The *Pesticides Monitoring Journal* is published quarterly under the auspices of the WORKING GROUP, Subcommittee on Pesticides, President's Cabinet Committee on the Environment, and its Panel on Pesticide Monitoring as a source of information on pesticide levels relative to man and his environment.

The WORKING GROUP is comprised of representatives of the U. S. Departments of Agriculture; Defense; the Interior; Health, Education, and Welfare; State; and Transportation.

The Pesticide Monitoring Panel consists of representatives of the Agricultural Research Service, Consumer and Marketing Service, Federal Extension Service, Forest Service, Department of Defense, Fish and Wildlife Service, Geological Survey, Federal Water Pollution Control Administration, Food and Drug Administration, Environmental Health Service, Department of Defense, National Science Foundation, and Tennessee Valley Authority.

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Pesticide monitoring activities of the Federal Government, particularly in those agencies represented on the Pesticide Monitoring Panel which participate in operation of the national pesticides monitoring network, are expected to be principal sources of data and interpretive articles. However, pertinent data *in summarized form*, together with interpretive discussions, are invited from both Federal and non-Federal sources, including those associated with State and community monitoring programs, universities, hospitals, and nongovernmental research institutions, both domestic and foreign. Results of studies in which monitoring data play a major or minor role or serve as support for research investigation also are welcome; however, the *Journal* is not intended as a primary medium for the publication of basic research. Manuscripts received for publication are reviewed by an Editorial Advisory Board established by the Monitoring Panel. Authors are given the benefit of review comments prior to publication.

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EDITORIAL

Change in Sponsorship for the Pesticides Monitoring Journal

Readers will see reflected in this issue a change in organizational support for the Pesticides Monitoring Journal. The stage was set for this change by a White House announcement of November 20, 1969, stating that the Federal Committee on Pest Control (FCPC) would be replaced by a working group of a cabinet-level Committee on Pesticides. Rapid developments early in 1970 resulted in the announced organization, with certain name redesignations. Thus, the FCPC was replaced by the Working Group of the Subcommittee on Pesticides of the Cabinet Committee on the Environment.

The FCPC (originally the Federal Pest Control Review Board) was formed in 1961 through the agreement of the Secretaries of USDA, USDI, DHEW and DOD. Its role was purely advisory. It operated by consensus, with the deliberateness inherent in that approach. No credit was sought, lest it detract from the pride of accomplishment by participating agencies; if there was an exception to this policy of reticence, it was the FCPC's name on this Journal.

The White House announcement described the general functions of the Working Group as provision of day-to-day coordination and the development of program and policy proposals. Its charter will be published in the Federal Register. The organizational position of the Working Group is obviously quite different from that of the former FCPC. While too early for a definitive assessment of its effectiveness, a direct pathway for action has been afforded.

The Working Group has to date taken several forthright actions. One of the first was a review of the status of the Pesticides Monitoring Journal and consideration of its continued support. Based on well-documented data presented by Mrs. Sylvia O'Rear, Editorial Manager, the Working Group agreed to sponsor continued publication of the Journal. Also, the former Subcommittee on Monitoring is to be continued as the Panel on Pesticide Monitoring of the Working Group; its first task will be a presentation to the Working Group of a somewhat revised National Pesticide Monitoring Program.

The importance of monitoring for pesticides has been evidenced in a number of reports, including two issued in 1969: the Report of the Committee on Persistent Pesticides (Jensen) and the Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health (Mrak). Work is underway for a global network for environmental monitoring under the auspices of the International Biological Program. Public and Congressional interest in the quality of the environment is more acute than ever before. The Working Group will make every effort to be responsive to this climate and to support the objectives of the Pesticides Monitoring Journal.

George L. Hutton
Chairman
Working Group

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Insecticides in the Big Bend National Park

Howard G. Applegate [1]

ABSTRACT

Soil, vegetation, birds, rodents, and lizards from the Big Bend National Park, Texas, were analyzed for insecticides. All samples from campground areas had significant concentrations of insecticides; however, in other areas sampled, only migratory birds were found to have significant concentrations.

Introduction

National parks are unique areas in which to study the movement of pollutants. The State of Texas presented to the U. S. Government 707,895 acres of land in the Big Bend Region of Texas. The area was then designated as the Big Bend National Park and closed to all commercial enterprises in 1944. Prior to this time the area had been used for cattle grazing. Although no records are available, based on present day operations of ranches in the region, it can be assumed that few pesticides were used in the area. No chlorinated hydrocarbons have been used in the Park since its establishment in 1944. Malathion was used in limited amounts at two sites in the Park (Boquillas and Castolon) in 1966. Since chlorinated hydrocarbons are ubiquitous, it seemed of interest to determine if these as well as other compounds could be found in the Big Bend National Park. Publication of these data now will establish a base that will permit future investigations to show either an increase or decrease in pollution.

Lizards, birds, and rodents and samples of soil and vegetation were collected from the following sites in the Park (Fig. 1): Boquillas and Chisos Basin campgrounds, Castolon, Croton Spring, Glenn Spring, Maverick, Persimmon Gap, and Tornillo Flats. Another collection site, Lajitas, was located approximately 3 miles west of the Park. The sites were selected both to be representative of various ecosystems in the Park and to provide coverage of the entire Park. All collections were made in June, July, and August of 1968.

The nearest areas using insecticides are all in Mexico. They are: Santa Helena (76.3 acres), San Carlos (12.5 acres), Paso San Antonio (47.5 acres), Alamos San Antonio (57.5 acres), and Boquilla San Isidro (7.3 acres). These are all located 5-10 miles southwest of the Lajitas and Castolon sites with the exception of Santa Helena which is situated just 1 mile west of Castolon (Fig. 1). The following pesticides have been applied to cotton fields in these areas: methyl parathion, ethyl parathion, malathion, ozinphosmethyl, and DDT. The nearest area using insecticides in the United States is Redford, Tex., 80 miles northwest of the Park.

