	14 19	47 54				1.39 1.62	Saylor and Leach, 1980
Sheep	2 g/kg	ZnO	49 or 72 d	Diet or stomach tube		2,130[b]	1,671[b]	1,440[b]				Allen et al., 1983
Sheep	731 mg/kg 1.41 g/kg	ZnO	49 d	Diet		671[b] 2,755[b]	378[b] 1,102[b]	471[b] 2.555[b]				Ellis et al., 1984
Sheep	1,000 mg/kg	$ZnCO_3$	56 d	Diet		578[b]	166[b]					Rosa et al., 1986
Sheep	700 1,400 2,100 mg/kg	$ZnSO_4 \cdot 7H_2O$	Up to 30 d	Diet	164[b] 134[b] 136[b]	315[b] 582[b] 1,236[b]	202[b] 269[b] 535[b]		171[c] 188[c] 182[c]			Henry et al., 1997
Sheep	700 1,400 2,100 mg/kg	$ZhSO_4 \cdot 7H_2O$	23 d	Diet		659[b] 1,148[b] 1,543[b]	464[b] 577[b] 612[b]	643[b] 1,171[b] 1,203[b]				Sandoval et al., 1997b
Sheep	700 1,400 2,100 mg/kg	$ZnSO_4 \cdot 7H_2O$	21 d	Diet		528[b] 1,164[b] 1,519[b]	255[b] 374[b] 436[b]	305[b] 1,080[b] 1,157[b]				Cao et al., 2000b
Fish, tilapia	1 mg/L 10 mg/L	$ZnCl_2 \cdot H_2O$	10 d	Water	24[b] 29[b]		33[b] 46[b]					Kargin and Cogun, 1999
Fish, rainbow trout	1.52 mg/L 10 mg/L	$ZnSO_4 \cdot 7H_2O$	72 h	Water	30[b] 50[b]	160[b] 200[b]	140[b] 150[b]					Lovegrove and Eddy, 1982

[a]Data are on a fresh tissue basis.
[b]Data are on a dry tissue basis.
[c]Data are mg/g of ash.
[d]Meta-analysis (combined analysis of several datasets).
[e]Not provided.

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Other Minerals

INTRODUCTION

There are several elements that are unlikely to be of toxicological concern under natural conditions to domestic animals, and therefore are not reviewed in separate chapters in this document. However, the tolerable levels of these elements may be of interest because of possible exposure through supplements promoted in response to findings suggesting essentiality (rubidium, tungsten), growth stimulation (rare earths, titanium), or some other beneficial effect (lithium, germanium, strontium); pharmaceutical formulations (antimony, silver); or anthropogenic sources (uranium). Thus, succinct information related to toxicity about these elements is presented in this chapter. To keep this chapter to a reasonable length, some sections included in other chapters (e.g., methods of analysis) have been omitted from this chapter.

ANTIMONY

Antimony (Sb) is a lustrous, silver-white metal with a bluish tinge that is commercially derived mostly from stibnite ore (Sb_2S_3). Abundant deposits are found in China, Mexico, Bolivia, South Africa, and Tajikistan. The estimates of the abundance of antimony in the Earth's crust range from 0.2 to 0.5 mg/kg (USGS, 2004). The major use of antimony metal is as a hardener of lead in storage batteries; other alloy uses of the metal include solder, sheet and pipe metal, bearings, castings, and pewter. Antimony oxide is primarily used in flame-retardant formulations for children's clothing, toys, aircraft, and automobile seat covers; other uses for the oxide include paints, ceramics, and fireworks, and as enamels for plastics, metal, and glass.

Biological interest in antimony developed when the trivalent antimony compound potassium antimonyl tartrate ($KSbC_4H_4O_7 \cdot {}^1/_2H_2O$) was found to be an effective treatment for schistosomiasis, or bilharziasis, and subsequently for leishmaniasis. Treatment of schistosomiasis requires the intravenous administration of potassium antimonyl tartrate at a near lethal dose of 36 mg/kg (Dieter et al., 1991). Currently, treatment of leishmaniasis requires parenteral administration of pentavalent antimony compounds (Croft and Yardley, 2002). Other organic drugs that can be taken orally have been or are being developed to replace antimony compounds that need invasive methods of administration. Antimony taken orally has no known essential or beneficial metabolic function in living organisms.

Sources and Metabolism

Potentially toxic doses of antimony may happen as a consequence of industrial processing and use of antimony, preparation or storage of food in containers improperly glazed with enamels containing antimony, and accidental or intentional ingestion of excessive amounts of antimony compounds. Enamel glazes utilizing antimony trioxide as an opacifier, particularly if low in silica, are readily attacked by food acids. Monier-Williams (1925, 1934) reported that a 1 percent citric acid solution dissolved 10 mg Sb/L from an enameled container. Reported reliable values for antimony in foods and feedstuffs are few. Most human foods contain no more than a few µg Sb/kg (Nielsen, 1986). Nuts are relatively high in antimony (50–300 µg/kg DW) (Furr et al., 1979). Clemente et al. (1978) reported that the daily dietary intake of Italians was 1.5 µg of antimony. Becker et al. (1975) found that the antimony content of swine feeds, piglet starter rations, fishmeals, and various mixed feeds was generally between 20 and 60 µg/kg. An occasional feed sample contained over 100 µg/kg.

Soluble antimony compounds, such as antimonites and tartrates, are poorly absorbed. For example, only 2 percent of initial body burden of either trivalent or pentavalent antimony tartrate was present 4 days after a gavage administration, and over 60 percent of this was in the gastrointestinal tract (Felicetti et al., 1974). In most rodents, trivalent antimony is excreted primarily in the feces and pentavalent anti-

mony primarily in the urine (Otto and Maren, 1950). In humans, trivalent antimony is excreted in the bile after conjugation with glutathione (Bailly et al., 1991), and both valence states are excreted in the urine (Otto et al., 1947). A review (NRC, 1980a) found that trivalent antimony concentrates in the liver of all species studied and in the erythrocytes of many species, including humans. Pentavalent antimony has a lesser affinity for the liver than trivalent antimony and concentrates more in the spleen. Human erythrocytes are almost impermeable to pentavalent antimony.

Metabolic Interactions and Mechanisms of Toxicity

Thiol-containing enzymes are inhibited in vitro by antimony salts. Thus, enzyme inhibition may be one mechanism through which antimony is toxic. Antimony has been shown to suppress in vitro arsenic genotoxicity (Gebel, 1998).

Toxicosis and Maximum Tolerable Levels

Generally, compounds containing trivalent antimony are more toxic than pentavalent antimony. Regardless of valence state, however, the inherent oral toxicity of antimony compounds is low. Lifetime studies with mice fed drinking water supplemented with antimony potassium tartrate to provide 5 mg/L revealed no demonstrable toxic effects on males and only a slight decrease in life span and longevity and some suppression of growth of females (Schroeder et al., 1968b). A similar lifetime study with rats found no effect on growth but a decrease in life span in both sexes (Schroeder et al., 1970). In 14-day studies, drinking water doses of antimony potassium tartrate estimated at 0,16, 28, 59, 94, and 168 mg/kg BW in rats and 0, 59, 98, 174, 273, and 407 mg/kg BW in mice were poorly absorbed and relatively nontoxic (Dieter et al., 1991). The NOAEL was determined to be 2,500 mg antimony potassium tartrate/L drinking water in both rats and mice (Lynch et al., 1999). Diets containing 1,000, 5,000, and 20,000 mg/kg antimony trioxide fed for 90 days had no effect on growth, feed consumption, and clinical variables (Hext et al., 1999). Poon et al. (1998) provided rats with drinking water containing 0.5, 5, 50, and 500 mg/L potassium antimony tartrate to male and female rats for 13 weeks and found toxicological signs at the highest concentration. These signs included depressed body weight; hematuria; decreased red blood cell and platelet counts; increased corpuscular volume; and mild histological changes in the thyroid, liver, pituitary gland, spleen (males), and thymus (females). Rabbits may have a lower tolerance to antimony. After 5 to 20 days, some rabbits fed 15 mg of potassium antimonyl tartrate (5.5 mg antimony) per kg BW exhibited fatty degeneration and parenchymal necrosis of the liver (Pribyl, 1927). Rabbits fed 2 to 6 mg potassium antimonyl tartrate (0.7 to 2.2 mg antimony) per kg BW exhibited no pathology.

Acute toxicity induced by feeding relatively high amounts of potassium antimonyl tartrate was examined in rabbits (Oelkers, 1937). A single oral dose of 125 mg per kg BW (46 mg/kg antimony) was fatal in all cases; 120 mg/kg BW (44 mg/kg antimony) was almost certainly fatal in 24 to 36 hours; and 115 mg/kg BW (42 mg/kg antimony) was fatal to 50 percent of the animals. Bradley and Fredrick (1941) found the minimum lethal dose of potassium antimonyl tartrate expressed as antimony was 300 mg/kg BW for rats. After drinking lemonade containing 0.013 percent antimony, 70 people became acutely ill with burning stomach pains, colic, nausea, and vomiting; most recovered within three hours (Dunn, 1928; Monier-Williams, 1934). A person consuming 300 mL of lemonade would have received a dose of approximately 36 mg of antimony, or approximately 0.5 mg/kg for a 70-kg adult.

The signs of antimony toxicity caused by non-oral routes are extensive and have been reviewed (Winship, 1987).

Setting maximum tolerable limits for domestic animals is not possible because of the lack of data. The rabbit data suggest that a daily intake of 3 mg Sb/kg BW is an appropriate conservative limit. The previous edition of this book (NRC, 1980a) suggested a maximum tolerable limit of 70–150 mg Sb/kg dry diet for the rabbit.

Tissue Levels

Antimony occurs in low amounts in animal and human tissue. Smith (1967) found that median value for a variety of human organs fell between 0.05 and 0.15 mg/kg dry weight. Yukawa et al. (1980) found that means of human organs except the lung ranged from 0.01 to 0.03 mg Sb/kg fresh weight. Sumino et al. (1975) found 0.01 and 0.11 mg Sb/kg fresh tissue, with the highest amounts being in the skin and adrenal gland. Hamilton et al. (1972/1973) found antimony in all human tissues but in lower amounts than those above; mean concentrations were between 0.005 and 0.02 mg/kg fresh tissue. A review of reported blood concentrations for humans indicated that the normal concentration apparently is less than 1 μg/L (Nielsen, 1986).

GERMANIUM

Germanium (Ge) is a lustrous, gray-white brittle metalloid with a diamond-like crystalline structure (Furst, 1987). It is similar in chemical and physical properties to silicon and obtained chiefly from germanite, an ore that contains about 7 percent germanium and 22 other elements. Varying average abundances of germanium in the Earth's crust have been reported, including 1.5 mg/kg (Furst, 1987) and 7 mg/kg (Chase et al., 2003). Germanium is used in transistors and in integrated circuits, alloys, and glass (where it increases the index of refraction).

Biological interest in germanium was stimulated when it was discovered that some of its organic complexes inhibit tumor formation in animal models (Sato et al., 1985). The suggested mechanism behind tumor inhibition is that

germanium enhances immune function. This has resulted in the promotion of over-the-counter supplements containing germanium (e.g., germanium-132 or carboxyethyl germanium sesquioxide and lactate-citrate-germanate) as anticancer agents. Additionally, germanium is promoted for the treatment of numerous conditions that could be affected by improved immune function including rheumatoid arthritis, osteoarthritis, candidiasis, and chronic viral infections (Chase et al., 2003). Recently, carboxyethyl germanium sesquioxide supplementation (18 mg/kg diet) was found to improve transverse bone strength and bone mineral density in rats with experimental osteoporosis (Matsumoto et al., 2002). Germanium is not considered essential, but low dietary germanium compared to more normal intakes alters bone and liver mineral composition and decreases tibial DNA in rats (Seaborn and Nielsen, 1994). Compared to when the diet was supplemented with 1 mg Ge/kg diet, 10 mg Ge/kg diet as germanium dioxide stimulated growth in rats (Venugopal and Luckey, 1978) and chickens (Li et al., 1993).

Sources and Metabolism

Because of the limited nutritional interest in germanium, very few reports have appeared that indicate the germanium content of animal feedstuffs. The most extensive germanium analysis of foods was performed by a colorimetric method almost 40 years ago (Schroeder and Balassa, 1967a). Based on recent animal tissue analyses by more modern techniques, the values reported by Schroeder and Balassa (1967a) provide a reasonable idea of germanium intakes by animals and humans. Almost all of the 125 foods and beverages they analyzed contained detectable amounts of germanium, but only 4 of them contained more than 2 mg Ge/kg wet weight, and only 15 others contained more than 1 mg Ge/kg. The values they reported for grains included (in mg/kg wet weight): rye, 0.64; wheat, 0.64; oats, 0.20; brown rice, 0.10; and degermed cornmeal, 0.41. Purina cat chow contained 0.33 mg Ge/kg, and a rat diet composed of skim milk, corn oil, and rye contained 0.32 mg/kg. The findings of Schroeder and Balassa (1967a) indicate that most diets of domestic animals would contain less than 1 mg Ge/kg and human diets would provide about 1.5 mg/day. Water is not a significant source of germanium.

Over 96 percent of an oral dose of 6.94 mg germanium as germanium dioxide given to rats by stomach tube was rapidly absorbed from the gastrointestinal tract and excreted mainly via urine (Rosenfeld, 1954). Less than 5 percent was excreted through the bile. Little is known about the metabolism of germanium ingested with ordinary diets. However, based on their analysis of foods and urine, Schroeder and Balassa (1967a) concluded that, like in rats, dietary germanium is well absorbed in humans and excreted largely via the kidneys. They calculated that adults ingest about 1.5 mg Ge/day, and 1.4 mg appears in the urine and 0.1 mg in the feces.

Metabolic Interactions and Mechanisms of Toxicity

The mechanisms through which germanium has toxic effects, including causing nephropathy and peripheral nerve disorders, have not been precisely defined. Arginine and enalapril prevent nephropathy caused by long-term germanium dioxide intake (Nodera and Yanagisawa, 2003); this suggests that oxidative stress involving nitric oxide might have a role in germanium toxicity. High dietary germanium can affect the metabolism or tissue distribution of several other mineral elements in animals; this may be the basis for some of the biological effects of germanium. Providing 5 mg Ge/L drinking water for life depressed the chromium concentration in heart, kidney, and spleen and increased the copper concentration in livers of rats (Schroeder and Nason, 1976). High doses of germanium protected against acute selenium toxicity and increased urinary selenium excretion (Paul et al., 1989). Zinc administration prevented the decrease in bone alkaline phosphatase activity and DNA content induced in rats by an oral dose of 2.2 mg Ge/100 g BW for 3 days (Yamaguchi and Uchiyama, 1987). Germanium supplementation has been found to reverse changes in rats caused by a silicon deprivation (Seaborn and Nielsen, 1994).

Toxicosis and Maximum Tolerable Levels

Germanium has a low order of toxicity. The oral LD_{50} of germanium dioxide is 3.7 g/kg for male rats, and 6.3 g/kg for male mice (Hatano et al., 1981). Germanium dioxide toxicity in mice and rats results in tremors, sedation, cyanosis, vasodilation, hypothermia, and respiratory failure resulting in death. The oral LD_{50} for germanium sesquioxide is 11.7 g/kg and 11.0 g/kg for male and female rats, respectively, and 12.5 g/kg and 11.4 g/kg for male and female mice, respectively (Tao and Bolger, 1997). In order to induce chronic germanium toxicity in rodents, more than 100 times the normal concentration of usually less than 1 mg Ge/kg diet is required. Germanium dioxide in amounts of 10 mg Ge/kg diet stimulated growth, 100 mg Ge/kg had little or no effect, and 1,000 mg Ge/kg was toxic to rats (Venugopal and Luckey, 1978) and chicks (Li et al., 1993). Nakano et al. (1987) provided rats with drinking water containing 0, 100, or 500 mg germanium dioxide/L; the rats provided the highest amount had pathological changes in the kidneys, skeletal muscles, and myocardium. Rats fed 150 mg GeO_2/kg/day for 13 weeks exhibited decreased body weight and pathological changes in the kidney, liver, and heart (Sanai et al., 1990). No toxic effects or renal histological abnormalities were seen in rats fed 120 mg germanium sesquioxide/kg BW for 24 weeks, but toxic effects were seen in rats given an equal amount of germanium as germanium dioxide (75 mg/kg BW) (Sanai et al., 1991a). The germanium dioxide group exhibited increased blood urea nitrogen and serum phosphate, decreased creatinine clearance, weight loss, anemia, and liver dysfunction. Tubular degeneration and tubulo-

interstitial fibrosis were seen in the kidney. In contrast to the study of Sanai et al. (1991a), Chase et al. (2003) described a study in which both germanium dioxide and germanium sesquioxide fed at a level of 50 mg/kg diet resulted in some renal pathology. The only effect a lifetime exposure to 5 mg Ge/L in their drinking water had on mice and rats was a slightly shortened life span (Schroeder and Balassa, 1967b; Schroeder et al., 1968a). The germanium exposure slightly reduced the incidence of tumors in rats. The major concern about germanium toxicity in humans is that consuming large doses in supplements over extended periods of time can lead to nephrotoxicity and death. At least 31 cases of germanium-associated nephrotoxicity, including 9 fatalities, have been reported (Chase et al., 2003). Anorexia, weight loss, fatigue, nausea, vomiting, anemia, and muscle weakness accompanied renal failure. In most of these cases, the individual consumed a few hundred grams of a germanium compound over several months.

Based on the findings with rodents, the maximum tolerable level of germanium is greater than 10 mg/kg diet but is less than 50 mg/kg diet for domestic animals. Sanai et al. (1991b) has indicated 37.5 mg germanium dioxide/kg BW/day is tolerated by rats.

Tissue Levels

Because of the lack of reports, germanium analyses of foods and of human and rodent tissues have to be used to estimate the germanium content of tissues from domestic animals. These analyses indicate that the germanium concentration in most tissues is relatively low with normal dietary intakes. Schroeder and Balassa (1967a) found the following concentrations (mg/kg wet weight) in foods: pork chops, 0.75; chicken liver, 0.20; beef liver, 0.36; ground beef, 0.47; lamb liver, 0.15; haddock filet, 0.28; codfish filet, 0.42; salmon, 1.23; and milk, 1.51. Hamilton et al. (1972/1973) found the following concentrations in human tissues (mg/kg wet weight): liver, 0.04; kidney, 9; and muscle, 0.03. Mice fed a diet containing 0.32 mg Ge/kg and provided drinking water containing 5 mg Ge/L as sodium germanate for life had increased concentrations of germanium (mg/kg wet weight) in liver (3.12 versus 0.12) and kidney (2.63 versus 0.49) compared to controls fed regular drinking water (Schroeder and Balassa, 1967a). The increase in germanium concentrations was not as marked in rats treated the same as the mice, with kidney being 0.62 vs. 0.18 and liver being 0.27 vs. 0.14 mg Ge/kg wet weight (Schroeder et al., 1968b). These rodent results indicate that tissue levels found in domestic animals with elevated intakes of germanium would not produce foods high enough in germanium to be of toxicological concern for humans.

LITHIUM

Lithium (Li) is a soft, silver-white alkali metal that has only half the density of water (Wikepedia, 2004a). With a mean concentration of 50–65 mg/kg, lithium is 27th in elemental abundance in the Earth's crust. The minerals lepidolite, spodumene, petalite, and amblygonite are the more important sources of the lightest metal lithium. Lithium is primarily used in heat transfer alloys, batteries, lubricants, and mood-stabilizing drugs.

Biological interest in lithium focuses mainly around its use as a mood-stabilizing drug (Birch, 1995), but some interest stems from findings suggesting that it has other beneficial, perhaps essential, functions in higher animals, and can be used as a food aversion substance for grazing animals. Lithium deprivation (less than 1.5 mg/kg diet) has been reported to depress fertility, birth weight, lifespan, liver monoamine oxidase activity, and the activity of several liver and blood enzymes used in the citrate cycle, glycolysis, and nitrogen metabolism in goats (Anke et al., 1991). In rats, lithium deprivation (5–15 μg/kg diet) depressed fertility, birth weight, litter size, and weaning weight (Patt et al., 1978; Pickett and O'Dell, 1992). In addition, the lithium content was depressed in testes, seminal vesicles, and epididymis. Lithium concentrations were relatively high in the pituitary and adrenal glands and remained constant through two generations regardless of dietary lithium. These findings suggest lithium may have a role in the regulation of some endocrine function. Large doses of lithium chloride given by gavage while providing a toxic plant material for consumption result in an aversion to the consumption of those materials by grazing cattle and sheep. For example, a 200 mg/kg BW dose of lithium chloride created aversion to larkspur (Ralphs, 1997) and locoweed (Ralphs et al., 1997) in cattle, and a 160 mg/kg BW dose resulted in aversion to vermeerbos by sheep (Snyman et al., 2002). In humans, low lithium intakes from water supplies (little or no lithium compared to 70–170 μg/L) have been associated with increased rates of suicides, homicides, drug use, and other crimes (Schrauzer and Shrestha, 1990; Schrauzer, 2002). Lithium also has been shown to have insulinomimetic (Rossetti et al., 1990) or antidiabetic (Hu et al., 1997) actions.