Materials and Methods

Birds were captured by shooting, and lizards and rodents were trapped. Soil samples were gathered by taking the top 2.5 cm of 1 square meter of ground surface; all rocks and organic debris were discarded and the remaining soil mixed. Plants were cut at the soil line, their leaves stripped and placed in bags. Each sample was placed in an ice chest as quickly as possible after collection. The samples were taken to Presidio, Tex., (120 miles) 3-5 days later where they were placed in deep freezers and held for 10-14 days until processing.

Just prior to processing, each sample was thoroughly mixed (soil) or diced (flora and fauna). The samples were then weighed into two equal portions of 10 g each. Tissues from several organisms were pooled to obtain the required weight. One portion was spiked by adding

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10 ml of hexane containing 5 ppm (w/v) of the insecticides that were expected to be found; 10 ml of hexane was added to the remaining portion. Both portions were then treated identically. A total of four individual samples of each specimen type were prepared from each site for analysis by gas chromatography.

The following extraction procedures were used (*3*):

SOIL

Ten grams of soil was placed in a flask, and 50 ml of hexane:acetone solution (9:1 v/v) added; after 1 hour on a wrist shaker, the solution was filtered off and the soil re-extracted with another 25 ml of the solution; the solutions were combined, concentrated in a Kuderna-Danish concentrator, cleaned up by sweeping through a Kontes codistiller, and then made to volume (1 ml of liquid = 10 g of sample). Extraction efficiency based on spiked samples ranged from 81-92% for DDT; 90-95% for DDE; 85-94% for TDE; 87-95% for methyl parathion; and 93% for ethyl parathion.

FIGURE 1.—*Map of Big Bend National Park (Some of the areas in Mexico are oversized relative to other map features to show their shape and location.)*

Name	*Elevation (Ft)*	*Name*	*Elevation (Ft)*
Lajitas	2200	Chisos Mountains	6705–7835
Castolon	2734	Chilicotal Mountain	4104
Maverick	2745	Talley Mountain	3800
Croton Spring	3504	Mariscal Mountain	3940
Chisos Basis campground	5560	Santa Helena	2734*
Glenn Spring	2605	San Carlos	2800*
Solis Spring	2135	Paso San Antonio	2800*
Boquillas campground	1873	Alamos San Antonio	2800*
Tornillo Flats	2814	Boquilla San Isidro	2800*
Persimmon Gap	2971	* Approximate elevation	

MEAT

Ten grams of meat was ground with 10 g of anhydrous sodium sulfate; the mash was extracted first with benzin and then with acetonitrile; the benzin layer was drawn off and the mash re-extracted three more times with acetonitrile (the bottom layer was drawn off each time and combined with the benzin; the combined extract was extracted three times with 2% sodium chloride; the benzin layer was then filtered through anhydrous sodium sulfate, concentrated in a Kuderna-Danish concentrator, cleaned up by sweeping through a Kontes codistiller, and then made up to volume (1 ml of liquid = 10 g of sample). Extraction efficiency based on spiked samples ranged from 85-90% for DDT; 89-95% for DDE; 84-94% for TDE; 85-95% for methyl parathion; and 85-93% for ethyl parathion.

LEAVES

Ten grams of leaves was blended with ethylacetate, filtered through glass wool, and the filtrate treated with 5 g of Nuchar; after filtering, crystalline sodium chloride was added to the filtrate and the top layer concentrated in a Kuderna-Danish concentrator, cleaned up by sweeping through a Kontes codistiller, and then made up to volume (1 ml of liquid = 10 g of sample). Extraction efficiency based on spiked samples ranged from 93-95% for DDT; 94-95% for DDE; 90-94% for TDE; 91-95% for methyl parathion; and 89-93% for ethyl parathion.

Solvents were cleaned up by the methods of Burke and Giuffrida (*4*). All values reported in this paper were corrected for percentage loss during extraction and clean-up.

Two gas chromatographs were used in this study. The MicroTek 2500R was equipped with a nickel-63 detector. The operating parameters were: nitrogen gas flow—50 ml/minute; temperatures—column 190 C, inlet 200 C, outlet 230 C, detector 270 C; power supply voltage, 54 v; pulse width, 9 microseconds; pulse rate, 30 microseconds. The column was ¼" x 6', glass, packed with a 50:50 (w/w) mixture of 7% OV-17 and 9% QF-1 on 80/100 acid-washed, silane-treated Chromosorb W. The Barber-Colman was equipped with an automatic injection device. The device and all operating conditions have been described previously (*2*). Quantification for both instruments was by use of a digital integrator. In a 2-μl injection, 0.05 ppm could be quantified.

Samples to be analyzed by mass spectrometry were collected off the gas chromatograph. After establishing retention times for the compounds, the column was detached from the detector. A sample was injected and, at the appropriate time, a glass-wool collecting device placed on the open end (*8*). The samples were washed from the glass wool with hexane. The hexane was evaporated to near dryness and collected in capillary tubes. A model 21-110B Consolidated Electrodynamics Corporation Mass Spectrometer was used for analysis of standard and collected samples.

Thin-layer chromatography was used as an additional confirmatory tool. Extracts were spotted or streaked on silica gel HF 254 and hexane:ether (100:1 v/v) was used as the solvent. Compounds were located under ultraviolet radiation and their R_f's compared to those of standards on the same thin-layer plate. Silica gel containing the sample was scraped off, eluted with hexane, and the eluate injected into the gas chromatographs, both "as is" and spiked with standards.

Results

The data for soils are presented in Table 1. Lajitas had by far the highest concentrations. Boquillas campground, Castolon, and the Chisos Basin campground had lower concentrations; all three sites had similar values. Soils from other sites had only trace concentrations of the insecticides.