Sources and Metabolism

Lithium is taken up by all plants. However, because it apparently is not required for growth and development, the uptake is dependent upon the available lithium in the soil. Thus, similar foods and feeds grown on different soils can have widely different lithium concentrations. This variance probably explains the large range (8.6 to 546 μg/day) for reported daily lithium intakes by humans (Van Cauwenbergh et al., 1999). The limited number of reports that give the lithium concentration in materials used as animals feeds indicates that forage and grains generally are good sources of lithium but vary with the soil on which they are grown. For example, Anke et al. (1991) reported the following concentrations (mg/kg DW) for feedstuffs grown on lithium-rich and lithium-poor soils, respectively: red clover, 3.0 and 1.4;

rye, 4.1 and 1.0; wheat, 2.9 and 0.7; barley, 1.1 and 0.7; and oats, 1 and 0.5. These data indicate that animal diets normally contain a few milligrams of lithium per kilogram. However, it should be noted that there are some plants that can accumulate lithium in very high concentrations; for example, up to 1,000 mg Li/kg can occur in nightshade species (Schrauzer, 2002). Drinking water also can be a significant source of lithium as some ground water may reach 0.5 mg/L and up to 100 mg Li/L are found in some natural mineral waters (Schrauzer, 2002). Concentrations of 1.5 and 5.2 mg Li/L have been found in river water from lithium-rich regions of northern Chile (Zaldivar, 1989). Most drinking water contains less than 25 µg/L (Anke et al., 1997).

Ingested lithium in the form of soluble salts is essentially 100 percent absorbed by the small intestine and is excreted primarily by the kidneys. Several studies have indicated that lithium transfer in the gastrointestinal tract occurs by paracellular transport via the tight junctions and pericellular spaces and not by passage through the cell (Birch et al., 1994). Lithium is not protein-bound and distributes throughout body water with only small differences between extracellular and intracellular concentrations (Schrauzer, 2002). Lithium distribution and excretion is similar to that of sodium. About 90 percent of lithium excretion occurs via urine; most of the rest is excreted via the feces, with about 20 percent arising from the bile and the remainder through the intestinal wall (Anke et al., 1997).

Metabolic Interactions and Mechanisms of Toxicity

Probably the most important interaction involving lithium is that with sodium. The replacement of lithium with sodium apparently can have undesirable consequences. Pickett and O'Dell (1992) reported that increased sodium in the diet exacerbated the impaired reproductive performance induced by lithium deprivation in rats.

Other possible interactions in addition to that with sodium may be involved in lithium toxicity. Lithium may displace potassium, magnesium, and calcium from membrane or enzyme sites with resultant impaired functions. For example, the Li^{2+} ion has been shown to interfere with numerous magnesium-dependent enzymes including adenylate cyclase, Mg^{2+}-ATPase, cholinesterase, DNA polymerase, pyruvate kinase, tyrosine aminotransferase, and tryptophan hydroxylase (Geisler and Mork, 1990). Magnesium enzymes involved in the inositol signaling pathway (which also involves calcium) are inhibited by lithium. It has been stated that the biochemical and toxicological effects of lithium can be explained by its selective interference with the phosphoinositide cycle (Ragan, 1990; Anke et al., 1997).

Toxicosis and Maximum Tolerable Levels

The toxicity of oral lithium for domestic animals has been studied mostly in Germany by Anke and colleagues (1991, 1997). Food consumption was reduced by 50 mg Li/kg diet by chickens, 125 mg Li/kg diet by pigs, and 100 mg Li/kg diet by cattle (Anke, 1991). Other signs of toxicity found by Anke et al. (1991) included depressed weight gain, egg production, and egg weight in chickens fed 100 or 150 mg Li/kg diet; less weight gain in cattle fed 100 or 200 mg Li/kg diet; and less weight gain and enormous water consumption in pigs fed 500 mg Li/kg diet. Pigs fed 1,000 mg Li/kg diet died within 92 days. No significant toxicity signs were seen in chickens fed 25 mg Li/kg diet, or in cattle fed 10 or 50 mg Li/kg diet. Pigs fed 17 to 84 mg Li/kg diet showed increased serum lithium concentrations and less biting activity among unacquainted pigs, but weight gain and feed efficiency were not affected (McGlone et al., 1980). Regius et al. (1993) found that 25 mg Li/kg diet tended to improve growth and reduce mortality of lambs, but 50 mg Li/kg diet depressed growth of lambs.

The clinical control of bipolar illness in humans is achieved by doses of 900 to 1,500 mg lithium carbonate/day or 169 to 282 mg Li/day. These dosages elevate the normal serum lithium concentration from 2–20 µg/L to 2,780–55,550 µg/L. Higher blood lithium concentrations can have toxic side effects of tremor, dizziness, drowsiness, and diarrhea (Weiner, 1991).

Based on the findings with chickens, cattle, pigs, and sheep, the maximum tolerable lithium level for domestic animals is about 25 mg/kg diet. This amount apparently does not cause food aversion and thus decreased food intake, nor does it cause apparent toxicity signs not related to decreased food intake.

Tissue Levels

The concentration of lithium in animal and human tissue and fluids is very dependent upon lithium intake. For example, the following mean concentrations were found in tissues (µg/kg DW) and fluids (mg/L) from rats fed 2 or 500 µg Li/kg diet as lithium carbonate, respectively, for three generations: liver, 1.6 and 12; heart, 2.3 and 25; skeletal muscle, 4.6 and 34; kidney, 2.9 and 40; bone, < 7 and 304; whole blood, 0.9 and 35; and blood serum, 2.3 and 67 (Patt et al., 1978; Pickett and O'Dell, 1992). Tissue lithium concentrations in domestic animals fed normal diets probably are similar to those of rats fed the diet containing 500 µg Li/kg.

RARE EARTHS

The rare earths are a relatively abundant group of 17 elements composed of scandium (Sc), yttrium (Y), and the lanthanides cerium (Ce), dysprosium (Dy), erbium (Er), europium (Eu), gadolinium (Gd), holmium (Ho), lanthanum (La), lutetium (Lu), neodymium (Nd), praseodymium (Pr), promethium (Pm), samarium (Sm), terbium (Tb), thulium (Tm), and ytterbium (Yb). "Rare" earths is a historical misnomer; persistence of the term reflects unfamiliarity rather

than true rarity. Some rare earth elements are as abundant in the Earth's crust as more familiar metals such as chromium, nickel, copper, zinc, molybdenum, tin, tungsten, and lead. The abundances of the elements in the Earth's crust range from 60 mg/kg for cerium (25th in abundance of the 78 common elements) to 0.5 mg/kg for thulium and lutetium (Hedrick, 2001). The elemental forms of rare earths are iron gray to lustrous metals that are typically soft, malleable, ductile, and usually reactive, especially at elevated temperatures or when finely divided. The principal economic sources of rare earths are the minerals bastnasite, monazite and loparite, and the lateritic ion-adsorption clays. The rare earth elements are used in a wide variety of applications including glass polishing and ceramics; petroleum refining catalysts; automotive catalytic converters; metallurgical additives and alloys; rare earth phosphors for lighting, televisions, computer monitors, radar, and x-ray intensifying film; and permanent magnets (Hedrick, 2001).

Biological and toxicological interest in the rare earth elements have arisen from the common practice in mainland China to add their soluble salts, especially lanthanum and cerium salts, to fertilizers for the purpose of increasing crop yields (Guo, 1988). The rare earth elements have been shown to increase in crops the rates of photosynthetic light reactions, chlorophyll content, and transport of photosynthetic end products from leaves to seeds (Chen et al., 2001; Fashui et al., 2002). A review of the literature by He et al. (1999) indicates that appropriate supplementation of rare earth elements can increase the feed conversion and weight gain of beef cattle, sheep, pigs, rabbits, chickens, and ducks; the milk production of dairy cattle; the egg production of hens; and the output, survival rate, and feed conversion of grass carp and prawn. He and Rambeck (2000) suggested that rare earth elements may be useful as safe and inexpensive alternatives to antibiotics as growth promoters in animal feed. Horovitz (1993) has suggested that scandium and yttrium have beneficial effects in animals and humans. Cerium has been found to stimulate collagen and noncollagen protein synthesis in rat hearts (Prakash Kumar et al., 1995). Whether this stimulation is beneficial or not is uncertain because it has been speculated that this could lead to cardiac fibrosis (Prakash Kumar and Shivakumar, 1998).

Sources and Metabolism

Surprisingly little has been published about the concentration of rare earth elements in foods and feedstuffs considering their use to increase crop production in some countries, especially China. Iyengar (1998) indicated that the human daily dietary intake of cerium was less than 15 μg and scandium was less than 0.5 μg. Kavas-Ogly et al. (1998) reported that various Uzbek foods made mostly from grains contained 1.1 to 1.2 μg Sc/kg. The scandium concentration in 27 different Uzbek food dishes ranged from 0.8 to 5.9 μg/kg. The concentration of scandium in Brazilian diets was found to range between 1.0 and 1.8 μg/kg (Fávaro et al., 1997).

Very limited information is available on the metabolism of the rare earth elements. They apparently are poorly absorbed (less than 1 percent) and therefore can be used as nonabsorbable fecal markers (Fairweather-Tait et al., 1997). A study with mice indicated that absorbed rare earth elements are rapidly accumulated in teeth and bones (Zhang et al., 1988). After 73 days of feeding 0.83 μg Y + Yb/day, these rare earth elements ranged from nondetectable to traces in most other organs (Zhang et al., 1988). An exception was gallbladder, which contained 22.68 mg of Y + Yb/kg; this high concentration suggests that the bile is a significant excretory route for the rare earth elements.

Metabolic Interactions and Mechanisms of Toxicity

The ionic forms of rare earth elements share biologically important properties with divalent calcium. Because they have similar ionic radii, coordination chemistry, and affinity for oxygen donor groups, rare earth ions strongly interact with Ca^{2+}-binding sites on a wide range of proteins (Enyeart et al., 2002). Thus, the toxicological and beneficial effects of the rare earth elements probably result from them displacing or being a surrogate for calcium in various biological functions. For example, in the rat model of chronic renal failure, there is an association between lanthanum accumulation and mineralization defects characteristic of osteomalacia (Vanholder et al., 2002). Findings with plants suggest that Eu^{3+} can replace Ca^{2+} in the calcium/calmodulin-dependent phytochrome signal transduction system and promote plant development by enhancing the transport of calcium across the plasma membrane (Zeng et al., 2003).

Toxicosis and Maximum Tolerable Levels

The rare earth elements are relatively nontoxic to animals. When rats were administered daily oral doses of 0, 40, 200, and 1,000 mg $LaCl_3 \cdot 7H_2O$/kg BW for 28 days, only the 1,000 mg/day dose irritated the stomach mucosa and changed some liver enzymes suggestive of a hepatotoxic effect (Ogawa, 1992). When rats were gavaged with daily doses of 0, 40, 200, and 1,000 mg $EuCl_3 \cdot 6H_2O$/kg BW for 28 days, both the 200 and 1,000 mg/kg doses significantly decreased body weight gain because of a reduction in food consumption (Ogawa et al., 1995). However, hyperkeratosis of the forestomach and eosinocyte infiltration of the stomach mucosa occurred only in rats dosed with 1,000 mg/kg BW/day. Based on these findings, it was concluded that NOAEL for the europium salt was 200 mg/kg BW/day. Feeding a rare earth mixture containing mostly chlorides of lanthanum, cerium, and praseodymium such that the final dietary concentration of lanthanum was 36–43 mg/kg and cerium was 49–57 mg/kg had only beneficial effects in pigs (He and Rambeck, 2000). Considering that the mixture contained other rare earth

elements, these findings indicate that diets containing over 100 milligrams of rare earth elements per kilogram are safe. Subchronic and chronic toxicity studies showed no abnormal or specific pathological changes in monkeys dosed with 100 mg/kg BW or in rats dosed with 200 or 1,800 mg/kg BW of a mixture of rare earth nitrates (Ce, La, Nd, Pr, and Sm) (Ji and Cui, 1988) Rat fetuses did not show any teratogenicity when dams were fed up to 330 mg of the nitrate mixture/kg BW. The oral LD_{50} for the mixture ranged from 1,397 to 1,876 mg/kg BW for mice, rats, and guinea pigs.

Based on the limited toxicity studies done, the maximum tolerable level for the rare earth elements is high. Diets containing 100 mg/kg should be considered safe. Because feedstuffs apparently contain low amounts of the rare earth elements, they are of toxicological concern only when supplemented in excessive amounts.

Tissue Levels

The concentrations of most rare earths in animal and human tissue and fluids are quite low. Fujimori et al. (1996) found that the lanthanum, cerium, and neodymium concentrations in human serum were 360, 584, and 212 ng/L, respectively. The serum concentrations of praseodymium, samarium, gadolinium, dysprosium, europium, and ytterbium ranged from 34.6 to 53.2 ng/L, and the concentrations of europium, terbium, holium, thulium, and lutetium ranged between 4.6 and 10.5 ng/L. Katoh et al. (2002) found lanthanum and scandium arithmetic mean concentrations (µg/kg DW) were, respectively, in human heart, 32 and 5; kidney, 64 and 4; liver, 285 and 5; and muscle, 55 and 4. Beneš et al. (2000) found cerium and lanthanum geometric mean concentrations (µg/kg wet weight) were, respectively, in human kidney, 4 and 3; liver, 43 and 27; and bone, 49 and 27. The lanthanum concentration in human milk was found to range between < 0.05 to 3.7 µg/L (Krachler et al., 1998; Rossipal et al., 1998). After 73 days of feeding 0.83 µg Y + Yb/day to mice, Zhang et al. (1988) found the following concentrations of the combined elements (mg/kg wet weight): in mice liver, 0.229; kidney, 0.521; heart, 0.929; blood, 0.942; muscle, below detection limit; and bone, 2.464.

RUBIDIUM

Rubidium (Rb) is a soft, silvery-white alkali metal that can be liquid at room temperature, ignites spontaneously in air, and reacts violently with water (Wikipedia, 2004b). Rubidium is the 16th most abundant element in the Earth's crust (310 mg/kg) and is commercially obtained from the minerals lepidolite and pollucite. Rubidium compounds are used for chemical and electronic applications, and in fireworks for a purple color.

Biological interest in rubidium has resulted from findings suggesting that it is beneficial, or possibly essential, for higher animals. Compared to goats fed 1 or 10 mg Rb/kg diet, goats fed less than 0.28 mg Rb/kg diet exhibited decreased food intake, growth, milk production, and life expectancy, and increased spontaneous abortions (Anke et al., 1993). These rather general deprivation findings have not been confirmed in another research setting and do not indicate a possible biochemical function. Rubidium has a physiochemical relationship to potassium and thus may act beneficially through being a nutritional substitute for potassium. Evidence for this is that adding rubidium to potassium-deficient diets prevented the occurrence of characteristic lesions in kidneys and muscles of rats (Follis, 1943).

Sources and Metabolism

Very little reported rubidium analyses of animal feeds exist. Becker et al. (1975) found that rubidium concentrations in swine feeds, piglet starter rations, and various mixed feeds ranged from 2.6 to 26.1 mg/kg DW. The rubidium concentrations in most animal feedstuffs probably are in this range based on analysis of human foods that are similar to those fed to animals (Varo et al., 1980a,b; Anke and Angelow, 1995).

The absorption, distribution, and excretion of rubidium in animals are similar to potassium. Findings from studies using brush border membrane vesicles isolated from rabbit jejunum indicate that rubidium and potassium use the same transport system (Gunther and Wright, 1983). Thus, rubidium is rapidly and highly absorbed by mammals (Schäfer and Forth, 1983). Also, human studies indicate that rubidium is actively transported from the mother to fetus (Krachler et al., 1999). The major route of rubidium excretion is through urine, with a kidney clearance rate slightly less than potassium (Kunin et al., 1959). The intestine also is involved in rubidium excretion. Schäfer and Forth (1983) found that rubidium was excreted against a concentration gradient from blood into the lumen of both the small and large intestine of rats. Anke and Angelow (1995) reported that 30 percent of ingested rubidium is excreted through the feces and 70 percent through the urine by humans.

Metabolic Interactions and Mechanisms of Toxicity

In addition to absorption and excretion findings, a deprivation study also indicated that the major metabolic interaction involving rubidium is that with potassium. Yokoi et al. (1994) found that rats fed diets containing 0.54 mg Rb/kg, compared to those fed 8.12 mg Rb/kg, had increased potassium in the plasma, kidney, and tibia, but decreased potassium in testis. In addition, the rubidium deprivation decreased phosphorus in the heart and spleen, decreased calcium in the spleen, and increased magnesium in the tibia. These changes suggest that rubidium intake can affect phosphorus, calcium, and magnesium metabolism. Rubidium also might interact with selenium. It has been suggested that high dietary selenium may depress rubidium absorption because

rubidium concentrations decreased as selenium concentrations increased in serum, erythrocytes, and breast milk of lactating women in seleniferous areas of Venezuela (Negretti de Brätter et al., 2000).

The mechanism for rubidium toxicity most likely is the inefficient replacement of potassium in critical biochemical functions. For example, when injected in a dose that substitutes for more than 40 percent of intracellular potassium, rubidium may cause cardiac arrhythmia, generalized convulsions, and death (Meltzer, 1991). Because it mimics potassium, rubidium may be toxic through the same mechanisms as those for potassium toxicity.

Toxicosis and Maximum Tolerable Levels

The most significant oral toxicity study is that performed by Glendening et al. (1956). They found that rats fed diets containing 0.1 percent rubidium (1,000 mg/kg) had slightly depressed growth and gave birth to young that did not survive when fed the same diet. The growth of rats fed diets containing 0.2 percent rubidium (2,000 mg/kg) was severely depressed, and the rats did not reproduce. Other signs of toxicity with dietary rubidium at 0.1 percent and above were poor hair coat, sore noses, sensitivity, extreme nervousness leading to convulsions in advanced stages, and finally death. Purified diets containing 0.02 percent (200 mg/kg) rubidium were not toxic to rats. Rubidium chloride injected intraperitoneally induces irritability and aggressive behavior in rats (Stolk et al., 1971) and monkeys (Meltzer et al., 1969). The rubidium treatment increases the release and turnover of brain stem norepinephrine (Stolk et al., 1970).

The rat study of Glendening et al. (1956) indicates that the maximum tolerable level for rubidium is somewhere between 0.02 percent and 0.1 percent (200 and 1,000 mg/kg) of the diet and would be between 20 to 100 times greater than levels normally found in animals diets. Thus, rubidium is not a toxicological concern for animals. Toxicological intakes of rubidium would only occur with intentional dosing or supplementing with high amounts of rubidium salts.

Tissue Levels

Rubidium occurs in relatively high amounts in tissues and fluids. Ward and Abou-Shakra (1993) summarized the reported concentrations of rubidium in human serum and plasma; the means ranged from 150 to 265 µg/L. They also analyzed normal human tissues and found the following mean rubidium concentrations (mg/kg WW): liver, 8; kidney, 6.5; and bones, 1. Yokoi et al. (1994) found that dietary rubidium affected tissue and fluid rubidium concentrations in rats. They found the following rubidium concentrations (mg/kg WW) in rats fed 0.54 and 8.12 mg Rb/kg diet, respectively: heart, 0.74 and 9.83; liver, 1.49 and 20.5; kidney, 1.09 and 13.8; muscle, 1.18 and 15.0; tibia, 0.97 and 4.27; and blood (mg/L), 0.46 and 5.5. Anke and Angelow (1995) found the following mean concentrations of rubidium (µg/g DW) in liver and kidney, respectively, for cattle, 36 and 31.8; sheep, 33.6 and 40.3; pigs, 13.6 and 14.3; and cats, 16.4 and 17. Toxic intakes of rubidium markedly increase the rubidium concentration in tissues. Glendening et al. (1956) found that 0.25 percent (2,500 mg/kg) compared to less than 0.02 percent (200 mg/kg) dietary rubidium increased rubidium concentrations from 100 to 200 mg/kg DW to 8,000 to 17,000 mg/kg DW in liver, heart, muscle, and kidney of rats.

SILVER

Silver (Ag) is a white lustrous metal whose concentration in the Earth's crust is about 0.1 mg/kg. Silver of commercial value is primarily a by-product of the mining of nonferrous base metals such as copper, lead, and zinc. Major uses of silver are in the manufacture of tableware, jewelry, decorative items, and coinage. Industrial and technical uses include photographic materials, electrical and electronic products, catalysts, brazing alloys, dental amalgam, and bearings (Hilliard, 2003). Use in coinage and photographic materials has declined markedly recently.

No essential metabolic function for silver has been identified in animals. However, Brauner and Wood (2002) found that 1 compared to 0.1 µg Ag/L as silver nitrate in moderately hard water increased the rate of growth and ionoregulatory development in rainbow trout. Biological and toxicological interest in silver mainly arose from findings showing that it affects vitamin E, selenium, and copper metabolism and has possible use as a drinking water disinfection agent. Recently there has been a revival in the promotion of colloidal silver supplements for the prevention and treatment of many human diseases.

Sources and Metabolism

There are no reports showing the silver concentrations in common feedstuffs. Bowen (1966) indicated that land plants in general have a mean concentration of 60 µg Ag/kg. Thus, diets apparently are not a significant source of silver for animals or humans.

Studies on the metabolism of ingested silver are limited. Scott and Hamilton (1950) found that a radiotracer dose of silver administered by stomach tube was 99 percent excreted in the feces by rats at 4 days post-dosing. Intramuscularly and intravenously administered radiosilver were also eliminated via the feces. Recent findings show that silver is excreted in the bile and indicate that silver and copper share a common transport system for their hepatobiliary removal (Dijkstra et al., 1996). A study of the distribution of silver in mice that were provided drinking water containing 0.03 mg Ag/L as radiolabeled silver nitrate for one to two weeks found that the highest concentrations of the radiolabel occurred in musculus soleus, cerebellum, spleen, duodenum,

and myocardial muscle (in rank order) (Pelkonen et al., 2003).

Metabolic Interactions and Mechanisms of Toxicity

In the 1950s, silver, provided as silver nitrate in drinking water (Shaver and Mason, 1951) of rats or as silver acetate (20 mg Ag/kg) in the diet of chicks (Dam et al., 1958), was found to be more toxic to vitamin E-deficient than vitamin E-adequate animals. These observations led to studies showing an antagonistic relationship between silver and selenium. Diplock et al. (1967) found that providing vitamin E-deficient rats with drinking water containing 0.15 percent silver acetate resulted in liver necrosis and mortality that could be totally prevented by tocopherols, partially prevented by adding 1 mg Se/kg diet, and only marginally alleviated by an additional 0.15 percent dietary methionine. Bunyan et al. (1968) found that a diet containing lard and combined with drinking water containing 0.15 percent silver as silver acetate resulted in green exudates in chicks that were prevented by either vitamin E or selenium. Peterson and Jensen (1975a) found that 1 mg Se/kg diet or 100 IU vitamin E/kg diet prevented depressed growth and mortality in chicks caused by 900 mg Ag/kg diet as silver nitrate. Adding 0.15 percent cystine to the diet stimulated growth but did not prevent the mortality, largely from exudative diathesis. Jensen (1975) found that 1,000 mg Ag/kg diet as silver nitrate prevented growth depression and mortality caused by 80 mg Se/kg diet. Radiotracer studies and biochemical changes indicate that silver interferes with the absorption of selenium. Feeding 250 mg Ag/kg diet as silver acetate decreased blood selenium and glutathione peroxidase activity in rats (Yoshida, 1993); silver also increased serum thyroxine concentration.

Hill et al. (1964) reported that 25 mg Cu/kg diet were totally effective in preventing mortality and depressed growth, hemoglobin, and aorta elastin content caused by feeding 200 mg Ag/kg diet to chicks.

Peterson and Jensen (1975b) observed that increased heart weight, mortality, and decreased aorta elastin induced by 900 mg Ag/kg practical diet was effectively prevented by a supplement of 50 mg Cu/kg; depressed growth was only partially corrected. In turkeys, 900 mg Ag/kg diet depressed growth, reduced packed cell volume, increased heart size, and caused gizzard musculature degeneration. The gizzard degeneration was completely prevented by the addition of 1 mg Se/kg diet and partially prevented by the addition of 50 IU vitamin E/kg diet (Jensen et al., 1974). The microcytic, hyperchromic anemia was prevented by the addition of 50 mg Cu but not 5 mg Cu per kg diet; both copper treatments reduced heart size.

Silver apparently antagonizes copper metabolism by interfering with the copper-transporting and oxidase functions of ceruloplasmin. Developmental abnormalities and prenatal deaths caused by feeding silver chloride to rat dams throughout term were prevented by injections of ceruloplasmin (Shavlovski et al., 1995). Findings have been obtained that indicate silver competes with copper for incorporation into the site of apo-ceruloplasmin that results in the active holo-ceruloplasmin (Hirasawa et al., 1994, 1997; Sugawara and Sugawara, 2000).

The antagonistic relationships between silver and selenium and between silver and copper indicate that the mechanism through which silver has toxic effects in animals is by interfering with copper and selenium uptake and function. Also, because silver is easily reduced, it could initiate peroxidation that requires higher intakes of nutrients, such as copper, selenium, and vitamin E, involved in protection from reactive oxygen species (Whanger and Weswig, 1978). The manifestations of acute silver toxicity in fish are the result of the failure to maintain constant concentrations of sodium and chloride ions in blood plasma (Hogstrand and Wood, 1998). For freshwater fish, this apparently occurs by ionic silver preventing active Na^+ and Cl^- uptake by inhibiting gill basolateral Na^+, K^+-ATPase activity (Wood et al., 1999). In marine fish, the intestine appears to be the primary toxic site of action.

Toxicosis and Maximum Tolerable Levels

Feeding a diet containing 100 mg Ag/kg as silver sulfate to one-day-old chicks did not adversely affect growth, mortality, hemoglobin concentration, and elastin content of the aorta (Hill et al., 1964). Higher dietary concentrations of silver induced signs associated with copper and selenium deficiency including depressed growth, hemoglobin and aortic elastin, increased mortality and heart weight, and exudative diathesis in chicks (Hill et al., 1964; Bunyan et al., 1968; Petersen and Jensen, 1975a,b). In turkeys, 300 mg Ag/kg diet depressed growth, and 900 mg Ag/kg diet induced gizzard musculature dystrophy, enlarged hearts, and decreased packed red blood cell volume, in addition to depressed growth (Jensen et al., 1974). In growing rats, signs of silver toxicity included depressed growth, increased mortality, liver necrosis, and a generalized deposition of silver in tissues (argyrosis) (Shaver and Mason, 1951; Diplock et al., 1967). A lifetime administration of 1,000 mg Ag/L of drinking water produced an intense pigmentation of many tissues in rats (Olcott, 1948, 1950). The argyrosis was most marked in the basement membrane of the glomeruli, the portal vein, and other parts of the liver; the choroid plexus of the brain; the choroid layer of the eye; and in the thyroid gland. Argyria in people caused by the use of colloidal silver still occurs (Gulbranson et al., 2000).

Rainbow trout fed 3.1 mg/kg diet as biologically incorporated silver in trout meal showed no adverse effects when compared to trout fed 0.05 mg Ag/kg diet (Galvez et al., 2001). Ionic silver in water is highly toxic to fish, with freshwater fish more sensitive to the silver ion than marine fish (Hogstrand and Wood, 1998). The 96-hour LC_{50} concentration for rainbow trout in synthetic soft water was determined

to be 13.3 µg total Ag/L and 3.3 µg dissolved Ag/L (Morgan and Wood, 2004).

Silver is a relatively nontoxic element when ingested with a diet that contains rich amounts of copper, selenium, and vitamin E. Because no adverse effects were seen in chicks fed 100 mg Ag/kg diet, the maximum tolerable level for domestic animals probably is about this amount. The World Health Organization allows silver ions up to 0.1 mg/L in drinking water for disinfection. A maximum tolerable limit for fish is probably higher than 3 mg Ag/kg diet based on the study of Galvez et al. (2001) and about 1 µg ionic Ag/L in water (Brauner and Wood, 2002).

Tissue Levels

Only limited information is available about silver concentrations in tissues and fluids of domestic animals. However, they are probably very low under normal conditions. Cow's milk was found to contain 27 to 54 µg Ag/L (Murthy and Rhea, 1968). Hamilton et al. (1972/1973) found the following mean silver concentrations (mg/kg fresh weight) in human tissues: kidneys, 0.002; liver, 0.006; muscle, 0.002; and whole blood, 0.008. Versieck and Cornelis (1980) found that reported values for the silver concentrations in plasma and serum varied widely from 0.68 to 113 µg/L. They concluded, however, that the normal concentration is less than 1 µg/L.

STRONTIUM

Strontium (Sr) is a soft silver-white or yellowish alkaline earth metal whose concentration in the Earth's crust averages 0.04 percent, which makes it the 15th element in abundance (Ober, 2002). Strontium is highly reactive and turns yellow with the formation of oxide when exposed to air. Strontium is recovered from strontianite ($SrCO_3$) and celestite ($SrSO_4$), its two principal naturally occurring mineral forms. Strontium is used mainly in color television picture tube faceplates; other important uses for strontium are ferrite ceramic magnets and pyrotechnics (Ober, 2002).

Strontium has not been shown to be essential for either plants or animals. Rygh (1949) reported that strontium deprivation depressed growth, impaired the calcification of bones and teeth, and increased dental caries incidence in rats and guinea pigs, but these findings have not been confirmed. Biological interest in strontium was small until it was established that ^{90}Sr, a by-product of nuclear fission and a beta-emitter with a long half-life (28.78 years), can substitute for calcium in bone. This substitution occurs because strontium naturally exists as a divalent cation and has an atomic radius similar to calcium. Recently, interest in strontium has focused on its ability, especially in the form strontium ranelate (a compound containing the organic acid, ranelic acid, and two atoms of strontium), to increase bone formation and uncouple bone formation from bone resorption (Marie et al., 1993, 2001). Strontium ranelate is a promising pharmaceutical for the treatment of postmenopausal osteoporosis (Meunier et al., 2002).

Sources and Metabolism

In general, foods and feedstuffs of plant origin are appreciably richer sources of strontium than are animal products, except where the latter include bone. Strontium tends to be concentrated in the bran rather than in the endosperm of grains. The strontium content is higher in leafy dicotyledons than in monocotyledons; thus, for animals, strontium intakes would be much higher from leguminous than from gramineous forages. Mitchell (1957) reported that the strontium concentration (mg/kg DW) in red clover growing on different soils ranged from 53 to 115 (mean 74) and ryegrass ranged from 5 to 18 (mean of 10). Schroeder et al. (1972) determined the strontium concentrations (mg/kg) in several items that are used as animal feeds; these included wheat, 3.46; oats, 3.01; millet, 1.29; buckwheat, 3.84; barley, 0.98; and hay, 9.4. As reviewed by Nielsen (1986) and Anke et al. (2000b), the human daily strontium intake from food is usually no more than a few milligrams. This intake is consistent with the strontium analyses of foods performed by Schroeder et al. (1972), who found most foods contained between 0.5 and 5.0 mg Sr/kg. Water may significantly add to the intake of strontium. Schroeder et al. (1972) estimated that water provides 10 percent of the daily intake of strontium for humans. A review by Wasserman et al. (1977) indicated that most drinking water contains less than 1 mg Sr/L, but higher concentrations (e.g., 1.6 mg/L) have been found in some waters (Wolf et al., 1973).

The metabolism of strontium is similar to calcium. Strontium and calcium use the same mechanisms for absorption from the gastrointestinal tract, concentrate in bone, and are excreted mainly in the urine after absorption. Strontium can substitute for calcium in a variety of physiological processes including blood clotting, bone formation, and muscular contraction. However, these processes are slowed when strontium is substituted for calcium. Also, wherever there is a metabolically controlled passage of ions across a membrane (e.g., gastrointestinal absorption, renal excretion, lactation, and placental transfer), calcium is transported more effectively than strontium (Comar and Wasserman, 1964). This discrimination between calcium and strontium apparently develops as an organism matures. Mechanisms discriminating against the uptake of strontium in favor of calcium develop gradually in rats (Sugihira et al., 1990), and there is a discrimination against strontium in favor of calcium in the tubular reabsorption of these elements that is not fully developed in young rats before weaning (Sugihira and Suzuki, 1991).

Seifert et al. (2002) found an apparent strontium absorption of 20 percent from a mixed diet by humans. This apparent absorption is similar to the 20 to 25 percent range found by Spencer et al. (1960) using a tracer dose of ^{85}Sr. A vegetarian

diet will markedly reduce the apparent absorption of strontium (Anke et al., 2000b). Although absorbed strontium is excreted via the kidney, some apparently is excreted in the bile. Schäfer and Forth (1983) found that strontium is excreted against a considerable concentration gradient from blood into bile. Sweat also may be a significant route of excretion of strontium (Consolazio et al., 1964).

Strontium is poorly retained by humans. In adults, net retention is essentially zero, or a steady state exists (Wasserman et al., 1977). The small amount of strontium retained by the body is incorporated in bones and teeth. Strontium incorporation occurs mainly by exchange with calcium on bone crystal surfaces and is dependent upon the amount ingested (Dahl et al., 2001). However, findings with rats, monkeys, and humans indicate that the incorporation of strontium in bone reaches a plateau during repeated oral administration of high amounts as strontium ranelatae or strontium chloride. Stopping the strontium administration results in a rapid decrease in bone strontium content because that on the crystal surface is rapidly eliminated (Dahl et al., 2001). In new bone, only a few strontium atoms may be incorporated into the crystal by ionic substitution for calcium (Dahl et al., 2001).

Metabolic Interactions and Mechanisms of Toxicity

Strontium has a low order of toxicity; it is less toxic than calcium. Ineffectively substituting for calcium in physiological processes apparently is the mechanism of strontium toxicity. Thus, actions that would promote this substitution would exacerbate, whereas those that would inhibit the substitution would alleviate, strontium toxicity. For example, when young or growing animals are fed high dietary strontium in combination with low dietary calcium, they develop a condition known as "strontium rickets" (Bartley and Reber, 1961; Colvin and Creger, 1967; Colvin et al., 1972). Ethanol ingestion decreases bone strontium content and increases the urinary excretion of strontium (González-Reimers et al., 1999), whereas low dietary protein increases bone strontium content (González-Riemers et al., 2002). Strontium absorption is decreased by dietary fiber (Momčilovič and Gruden, 1981) and vitamin D deprivation (Moon, 1994). There is evidence for an antagonism between strontium and fluoride (Liu and Min, 1999).

Toxicosis and Maximum Tolerable Levels

Extremely high oral doses of strontium relative to normal intakes are needed to elicit toxic effects in animals and humans. Dosing adult monkeys with 100, 275, and 750 mg strontium ranelate/day for six months had no deleterious effects on bone formation (Buehler et al., 2001); it did decrease indices of bone resorption, which was considered advantageous in regards to being therapeutic for osteoporosis. Findings from a study in which osteoporotic women were supplemented with 0.5, 1, and 2 g of strontium ranelate/day for 2 years indicated all doses were well tolerated (Meuneir et al., 2002). The 2 g/d dose was considered to offer the best combination of safety and efficacy against osteoporosis. Rats given drinking water containing 0.19 percent $SrCl_2$ exhibited no adverse effects (Grynpas and Marie, 1990). However, 0.40 percent $SrCl_2$ in drinking water induced bone hypomineralization and inferior bone apatite crystals. Generally, toxic effects on bone cell or mineralization are not seen in rats and mice fed diets containing less than 1 percent (10,000 mg/kg) strontium (Marie et al., 2001). Based on calculations from available literature, Marie et al. (2001) considered 350 mg/kg/day a low nontoxic dose of strontium. Knight et al. (1967) found that feeding diets containing 2,000 mg Sr/kg for 100 days had no adverse effect on growth or feed efficiency of growing beef cattle.

As indicated above, excessive strontium disturbs calcium metabolism. Thus, the intake of strontium that induces signs of toxicity is dependent upon calcium intake. In experiments where pigs aged 3 weeks were fed diets containing 6,700 mg Sr/kg for 5 weeks, only mild effects of weakness and incoordination were seen when the diet contained 0.89 percent calcium; when the diet contained only 0.16 percent calcium, severe incoordination and weakness that occurred by the second week progressed to complete paralysis by the end of the third week (Bartley and Reber, 1961). Weber et al. (1968) fed diets containing either 3,000 or 6,000 mg Sr/kg diet and either 0.72 percent or 1 percent calcium to chicks for 4 weeks. No adverse effects were seen in chicks fed the diet containing 3,000 mg Sr/kg. Feeding 6,000 mg Sr/kg diet depressed growth and calcium retention more markedly in chicks fed the lower calcium diet. Hens fed up to 30,000 mg Sr/kg diet containing 2.9 percent calcium for 4 weeks exhibited no adverse effects, but 50,000 mg Sr/kg diet reduced egg weight and production and feed consumption (Doberenz et al., 1969). In a study to determine the distinction between pharmacological and toxic doses of strontium, rats were fed diets containing 0, 0.05 percent, 0.10 percent, and 0.50 percent strontium (about 0, 7.67, 15.3, and 76.67 mg Sr/day). All calcium metabolic variables determined, including decreased calcium in bone and serum, were markedly depressed by the 0.5 percent (5,000 mg/kg) strontium diet (Morohashi et al., 1994). The calcium content of bone was increased by the 0.05 percent (500 mg/kg) strontium diet. The study indicated that diets containing less than 0.1 percent strontium (providing about 15.3 mg Sr/day) do not have a toxic effect on calcium metabolism in rats.

As indicated above, the maximum tolerable level for strontium is affected by the intake of calcium. When dietary calcium is adequate, animals have a high tolerance for strontium. Mature animals can tolerate higher levels than young. The findings above indicate that cattle and swine can tolerate 2,000 mg Sr/kg diet (0.2 percent), chicks can tolerate 3,000 mg Sr/kg (0.3 percent), and hens can tolerate 30,000 mg Sr/kg diet (3.0 percent) when dietary calcium is adequate.

These amounts are 100 to 1,000 times greater than those normally found in animal diets. Thus, strontium is not a toxicological concern for animals. Toxicological intakes of strontium by animals and humans would only occur with intentional dosing or supplementing with high amounts of strontium salts.

Tissue Levels

More than 70 years ago, Gerlach and Muller (1934) reported that the strontium concentrations in a wide variety of animal tissues ranged from 0.01 to 0.10 mg/kg, with no evidence of accumulation in any particular species, soft organ, or tissue. Forty years later, Hamilton et al. (1972/1973) found similar concentrations in human tissues; values included (in mg/kg fresh tissue) kidney, 0.1 ± 0.02; liver, 0.1 ± 0.03; and muscle, 0.05 ± 0.02. A biology data book (Altman and Dittmer, 1973) has indicated that the concentration of strontium in serum is 0.057 mg/L and that in blood is 0.033 mg/L.

TITANIUM

Titanium (Ti) is a dark, gray metal whose concentration in the Earth's crust is estimated to be 0.43 percent. Titanium forms compounds in which it has an oxidation state of +2, +3, or +4. Titanium is used to make aluminum, tin, and vanadium alloys, and ferrotitanium for the steel industry. Titanium oxide is used as a white pigment for paints. Titanium compounds are constituents of glass and ceramics and used as a mordant for the dyeing industry. Titanium metal is used to make dental and orthopedic implants. Titanium dioxide is used as a color additive in human and pet foods.

No essential metabolic function in animals has been identified for titanium. However, some biological interest has been recently promoted by findings showing some physiologically soluble titanium compounds have beneficial actions in animals and plants. Pais et al. (1977) synthesized water-soluble Ti IV ascorbate (Ti-ascorbate, trade name TITAVIT) by combining ascorbic acid with $TiCl_4$ in the presence of gaseous HCl. Ti-ascorbate is stable up to pH 8 and apparently is not toxic to living systems. Pais (1983) found that Ti-ascorbate increased the yield of various crops. The beneficial action is hypothesized to be the result of titanium promoting better utilization of magnesium and iron in photosynthetic activity (Carvajal and Alcaraz, 1998). Ti-ascorbate supplementataion (30–60 mg Ti/kg diet) improved reproduction in pigs (Pais et al., 1989) and increased egg production, weight, and shell hardness of laying hens (Halmágyi-Valter et al., 1988). Schroeder et al. (1963) found that potassium titanium oxalate increased growth of mice. Yaghoubi et al. (2000) also reported that the titanyl ion (TiO^{2+}) in the form of titanyl oxalate enhanced the growth of mice. It was hypothesized that titanium was acting as an antibacterial and/or antiviral agent (Yaghoubi et al., 2000; Schwietert et al., 2001).