TABLE 1.—*Concentrations of insecticides in surface soil*

Site	Residues in PPM [1]				
	Methyl Parathion	Parathion	DDE	TDE	DDT
Boquillas	0.49	0	1.00	0.99	0.81
Castolon	0.15	0	0.84	0.37	0.70
Chisos Basin	0	0	0.99	0.48	2.39
Croton Spring	0	0	0	0	0
Glenn Spring	0	0	0.01	0.01	0.01
Lajitas	6.34	0	8.55	8.04	10.47
Maverick	0.19	0	0.14	0	0.11
Persimmon Gap	0.01	0.01	0.01	0.01	0.01
Tornillo Flats	0.01	0	0.03	0	0.04

[1] Results are the means of four samples per site.

The data for vegetation, leatherstem leaves, *Jatropha dioica* var. *dioica* Sesse *ex* Cerv. are presented in Table 2. The same pattern of distribution appeared as for the soils. Lajitas had the highest concentrations followed by Boquillas campground, Castolon, and the

TABLE 2.—*Concentrations of insecticides in leatherstem*

Site	Residues in PPM [1]				
	Methyl Parathion	Parathion	DDE	TDE	DDT
Boquillas	0.04	0.01	0.99	0.88	1.15
Castolon	0	0	0.91	0.80	1.07
Chisos Basin	0	0	0.93	0.80	0.84
Croton Spring	0	0	0	0	0
Glenn Spring	0	0	0.01	0.03	0.06
Lajitas	0.09	0	3.09	2.07	2.08
Maverick	0.01	0	0.51	0.03	0.04
Persimmon Gap	0	0	0.01	0.02	0.01
Tornillo Flats	0.01	0	0.03	0	0.04

[1] Results are the means of four samples per site.

Chisos Basin campground with closely grouped values. The remaining sites had trace amounts except for Maverick which contained 0.51 ppm DDE.

The data for the rodents are for muscle tissue only (Table 3). Several species of rodents were collected: *Perognathus penicillatus* Woodhouse, *Sigmodon hispidus* Say and Ord, *Citellus variegatus* Erxleben, and *Citellus mexicanus* Erxleben. Not unexpected, the pattern of distribution followed the patterns for soils and leatherstem. Lajitas had by far the greatest concentrations. Closely grouped with lesser values were Boquillas campground, Castolon, and the Chisos Basin campground. The remaining sites had only trace amounts.

Data for concentrations in whole lizards are presented in Table 4. The following species were collected: *Uta*

TABLE 3.—*Concentrations of insecticides in muscle tissue of rodents*

Site	Number of Specimens	Residues in PPM				
		Methyl Parathion	Parathion	DDE	TDE	DDT
Boquillas						
P. penicillatus	3	1.24	0.04	1.05	1.10	1.37
C. mexicanus	1	1.20	0	0.95	1.16	1.43
Castolon						
P. penicillatus	2	0.34	0	1.25	1.35	1.06
C. mexicanus	1	0.40	0	1.27	1.40	1.06
S. hispidus	1	0.39	0	1.24	1.30	1.16
Chisos Basin						
S. hispidus	2	0	0.11	1.66	1.86	1.00
C. variegatus	2	0.06	0	1.65	1.94	1.12
Croton Spring						
P. penicillatus	3	0.88	0	0.08	0.01	0.01
C. mexicanus	1	0	0	0	0	0
Glenn Spring						
P. penicillatus	2	0	0	0	0	0.01
C. mexicanus	2	0.11	0	0.16	0	0
Lajitas						
P. penicillatus	3	3.19	0	3.26	3.99	4.31
C. mexicanus	1	3.23	0	3.00	3.81	4.13
Maverick						
S. hispidus	2	0	0	0.09	0.01	0.04
C. variegatus	1	0.01	0	0	0	0.04
C. mexicanus	1	0	0	0	0	0
Persimmon Gap						
P. penicillatus	2	0.07	0	0.04	0	1.39
C. variegatus	1	0	0	0.06	0.04	0
C. mexicanus	1	0	0	0.05	0.04	0
Tornillo Flats						
P. penicillatus	4	0	0	0.01	0.01	0.06

TABLE 4.—*Concentrations of insecticides in whole lizards*

Site	Number of Specimens	Residues in PPM				
		Methyl Parathion	Parathion	DDE	TDE	DDT
Boquillas						
C. tigris	3	0.55	0.01	0.93	0.77	0.84
U. ornatus	1	0	0	1.05	0.69	0.82
Castolon						
U. stansburiana	4	0.07	0	0.77	0.54	0.16
Chisos Basin						
C. tigris	2	0.51	0	0.70	0.58	0.66
C. septemvittatus	1	0	0.01	0.61	0.63	0.62
U. ornatus	1	0	0	0.69	0.71	0.57
Croton Spring						
U. stansburiana	4	0	0	0	0.03	0.01
Glenn Spring						
C. tigris	2	0.05	0	0.04	0.06	0.04
C. texanus	2	0.05	0	0.10	0.04	0
Lajitas						
U. ornatus	3	0.70	0.10	1.61	1.40	1.50
C. texanus	1	0.52	0	1.69	1.50	1.32
Maverick						
C. tigris	2	0.01	0	0.27	0.18	0
U. ornatus	1	0	0	0	0	0.64
C. septemvittatus	1	0	0	0	0	0
Persimmon Gap						
U. stansburiana	2	0	0	0.13	0	0
C. septemvittatus	1	0.01	0	0	0	0
C. tigris	1	0	0	0	0	0
Tornillo Flats						
C. texanus	3	0	0	0.12	0	0.18
C. tigris	1	0	0	0	0	0