Sources and Metabolism

Most titanium consumed by domestic animals probably comes from soil contamination; soil concentrations are about 10,000 times greater than those in uncontaminated herbage (Swaine, 1955). The titanium concentrations in a variety of plants were determined by Bertrand and Varonca-Spirt (1929a,b). The concentrations ranged from 0.1 to 5 mg/kg DW with a majority near 1 mg/kg. Mitchell (1957) found similar concentrations in red clover (mean 1.8 and range 0.7–3.8 mg/kg DW) and in ryegrass (mean 2 and range 0.9–4.6 mg/kg DW) grown on different soils. A brief summary of titanium concentrations of human foods indicates that they range from 0.2 to 6 mg/kg DW (Nielsen, 1986); exceptions are taro (80 mg/kg) and yam (15 mg/kg). English total diets were found to provide about 800 µg/day (Hamilton and Minski, 1972/1973).

Very little is known about the metabolism of titanium. It is generally believed that most titanium, especially that from soil contamination, is poorly absorbed. Titanium that is absorbed is not extensively retained by either plants or animals. However, if dietary titanium is in a soluble form in the gastrointestinal tract, it apparently is absorbed to some extent. Evidence for this is that Tipton et al. (1966) found about equal amounts of titanium in feces and urine of individuals consuming an average 0.37 and 0.41 mg titanium per day for a month in their diet. Also, after the administration of titanyl ion (TiO^{2+}), the ion is found in breast milk (Schwietert et al., 2001).

Metabolic Interactions and Mechanisms of Toxicity

It is questionable whether a specific toxicity of titanium has been demonstrated. Thus, no mechanism of toxicity can be described. As mentioned above, titanium is thought to have beneficial effects in plants through promoting magnesium and iron metabolism.

Toxicosis and Maximum Tolerable Levels

Titanium is essentially nontoxic in the amounts and forms that usually are ingested. Thus, no specific oral toxicity of titanium has been described and a maximum tolerable limit cannot be suggested for any domestic animal.

Tissue Levels

The reported concentrations of human and animal tissues are variable, with high amounts commonly found in lungs, probably as a result of dust inhalation. Most reported values for human tissues are between 0.2 and 1.4 mg/kg fresh tissue. Hamilton et al. (1972/1973) reported the following mean values in mg/kg fresh tissue for human organs: muscle, 0.2 ± 0.01; kidney cortex, 1.3 ± 0.2; kidney medulla, 1.2 ± 0.2; liver, 1.3 ± 0.2; brain, 0.8 ± 0.05; and lung, 3.7 ± 0.9.

Schroeder et al. (1964) found that the titanium concentrations in a variety of mouse organs were increased from 0.13–1.10 to 1.66–8.80 mg/kg fresh tissue when dietary (water and food) titanium was increased from 0.03 to 5.03 mg/kg.

TUNGSTEN

Tungsten (W) is a whitish-gray metal used in a wide variety of commercial, industrial, and military applications (Shedd, 2002). The largest use is as tungsten carbide in cemented carbides, which are wear-resistant materials used by the metalworking, mining, and construction industries. Tungsten alloys are used to make armaments, tool steels, and wear-resistant materials and coatings, and as a substitute for lead in bullets and shot. Tungsten metal wires, electrodes, and contacts are used in lighting, electronic, electrical, heating, and welding applications. Tungsten chemicals are used to make catalysts, inorganic pigments, high-temperature lubricants, and semiconductors. Tungsten is one of the rarer elements in the Earth's crust; it occurs in concentrations that average 5 mg/kg (Standen, 1970).

Biological interest in tungsten developed when De Renzo (1954) found that it was antagonistic to molybdenum metabolism. Recently, biological interest has centered on its enzymatic functions and pharmacological antidiabetic action. More than a dozen tungstoenzymes in three functionally and phylogenetically distinct families have been identified and characterized in lower forms of life (Roy and Adams, 2002). All known tungstoenzymes catalyze reactions involving oxygen atom transfer and coupled electron proton transfer, similar to reactions catalyzed by molybdoenzymes (Roy and Adams, 2002). Both sodium tungstate (Barbera et al., 2001) and ammonium paratungstate (Palanivel et al., 2001) have antidiabetic action in streptozotocin-induced diabetes in rats. However, sodium tungstate only transiently alleviated the streptozotocin-nicotinamide rat model of diabetes that shares several features with human type 2 diabetes (Fierabracci et al., 2002). Although attempts have been made, no findings have been obtained that suggest tungsten is essential for animals and humans. After comparing intakes of 60 vs. 1,000 μg W/kg diet in six experiments with goats, Anke et al. (1983a) stated that their findings indicated that tungsten is either not essential for or that 60 μg W/kg diet meets the requirement of higher animals.

Sources and Metabolism

Anke et al. (1983b) found that normal feedstuffs of ruminants contained average tungsten concentrations between 118 and 478 μg/kg dry weight.

The metabolism of tungsten has been recently reviewed by Lagarde and Leroy (2002). Their review disclosed that a relatively high percentage of tungsten is rapidly absorbed after being ingested by a variety of animals. Beagle dogs absorbed 57–74 percent of tungsten from a solution containing 25 or 50 mg sodium tungstate/kg; absorption was 25 percent from a weakly acidic aqueous solution of tungstic oxide. Rats gave similar results, absorbing 40–92 percent of tungsten administered as tungstate, but only 1 percent as tungstic acid. Dairy cows absorbed 25 percent of a dose of ^{181}W-labeled tungstate. Swine absorb at least 75 percent and sheep absorb 44–65 percent of orally administered tungsten as tungstate. Urine apparently is the major excretory pathway for absorbed tungsten. The major site of long-term retention of tungsten is bone, with retention greater in growing rather than mature bone. Generally, kidney and liver are the soft tissues that are highest in tungsten. Lagarde and Leroy (2002) suggested that the liver retention of tungsten may be explained by its ability to replace molybdenum in molybdoenzymes.

Metabolic Interactions and Mechanisms of Toxicity

Tungsten and molybdenum have almost identical atomic and ionic radii and similar chemical properties. Thus, it is not surprising that studies with chickens, rats, goats, and cows have shown that tungsten is an antagonist of molybdenum (Lagarde and Leroy, 2002). High dietary tungsten decreased the activity of sulfite and xanthine oxidase (two liver molybdenum enzymes) and the concentration of molybdenum in liver. Lagarde and Leroy (2002) suggested that the most likely mode of action of tungsten was that it replaced molybdenum in enzymes. However, they indicated other modes of action were possible. These actions included tungsten preventing the incorporation of molybdenum in enzymes without itself being incorporated, and inhibiting the transport of sulfate, molybdate, sulfite, and thiosulfate in the gut. Like molybdenum, tungsten can replace phosphate in bone and, in rats, induce signs of copper deficiency, including causing a progressive decline in ceruloplasmin oxidase activity (Lagarde and Leroy, 2002).

Toxicosis and Maximum Tolerable Levels

Toxicological studies on laboratory animals indicate that tungsten has a relatively low order of toxicity. Schroeder and Mitchener (1975) did not observe any adverse effects in rats given drinking water containing 5 mg W/L as sodium tungstate for a lifetime. Kinard and Van de Erve (1941) found that 1,000 mg W/kg diet as tungstic oxide, sodium tungstate, and ammonium paratungstate fed to rats for 70 days slightly depressed growth. However, much higher doses (5,000 mg W/kg diet or greater for tungstic oxide and sodium tungstate, 20,000–50,000 mg W/kg diet for ammonium paratungstate) produced extensive mortality. Karantassis (1924) found that the acute lethal oral dose for guinea pigs was 550 mg tungstate; the guinea pigs exhibited anorexia, colic, disorganized movements, trembling, and dyspnea.

Toxicological studies of tungsten in domestic animals are few. Owen and Proudfoot (1968) found that 37.5 mg/kg BW administered in 2 or 3 separate doses as sodium tungstate

over a period of 3 weeks had no adverse effects on milk production but markedly decreased milk xanthine oxidase activity. Similar effects were found with goats administered 56 mg tungsten as 2 separate doses of sodium tungstate. Higgens et al. (1956a,b) found that feeding 45 and 94 mg W/kg diet (low molybdenum) as sodium tungstate for 35 days decreased the growth rate and increased the death rate in 1-day-old chicks. These adverse effects of tungsten were completely reversed by increasing the molybdenum content of the diet. Older chickens may be less sensitive to tungsten toxicity. Feeding 250 or 500 mg W/kg diet as sodium tungstate for 30 days had no adverse effects on egg production and hatchability of breeder chickens, but 500 mg W/kg diet did decrease xanthine oxidase activity in tissues (Teekell and Watts, 1959). The form of tungsten also affects its toxicity. Mitchell et al. (2001a,b) found that eight #4 tungsten-iron shot, or #4 tungsten-polymer shot administered orally on days 0, 30, 60, 90, and 120 of a 150-day trial had no adverse effects on mortality, body weights, organ weights, liver and kidney histology, or reproduction.

The chicken studies of Higgens et al. (1956a,b) indicate that maximum tolerable level of tungsten is quite dependent upon the molybdenum content of the diet. However, the lifetime rat study of Schroeder and Mitchener (1975) suggests that 5 mg W/L of water is a tolerable intake. If one accepts that a slight decrease in tissue and milk xanthine oxidase is not of toxicological concern, the recommendation in the previous edition of this document (NRC, 1980b) of 20 mg/kg diet may be appropriate.

Tissue Levels

There is a lack of information about the normal amount of tungsten in animal tissue. Kinard and Aull (1944) found that after 100 days of consuming a diet containing 1,000 mg W/kg as tungstic oxide, sodium tungstate, or ammonium paratungstate, appreciable amounts of tungsten accumulated in the bone, skin, and spleen (20–120 mg/kg fresh weight). All other tissues contained trace quantities (i.e., less than 10 mg/kg).

URANIUM

Metallic uranium (U) is a silver-white, lustrous, dense, and weakly radioactive material. The average concentration of uranium in the Earth's crust is about 3 mg/kg (Anke et al., 2000a). Uranium does not occur in concentrated deposits, and much of the ore from which it is extracted contains less than 0.1 percent uranium. Uranium occurs in both North Carolina and Florida marine sedimentary phosphate minerals and in igneous phosphate minerals from western U.S. states in concentrations up to 250 mg/kg. Depleted uranium is a by-product of the ^{235}U radionuclide enrichment process in making fuel for nuclear reactors. Depleted uranium contains approximately 40 percent of the radioactivity and retains all the chemical properties of natural uranium (Sztajnkrycer and Otten, 2004). Depleted uranium has military uses (WHO, 2003) because its high density, hardness, and pyrophoric properties (it ignites on impact if the temperature exceeds 600°C) make it superior to classical tungsten in armor-piercing munitions. Because of its high density, depleted uranium is used as counterweights in aircraft and in radiation shielding applications. Military use of depleted uranium has recently become of biological concern because of possible hazards arising from its radioactivity and chemical toxicity. There is no evidence that uranium has an essential or a beneficial metabolic function in animals.

Sources and Metabolism

Very little has been reported about the uranium content of animal feedstuffs and diets. Bowen (1966) indicated that most plants contain less than 0.04 mg/kg. The low uranium content in plants used as animal feeds was confirmed by Anke et al. (2000a) who found concentrations between 0.8 and 15 mg U/kg dry weight in lucerne, wheat, rape, red clover and white clover, and means of 0.58 and 0.71 mg/kg dry weight in wheat grain and rape seed, respectively. The uranium concentration in corresponding plant material from a uranium mining area was several times higher and the uranium concentration in plants decreased as they matured. Because of the high concentrations found in some phosphate deposits, commercial feed grade phosphate supplements may be a major contributor to the uranium intake by domestic animals. The uranium concentrations in commercial feed grade phosphates containing about 18 percent phosphorus range from 70 to 180 mg/kg (Reid and Sackett, 1977). It was calculated that such supplements would provide 0.7 to 1.8 mg U/kg diet for farm livestock (NRC, 1980c). Drinking water also may be a major source of uranium for domestic animals. One study of drinking water from drilled wells found a median concentration of 28µg U/L with an interquartile range of 6–135 µg and a maximum concentration of 1,920 µg/L (Kurttio et al., 2002). However, Anke et al. (2000a) found that drinking water in Germany ranged from 0.28 to 2.4 µg/L; some mineral waters were high in uranium, up to 24.5 µg/L. The higher concentrations in drinking water indicate that higher intakes are possible for adult humans than the average intake of 500 µg/year (less than 1.5 µg/day) through the ingestion of food and water estimated by the World Health Organization (2003). Anke et al. (2000a) reported that the average intake of people in Germany was 2.7 µg/day.

Typical gut absorption by humans of uranium from food and water is about 2 percent for soluble and about 0.2 percent for insoluble uranium compounds. After reviewing and analyzing the literature, Wrenn et al. (1985) concluded that the average gastrointestinal absorption of ingested uranium is 1–2 percent, and reasonably independent of age and amount of uranium ingested. Anke et al. (2000) provided

evidence suggesting that absorption of uranium from the diet was about 6 percent. Absorbed uranium is excreted mainly in the urine. Uranium does not accumulate to any extent in the body. The small amount retained is found mostly in the skeleton, liver, and kidneys (WHO, 2003).

Metabolic Interactions and Mechanisms of Toxicity

Oral administration of a biphosphonate (ethane-1-hydroxy-1,1-biphosphonate, EHBP) reduced the lethality and kidney histopathology induced by a single orally administered toxic dose of uranyl nitrate (350 mg/kg BW) (Martinez et al., 2000). There is some evidence suggesting that inorganic mercury can enhance the nephrotoxicity of uranium (Sanchez et al., 2001).

By the late 1950s the mechanism of uranium toxicity in the kidney was thought to be the binding of uranium to the luminal membranes of renal tubular cells, which interfered with the reabsorption of critical substances for cell respiration and resulted in slow cell death (Leggett, 1989). Bone formation depression caused by uranium toxicity was also suggested to be caused by the binding of uranium to cell membranes resulting in damage to osteoblasts (Ubios et al., 1991).

Toxicosis and Maximum Tolerable Levels

Early studies of uranium toxicity suggested that it is not very toxic. Mice fed uranyl nitrate hexahydrate in amounts ranging from 2 to 2,370 mg/kg diet for 48 weeks exhibited no signs of toxicity except for a decrease in growth at the highest concentration (Tannenbaum and Silverstone, 1944). When mice were fed 1 percent uranyl nitrate hexahydrate (4,740 mg/kg) in the diet, after a few days they ate less food, failed to grow or lost weight, were cold to the touch, and had ruffled fur and arched backs. When necropsied during the second or third week, the kidneys were enlarged, pink-gray, and exhibited an acute necrotizing nephrosis (Tannenbaum and Silverstone, 1944). Some animals died but those that survived recovered with normal or near-normal growth rates and a return of normal size and appearance of the kidney; these findings suggest that animals can acquire a tolerance to uranium. Hodge (1953) fed rats diets containing 474, 2,370 or 9,480 mg U/kg as uranyl nitrate hexahydrate. Rats fed 474 mg U/kg diet did not differ from controls. At 2,370 mg U/kg diet a slight growth depression was observed. However, rats fed diets containing 9,480 mg U/kg diet had markedly reduced growth and a high mortality. The body weights of dogs fed 100 mg uranyl nitrate hexahydrate/kg BW/day were not affected, but kidneys had characteristic histological changes associated with uranium toxicity when the dogs were fed diets providing as low as 20 mg/kg BW/day (Hodge, 1953). More recent studies indicate that much lower amounts of ingested uranium are toxic to experimental animals. Gilman et al. (1998a) found that 0.96 mg uranyl nitrate hexahydrate/L of drinking water produced renal lesions in rats. The histopathological lesions included apical nuclear displacement and vesiculation, cytoplasmic vacuolation, and dilation of the tubules; capsular sclerosis of the glomeruli; and reticulin sclerosis and lymphoid cuffing of the interstitium. Gilman et al. (1998b) also provided 0.96, 4.8, 24, 120, or 600 mg uranyl nitrate/L drinking water to rabbits. Based on changes in renal tubules, they concluded that the LOAEL in males was 0.96 mg U/L for male rabbits, and 4.8 mg U/L for female rabbits. Dose- and duration-dependent histopathologies were found in the liver and kidney of lake whitefish fed for 10, 30, and 100 days a commercial diet contaminated with 100, 1,000, and 10,000 mg U/kg (Cooley et al., 2000). Lesions in the liver were focal hepatocyte necrosis and alterations in the bile ductule epithelium. Lesions in the kidney included tubular necrosis, inflammation, hemorrhaging, depletion of hematopoietic tissues, alterations of distal tubules and collecting ducts, tubule dilation, pigmented macrophage proliferation, and glomerular lesions. The toxicity of uranium in water for several tropical freshwater fish was determined by Bywater et al. (1991). The 96-hour LC_{50} (in mg/L) were 1.7 and 1.9 for 7- and 90-day-old black banded rainbow fish, 1.22 for Mariana's hardyhead, 0.73 for delicate blue-eyes, 0.8 for reticulated perchlet, and 1.11 and 1.46 for 7- and 90-day-old purple-spotted gudgeon.

Recent studies with mice showed that oral uranium at relatively low doses is a reproductive, maternal, and developmental toxicant (Domingo et al., 1989; Domingo, 2001). The NOAEL for maternal and fetal toxicity was found to be below 5 mg/kg BW/day when uranyl acetate dihydrate was gavaged to mice on gestation days 6 to 15 at 0, 5, 10, and 25 mg/kg/day. Male reproduction variables were not as sensitive. Testicular function/spermatogenesis was not affected by doses of uranyl acetate dihydrate as high as 80 mg/kg BW/day given in drinking water for 64 days (Llobet et al., 1991). However, at 80 mg/kg/day interstitial alterations and vacuolization of Leydig cells were seen.

Based on acute toxicity studies with purple-spotted gudgeon and chequered rainbow fish, Holdway (1992) calculated the threshold value of 200 µg/L water for uranium toxicity using growth as the most sensitive response. He suggested that 70 µg U/L would be a safe uranium exposure for fish, based on the lower 95 percent fiducial limit of the lowest lethal concentration. Kurtitio et al. (2002) concluded that the safe concentration of uranium in drinking water for humans may be within the range of 2–30 µg/L. The WHO (2003) concluded that a tolerable intake of uranium (based on its chemical toxicity) from insoluble uranium compounds with a very low absorption rate may be 5 µg/kg BW/day (about 300–350 µg/day). When solubility characteristics of uranium compounds are unknown, it would be prudent to apply a tolerable intake of 0.5 µg/kg BW/day (about 30–35 µg/day) for ingestion. Because there is no information about the toxicity of uranium in domestic animals, it is difficult to suggest a maximum tolerable level. However, it should be

below 568 µg/L because 960 µg uranyl nitrate hexahydrate/L water caused renal histopathology in rats and male rabbits (Gilman et al., 1998a,b). Hodge (1953) found that an oral intake of 474 mg U/kg diet was not toxic to rats, and Tannenbaum and Silverstone (1944) found no adverse effects of 237 mg/kg diet in mice; however, renal histopathology was not an endpoint in these studies. The fish study of Cooley et al. (2000) indicates that the maximum tolerable limit for fish is less than 100 mg/kg diet. Thus a maximum tolerable intake for domestic animals is probably between 100 and 400 mg/kg diet. This intake indicates that uranium toxicity is not a concern for domestic animals because most diets probably do not exceed 3–4 mg/kg.

Tissue Levels

On average, the human adult body contains about 90 µg of uranium with 66 percent in the skeleton, 16 percent in liver, 8 percent in kidneys, and 10 percent in other tissues (WHO, 2003). This indicates that most tissue uranium concentrations in animals would be quite low. Low tissue uranium concentrations were confirmed by the human food analyses of Anke et al. (2000a). They found uranium concentrations (in µg/kg fresh weight) of 4.1 for chicken eggs, 2.1 for cattle kidney, 1.5 for trout and herring fillets, 1 for mutton, 0.8 for beef muscle, 0.4 for pork muscle, and 0.2 for milk.

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33

Minerals and Acid-Base Balance

INTRODUCTION

The maintenance of a stable pH in the fluids of the body is essential to life. The pH of the extracellular fluids is maintained at about 7.4 and generally varies less than ± 0.05 pH units. When considering the myriad of mechanisms involved in acid-base balance, it is important to remember that the core problem faced by the body is maintenance of the hydrogen ion (H^+) concentration of the extracellular fluids. Proteins within cell membranes and the proteins comprising enzyme systems are extremely sensitive to even small changes in H^+ concentration of the fluids bathing them. Blood pH below 7.25 or above 7.5 can alter the tertiary structure of these proteins, severely testing the ability of most animals to survive. Less severe perturbations in blood pH over a long term can cause pathological changes in body tissues involved in buffering blood. For example, during prolonged acidosis, calcium is released from bone tissues to raise the pH of the blood. Over time this leads to a diminution of bone strength.

DETERMINATION OF ACID-BASE BALANCE

Each animal species has a normal blood pH. Simple measurement of whole blood pH can quickly identify changes in blood pH that might be considered a threat to the health and productivity of an animal.

At its simplest, accurate measurement of blood pH can be made with a pH probe inserted into whole blood that is collected and maintained anaerobically until the pH measurement is made. Collecting or pouring whole blood into an open test tube and inserting a standard laboratory pH meter is inappropriate, because exposure to the atmosphere allows exchange of carbon dioxide and oxygen, rapidly altering the pH of the sample. Blood gas analyzers, on the other hand, have a special port that allows contact between whole blood and the pH probe without exposure to the atmosphere. Further procedures are necessary to further define the biological basis for changes in blood pH, and their interpretation can become quite complicated.

A relatively simple method that can be employed with most commercial blood gas analyzers is to determine blood carbon dioxide content using a carbon-dioxide-sensitive electrode in conjunction with blood pH determination. If blood hemoglobin level is also determined (or estimated from normal values for that species), it is possible to determine blood base excess from blood pH, carbon dioxide, and hemoglobin concentrations using a mathematical formula. Blood base excess is defined as the amount of a strong acid (such as hydrochloric acid) needed to titrate the pH of 100-percent-oxygenated blood with a partial pressure of 40 mm Hg from carbon dioxide, to a pH of 7.40 at 37°C. This value gives a suitable index for assessing the nonrespiratory component of an acid-base abnormality. Negative values suggest a metabolic acidosis due to accumulation of anions in the blood of the animal, while abnormally positive values indicate metabolic alkalosis due to excessive accumulation of cations in the blood.

When determining acid-base balance, it is preferable to use arterial whole blood. However, venous blood can also be used, bearing in mind that the acidity of venous blood is normally greater than that of arterial blood. More refined assessments and interpretations of acid-base status can be made and are often necessary if the respiratory system is compromised in any way (Constable, 1999).

Dietary Minerals and Acid-Base Physiology

The role of minerals in acid-base balance is best understood by a review of the Strong Ion Difference Theory of acid-base physiology (Stewart, 1983). The basic tenet of this theory is that the number of moles of positively charged particles (cations) in any given solution (including body fluids) must equal the number of moles of negatively charged particles (anions) in the solution, and that the product of the concentration of hydrogen ions and hydroxyl ions is always

equal to the dissociation constant of water, approximately 1×10^{-14}.

1. number of moles cations = number of moles anions
2. $[H^+] \times [OH^-] = 1 \times 10^{-14}$

Both equations must be satisfied simultaneously. Since pH is the negative log of the concentration of hydrogen ions, this essentially means that the pH of a solution is dependent on the difference between the number of negatively and positively charged particles in the solution. If positively charged particles are added to a solution such as plasma, the number of H^+ cations will decrease and the number of OH^- anions will increase to maintain the electroneutrality of the solution (the solution becomes more alkaline). Conversely, adding anions to a solution causes an increase in H^+ and a decline in OH^- to maintain electroneutrality, and the pH decreases (the solution becomes more acidic).

The primary cation and anion charges in the blood occur as bicarbonate anions, nonmetabolizable anions and cations, and blood proteins. Each contributes to blood and body fluid pH.

Bicarbonate Anions [HCO3$^-$]

The blood HCO_3^- concentration is essentially determined by the concentration of CO_2 in the blood as predicted by the Henderson-Hasselbach equation, pH = pKa (6.1) + log $[HCO_3^-]/[H_2CO_3]$. Blood CO_2 concentration is under the control of the respiratory system and allows minute-by-minute fine tuning of blood pH. When respiratory function is depressed, blood CO_2 concentrations increase, increasing the concentration of HCO_3^- anions, causing blood pH to decline. Conversely, when respiratory rate is elevated (as can occur in heat stress), blood CO_2 declines, blood HCO_3^- declines, and pH increases.

Concentration of Nonmetabolizable Anions and Cations

The difference between the total number of nonmetabolizable cations and anions in the blood is referred to as the Strong Ion Difference. Strong ions enter the blood from the digestive tract, making the cation-anion difference of the diet the ultimate determinant of blood Strong Ion Difference. Once Strong Ions are absorbed, their concentration in the blood is regulated by the kidneys. Adjustment of the Strong Ion Difference of the blood is slower than respiratory control of blood pH but is capable of inducing much greater changes in blood pH.

In theory, all cations and anions in the diet are capable of exerting an influence on the Strong Ion Difference of the blood. The major cations present in feeds and the "charge" they carry are sodium (+1), potassium (+1), calcium (+2), and magnesium (+2). The major anions and their charges found in feeds are chloride (–1), sulfate (–2), and phosphate (assumed to be –3). Sulfate is also released to body fluids during catabolism of cysteine and methionine, and high protein diets have long been recognized as decreasing blood and urine pH. Most carnivores, therefore, are in a state of mild metabolic acidosis and produce urine with a pH below 6.8 as the kidneys remove the anions from the blood (returning blood pH toward normal) and place them into the renal tubular fluids (reducing the pH of the glomerular filtrate). Herbivores tend to ingest forages where the predominant minerals are usually potassium and calcium. Since nearly all of the dietary potassium cations ingested are absorbed across the digestive tract, high forage diets place most herbivores in a state of mild metabolic alkalosis, causing them to produce alkaline urine.

Cations or anions present in the diet will only alter the Strong Ion Difference (electrical charge) of the blood if they are absorbed into the blood. Under normal circumstances, the trace elements present in most diets are absorbed in such small amounts that they are of negligible consequence to acid-base status. However, improper supplementation with very high amounts of trace minerals could affect acid-base balance. Amino acid supplements are often prepared as the salt of the amino acid (lysine hydrochloride, monosodium glutamate, cysteine hydrochloride). Large doses of these compounds can be toxic due to effects on systemic acid-base balance (as opposed to toxicity from the amino acid itself). Organic acids within the ingesta, such as the volatile fatty acids, are generally absorbed into the bloodstream in the undissociated form so that they carry both a positive and negative charge into the blood. They also are rapidly metabolized within the liver so they have only a small effect on blood pH under most circumstances. However, if an organic acid is consumed or produced at levels that exceed the liver's ability to metabolize it, as in the case of rumen lactic acidosis, the anion (lactate) can build up in the blood of the affected animal and cause severe metabolic acidosis.

The difference between the number of cation and anion particles absorbed from the diet determines the pH of the blood. The cation-anion difference of a diet is commonly described in terms of mEq/kg of just sodium, potassium, chloride, and sulfate as follows:

Dietary Cation-Anion Difference (DCAD, mEq) = (mEq Na^+ + mEq K^+) – (mEq Cl^- + mEq S^-)

Generally, DCAD is calculated on the basis of 100 g or 1 kg of diet. This equation is useful, though it must be kept in mind that Ca, Mg, and P absorbed from the diet will also influence blood pH. It might be more proper to rewrite the common equation as DCAD = (Na + K + Ca + Mg) – (Cl + S + P). Trace minerals could also be included but generally the amount (percent of diet) of any one trace mineral needed to cause an acid-base imbalance would be well beyond the toxic levels as described in the individual mineral chapters in this book. The problem with any of these equations is that they assume all of the cations and anions present in the diet are absorbed in equal amounts into the blood of the animal.

This simply is not true. Approximately 90 percent of ingested sodium, potassium, and chloride are absorbed into the blood. But most other minerals are not as well absorbed and their rate of absorption can vary with form or source of the mineral (solubility, particle size, etc.) and the physiological state of the animal. Unfortunately, few data exist to allow specification of a coefficient of absorption in front of each element of the DCAD equation for most species.

Concentration of Proteins in the Blood

Proteins, such as hemoglobin and albumin, tend to be negatively charged and are considered as anions. Their concentration in blood is generally dependent on liver function. Blood protein levels are fairly constant unless there are large changes in liver function or plasma volume. Mineral toxicities affecting liver function could therefore alter blood pH.

TOXICOSIS

Acute

If the amount of cations absorbed from the diet far exceeds the amount of anions absorbed from the diet, a severe metabolic alkalosis can develop in the animal. Conversely, ingestion of a diet in which the absorption of the anions in the diet far exceeds the absorption of the cations in the diet can induce a severe metabolic acidosis in the animal.

Uncompensated Metabolic Acidosis or Alkalosis

When highly excessive amounts of readily absorbable cations or anions are ingested, the ability of the kidneys and respiratory system to compensate for the added cations (in the case of metabolic alkalosis) or anions (in the case of metabolic acidosis) is exceeded. The animal now enters a state of uncompensated metabolic acidosis or alkalosis. Both situations are acutely toxic and are often lethal. The acidotic animal will exhibit severe respiratory exertion to regain control of blood pH by removing carbon dioxide as it attempts to reduce blood bicarbonate anions. In alkalosis, respirations will be slowed to permit carbon dioxide and bicarbonate anions to build up in the blood. Metabolic alkalosis is more quickly lethal than is metabolic acidosis. These are acute toxicoses and animals can die from the acid-base imbalance. At the very least, the animals very quickly reduce intake of the diet, and performance quickly deteriorates simply from lack of feed intake.

Chronic

Compensated Metabolic Acidosis or Alkalosis

Mild imbalances in dietary cation-anion difference cause mild acid-base imbalances that can often be accommodated by increased renal excretion of cations or anions. The respiratory system can also be used to raise or lower the concentration of bicarbonate anion in the blood to help restore blood pH to normal. Though these actions spare the animal's life, the chronic imbalances in acid-base can cause a reduction in the animal's performance.

Bone is a large depot of buffer for the body. During metabolic acidosis, calcium, and to a lesser extent, other cations such as potassium and magnesium leave bone to help buffer the acid (excess anion) in the blood. If prolonged, metabolic acidosis will result in bone loss and increased susceptibility to fracture. Conversely, calcium, magnesium, and potassium can be deposited into the extracellular fluids of bone during periods of metabolic alkalosis, reducing the cation load of the blood and reducing blood pH.

Prolonged changes in acid-base status can also contribute to urolithiasis in several species. Metabolic alkalosis has been implicated as a contributing factor in formation of struvite (magnesium ammonium phosphate) urethral stones in cats, and it is common to add absorbable anions to the diet to acidify the urine and help prevent their occurrence (Allen et al., 1997). Unfortunately, as the urine is acidified, the amount of calcium excreted in the urine increases, which may increase susceptibility to formation of calcium oxalate crystals and perhaps calcium phosphate crystals (Osborne et al., 1996).

MAXIMAL TOLERABLE DIETARY CATION-ANION DIFFERENCE FOR ANIMAL HEALTH AND PRODUCTIVITY

It is impossible to determine the level of a single cation or anion in the diet that can cause metabolic alkalosis without considering the dietary content of other cations or anions that might also contribute to the blood pH of the animal. Therefore, the total cation-anion difference of a diet must be considered. Unfortunately, there is no consensus on the type of equation needed to describe cation-anion difference of a diet.

In general, the research conducted with poultry uses the equation (Na + K) – Cl to describe diet "electrolyte balance." Optimal growth and performance was reported when electrolyte balance was between +200 and +300 mEq/kg diet (Johnson and Karunajeewa, 1985; Oviedo-Rondon et al., 2001; Borges et al., 2003). Low (<180 mEq/kg) or high (>300 mEq/kg) dietary electrolyte balance reduced growth (Johnson and Karunajeewa, 1985). Early research with poultry described electrolyte balance as the equivalents of Na + K/Cl (Sauveur and Mongin, 1978). Broiler performance was reduced when Na + K/Cl was less than +30 mEq/kg diet, and significantly reduced performance of hens was observed when Na + K/Cl was <10 or >50 mEq/kg (Mongin, 1981). However, other minerals (such as calcium, magnesium, phosphate, and sulfate) are also known to contribute to acid-base balance in birds. For example, Halley et al. (1987) found

that the incidence of bone problems in broilers increased as the ratio of calcium + magnesium to phosphorus + chloride + sulfate decreased.

Most mammalian research into acid-base balance has used the DCAD equation (Na + K) – Cl, until more recently, when the contribution of sulfur was recognized, resulting in the current DCAD equation (Na + K) – (Cl + S). In lactating dairy cows, milk production and feed intake are greatest when the diet fed to lactating cows has a DCAD of +380 mEq/kg diet (using the formula (Na + K) – Cl) (Sanchez et al., 1994). There is a significant decrease in milk production when DCAD falls below +200 mEq/kg or rises above +550 mEq/kg diet using the equation (Na + K) – (Cl + S) (Ghorbani et al., 1995). Prior to parturition, lowering the DCAD (Na + K) – (Cl + S) below zero has proved to be an effective means of preventing the development of hypocalcemia at parturition. At the lower blood pH induced by these diets, calcium homeostasis is improved, apparently through increased sensitivity of bone and kidney tissues to parathyroid hormone (Gaynor et al, 1989).

Acid-base status can also affect tissue responsiveness to hormones not involved in calcium metabolism. For instance, metabolic acidosis impairs glucose tolerance and tissue response to insulin (Cuthbert and Alberti, 1978; Bigner et al., 1996) and growth hormone (Kuemmerle et al., 1997). However, it is difficult to discern the DCAD at which these phenomena occur.

A negative DCAD, using (Na + K) – Cl, reduces growth in pigs (Dersjant-Li et al., 2001), while DCAD between +200 and +500 seems well tolerated. Golz and Crenshaw (1991) noted increased ammonium excretion when DCAD was reduced by dietary anion addition and concluded that alterations in growth caused by changes in dietary K and possibly Cl levels were mediated via mechanisms involving renal NH_4^+ metabolism during protein catabolism.

In horses, addition of cations to the diet or by oral gavage may increase the tolerance of the animal to anaerobic buildup of lactic acid during exercise (Hyyppa and Posa, 1998). Greenhaff et al. (1990) suggested the horse can tolerate as much as 0.3 g sodium bicarbonate / kg BW, given as a single oral dose.

Water pH can affect dissolved oxygen content of water affecting aquaculture. Warm water, freshwater species can tolerate water with a pH between 6 and 10. Cold water, freshwater fish can tolerate water with a pH between 6.5 and 8.5 (Colt, 1991). The interaction between diet cation-anion balance and blood pH and performance of fish is largely unknown, however.

The total cation-anion load in a diet can also be toxic, independent of effects on blood pH (Bennett et al., 2003). These osmotic effects are discussed in the chapter on sodium chloride (NaCl).

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34

Nitrates and Nitrites

INTRODUCTION

Nitrates (NO_3) and nitrites (NO_2) are salts or esters of nitric acid (HNO_3) and nitrous acid (HNO_2), respectively. Common inorganic nitrates and nitrites include ammonium nitrate (NH_4NO_3), calcium nitrate ($Ca(NO_3)_2$), calcium nitrite ($Ca(NO_2)_2$), magnesium nitrate ($Mg(NO_3)_2$), potassium nitrate (KNO_3), potassium nitrite (KNO_2), sodium nitrate ($NaNO_3$), and sodium nitrite ($NaNO_2$). Nitrate and nitrite compounds range in color from colorless to white or slightly yellow and are generally very soluble in water. Calcium nitrate (Norwegian saltpeter) and sodium nitrate (Chile saltpeter) are used in fertilizers. Sodium nitrate and nitrite are used to cure, color, and preserve meat. Nitrates and nitrites are used as a corrosion inhibitor and in diesel fuels. They are used to make nitrous oxide, freezing mixtures, safety explosives, matches, gunpowder, and radio tubes (Budavari, 1996).

Nitrates and nitrites are also formed naturally through the nitrification process in the biological nitrogen cycle. Ammonia in the soil is oxidized by aerobic bacteria to nitrite and then further to nitrate. Although plants can use ammonia directly, most of the ammonia in soil is converted to nitrate where plants assimilate it and convert it into amino acids and proteins during growth (Campbell, 1990).

ESSENTIALITY

There is no known essentiality of nitrates or nitrites by mammals. Nitrates and nitrites are an essential component of the nitrogen cycle (Campbell, 1990). Plants cannot convert atmospheric nitrogen (N_2) directly and therefore rely on prokaryotes to reduce nitrogen to ammonia, and on symbiotic bacteria on root nodules and nonsymbiotic bacteria in the soil to convert ammonia to nitrate. Nitrate is the major nutrient form of nitrogen in most soils and is often the rate-limiting nutrient factor for plant growth (Wright and Davison, 1964).

DIFFICULTIES IN METHODS OF ANALYSIS AND EVALUATION

Several methods have been developed to determine nitrate or nitrite content in biological fluids (Greweling et al., 1964; Shechter et al., 1972; Schneider and Yeary, 1973; Green et al., 1982); food (Sanderson et al., 1991); plant material (Cataldo et al., 1975; Bedwell et al., 1995); water (Hubble and Harper, 2000); and soils (Kuchnicki and Webster, 1986; Szekely, 1991). Primarily, methods should be evaluated based on accuracy, precision, sensitivity, and the potential for interference by other ions. Other practical considerations may include the level of difficulty, time required, costs, and sample size.

Nitrate or nitrite analytical results may vary depending on the method of analysis. Bedwell et al. (1995) compared the diphenylamine spot plate (SPOT), spectrophotometric (SPEC), nitrate selective electrode (NSE), and high performance liquid chromatographic (HPLC) methods for nitrate analysis in forages. The SPEC and NSE methods had similar, and the most accurate, nitrate recoveries. The SPOT method overestimated the amount of nitrate and was the least accurate. The HPLC method overestimated nitrate content and resulted in a number of outlier values. The NSE method was repeatable and had a low ($P < .05$) coefficient of variation compared to the SPOT, SPEC, and HPLC methods. The HPLC method has also been compared to the phenoldisulfonic acid (PDA) and hydrazine sulfate (HS) methods for analysis of nitrates in soil (Kuchnicki and Webster., 1986). Organic matter interfered with nitrate recovery using the PDA method. The HS method was more precise than the PDA method; however, negative values were often obtained when analyzing soil with low nitrate addition. When one soil sample was excluded, the HPLC method had equal precision and greater accuracy than the HS method. The recovery of 100 mg/kg of nitrate resulted in a range of 97.5–112 percent, 87.9–93.6 percent, and 71.4–109 percent for the HPLC, HS, and PDA methods, respectively.

Sanderson et al. (1991) compared the HPLC method to the cadmium reduction-Griess (Cd-G) method when using fresh and cured meats. The precision was similar between methods; however, the accuracy was greater for the HPLC method. Using one sample, the nitrate percent recovery was 80.1 percent for HPLC and 31.1 percent for Cd-G. Overall, the nitrate values were higher ($P < .05$) for the HPLC as compared to the Cd-G method. Nitrate values for fresh meats ranged from 0.384 to 1.66 mg/kg and from 0 to 1.07 mg/kg, while cured meats ranged from 7.69 to 190 mg/kg and from 0.583 to 60 mg/kg for HPLC and Cd-G, respectively. Nitrate analysis by nitration of salicylic acid (NSA), dissimilatory nitrate reductase from *Escherichia coli* (DNR), and the PDA methods were compared by Cataldo et al. (1975). Using corn and oat plant samples, the NSA and DNR methods gave similar nitrate values. The PDA method was deemed adequate when samples contain more than 1,000 mg/kg nitrate-N. Interference by chloride (particularly at levels greater than 40 µg per assay [equivalent to 2 percent Cl^- in plant tissue]), nitrite, and ammonium ions occurred with analysis of nitrate using the NSA method.

Nitrate or nitrite can be expressed in a variety of ways. The reported form of nitrate or nitrite should be considered when interpreting or comparing results. The following are formulas that can be used for conversion of nitrate and nitrite forms.