TABLE 5.—*Concentrations of insecticides in bird muscle*

Site	Number of Specimens	Residues in PPM				
		Methyl Parathion	Parathion	DDE	TDE	DDT
Boquillas						
N. borealis—M [1]	2	7.54	0	4.64	0.31	2.29
Dendrocia spp.—M	1	7.00	0	4.44	0.58	2.73
C. squamata—P [1]	1	0.01	0	0.01	0	0.01
Castolon						
A. bilineata—P	4	0	0	0.09	0.14	0.19
Chisos Basin						
P. sinuata—P	4	0.03	0.03	0.11	0.03	0.03
Croton Spring						
C. mexicanus—P	2	0	0	0.19	0.04	0.08
A. ultamarina—P	2	0	0	0	0	0.12
Glenn Spring						
C. mexicanus—P	4	0	0	0.03	0	0.01
Lajitas						
P. pyrrhonota—M	1	0.04	0.04	10.80	9.93	7.35
P. sinuata—P	2	0	0	0.92	0	0.33
A. bilineata—P	1	0.02	0.02	0.72	0.43	0.41
Maverick						
I. parisorum—M	1	0.11	0.21	1.05	10.89	9.37
A. bilineata—P	3	0.08	0.12	0.54	0.46	0.47
Persimmon Gap						
P. sinuata—P	1	0	0	0.01	0	0
C. mexicanus—P	1	0	0	0	0	0.08
A. ultramarina—P	1	0	0	0	0	0
C. squamata—P	1	0.01	0	0	0	0
Tornillo Flats						
Dendrocia spp.—M	3	0.11	0.01	0.91	0.52	2.57
C. mexicanus—P	1	0	0	0.11	0	0.12

[1] M—Migrant; P—Permanent.

stansburiana Baird and Girard, *Urossaurus ornatus* Baird and Girard, *Cophosaurus texanus* Trosche, *Cnemidophorus tigris*, and *Cnemidophorus septemvittatus*. Once again, specimens from Lajitas had the greatest concentrations. Specimens from Boquillas campground, Castolon, and the Chisos Basin campground had similar concentrations which were less than those found at Lajitas. Specimens from the remaining sites had only trace amounts.

Data for concentrations in bird muscle are presented in Table 5. Migrant species contained the higher concentrations; those collected were *Nuttallornis borealis* Swainson, *Dendroica* spp., *Petrochelidon pyrrhonota* Vieillot, *Icterus parisorum* Bonaparte. Those species which were permanent residents in the Park had low concentrations; they were *Amphispiza bilineata* Cassin, *Pyrrhuloxia sinuata* Bonaparte, *Carpodacus mexicanus* Muller, *Aphelocoma ultramarina* Bonaparte, and *Callipepla squamata* Vigors. Results for resident birds showed that Lajitas, the Boquillas and Chisos Basin campgrounds, Castolon, as well as Maverick had organisms with higher concentrations of insecticides than the remaining sites.

Discussion

In this study very few specimens were gathered over a very large area. Obtaining and storing enough ice to keep the samples frozen until they could be placed in deep freezers was the chief limitation. Some of the collecting sites were 100 miles (round trip) from the nearest ice source. The desert temperatures were over 100 F in most afternoons during the collecting period.

When the sites were selected, it was anticipated that Lajitas and Castolon would have the highest concentrations due to their proximity to sprayed areas. It was also felt that all other sites would show trace concentrations if any insecticide residues were found at all, based on the distances of the sites from areas of insecticide application and orography of the study Park areas.

Our data show that Lajitas or Castolon usually contained more insecticides per sample than all other sites combined. Concentrations found at Boquillas campground and the Chisos Basin campground were unexpected. Although the elevation and ecosystem of the Boquillas campground is similar to Lajitas and Castolon, Boquillas is located some distance from the cotton-growing areas in Mexico where insecticides were used. As can be seen from Fig. 1, the Chisos Basin campground is literally a basin with a rim 2,000 feet above it that would serve as a shield from pesticides applied 3,000 feet below.

All four sites are used by people. They either contain camping sites (the Park areas) or a trading post (Lajitas). The Chisos Basin is heavily used during the warm months, while Boquillas and Castolon are used during the colder months. Lajitas is used all year long with heavy tourist use in the summer months. It would seem, therefore, that the presence of insecticides can be linked to the presence of people and not to drift from cotton fields.

It is possible that the insecticides found were applied by campers. The amounts needed to be used by the campers to reach the DDT soil concentrations reported here can be calculated if it is assumed that the insecticide values are characteristic for the total camping area; that an acre of soil 3 inches deep weighs 1 million pounds (a figure commonly used in agronomy texts); and that a pressurized spray can contains 0.9% DDT. (The last assumption is based on a survey made at local markets.) The size of the three camping sites and the number of persons using each one during the camping season of June, July, and August 1968 are as follows: Chisos Basin—10 acres and 29,288 persons; Castolon—6.5 acres and 738 persons; Boquillas—15 acres and 13,895 persons.

The following data were calculated: Chisos Basin contained 17,458 g of DDT in the soil which would require 4,367 cans containing 0.9%, or 1.4 cans per person. Castolon contained 5,630 g of DDT in the soil which would require 1,408 cans containing 0.9% DDT, or 1.9 cans per person. Boquillas contained 19,050 g of DDT in the soil which would require 4,762 cans containing 0.9% DDT, or 0.3 cans per person. No information is available on family groups versus lone campers or trailer users versus open-air campers.

No data are available on the people who visited the Lajitas trading post (population 15). Undoubtedly, several hundred tourists per year stop while on their way to or from the ghost town of Terlingua. Lajitas is situated in a cul-de-sac opening to the southwest. The prevailing winds are southerly and southeasterly. While it is possible that drift from the cotton fields blew over the mesas to Lajitas, it does not seem probable. Lajitas is used by Mexicans; however, it is not an authorized Mexican port of entry for insecticides. Unauthorized movement of insecticides across the border may have occurred; if so, then haste in handling the sacks at night may have resulted in spillage.

The amounts of insecticides found at Lajitas are similar to those found adjacent to cotton fields at Presidio, Tex. (*1,5,6,7*). Concentrations found at Boquillas campground, Castolon, and the Chisos Basin campground are similar to those found 3 miles from the cotton fields at Presidio, while concentrations at the other sites in the Big Bend National Park are similar to those found more than 9 miles from the cotton fields at Presidio. The relative proportions of TDE to DDE and DDT are also similar to those found in the Presidio Basin.

The differences in concentrations between migratory and resident birds in the Park may indicate that the migratory birds ingested the pesticides elsewhere. However, the listed migratory species are predominantly insectivorous while the species listed as resident are predominantly seed eaters. Thus, the concentration differences may reflect dietary habits. If this is true, then the compounds could have been ingested in the Park.

The data showing no species differences in insecticide concentrations in the lizards and the rodents supports data gathered from the Presidio Basin. Previous studies have shown that organisms occupying similar niches within an ecosystem had similar insecticide concentrations when similar tissues were compared (*5,6*). Care must be used in interpreting data from whole organisms; whole gravid female lizards had higher insecticide concentrations than whole non-gravid female lizards. However, when muscle versus muscle comparisons were made, there were no differences between a gravid and a non-gravid female lizard. Results of an earlier study showed that higher concentrations in the whole gravid lizards were due solely to insecticide accumulations in the lipids of the developing eggs (5).

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See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(*1*) *Applegate, H. G. 1966.* Pesticides at Presidio. II. Vegetation. Tex. J. Sci. 18:266-271.

(*2*) *Applegate, H. G. and G. Chittwood. 1968.* Automation of pesticide analysis. Bull. Environ. Contamination Toxicol. 3:211-226.

(*3*) *Burchfield, H. P. and D. E. Johnson. 1965.* Guide to the Analysis of Pesticide Residues. U. S. Dep. of Health, Educ. and Welfare, Public Health Service, Office of Pesticides, Washington, D. C. 20204.

(*4*) *Burke, J. and L. Guiffrida. 1964.* Investigations of electron capture gas chromatography for the analysis of multiple chlorinated pesticide residues in vegetables. J. Ass. Agr. Chem. 47:326-342.

(*5*) *Culley, D. D. and H. G. Applegate. 1967.* Pesticides at Presidio. IV. Reptiles, Birds, and Mammals. Tex. J. Sci. 19:301-310.

(*6*) *Culley, D. D. and H. G. Applegate. 1967.* Insecticide concentrations in wildlife at Presidio, Texas. Pesticides Monit. J. 1:21-28.

(*7*) *Lahser, C. and H. G. Applegate. 1966.* Pesticides at Presidio. III. Soil and Water. Tex. J. Sci. 18:387-395.

(*8*) *Mumma, R. O. and T. R. Kantner, 1966.* Identification of halogenated pesticides by mass spectroscopy. J. Econ. Entomol. 59:491-492.

Occurrence of Pesticides in Whales

Allen A. Wolman [1] and Alfred J. Wilson, Jr.[2]

ABSTRACT

Organochlorine pesticides were found in tissue samples of brain, blubber, and liver from 6 of 23 gray whales, Eschrichtius robustus, *and in each of 6 sperm whales,* Physeter catodon, *collected near San Francisco, Calif., in 1968 and 1969. Concentrations of DDT or its metabolites ranged up to 0.36 ppm in blubber tissue of gray whales, and 6.0 ppm in blubber tissue of sperm whales. The highest dieldrin concentrations were 0.075 ppm in gray and 0.019 ppm in sperm whales.*

Introduction

Residues of the chlorinated hydrocarbon insecticides, DDT and dieldrin, have been found in various forms of wildlife, mainly in fatty tissues (*5, 7*); these compounds are resistant to chemical breakdown by digestive and physiological processes in mammals, birds, and fish (*3*). Pesticide residues have been found in blubber, brain, and other body tissues of marine mammals in Antarctica (*4, 12*) and in gray seals, *Halichoerus grypus;* common seals, *Phoca vitulina;* and harbor porpoises, *Phocoena phocoena,* from the coasts of Scotland (*7*). In addition, juvenile and adult harp seals, *Phoca groenlandica,* on the Canadian Atlantic coast contained pesticides, principally of the DDT group (*7*), as did porpoises examined by Wilson in Florida (*unpublished data*). Anas and Wilson (*1*) found DDT and its metabolites and dieldrin in brain and liver samples from the northern fur seal, *Callorhinus ursinus.* Koeman and van Genderen (*8*) found 9.6 to 27.4 ppm of DDT and 0.07 to 2.30 ppm of dieldrin in harbor seals in the Netherlands. These chemicals, although they mainly affect the nervous system, may after long-term accumulation cause sterility and mortality in adults or mortality among progeny, as observed in pelicans and cormorants (*2*). They may interfere with steroid hydroxylation, resulting in the lowering of calcium deposition in eggshells (*6*); interact in polyunsaturated fatty acid metabolism, resulting in riboflavin deficiency (*13*); and cause degenerative changes in rat liver tissue (*10*). This report records the amounts of DDT and its metabolites and dieldrin found in the tissues of gray whales, *Eschrichtius robustus,* and sperm whales, *Physeter catodon.*

Sampling

Samples of brain, blubber, and liver tissues were collected from 23 gray whales taken during migration seasons in March-April 1968, December 1968-January 1969, and March 1969. Similar samples from six sperm whales were taken during May and November 1968. All of the collections were made off San Francisco, Calif. About 100 g of each tissue was collected from each animal. Tissues were frozen from the time of collection until time of analysis. Maturity and reproductive condition were determined by histological examination of the testes and mammary glands and examination of ovaries for corpora lutea and corpora albicantia.

Analytical Procedures

Liver, brain, and blubber tissues were analyzed for BHC, heptachlor, aldrin, heptachlor epoxide, toxaphene, methoxychlor, dieldrin, endrin, and the *o,p'* and *p,p'* isomers of DDE, DDD (TDE), and DDT. Tissues were thawed and mixed with anhydrous sodium sulfate in a blender. The mixture was extracted for 4 hours with petroleum

[1] Bureau of Commercial Fisheries, Marine Mammal Biological Laboratory, Seattle, Wash. 98115.