- Nitrate nitrogen (NO_3-N) × 4.43 = Nitrate (NO^-_3)
- Potassium nitrate (KNO_3) × 0.613 = Nitrate (NO^-_3)
- Sodium nitrate ($NaNO_3$) × 0.729 = Nitrate (NO^-_3)
- Nitrite nitrogen (NO_2-N) × 3.29 = Nitrite (NO^-_2)
- Sodium nitrite ($NaNO_2$) × 0.667 = Nitrite (NO^-_2)

REGULATION AND METABOLISM

Nitrate and nitrite absorption, distribution, and excretion data have been reviewed by Walker (1990). In humans and rats, the majority of ingested nitrate is absorbed from the upper small intestine. A very small amount of nitrate is absorbed from the stomach, distal ileum, cecum, or proximal colon. In ruminants, nitrates can be absorbed from the rumen, most likely occurring via the Cl^-/HCO_3^- exchange mechanism (Bruning-Fann and Kaneene, 1993). The absorption of nitrates is rapid. Witter and Balish (1979) detected N-labeled nitrate and nitrite throughout the entire gastrointestinal tract of rats as soon as 15–30 minutes following gavaged dose. They noted a similar pattern in the progression of N-labeled nitrate and nitrite through the gastrointestinal tract, although N-labeled nitrite remained in the stomach for a longer period of time then nitrate.

Following absorption, nitrate quickly equilibrates with interstitial fluids (Walker, 1990). Parks et al. (1981) administered 15 µl of radioactive nitrogen-13, from nitrate or nitrite, intravenously to rabbits. Intravascular and extravascular $^{13}NO_3^-$ and $^{13}NO_2^-$ equilibrium occurred within 5 minutes of injection and nitrogen-13 activity was distributed evenly throughout most organs. This study was repeated using mice and intratracheal administration of nitrogen-13; results were consistent. In humans and laboratory animals other than rats, absorbed nitrate is selectively transported from the blood and secreted into saliva (Walker, 1990). Bartholomew and Hill (1984) orally dosed humans with 25, 50, 100, or 170 mg of potassium nitrate in distilled water. For all dosage levels, maximum salivary nitrate concentration was reached within 1 hour of nitrate ingestion. The proportion of orally ingested nitrate that will be secreted in the saliva has been estimated at 25 percent (Walker, 1990).

Literature about measuring the elimination of nitrate and nitrite from plasma indicates that plasma nitrite elimination half-lives are less variable between animal species as compared to nitrate elimination half-lives (Lewicki et al., 1994). Schneider and Yeary (1975) reported that the half-lives of nitrite for dogs, sheep, and ponies were 0.499, 0.475, and 0.566 hours, while nitrate half-lives were 44.681, 4.233, and 4.821 hours, respectively. Lewicki et al. (1994) reported similar values for sheep, with plasma nitrite and nitrate elimination half-lives of 0.49 hours and 4.5 hours, respectively. A plasma nitrate elimination half-life of 22 hours was reported for milk-fed calves orally administered nitrate, nitrite, or nitrate combined with nitrite (Hüsler and Blum, 2001).

The excretion of nitrates or nitrites has been investigated using a variety of species. In general, nonruminant animals excrete more urinary nitrate than ruminant animals and horses excrete nitrate intermediate to nonruminant and ruminant animals (Bruning-Fann and Kaneene, 1993). Early studies using dogs, rabbits, or goats as summarized by Wang et al. (1981) found 30–90 percent of the ingested nitrate was excreted in urine. Dull and Hotchkiss (1984) used ferrets and reported that 36 percent of the nitrate ingested was excreted in urine. Wang et al. (1981) used ^{15}N-labeled sodium nitrate or sodium nitrite in a single dose and multiple dose feeding study with rats. They reported that within 72 hours of the final dose, 60–70 percent of ingested ^{15}N was excreted in the urine and 10–20 percent was excreted in the feces. In addition, at least 50 percent of the ^{15}N excreted in the urine and feces was not excreted as nitrate or nitrite. Schultz et al. (1985) reported that 55 percent of ingested ^{15}N was excreted as nitrate in the urine of rats and 11 percent of ingested ^{15}N was excreted as urea or ammonia. Lewicki et al. (1994) administered sodium nitrate or sodium nitrite to sheep intravenously. Within 30 hours of the sodium nitrite dose, nitrate and nitrite were excreted in the urine at 13.8 percent and 0.29 percent of the administered dose, respectively. Within 30 hours of administered nitrate, nitrite was not observed in the urine and nitrate was excreted at 16.12 percent of the administered sodium nitrate dose.

The amount of nitrate an animal will excrete is dependent on the degree of microbial nitrate reduction that occurs within the gastrointestinal tract. Nitrate reduction can occur through the assimilatory nitrate reduction pathway, where

nitrate is reduced to nitrite and further converted to ammonia, or the denitrification pathway, where nitrate is reduced to nitrite, which is further reduced to nitric oxide, nitrous oxide, and finally nitrogen gas (Russell, 2002). Rapid bacterial reduction of nitrate to nitrite occurs in the rumen of ruminants; however, minimal nitrate reduction occurs in nonruminant animals. The exception is the horse, which has an enlarged cecum and colon allowing bacterial nitrate reduction that is intermediate to other nonruminant and ruminant animals (Bruning-Fann and Kaneene, 1993).

SOURCES AND BIOAVAILABILITY

In humans and animals, exposure to nitrates or nitrites occurs primarily through the ingestion of food and water.

Crops

Nitrate is the primary nitrogen source available to plants in the soil (Garrett and Grisham, 1999) and under normal environmental conditions, minimal nitrate is accumulated in plants. Instead, nitrate absorbed by the roots is reduced to ammonia and combined with carbohydrates to form amino acids (Pfister, 1988). The reduction of nitrates to ammonia in plants is called nitrogen assimilation and occurs in a two-step process: (1) nitrate is reduced to nitrite via nitrate reductase and (2) nitrite is further reduced to ammonia via nitrite reductase (Garrett and Grisham, 1999). The rate-limiting enzyme for nitrogen assimilation is nitrate reductase (Ferrario-Méry et al., 1997).

Many plants have the potential to accumulate nitrate at toxic levels. Important forage crops that have been reported to accumulate nitrate under certain conditions include sorghum, Sudan grass, Johnson grass, oats, corn, and wheat. Weeds that are nitrate accumulators include pigweed, kochia, thistle, lambsquarter, bindweed, and ragweed (Sonderman, 1993). Cases of nitrate poisoning from forages have been reported by Nicholls (1980) and Carrigan and Gardner (1982).

The capacity to accumulate nitrates is dependant on a variety of environmental and endogenous factors. Drought conditions are commonly associated with nitrate accumulation in plants. When soil moisture is limiting, nitrate assimilation is depressed due to a reduction in nitrate reductase activity and photosynthesis (Pfister, 1988). Reduced light intensity also decreases nitrate reductase activity, thereby increasing nitrate accumulation (Maynard et al., 1976; Blom-Zandstra and Lampe, 1985; Pfister, 1988). Under normal environmental conditions, increasing availability of nitrogen through fertilization may also increase nitrate accumulation (George et al., 1973; MacLeod and MacLeod, 1974; Reid and Strachan, 1974; Vieira et al., 1998). The impact of plant genetics on nitrate accumulation is variable. Although the tendency to accumulate nitrate is more commonly noted for some forage species, nitrate accumulation is highly variable even between cultivars of the same species (Wright and Davidson, 1964; Maynard et al., 1976; Cárdenas-Navvaro et al., 1999). Herbicide application has been shown to affect nitrate accumulation in plants; however, the response varies by herbicide and plant species (Williams and James, 1983). Phosphorus deficiency reduces nitrate uptake (Jeschke et al., 1997). Molybdenum and manganese deficiency in plants is associated with increased nitrate accumulation (Maynard et al., 1976). Stems normally contain the highest levels of nitrates, followed by roots, leaves, and flowers (Wright and Davidson, 1964), and older leaf blades contain a higher concentration of nitrate than younger leaf blades (Cárdenas-Navarro et al., 1999).

Ensiling plants has been suggested as a method of reducing plant nitrate content (Ataku et al., 1982). However, the extent of nitrate degradation is dependent on factors that affect the rate of silage fermentation and therefore silage quality (Spoelstra, 1985). Ensiling processes that result in rapid fermentation, reaching a stable pH within a couple of days, result in less degradation of nitrate than extended fermentation, which occurs over several days to weeks. Silage nutrient quality is better retained and "preserved" with rapid fermentations than with a slow extended fermentation. The following factors contribute to rapid fermentation and decreased nitrate degradation: high plant sugar content; wilting, lacerating, and chopping of plants; the addition of fermentable substrates such as starch or sugar; or the addition of fermentation inhibition products such as acids (Spoelstra, 1985). Ammonia, urea, or calcium carbonate, when added to silages, will extend silage fermentation time (Li et al., 1992). Spoelstra (1985) reported that adding these products to silages reduced nitrate concentration but did not quantify the extent of reduction. Li et al. (1992) evaluated the effects of ammonium hydroxide, urea, calcium carbonate, or a microbial inoculant on nitrate reduction over 28 days of fermentation. In experiment 1, ammonium hydroxide, calcium carbonate, microbial inoculant, or no additive along with 0; 2,000; 4,000; and 6,000 mg/kg nitrate N were added to chopped corn plants. In experiment 2, urea, calcium carbonate, or no additive along with 0; 2,380; 4,770; or 9,530 percent nitrate N were added to chopped corn plants. After 28 days of ensiling, the additives in experiment 1 did not significantly reduce nitrate N. In experiment 2, nitrate was reduced with the application of urea (15 percent) or calcium carbonate (13 percent).

Food

Reviews by Wright and Davidson (1964), Walker (1975, 1990), Maynard al. (1976), and Van Diest (1986) reported a range of nitrate and nitrite levels in food. Vegetables that commonly have nitrate levels of 1,000 mg/kg or greater include spinach, lettuce, beets, radish, and turnips. Fruits generally contain less than 10 mg/kg nitrate, with the highest nitrate levels ranging from 25 to 150 mg/kg for bananas,

strawberries, and tomatoes (Walker, 1975, 1990). Nitrite concentrations in fruits and vegetables are generally low. Fresh meats usually have low levels of nitrates and nitrites, while a wide range of values have been reported for cured meats. Walker (1990) reported the nitrate and nitrite in cured meats ranged from 19 mg/kg to 670 mg/kg and from 0 mg/kg to 96 mg/kg, respectively.

Water

Nitrate has been reported to be a common well water contaminant in rural areas and in urban areas with a high concentration of septic tanks (NRC, 1974). Squillace et al. (2002) analyzed 1,497 wells, which were distributed across the United States, and reported that 28 percent of them had a nitrate-N concentration greater than 3 mg/L with 11 percent exceeding the current EPA drinking water maximum contaminant level of 10 mg/L. Nitrate-nitrogen levels greater than 3 mg/L were associated with higher dissolved oxygen concentrations, shallow well depth, unconfined aquifers, agricultural land use, and population density. Nitrate was also detected in combination with atrazine in 13 percent of samples. Combinations of nitrate and atrazine or nitrate, atrazine, and aldicarb have been shown to significantly affect endocrine function and alter the aggression levels of mice when added to water at 28 mg/L nitrate-nitrogen and .01 mg/L atrazine and aldicarb (Porter et al., 1999). Nitrate with atrazine also significantly affected mouse immune function. In 13 groundwater studies summarized by Spalding and Exner (1993), between 3 and 35 percent of water samples contained nitrate-N levels greater than 10 mg/L.

TOXICOSIS

Recent literature reviews on the toxicology of nitrates and nitrites in nonruminant and ruminant animals have been written by Pfister et al. (1988), Bruning-Fann and Kaneene (1993), and Gangolli (1999). Selected references on the effects of nitrate or nitrite exposure on animals are listed in Table 34-1. Nitrate toxicity is somewhat a misnomer as nitrate in itself is not considered to be highly toxic. Nitrate becomes toxic when reduced to nitrite because nitrite, through the oxidation of hemoglobin, can form methemoglobin. Unlike hemoglobin, methemoglobin is unable to transport oxygen in the blood (Burrows, 1980). In ruminants, bacteria in the rumen rapidly convert nitrate to nitrite and then to ammonia for assimilation into bacterial protein (Russell, 2002). Nitrate toxicosis occurs when the conversion of nitrite to ammonia is disrupted or when high levels of nitrate are fed. Nonruminant animals generally have to consume nitrite to induce methemoglobinemia and a toxic effect of acute nitrate levels is generally severe gastritis (Bruning-Fann and Kaneene, 1993). Clinical signs of methemoglobinemia occur at 30–40 percent methemoglobin and include rapid breathing and pulse rate, muscle tremors, increased urination, and a chocolate brown appearance of the blood. Death from methemoglobinemia occurs when methemoglobin levels are greater than 80 percent (Burrows, 1980; Bruning-Fann and Kaneene, 1993).

Acute—Ruminants

Nitrate poisoning in cattle consuming cornstalks was first reported by Mayo in 1895. Bruning-Fann and Kaneene (1993) summarized literature on the effects of nitrates and nitrites on animal health. Numerous cases of nitrate toxicosis were reported from 1950 to 1980. Acute poisoning occurred when forages exceeded 5,000 mg/kg nitrate on a DM basis. The LD_{50} of cattle drenched with nitrate was estimated to be 330 mg of nitrate/kg BW, however, when nitrate was consumed with feed, the LD_{50} tripled to 990 mg of nitrate/kg BW. Gangolli (1999) reported that the oral single LD_{50} in cows, using sodium nitrate, was estimated to be 328 mg of nitrate/kg BW, but the same dose spread over a 24-hour period increased the LD_{50} to 707–991 mg nitrate/kg BW.

Several factors affect the toxicosis of ingested nitrate (Bruning-Fann and Kaneene, 1993). Composition of the diet containing nitrate is an important determinant as to the microbial use of nitrates. Ruminants fed grain carbohydrates along with high nitrate forages are more tolerant and less prone to poisoning than when high nitrate forages are the only feed in the diet. Pattern of eating also is important. Small amounts of feed ingested slowly over an expanded period of time allow animals to adapt to nitrate and result in less toxicosis than the same amount ingested in a single feeding. According to Russell (2002), in vivo and in vitro studies indicate rumen bacteria are highly adaptive to gradual increases in nitrate. Monensin was reported to precipitate nitrate toxicosis on high forage diets as it possibly shifts the rumen bacteria population to more nitrite producers (Bruning-Fann and Kaneene, 1993).

Acute—Nonruminants

Gangolli (1999) reported the following oral LD_{50} values:

Mouse	1,808–4,556 mg nitrate/kg BW as sodium nitrate
Rat	3,543–6,561 mg nitrate/kg BW as sodium nitrate
Rat	1,899–3,736 mg nitrate/kg BW as ammonium nitrate
Rabbit	1,954 mg nitrate/kg BW as sodium nitrate
Rabbit	1,165 mg nitrate/kg BW as potassium nitrate

Acute nitrate toxicosis has been reported in pigs, dogs, turkeys, rats, and mice (Bruning-Fann and Kaneene, 1993). In almost all cases, in addition to methemoglobin, gastric lesions that were suggestive of salt poisoning were found. Most cases of nitrate poisoning reported by Bruning-Fann and Kaneene (1993) and Gangolli (1999) in nonruminant animals occurred through direct ingestion of nitrate com-

pounds, experimentally or accidentally, or through water. Most grains are low in nitrate content and therefore, nonruminant farm animals are at a lower risk of nitrate toxicosis than are ruminants where nitrate can accumulate in forages. Straw was considered to be the source of nitrate poisoning in one case in pigs (Bruning-Fann and Kaneene, 1993).

Chronic—Ruminants

Ruminant animals can use nitrate as a nonprotein nitrogen source and therefore, effects of chronic nitrate toxicosis, if they occur, are difficult to observe. Abortions have been reported in ruminants receiving high doses of nitrate and exhibiting clinical signs of nitrate toxicosis (Bruning-Fann and Kaneene, 1993). Chronic nitrate toxicosis effects on production parameters, BW gains, or milk production of ruminants are relatively unknown. Beef cattle receiving more than 10,000 mg/kg nitrate in their diet and sheep more than 30,000 mg/kg nitrate in the diet exhibited reduced feed intake (Bruning-Fann and Kaneene, 1993). Other possible effects of nitrate cited by Bruning-Fann and Kaneene (1993) were methemoglobin increases, possible changes in pituitary function, placental transfer of methemoglobin, transfer of some nitrate into milk, and questionable but frequently reported effects on vitamin A metabolism. No differences have been reported when measuring the occurrence of birth weight of offspring, number of services per conception, gestation, or length of estrous cycle (Bruning-Fann and Kaneene, 1993). Sonderman (1993) reported a higher incidence of abortions in 13 beef cows exhibiting signs of toxicosis when orally administered potassium nitrate at 0.270 mg nitrate/kg BW on one day followed by 540 mg nitrate/kg BW the second day during the third trimester of pregnancy. In 11 beef cows given one oral dose of potassium nitrate at 400 mg nitrate/kg BW, during the second trimester of pregnancy, signs of nitrate toxicosis were observed, but no abortions occurred. Pregnant sheep given water containing 3,000–12,000 mg/L nitrate from 21 to 49 days of pregnancy or heifers fed a diet containing 445–665 mg of nitrate/kg for 2 months reportedly increased methemoglobinemia, but no abortions were observed (Gangolli, 1999).

Significant changes in blood parameters due to nitrate ingestion have been reported. Sheep dosed intraruminally with sodium nitrite at 130 mg nitrite/kg BW exhibited increased erythrocyte counts, while at 200 mg nitrate/kg BW, they exhibited hypochromic anemia. Increased leukocyte counts, neutrophil and eosinophil percentages, and decreased lymphocyte percentages were reported at both sodium nitrite levels. Additional research in cattle and sheep has suggested that compensatory increases in hemoglobin concentration, packed red cell, and blood volumes occur due to extended periods of methemoglobinemia (Bruning-Fann and Kaneene, 1993).

Chronic—Nonruminants

Nitrates and nitrites have reduced the growth rates of nonruminant animals. Decreased BW gains have been reported for chickens treated with sodium nitrate at 3,100 mg nitrate/kg feed or sodium nitrite at 1,100 mg nitrite/kg diet for 4 weeks (Atef et al., 1991); rats treated with sodium nitrate at 2,916 mg/kg nitrate or nitrite at levels ranging from 1,334 to 3,335 mg/kg (Till et al., 1988; Grant and Butler, 1989); and chicks or turkey poults treated with 1,067 mg/kg nitrite in the form of sodium nitrite (Diaz et al., 1995). The effect of nitrates or nitrites on reproductive performance varies according to species. Increased fetal losses occurred when guinea pigs were treated with nitrate or nitrite; nitrite had a greater impact at lower levels (Sleight and Atallah, 1968). In rats, nitrite caused increased postpartum offspring mortality and a reduction in pup growth rates (Shuval and Gruener, 1972; Ema and Kanoh, 1983; Vorhees et al., 1984; Roth et al., 1987). Research with pigs has not found an effect of nitrate or nitrite on reproductive performance and an inconsistent response is reported in chickens (Bruning-Fann and Kaneene, 1993).

Changes in blood traits due to nitrate or nitrite have been shown in nonruminant animals. In pigs, nitrite increased hemoglobin, and packed cell volume caused lymphocytosis and resulted in anemia in pregnant rats (Bruning-Fann and Kaneene, 1993). Plasma vitamin E levels decreased when rats were treated with drinking water containing sodium nitrate at 1,458 mg/kg nitrate (Chow et al., 1980), and a dietary deficiency in vitamin E intensified the effects of nitrite on blood parameters of rats (Chow et al., 1984). The mutagenic and carcinogenic potential of nitrates and nitrites has been researched predominantly with rats and mice. A significant increase in the occurrence of chromosomal aberrations in bone marrow metaphase cells has been observed in adult rats and in the liver of transplacentally exposed rat embryos (El Nahas et al., 1984; Luca et al., 1985). Aoyagi et al. (1980) reported that rats fed 534 or 1,067 mg/kg nitrite as sodium nitrite, for almost 2 years, had an increased occurrence of liver tumors and at 1,067 mg/kg nitrite, 40 percent of the tumors were malignant. In another long-term feeding study, Grant and Butler (1989) fed sodium nitrite to rats at 1,334 and 3,335 mg/kg nitrite; however the control animals had the highest incidence of lymphoma, leukemia, and testicular interstitial cell tumors. A small number of studies indicate that nitrates or nitrites may negatively impact the motor development and learning behavior of rats (Vorhees et al., 1984; Markel et al., 1989).