[2] Bureau of Commercial Fisheries, Biological Field Station, Gulf Breeze, Fla. 32561.

ether in a Soxhlet apparatus. Extracts were concentrated and partitioned with acetonitrile. The acetonitrile was evaporated just to dryness and the residue eluted from a Florisil column (9). Sample extracts were then identified and quantified by gas chromatographs equipped with electron capture detectors. Column packing and operating parameters were as follows:

Columns:	5′ x ⅛″, glass, packed with 3% DC-200, 5% QF-1, and a 1:1 ratio of 3% DC-200 and 5% QF-1, all on 60/80 Gas Chrom Q	
Temperature:	Detector	210 C
	Injector	210 C
	Oven	190 C
Carrier:	Prepurified nitrogen at a flow rate of 40 ml/minute	

A few samples were analyzed using thin-layer chromatography. Laboratory tests gave the following recovery rates: *p,p′*-DDE, 80-85%; *p,p′*-DDD, 92-95%; *p,p′*-DDT, 91-95%; dieldrin, 85-90%. The lower limit of sensitivity was 0.010 ppm (mg/kg, wet weight). Data in this report do not include a correction factor for percentage recovery. Polychlorinated biphenyls (PCB's) reported by Holden and Marsden (7), were not detected in our samples. All values reported were calculated on a wet weight basis.

Discussion

DDT and its metabolites were found in 4 males and 2 females of the 23 (26%) gray whales examined (Table 1). All four of the whales taken during spring 1968 contained residues at levels ranging up to 0.058 ppm. In contrast, only 2 of 19 whales sampled during the 1968-69 migration contained pesticides, but DDT and its metabolites were about 5 times more concentrated in these 2 whales; the highest level was 0.36 ppm. One male and one female gray whale had trace amounts of DDE in the brain tissue. The liver tissue of all the gray whales was free of pesticides. Dieldrin was found in all the spring 1968 samples but was lacking in the 1968-69 migration series.

Gray whales feed during the summer in the Bering and Chukchi Seas, principally in large areas of shallow water with an abundant benthos. Their food is primarily benthic amphipods, mostly *Ampelisca macrocephala*, in addition to a number of benthic isopods, mysids, mollusks, polychaetes, and hydroids (*14*, *15*). While migrating between their northern summer grounds and their winter calving grounds off Baja California and during their stay on the wintering grounds they eat virtually no food (*11*). Body weight greatly decreases during the migration, resulting in a great use of stored body fat. Because they fast during migration, gray whales undergo considerable physiological stress, which is accentuated in lactating cows, whose calves suckle for about 6 months.

All of the sperm whales sampled contained DDT and metabolites in concentrations ranging from 0.010 to 6.0 ppm (Table 2). All had DDT or its metabolites in each of the three kinds of tissue examined. Concentrations ranged up to 0.12 ppm in brain tissue, 6.0 ppm in blubber, and 0.35 ppm in liver. Dieldrin was found in two samples of blubber taken in May 1968 but was absent in all samples from November 1968.

Sperm whales are in all the world oceans; they feed mainly on squid (mostly the larger species, such as *Moroteuthis robustus* off California), octopuses, and deepwater fishes, such as sharks, skates, hake, and to a lesser extent, rockfish.

In gray whales and sperm whales the concentrations of pesticides were highest in the blubber. Since a relatively high proportion of the body weight of whales is in the form of blubber, the animals may contain high total amounts of pesticides. For instance, the estimated weight of gray whale No. 1969-35 is 14,300 kg, and possible blubber proportion is 29% (*16*), or 4,147 kg. At a concentration of 0.36 ppm DDE in its blubber (Table 1), the whale might carry about 1.5 g of DDE in this tissue.

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(1) *Anas, R. E. and A. J. Wilson, Jr. 1970.* Organochlorine pesticides in fur seals. Pesticides Monit. J. 3(4):198-200.

(2) *Anderson, D. W., J. J. Hickey, R. W. Risebrough, D. F. Hughes, and R. E. Christensen. 1969.* Significance of chlorinated hydrocarbon residues to breeding pelicans cormorants. Can. Field-Natur. 83(2):91-112.

(3) *Gakstatter, J. H. 1967.* The uptake from water by several species of freshwater fish of *p,p′*-DDT, dieldrin, and lindane; their tissue distribution and elimination rate. Diss. Abstr. B27, 11, 3820.

(4) *George, J. L. and D. E. H. Frear. 1966.* Pesticides in the Antarctic. J. Appl. Ecol. 3 (Suppl):155-167.

(5) *Greenwood, R. J., Y. A. Greichus, and E. J. Hugghins. 1967.* Insecticide residues in big game mammals of South Dakota. J. Wildlife Manage. 31:288-292.

(6) *Hickey, J. J. (editor). 1969.* Peregrine falcon populations, their biology, and decline. Univ. Wisconsin Press, Madison, Wis., 596 p.

(7) *Holden, A. V. and K. Marsden. 1967.* Organochlorine pesticides in seals and porpoises. Nature 216(5122): 1274-1276.

(8) *Koeman, J. H. and H. van Genderen. 1966.* Some preliminary notes on residues of chlorinated hydrocarbon insecticides in birds and mammals in the Netherlands. J. Appl. Ecol. 3 (Suppl.):99.

(9) *Mills, Paul A., J. H. Onley, and R. A. Gaither. 1963.* Rapid method for chlorinated pesticide residues in nonfatty foods. J. Ass. Offic. Agr. Chem. 46:186-191.

(10) *Phillips, W. E. J. 1963.* DDT and the metabolism of Vitamin A and carotene in the rat. Can. J. Biochem. Physiol. 41:1793-1802.