Toxicosis—Aquatic Animals

Nitrite, an intermediate product in the nitrification of ammonia and the denitirification of nitrogenous compounds, is much more toxic to fish and aquatic organisms than is nitrate (Colt and Tchobanoglous, 1976; Pierce et

al., 1993; Basuyaux and Mathieu, 1999). The mechanism of nitrite toxicity in fish is the same as it is for land animals in that nitrite causes the oxidation of heme (Fe^{++} to Fe^{+++}) and the inability of hemoglobin to transport oxygen by converting it to methemoglobin (Jensen, 1999). Freshwater fish are more sensitive to nitrite than are saltwater fish as the chloride ions in salt water inhibit absorption of nitrite by actively competing with nitrites in the gills for absorption. There is considerable variation among and even within fish species in the sensitivity to nitrite with rainbow trout, perch, and pike more sensitive than carp, tench, and eel (Jensen, 1999).

Tiger shrimp were found to be much more tolerant of increasing nitrate concentrations in water as salinity of the water increased (Tsai and Chen, 2002). The 48-hour lethal concentrations (LC_{50}) were 2,876; 3,894; and 4,970 mg nitrate-nitrogen/L in water that contained 15 percent, 25 percent, and 35 percent seawater, respectively. Lin and Chen (2003) exposed white shrimp to water containing from 25 to 250 mg of nitrite-nitrogen/L and salinity concentrations of 15 percent, 25 percent, or 35 percent. Decreasing salinity from 35 percent to 15 percent increased susceptibility of shrimp to nitrite-nitrogen by 277 percent, 298 percent, 405 percent, 418 percent, and 421 percent after 24, 48, 72, 96, and 144 hours of exposure, respectively. Rainbow trout had a less than 10 percent survival rate within 24 hours of being in water that contained 32.2 mg/L nitrite and low concentrations of calcium (<4.0 mg/L) and chloride (<0.3 mM/L) (Bath and Eddy, 1980). However, when calcium was increased to 80.2 mg/L, survival increased to 50 percent, and increasing the chloride concentration to 21.3 mg/L resulted in a similar survival rate.

Basuyaux and Mathieu (1999) exposed abalone and sea urchins to water containing nitrite (0, 0.5, 1.0, 2.0, or 5.0 mg nitrite-nitrogen/L) or nitrate (0, 25, 50, 100, or 250 mg nitrate-nitrogen/L) for 2 weeks. No mortalities were observed. Nitrite or nitrate levels in water where growth of abalone was not reduced were ≤5 mg/L nitrite-nitrogen and ≤100–250 mg/L of nitrate-nitrogen. Growth rates of abalone were enhanced by adding up to 2 mg/L nitrite-nitrogen or 50 mg/L of nitrate-nitrogen to the water. Growth rate of sea urchin declined after water contained 1–2 mg/L of nitrite-nitrogen or more than 100 mg/L of nitrate nitrogen. Jensen (1999) indicated that elevated levels of nitrite in the body of aquatic animals not only lowered growth rates, but suppressed immune function. The survival rate of grass carp exposed to 1 or 1.6 mg/L of nitrite-nitrogen water for 15 days was 94 percent (Alcaraz and Espina, 1997). At 2.5 mg/L of nitrite-nitrogen in the water, the survival rate of the carp decreased to 75 percent. These sublethal concentrations of nitrite were found to decrease growth rate and alter energy metabolism (Alcaraz and Espina, 1997). Survival of silver perch was not affected by nitrite-nitrogen levels in water up to 16.2 mg/L, but growth was reduced when levels exceeded 1.43 mg/L (Frances et al., 1998).

TISSUE LEVELS

There is limited reported research on the effects of nitrates or nitrites in feed or water on tissue or milk nitrate and nitrite concentrations. However, due to the rapid excretion of nitrate and nitrites in urine and feces (Walker, 1990; Lewicki, 1994), accumulation in tissues is not expected. Available research reports no correlation between nitrate concentrations in diet or water and tissue nitrate levels in pigs (R = .1 and R = .2, respectively) (Eleftheriadou et al., 2002) and no correlation between nitrate concentration in water and the nitrate concentration of milk from cows (R = .2) (Nijhuis et al., 1982). Wright and Davidson (1964) dosed cows with nitrate up to 150 mg/kg BW/day for 6–12 months. Cows not dosed with nitrate and cows dosed at the highest nitrate level had milk nitrate concentrations of 1.1 and 4.5 mg/L of milk, respectively. Therefore, there is a very low risk of nitrate toxicosis, for adults or infants, from consumption of milk from cows fed moderate to high levels of nitrates.

MAXIMUM TOLERABLE LEVELS

Based on rat studies, the MTL of sodium nitrate or potassium nitrate in the diet, where no toxicological effects would be observed, is 1,823 mg nitrate/kg BW and 305 mg nitrate/kg BW, respectively (Gangolli, 1999). Establishment of MTL for other nonruminant species is difficult because of the wide array of diets and other conditions that affect nitrate toxicity. In ruminants, diets containing more than 5,000 mg/kg nitrate, DM basis, have the potential of resulting in nitrate toxicosis. The rumen bacteria act as an excellent buffer against nitrate toxicosis, but also complicate the derivation of a definitive MTL under all dietary conditions. However, for all species, the MTL is a sum of both diet and water concentration levels of nitrate. The EPA guideline of 10 mg/L of nitrate-N in drinking water appears to be a very safe MTL, as some studies have found no effects when animals were given water containing 200 times this level while other studies have reported reduced animal performance at only 20 times the EPA guideline. The National Academy of Sciences (1974) recommended an upper limit of 100 mg/kg nitrate-N and 10 mg/kg nitrite-N in the drinking water of livestock and poultry.

FUTURE RESEARCH NEEDS

Although nitrate accumulation in plants has been studied, additional research on important forage crops is needed to determine the capacity of new hybrids or varieties, including genetically modified plants, to accumulate nitrate. Additional research is needed to quantify the effects of ensiling on nitrate reduction and under what conditions maximum reduction occurs. Currently, research in this area is inconsistent. Further research on the transfer of nitrate or nitrite in consumed feed or water to tissues or milk is also needed.

SUMMARY

Animals do not require a source of nitrate or nitrite; however, they can consume a significant amount in the process of eating or drinking. Nitrate is a common water contaminant and can accumulate in plants at toxic levels when stress is encountered during growth. Nitrate itself is not very toxic; however, it becomes toxic when reduced to nitrite resulting in methemoglobinemia. Rumen bacteria rapidly convert nitrate to nitrite; therefore, ruminants are more susceptible to nitrate poisoning than nonruminants. Nitrate levels are generally low in most grains and therefore, nonruminants also are less exposed to naturally occurring nitrate toxicosis than are ruminants. Nitrate toxicosis is most likely to occur naturally from drinking of contaminated water and during periods of stressed plant (forage) growth such as a drought. The summation of consumed nitrate amounts from both feed and water need to be considered when encountering nitrate toxicity problems.

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TABLE 34-1 Effects of Nitrate or Nitrite Exposure in Animals

Animal	N[a]	Age	Weight	Quantity (Nitrate or Nitrite)	Source	Duration	Route	Effect(s)	Reference
Rabbits	4	8–10 mo	1–1.2 kg	Control		23–27 d	Oral, diet	Mild diarrhea	Mondal et al., 1999
	6			3,130 m nitrate/kg + 1.7 mg nitrite/kg diet	Nitrate Nitrite			Severe diarrhea, dehydration, BW loss and muscular weakness d 21–27; methemoglobin, plasma, and urinary nitrute and nitrite increased through d 27; thickening of stomach and small intestines along with degenerative changes in the liver and kidneys	
Mice	15		25–33 g	Control		12 d	Oral, water	No effect on reproductive performance or chromosomal aberrations	Shimada, 1989
	15			66.7 mg nitrite/L in water	$NaNO_2$			Average $NaNO_2$ intake = 0.81 mg/mouse/day	
	12			667 mg nitrite/L in water				Average $NaNO_2$ intake = 7.3 mg/mouse/day; no effect on reproductive performance or chromosomal aberrations	
Rats	24	8 wk	N/A[b]	Control	$NaNO_2$	646 d	Oral, diet	Liver tumors were not found	Aoyagi et al., 1980
				533.6 mg nitrite/kg diet				Liver tumors found in 4.5% of rats	
				1,067.2 mg nitrite/kg diet				Liver tumors found in 26.3% of rats and 40% of liver tumors were malignant	
Rats	9	2 mo	N/A[b]	Control		14 mo	Oral, water	Mortality rate was 44%; no effect on BW (556 g at 14 mo); milk lung lesions in 20% of rats	Chow et al., 1980
	12			1,334 mg nitrite/L in water	$NaNO_2$			Mortality rate was 60%; decreased BW at 14 mo (457 g); severe lung lesions in 100% of rats; blood vitamin E was 57% lower than control	
	10			2,916 mg nitrate/L in water	$NaNO_3$			Mortality rate was 58%; decreased BW at 14 mo (494 g); moderate lung lesions in 100% of rats; blood vitamin E was 29% lower than control	

continued

TABLE 34-1 Continued

Animal	N[a]	Age	Weight	Quantity (Nitrate or Nitrite)	Source	Duration	Route	Effect(s)	Reference
Rats	8	1 mo	N/A[b]	Control		16 wk	Oral, water	No effect on mortality rate, BW, liver, spleen, heart, or kidney weight	Chow et al., 1980
				133.4 mg nitrite/L in water	$NaNO_2$			Increase in lung weight	
				291.6 mg nitrate/L in water	$NaNO_3$			Increase in lung weight	
Rats	18	1 mo	N/A[b]	Control (vit E-deficient diet)		9 wk	Oral, diet	No effect on mortality; elevation of creatine phosphokinase, lactic dehydrogenase, glutamic oxalacetic transaminase, and pyruvic kinase	Chow et al., 1984
	22			667 mg nitrite/kg diet	$NaNO_2$			Mortality was 41%; marked muscular degeneration, tubular nephrosis, and eosinophilic enteritis; elevation of creatine phosphokinase, lactic dehydrogenase, glutamic oxalacetic transaminase, and pyruvic kinase	
	18			200 mg Vit E/kg diet	Vit E			No effect on mortality; mild muscular degeneration, tubular nephrosis, and eosinophilic enteritis	
	18			667 mg nitrite/kg + 200 mg Vit E/kg diet	$NaNO_2$, vit E			No effect on mortality	
Rats	6	15–20 wk	N/A[b]	Control		13 d	Oral, water	Occurrence of chromosomal aberrations in metaphase cells of bone marrow = 3.3% and 1.75% in transplacentally exposed embryos	El Nahas et al., 1984
	7			833.8 mg nitrite/L in water	$NaNO_2$			Significantly more chromosomal aberrations (chromatid breaks, centric fusions) in bone marrow metaphase cells in adults (9.6%) and in the liver of transplacentally exposed embryos (8.62%)	

Rats	20	5–6 wk	150–180 g	Control		2 yr	Oral, (diet) reduced protein in diet	Highest incidence of lymphomas, leukemias, and testicular interstitial cell tumors	Grant and Butler, 1989
	50			1,334 mg nitrite/kg diet	$NaNO_2$			Decreased red blood cell count, hematocrit, and hemoglobin	
	50			3,335 mg nitrite/kg diet				Decreased BW gain, red blood cell count, hematocrit, and hemoglobin	
Rats	6	10–12 wk	N/A[b]	Control	$NaNO_3$	14 d	Oral, water	Occurrence of chromosomal aberrations (chromatid and isochromatid gaps and breaks and acentric fragments) in bone marrow metaphase cells was 3.33%	Luca et al., 1985
				57.6 mg nitrate/L in water				Increased chromosomal aberrations in bone marrow metaphase cells (7%)	
				172 mg nitrate/L in water				Increased chromosomal aberrations in bone marrow metaphase cells (8.33%)	
				515.4 mg nitrate/L in water				Increased chromosomal aberrations in bone marrow metaphase cells (10.6%)	
				1,545.5 mg nitrate/L in water				Increased chromosomal aberrations in bone marrow metaphase cells (10.33%)	
Rats	100	8 wk	~170 g	Control		2 yr	Oral, water		Maekawa et al., 1982
				833.8 mg nitrite/L in water	$NaNO_2$			Total nitrite consumed = 17 g/rat; no effect on incidence of tumors	
				1,667.5 mg nitrite/L in water	$NaNO_2$			Total nitrite consumed = 30 g/rat; no effect on incidence of tumors	
				Control			Oral, diet		
				18,225 mg nitrate/kg diet	$NaNO_3$			Total nitrate consumed = 277 g/rat; no effect on incidence of tumors	
				36,450 mg nitrate/kg diet	$NaNO_3$			Total nitrate consumed = 575 g/rat; no effect on incidence of tumors	

continued

TABLE 34-1 Continued

Animal	N[a]	Age	Weight	Quantity (Nitrate or Nitrite)	Source	Duration	Route	Effect(s)	Reference
Rats	51 (pups)	1 d (pups)	N/A[b]	Control		Dams treated during pregnancy and lactation	Oral, water	No effect of dams' treatment on olfactory homing behavior, time of eye opening, or BW in pups	Markel et al., 1989
	49 (pups)			73.2 mg nitrate/L in water	KNO_3			Accelerated maturat on of reflexes function in pups; increased locomotor activity until pups were 20 d old; in adulthood, pups had a significant learning deficit	
	31 (pups)			146.4 mg nitrate/L in water		Pups remained on dams during treatment		Accelerated maturation of reflexes function in pups; increased locomotor activity until pups were 20 d old; in adulthood, pups had a significant learning deficit	
Rats	20	13 wk	119–148 g	Control		13 wk	Oral, water		Till et al., 1988
				54 mg nitrite/L in water	KNO_2				
				162 mg nitrite/L in water					
				540 mg nitrite/L in water				Decreased water intake throughout the trial; decreased red blood cell count	
				1,620 mg nitrite/L in water				Decreased BW gains starting wk 4 and decreased water intake throughout the trial; increased methemoglobin and decreased red blood cell count; increased kidney and liver weights	

Rats	35 adults, 215 pups	Adults and pups	210-g (adults)	Control		Dams treated during pregnancy and lactation	Oral, diet (9.0%)	Pup mortality at birth (1.3%); pup mortality from 2–24 d of age	Vorhees et al., 1984
				83.4 mg nitrite/kg diet	$NaN0_2$			Delayed pup swimming development; reduction in pup eye weights	
				166.8 mg nitrite/kg diet		Pups remained on dams treatment		Increased pup mortality at birth (6.2%); delayed pup swimming development; reduction in pup eye weights	
				333.5 mg nitrite/kg diet				Increased pup mortality from 2–24 d (14.8%); delayed pup swimming development	
Rats	8 dams, 64 pups		~ 190-g adults	Control		38 d, dams	Oral (water), dams treated and pups suckled dams	Pup mortality was 2% d 21	Roth et al., 1987
	10 dams, 80 pups			1,334 mg nitrite/L in water	$NaNO_2$			Decreased pup BW, hemoglobin, and red blood cell counts Increased pup mortality (10% d 21).	
	10 dams, 80 pups			2,001 mg nitrite/L in water				Decreased pup BW, hemoglobin, and red blood cell counts; increased pup mortality (30% d 21)	
Rats	5 dams, 50 pups		~ 190-g adults	Control		38 d, dams	Oral, (water) dams treated and pups suckled .dams	No effects.	Roth et al., 1987
	8 dams, 80 pups			333.5 mg nitrite/L in water	$NaN0_2$				
	8 dams, 80 pups			667 mg nitrite/L in water				Decreased hemoglobin	
	6 dams, 60 pups			1,334 mg nitrite/L in water				Decreased pup BW, hemoglobin, and red blood cell counts; no effect on pup mortality	

continued

TABLE 34-1 Continued

Animal	N[a]	Age	Weight	Quantity (Nitrate or Nitrite)	Source	Duration	Route	Effect(s)	Reference
Chickens	15	6 mo	312 g	Control		4 wk	Oral. diet	BW d 28 = 522 g	Atef et al., 1991
				3,061.8 mg nitrate/kg diet	$NaNO_3$			Decreased BW; BW d 28 492 g; increased methemoglobin d 28	
				1,133.9 mg nitrite/kg diet	$NaNO_2$			Decreased BW; BW d 28 377 g; increased methemoglobin, glutamic-pyruvic transaminase, creatinine and urea d 28	
Chickens	16	1 d	~40 g	Control	$NaNO_2$	35 d	Oral, diet	No effect on BW gains (d 35 1,600 g); no effect on pulmonary hypertension	Diaz et al., 1995
				133.4 mg nitrite/kg diet					
				266.8 mg nitrite/kg diet					
				533.6 mg nitrite/kg diet					
				800.4 mg nitrite/kg diet					
				1,067.2 mg nitrite/kg diet				Decreased BW (d 35 = 1403 g); increased methemoglobin d 7–21; no effect on pulmonary hypertension	
Turkeys	20	1 d	~60 g	Control	$NaNO_2$	14 d	Oral, diet	No effect on BW (d 14 = 218.1 g); no effect on occurrence of dilatory cardiomyopathy	Diaz et al., 1995
				133.4 mg nitrite/kg diet					
				266.8 mg nitrite/kg diet					
				533.6 mg nitrite/kg diet				Decreased hemoglobin d 14	
				800.4 mg nitrite/kg diet				Decreased hemoglobin d 14; increased methemoglobin d 7–14	
				1,067.2 mg nitrite/kg diet				Decreased BW (d 14 = 188.1 g); decreased hemoglobin d 14; increased methemoglobin d 7–14; no effect on occurrence of dilatory cardiomyopathy	

Turkeys	20	1 d	~60 g	Control	$NaNO_2$	14 d	Oral, diet	No effect on BW (d 14 = 218.1 g); no effect on occurrence of dilatory cardiomyopathy	Diaz et al., 1995
				133.4 mg nitrite/kg diet					
				266.8 mg nitrite/kg diet					
				533.6 mg nitrite/kg diet				Decreased hemoglobin d 14	
				800.4 mg nitrite/kg diet				Decreased hemoglobin d 14; increased methemoglobin d 7–14	
				1,067.2 mg nitrite/kg diet				Decreased BW (d 14 = 188.1g); decreased hemoglobin d 14; increased methemoglobin d 7–14; no effect on occurrence of dilatory cardiomyopathy	
Cattle	6	8 wk	80 kg	Control		3 d	Oral, milk	No effects	Hüsler and Blum, 2001
				12.4 mg nitrate/$kg^{.75}$ BW	$NaNO_3$			Increased plasma NO_3, peaking at 50.5 mg/L; increased urinary NO_3, peaking at 8.0 mg/L	
				9.3 mg nitrite/$kg^{.75}$ BW	$NaNO_2$			Increased plasma NO_3, peaking at 10.2 mg/L; increased plasma NO_2, peaking at 0.36 mg/L; increased urinary NO_3, peaking at 7.7 mg/L	
				6.2 mg nitrate/$kg^{.75}$ BW + 4.7 mg nitrite/$kg^{.75}$ BW	$NaNO_3$, $NaNO_2$			Increased plasma NO_3, peaking at 10.9 mg/L; increased urinary NO_3, peaking at 8.9 mg/L	
Cattle	6	8 wk	80 kg	24.8 mg nitrate/$kg^{.75}$ BW	$NaNO_3$	1 d	Oral, milk	Increased plasma NO_3, peaking at 9.9 mg/L; increased urinary NO_3; no effect on plasma or urinary NO_2	Hüsler and Blum, 2001
				18.7 mg nitrite/$kg^{.75}$ BW	$NaNO_2$			Increased plasma NO_3 peaking at 12.1 mg/L; increased plasma NO_2 peaking at 1.0 mg/L; increase in urinary NO_3; no effect on urinary NO_2	
Cattle	4	~d 147 of gestation	N/A[b]	Control		1 d	Oral, gavage	No effects; maximum methemoglobin levels ranged from 3–7%	Sonderman, 1993
	7			402.6 mg nitrate/kg BW	KNO_3			Clinical signs of nitrate toxicosis observed; maximum methemoglobin levels 51–73%; no effect on abortions	

continued

TABLE 34-1 Continued

Animal	N[a]	Age	Weight	Quantity (Nitrate or Nitrite)	Source	Duration	Route	Effect(s)	Reference
Cattle	6	3rd trimester of gestation	N/A[b]	Control		2 d	Oral, gavage		Sonderman, 1993
	7			268.4 mg nitrate/kg BW	KNO_3			After d 2, 4 cows exhibited clinical signs of nitrate toxicosis; maximum methemoglobin levels ranged 22–67%; 2 cows aborted; fetal ocular fluid 100 and 240 mg/kg nitrate	
	6			d 1 = 402.6 mg nitrate/kg BW d 2 = 536.8 mg nitrate/kg BW	KNO_3			After d 2, all cows had clinical signs of nitrate toxicosis; maximum methemoglobin levels 60–78%; 1 cow aborted and 1 cow died; fetal ocular fluid 50 mg/kg nitrate	
Sheep	14	~ d 61 of gestation	N/A[b]	< 200 mg nitrate/kg diet	Nitrate: Diets fed at 1.8 kg/diet/d	~ 61 d of gestation through lambing	Oral, (diet) sorghum, Sudan grass, and alfalfa	No clinical signs of toxicosis and effect on abortions; methemoglobin increased from 2.52–8.24% at trial end	Sonderman, 1993
				5,000–7,000 mg nitrate/kg diet				Methemoglobin increased from 1.64–7.32% at trial end	
				12,000–14,000 mg nitrate/kg diet				Methemoglobin increased from 1.42–8.16% at trial end	
				19,000–20,000 mg nitrate/kg diet				No clinical signs of toxicosis and no effect on abortions; methemoglobin increased from 2.57–8.59% at trial end	

[a]Number of animals per treatment group.
[b]N/A = information was not available.

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Water as a Source of Toxic Substances

INTRODUCTION

Water is a simple compound composed of two hydrogen atoms bonded to one oxygen atom. And yet, it is the nutrient most essential to sustain life. In most nonaquatic animals, water accounts for 50 to 70 percent of BW. In neonates and very young animals, water can account for more than 80 percent of the body mass. Water is found in both intracellular and extracellular spaces and provides the solvent for the movement of nutrients, waste products, and metabolic intermediates between body compartments. Water also functions in the maintenance of body temperature. Loss of 20 percent or more of the body's water content is generally fatal to most animals.

Body water is derived from water consumed directly by drinking and eating, or from the metabolic reactions related to the oxidation of carbohydrates and fats in the body. Under most situations, metabolic water is an insignificant source of water for poultry and livestock compared to water consumed by drinking or eating. Body water is lost through urine, feces, expiration of air, and perspiration.

The amount of water an animal requires depends on many physiological and environmental factors. For most terrestrial animals, water requirements and thus intakes are affected by body size; physiological state such as gestation, growth, and lactation; health; and environmental conditions. Lactating animals have particularly high water requirements to replace the water loss through milk production. Compared to animals in cool or cold climates, animals in climatic conditions where temperatures exceed their thermal neutral zone will consume more water per day to replace losses from sweating or evaporation from the lungs and to aid in the thermal regulation of internal body temperature. Sweating and evaporative water losses will be greater in hot arid conditions than in hot humid conditions and thus, water requirements will be greater in hot arid climates. Animals experiencing health problems resulting in diarrhea or excess urination can lose significant amounts of water from the body very quickly, resulting in possible life-threatening dehydration if water and electrolyte fluids are not replaced.

Thirst is a conscious desire to drink water. The thirst center is located in the anterior hypothalamus and regulates the release of vasopressin or antidiuretic hormone. Thirst is primarily triggered by an increase in the osmolarity of the blood but may be triggered by a decrease in blood volume. Osmoreceptors in the hypothalamus are particularly affected by changes in sodium chloride. A 1–2 percent increase in osmolarity will trigger a thirst sensation, which is partly exhibited by dryness of the mouth and throat. Losses in blood volume must be quite extensive, greater than 10 percent, to trigger thirst.

Because water is an essential nutrient for animals and an excellent solvent for minerals and other compounds, it can be a medium for consumption of excess minerals, undesirable minerals, and toxic substances. The purpose of this chapter is to make readers aware of the complexity of mineral forms that can be found in water, review the sources of minerals and toxic compounds in water, and briefly summarize water-mineral toxicities discussed in the various individual chapters throughout the book.

SOURCES OF MINERALS AND TOXIC SUBSTANCES IN WATER

Water contains many different elements and compounds beyond its basic hydrogen and oxygen structure. The high boiling point and heat of vaporization, a high surface tension, a density that is maximized at 4°C and not the freezing point, and a physical property of expanding on freezing are characteristics that make water an excellent solvent and reactive with the salts and polar molecules through which water passes. The exchange of elements between water and its environment is a very complex system involving atmospheric chemistry, water chemistry, sediment geochemistry, soil chemistry, kinetics, and residence time of the water in an aquifer or holding location. Thus, the elements found in

water will exist in a variety of oxidation states, protonated and nonprotonated forms, as free ions, and as complex ion forms. The mineral or element composition of ground water is affected by the composition of the rock through which water passes or in which it is stored, its residence time in the aquifer, the solubility of the mineral elements in the rock, the soil types through which the water passes to enter the aquifer, the original mineral composition of the rain water or water entering the aquifer, and the pH of the water. Mineral composition of surface water is affected by many of the same factors that affect ground water, as well as by airborne pollutants, dry windblown solid depositions, decaying organic matter, and removal of minerals by vegetation growth.

The sources and concentrations of many elements found in unpolluted fresh water and in sea water are presented in Table 35-1 and Table 35-2, respectively. However, as shown in many of the individual mineral chapters, the reactivity and toxic level of mineral elements are related to the form in which the mineral exists. Thus, knowing only the concentration of a mineral in water is an incomplete description as to the toxicity and the availability of the mineral.

The form of a mineral or element in water is referred to as speciation. An element can exist in water as a simple hydrated ion, as a molecule, as a complex with another ion or molecule, and as many other additional complexes (Stumm and Morgan, 1996). A list of the major mineral species found in fresh and salt water is given in Table 35-3. In fresh water, hydroxo and carbonate complexes are the predominant form of many minerals. While the assumption is that most minerals occur in a dissolved state in water, they often can exist as very minute, or colloidal, suspended particles. Many colloidal precipitates, such as $Fe(OH)_3$, are small enough to pass through filters and thus are not recognized as particulate matter, but as a dissolved substance.

Most water analyses only provide information related to the total amount of a mineral in the water and do not provide any indication as to the speciation.

For many of the major cation minerals, the concentration of the anions (S, Cl) and pH will provide an indication as to speciation. For example, magnesium is usually found in water in the ion state (Mg^{++}), but $MgSO_4$ predominates when sulfate exceeds 1,000 mg/L, and $MgOH^+$ occurs when the solution is very basic (pH >10). Iron is usually found in water as ferrous (Fe^{++}), but oxidation state and complexing with carbonate will vary with pH. At a pH <7, iron is commonly found as Fe^{+++}, $FeOH^{++}$, and $Fe(OH)_2^+$; at a pH of 9.5 iron is found as $Fe(OH)^+$; and at a pH >11, iron is found as $Fe(OH)_3^+$ and $HFeO_2^-$. For both iron and manganese, when sulfate levels in water increase above 200 mg/L, they increasingly complex with sulfate, and the major form of these two minerals in water will be $FeSO_4$ and $MnSO_4$.

The accuracy of the measurement of major minerals in water can be estimated by computing an Electro Neutrality (EN, percent) value. The sum of the positive and negative charges in water should balance as water must be at electrical neutrality. When concentrations of cations Na^+, K^+, Mg^{++}, and Ca^{++}, and anions Cl^-, HCO_3^-, SO_4^{2-}, NO_3^-, are expressed as mEq/L, the following formula can be used to check the accuracy of the analysis:

$$\text{EN, } \% = \left[\frac{(\text{Sum cations} + \text{Sum anions})}{(\text{Sum cations} - \text{Sum anions})}\right] \times 100$$

Differences in EN up to 2 percent are uncontrollable error, but errors above 5 percent indicate sampling or analytical problems. An exception may be when the water is very high in iron, ammonia, or acidity, as these factors are not considered in the formula (Appelo and Postma, 1999).

MINERALS AND TOXIC SUBSTANCES IN WATER

Terrestrial Animals

Table 35-4 lists the Environmental Protection Agency guidelines for human drinking water. These guidelines are divided into enforceable standards and secondary standards. Enforceable standards are levels that cannot be exceeded in water, and action to achieve lower levels must be taken. Secondary standards are guidelines and levels at which cosmetic (tooth or skin discoloration) or aesthetic (taste, odor, color) effects are apparent.

The human drinking water guidelines generally offer a very conservative assessment of water quality compared to the livestock guidelines presented in Table 35-4. Thus, use of human enforceable and secondary water quality guidelines should provide more than a safe guideline for livestock and poultry.

Based on the information contained in this publication, relatively few minerals naturally found in water are potentially toxic. Natural contamination levels of the materials arsenic, barium, iron, manganese, sodium chloride, sulfur, and nitrate can be high due to the geological environment through which the water passes or in which the water is contained. These levels could either be toxic or could contribute significantly to the toxicity of the mineral. Lithium, strontium, and uranium concentrations in water can also be elevated in isolated cases, but widespread high levels are not commonly found. In most situations, the naturally occurring minerals in water do not result in acute toxicosis, but lead to chronic conditions of poor animal performance or increased health problems. Naturally occurring levels of most other elements (aluminum, bismuth, boron, bromine, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, silicon, tin, and some rare earth elements) are low, and toxicosis or a significant contribution to toxicosis from water levels of these elements occurs from an exogenous contamination or pollutant. Required macro elements such as calcium, magnesium, phosphorus, and potassium are unlikely to be at levels that cause toxicosis, but are more likely

to result in aesthetic secondary standard effects. High levels of trace minerals in water (such as cobalt, copper, iron, manganese, and selenium along with salty water [sodium chloride]) have a greater potential of contributing to both a toxic level and a secondary standard aesthetic effect.

Fish

Metals are important natural components of the aquatic environment for fish and other organisms. Aquatic environments encompass both freshwater (lakes, rivers, pond, wetlands) and saline (oceans, estuaries, salt lakes, and marshes) conditions. These aquatic environments display a wide range of thermal regimes (in temperate latitudes, for example, seasonal thermal changes may be considerable, varying from freezing to 30°C or more), pH, salinity, and other chemical and physical characteristics. They are also influenced by the type and density of organisms that are found or cultured in these environments. A pond, for example, is a very different aquatic environment from a river or ocean.

Salinity is a measure of the total amount of salt found in water and is expressed as grams of salt per kg of water or parts per thousand (ppt, ‰). Freshwater salinity is usually less than 0.5 ppt. Water between 0.5 and 17 ppt is called brackish (e.g., estuaries, where river meets ocean). Seawater with salinity ranging from 30 to 40 and > 40 ppt is classified as euryhaline and hyperhaline, respectively. The dissolved ions (percentage of total salt) that make the water saline include sodium, 55.04; chlorine, 30.61; sulfate, 7.68; magnesium, 3.69; calcium, 1.16; potassium, 1.1; bicarbonate, 0.41; bromine, 0.19; borate, 0.07; strontium, 0.04; and fluorine, 0.003. Many of the ions found in seawater throughout the world are in nearly constant proportion despite the variation in total salinity. Freshwater discharge from rivers, as well as evaporation and freezing, may affect the salinity and concentration of various ions in seawater. Thus, the habitat of fish compared to terrestrial animals can vary more since the aquatic environment is subject to periodic changes in dissolved oxygen, pH, temperature, salinity, and mineral content.

Dissolved minerals in water can form complexes with inorganic and organic ligands. These complexes show a wide variation in their absorption by aquatic organisms. Some of the inorganic anionic ligands in natural waters include F^-, Cl^-, SO_4^{-2}, OH^-, and HCO_3^{-2}. In oxic waters, CO_3^{-2}, HPO_4^{-2} and NH^-_3 are important, whereas HS^- and S^{-2} are important in anoxic water. Free ions are highly soluble and toxic to fish. A direct correlation between the solubility of metal salts in seawater and toxicity to marine organisms has been demonstrated. Several elements such as vanadium, chromium, nickel, copper, zinc, arsenic, tin, and selenium can be present as environmental contaminants and at trace levels are toxic to fish in both freshwater and marine environments.

Metals enter the hydrosphere from either natural processes or through anthropogenic activities such as mining operations, burning of fossil fuels, and agriculture. The solubility of metals in natural waters depends upon the pH, type and concentration of ligands and chelating agents, oxidation states of the mineral components, and redox environment of the system. The soluble forms are usually ions (simple or complex) or nonionized organometallic chelates or complexes. Aquatic organisms absorb and retain these metals through the gills and body surfaces, and from ingestion of water. Exchange of ions occurs across the gills, skin, and oral epithelia in fish. Most aquatic organisms regulate the metabolism of minerals in tissues within a relatively narrow range except when the concentration reaches a nearly lethal amount. Toxicity mechanisms include the blocking of essential biological functional groups of enzymes, displacement of essential ions in the biomolecule (enzyme or protein), and modification of the active conformation of the biomolecule (Simkiss and Taylor, 1989). Sublethal effects of several minerals on fish have been listed in individual chapters.

FUTURE RESEARCH NEEDS

Water is the most critical essential nutrient for terrestrial animals and fish. Water is never pure in nature, but is a mixture of water and many dissolved or suspended minerals and other particles. Our knowledge of how these dissolved or suspended minerals affect animal or fish health and performance is very limited. Modern analytical methods have provided information on concentrations of minerals found in water, but future areas of research need to determine the availability of these minerals and their contributions to the metabolism of animals and fish. In addition, research is needed to determine if animals have the same sensitivity to the secondary water standards that are based on aesthetic and cosmetic effects as do humans. Finally, research is needed to determine if, and then to what degree, food-producing animals can concentrate potentially toxic minerals consumed through water in various tissues, especially tissues commonly consumed by humans.

SUMMARY

The impact of any mineral or compound in water is discussed in the chapter on that individual mineral or compound. Table 35-5 lists minerals and their common concentration in fresh and salt water. It does not represent toxic levels or any standards or recommended ranges of minerals, but is presented as a quick tabulated reference for evaluation of commonly reported ranges of minerals in water based on the information presented in this book.

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TABLE 35-1 Sources and Normal Ranges of Minerals in Unpolluted Fresh Water[a]

Mineral	Concentration (mg/L)	Source
Na^+	2.30–46.00	Feldspar, rock salt, zeolite, atmosphere
K^+	0.39–7.80	Feldspar, mica
Mg^{++}	1.20–49.00	Dolomite, serpentine, pyroxene, amfibole, olivine, mica
Ca^{++}	2.00–200.00	Carbonate, gypsum, feldspar, pyroxene, amfibole
Cl^-	1.80–71.00	Rock salt, atmosphere
HCO_3^-	0.00–12.00	Carbonates, organic matter
SO_4^{2-}	0.96–480.00	Atmosphere, gypsum, sulfides
NO_3^-	0.06–12.00	Atmosphere, organic matter
SiO_2	1.20–60.00	Silicates
Fe^{++}	0.00–28.00	Silicates, siderite, hydroxides, sulfides
PO_4-total	0.00–1.90	Organic matter, phosphates

[a]Appelo and Postma, 1999.

TABLE 35-2 Range or Mean of Mineral Concentrations Found in Sea Water[a]

Mineral	mg/L
Aluminum	0.0001–0.0084
Arsenic	0.0005–0.0037
Barium	0.002–0.063
Bismuth	0.000015–0.00002
Boron	4.44
Bromine	67.3
Cadmium	0.00001–0.0094
Calcium	412
Chloride	19,350
Chromium	0.0002–0.05
Cobalt	0.00001–0.0041
Copper	0.00005–0.0121
Fluorine	1.30
Iron	0.00003–0.07
Lead	0.00003–0.013
Magnesium	1,290
Manganese	0.00003–0.021
Mercury	0.00001–0.00022
Molybdenum	0.004–0.01
Nickel	0.00013–0.043
Phosphorus	0.06–0.088
Potassium	399
Selenium	0.000052–0.0002
Silicon	2.20–2.90
Salt (NaCl)	10,770
Sulfur	905
Tin	0.000002–0.00081
Vanadium	0.0009–0.0025
Zinc	0.0002–0.048
Strontium	7.00–8.50
Uranium	0.00004–0.006

[a]Bowen, 1979.

TABLE 35-3 Major Mineral Species Found in Fresh and Sea Water[a]

Condition	Element	Species
Hydrolyzed, anionic	B3+	H_3BO_3, $B(OH)_4^-$
	V5+	HVO_4^{2-}, $H_2VO_4^-$
	Cr6+	CrO_4^{2-}
	As5+	$HAsO_4^{2-}$
	Se6+	SeO_4^{2-}
	Mo6+	MoO_4^{2-}
	Si4+	$Si(OH)_4$
Predominantly free aquo ions	Li	Li^+
	Na	Na^+
	Mg	Mg^{2+}, $MgCO_3$
	K	K^+
	Ca	Ca^{2+}, $CaSO_4$
	Sr	Sr^{2+}
	Cs	Cs^+
	Ba	Ba^{2+}
Complexation with OH^-, CO_3^{2-}, HCO^-, Cl^-	Be2+	$BeOH^+$, $Be(OH)_2$
	Al3+	$Al(OH)_3$, $Al(OH)_2^+$, $Al(OH)_4^-$
	Ti4+	TiO_2, $Ti(OH)_4$
	Mn4+	MNO_2
	Fe3+	$Fe(OH)_3$, $Fe(OH)_2^+$, $Fe(OH)_4^-$
	Co2+	Co^{2+}, $CoCO_3$
	Ni2+	Ni^{2+}, $NiCO_3$, NiCl
	Cu2+	$CuCO_3$, $Cu(OH)_2$
	Zn2+	Zn^{+3}, $ZnCO_3$, ZnCl
	Ag1+	Ag^+, AgCl
	Cd2+	Cd^{2+}, $CdCO_3$, $CdCl_2$
	Hg2+	$Hg(OH)_2$, $HgCl_4^{2-}$
	Pb2+	$PbCO_3$, $PbCl^+$, $PbCO_3$
	Bi2+	$Bi(OH)_3$

[a]Stumm and Morgan, 1996.

TABLE 35-4 Drinking Water Standards for Humans and Livestock

Chemical (in mg/L)	EPA - Human MCL[a]	NRC - Livestock[b]	Canadian - Livestock[c]
		Enforceable Standards	
Arsenic	0.01	0.2	0.5
Barium	2.0		
Cadmium	0.005	0.05	0.02
Chromium	0.1	1.0	1.0
Cobalt		1.0	1.0
Copper	1.3	0.5	1.0 – cattle 0.5 – sheep 5.0 – swine
Lead	0.015	0.1	0.1
Mercury	0.002	0.001	0.003
Nitrate – nitrogen	10.0	440	100
Nitrite – nitrogen	1.0	33	10
Selenium	0.05		0.05
Chemical (in mg/L)	EPA - Human MCL[a]	NRC - Livestock[b]	Canadian - Livestock[c]
		Secondary Standards	
Aluminum	0.2		5.0
Chlorine	250		
Copper	1		
Fluoride	2	2.0	2.0
Iron	0.3		
Manganese	0.05		
Silver	0.1		
Sulfate	250		1,000
Total dissolved solids	500		3,000
Vanadium	0.01	0.01	0.01
Zinc	5	25.0	25.0
pH	6.5 – 8.5		

[a]Maximum Contaminate Level (MCL), EPA, 2004.
[b]NRC, 1974.
[c]Canadian Council of Ministries of the Environment, 1987.

TABLE 35-5 Range or Mean of Mineral Concentrations Found in Fresh and Salt Water[a]

	Fresh Water	Salt Water
Mineral	mg/L	
Aluminum	0.01–2.25	
Arsenic	1–10	
Barium	40–60	
Bismuth	< 0.02	0.001
Boron	< 0.4	4.6
Bromine		
Cadmium	< 0.001	
Calcium		
Chromium		
Cobalt		
Copper	0.001–0.02	0.015
Fluorine	0.02–1.5	1.2 –1.5
Iodine	0.002–0.004	0.04–0.06
Iron		
Lead	0.004	
Magnesium		
Manganese	0.016	
Mercury	0.001–0.003	0.0005–0.003
Molybdenum	0–0.004	0.008
Nickel	0.01	
Phosphorus		
Potassium		
Selenium	0.001–0.003	0–0.001
Silicon	0.8–44	
Salt (NaCl)		
Sulfur		
Tin	0.001–0.002	0.00025
Vanadium	< 0.003	< 0.003
Zinc		
Strontium	< 0.5	
Uranium	0.028	
Nitrate		

[a]Compiled from information reviewed in this publication.

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