(11) *Rice, D. W. and A. A. Wolman.* The gray whale *Eschrichtius robustus:* Life history and ecology. J. Mammal. Spec. Publ. 3 (in press).
(12) *Sladen, W. J. L., C. M. Menzie, and W. L. Reichel. 1966.* DDT residues in Adelie penguins and a crab-eater seal from Antarctica. Nature 210(5037):670-673.
(13) *Tinsley, I. J. 1966.* Nutritional interactions in dieldrin toxicity. Agr. Food Chem. 14:563-565.
(14) *Tomilin, A. G. 1937.* Kity dal-nego Vostoka [The whales of the far east]. Uch. Zap. Mosk. Gos. Univ., Ser. Biol. Nauk, 12:119-167. (Russian with English summary.)
(15) *Zenkovich, B. A. 1937.* The food of far-eastern whales. C. R. (Dokl.) Acad. Sci. URSS, 16(4):231-234.
(16) ———— *1937.* Esche o serom kalifornisskom kite *(Rhachianectes glaucus* Cope, 1864). [More on the gray California whale *(Rhachianectes glaucus* Cope, 1864).] Vestnik Akad. Nauk SSSR, Dal'nevostochnyi Fil., 23: 91-103.

TABLE 1.—*Pesticides in brain, blubber, and liver tissues of migrating gray whales*

Direction of migration and season collected	Field number	Date collected	Length (meters)	Sex	Reproductive condition	Residues in ppm (mg/kg, wet weight)[1]											
						DDT			DDD			DDE			Dieldrin		
						Brain	Blubber	Liver	Brain	Blubber	Liver	Brain	Blubber	Liver	Brain	Blubber	Liver
Northbound																	
Spring 1968	1968-57	3-11-68	10.68	♂	Immature	0	0.026	0	0	0.034	0	0	0.040	0	0	0.051	0
	1968-58	3-11-68	11.75	♂	Mature	0	0.022	0	0	0.034	0	0	0.041	0	0	0.044	0
	1968-60	4- 2-68	10.88	♂	Immature	0	0.033	0	0	0.029	0	0	0.046	0	0	0.055	0
	1968-64	4- 5-68	9.40	♀	Immature	0	0.028	0	0	0.058	0	0	0.046	0	0	0.075	0
Southbound																	
Winter 1968-69	1968-245	12-21-68	12.85	♀	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1968-247	12-22-68	11.30	♂	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1968-252	12-29-68	13.20	♀	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1968-256	12-30-68	11.85	♂	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-2	1- 2-69	12.15	♂	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-13	1- 5-69	13.30	♀	Mature	0	0.13	0	0	0.091	0	0.011	0.30	0	0	0	0
	1969-22	1- 7-69	11.60	♀	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-23	1- 7-69	13.40	♀	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-24	1- 8-69	12.15	♀	Mature	[2]	0	0	[2]	0	0	[2]	0	0	[2]	0	0
	1969-26	1- 8-69	11.30	♀	Immature	0	0	0	0	0	0	0	0	0	0	0	0
Northbound																	
Spring 1969	1969-30	3- 2-69	11.32	♂	Immature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-31	3- 2-69	10.92	♂	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-32	3- 5-69	12.37	♂	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-33	3- 9-69	11.92	♂	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-34	3- 9-69	12.44	♂	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-35	3-10-69	11.83	♂	Mature	0	0.13	0	0	0.19	0	0.011	0.36	0	0	0	0
	1969-44	3-15-69	12.65	♀	Mature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-45	3-15-69	11.93	♀	Immature	0	0	0	0	0	0	0	0	0	0	0	0
	1969-48	3-16-69	12.92	♀	Immature	0	0	0	0	0	0	0	0	0	0	0	0

[1] 0 = pesticides not detectable (<0.010 ppm).
[2] Brain tissue not sampled.

TABLE 2.—*Pesticides in brain, blubber, and liver tissues of sperm whales*

Field number	Date collected	Length (meters)	Sex	Reproductive condition	Residues in ppm (mg/kg, wet weight)[1]											
					DDT			DDD			DDE			Dieldrin		
					Brain	Blubber	Liver	Brain	Blubber	Liver	Brain	Blubber	Liver	Brain	Blubber	Liver
1968-119	5- 9-68	12.19	♂	Mature	0.026	2.6	0.042	0.010	0.83	0.066	0.12	6.0	0.33	0	0.016	0
1968-120	5-15-68	14.94	♂	Mature	0	0.86	0.11	0	0.22	0.029	0.014	0.74	0.15	0	0.019	0
1968-228	11- 7-68	10.26	♀	Mature	0	1.2	0.044	0	0.49	0.10	0.059	4.0	0.35	0	0	0
1968-229	11- 7-68	10.77	♀	Mature	0.013	1.3	0	0	0.48	0.053	0.054	3.3	0.23	0	0	0
1968-232	11- 7-68	11.20	♀	Mature	0.014	2.1	0.028	0.012	0.59	0.042	0.073	4.4	0.19	0	0	0
1968-233	11- 7-68	10.92	♀	Mature	0.022	1.9	0.016	0	0.50	0.056	0.074	3.3	0.18	0	0	0

[1] 0 = not detectable (<0.010 ppm).

Copper Sulfate in Flooded Cranberry Bogs[1]

Karl H. Deubert and Irving E. Demoranville

ABSTRACT

Cranberry bogs are treated with copper sulfate to control algal growth. In order to assess possible water pollution after release of treated floodwater into streams and ponds, the rate at which copper disappeared from the water after treatment was monitored in two separate bogs. In both bogs the concentration of copper 25 hours after application was higher than expected due to smaller volumes of floodwater. During the first 6 days after treatment, copper concentrations decreased rapidly, and after 10 days about 95% of the copper had disappeared. When floodwater was released about 4 weeks after treatment, the concentration of copper was at the same level found in the water prior to treatment.

Introduction

Cranberry bogs are potential sources of water pollution. Dieldrin and DDT have been shown to be very persistent in bog soil (*2, 4*), and adsorption of dieldrin on organic matter suggests that floodwater may remove small quantities of this insecticide from bogs with organic colloids (*1*).

The use of copper sulfate in cranberry bogs as an algicide is common practice. Growers apply copper sulfate at a rate of 4 lb/acre-foot of water. This amount is intended to yield about 0.4 ppm copper, an amount which is toxic to fish although the limiting concentrations may vary widely.

Since the fate of copper sulfate in floodwater on cranberry bogs was not known, a monitoring study was carried out to examine the disappearance of copper from water and assess the importance of this compound as a potential water pollutant. This study was conducted under practical field conditions.

Sampling Areas

Two bogs, located near East Wareham, Mass., were chosen for the monitoring study. Bog 1, a 10-acre bog planted in 1882, was divided by earth dikes into 4 bays. One bay, 2 acres in size, was treated with copper sulfate on April 22, 1969. Floodwater was taken from a nearby 18-acre pond and, after the treatment was finished, released into the same pond on May 20, 1969. Bog 2, a 2-acre bog about as old as Bog 1, received the water from a river. It was treated on April 22, 1969. Floodwater was released on May 27, 1969, into the same pond into which the water from Bog 1 was drained.

No special application or flooding techniques were used; application of the copper sulfate and depth of floodwater were the same as for a normal treatment.

Neither of the bogs was level, resulting in a variation of between 4 and 13 inches in the depth of the water. The average depth was estimated to be 6 to 7 inches. In each bog two sampling sites were chosen in shallow water and two sampling sites in the deeper water.

Sampling Methods

Water samples were taken in acid-washed 800-ml glass jars. For surface water samples, the jars were submerged until half of the opening was about ¼ inch below the water surface. To obtain subsurface water samples, covered jars (glass cover and rubber seal) were horizontally submerged to the bottom. The covers were then removed to fill the jars and put back in place before the jars were raised out of the water. This procedure allowed a distinction between surface and subsurface water only; the water was not deep enough to justify the use of different depths as reference points.

[1] From the University of Massachusetts, Cranberry Experiment Station, East Wareham, Mass. 02538.

The first samples were taken prior to the treatment. Sampling was continued 1, 3, 6, 8, 10, and 28 days after treatments. The last samples were taken 1 day before the bogs were drained. Pond water samples were examined at drainage and 1 and 3 days after.

Application of Copper Sulfate

The bogs were first flooded; then copper sulfate was applied at the rate of 4 lb /acre-foot of water. Distribution was made by placing the appropriate amount of copper sulfate in a burlap bag and dragging the bag through the water. This simple method assures a rather uniform distribution without excessive initial fixation by the soil.

Analytical Procedures

All water samples were analyzed within 15 to 20 minutes after they were taken. The samples were filtered through Whatman No. 44 paper to remove most suspended solids. Extraction and quantitation of copper was carried out using "bathocuproine" (7). At low residue levels (<0.04 ppm) 150 ml instead of 100 ml of water was extracted.

Standards were prepared as described in *APHA Standard Methods for the Examination of Water and Waste Water* (7). Recovery from 10 floodwater samples fortified to 0.3 ppm copper and then filtered through Whatman No. 44 was 94 ± 0.8%. Residue data reported in this paper are the means of two separate analyses and are corrected for the percentage of recovery.

Results and Discussion

Results of the monitoring studies on both bogs are given in Table 1. The pond and river water, prior to flooding and treatment in the bogs contained 0.02 ppm copper. The same amount was present 1 and 3 days after the bogs were drained back into the pond water.

At least during the first 3 days after treatment, the copper concentration in the treated floodwater will generally be greater than 0.4 ppm (the amount the applied dosage is intended to yield) for two reasons. First, the depth of water on a bog may vary considerably due to variations in grade, some bogs being as much as 2 feet higher at one end. The grower applies copper sulfate in relation to the depth of the water (4 lb/acre-foot of water) and not in relation to the surface area (4 lb/acre). Secondly, the volume of water may be reduced somewhat due to evaporation.

In the present study the copper concentration 24 hours after application was about twice as much as expected. After 6 days, however, about 91% of the copper disappeared leaving a concentration of 0.07 ppm in the floodwater. Ten days after treatment only 0.04 ppm copper was found, indicating that more than 95% of the copper had been "fixed."

The difference between levels in surface and subsurface water suggested that copper was adsorbed onto soil particles and that copper may disappear faster from the subsurface water.

TABLE 1.—*Disappearance of copper from floodwater*

Bog and Site No.	Copper Residues in ppm						
	Before treatment	Days after treatment					
		1	3	6	8	10	28
SURFACE WATER							
Bog 1							
Site 1	0.03	0.90	0.61	0.12	0.11	0.04	0.02
Site 2	0.02	0.77	0.42	0.09	0.05	0.05	0.02
Bog 2							
Site 1	0.02	0.77	0.52	0.10	0.05	0.05	0.02
Site 2	0.02	0.79	0.49	0.11	0.06	0.05	0.02
Mean	0.02	0.81	0.51	0.10	0.06	0.05	0.02
SUBSURFACE WATER							
Bog 1							
Site 1	0.03	0.98	0.09	0.06	0.05	0.04	0.02
Site 2	0.02	0.87	0.11	0.05	0.08	0.05	0.02
Bog 2							
Site 1	0.02	0.99	0.05	0.06	0.06	0.03	0.02
Site 2	0.02	0.94	0.07	0.07	0.05	0.03	0.02
Mean	0.02	0.94	0.08	0.06	0.06	0.04	0.02
Mean (both water levels)	0.02	0.87	0.29	0.08	0.06	0.04	0.02

The findings on the disappearance of copper from floodwater were in agreement with data obtained by other workers. Riemer and Toth (*5*) found that 90-100% of the copper applied as copper sulfate in various concentrations to small ponds disappeared from surface water 10 days after the treatment. Tobia and Hanna (*6*) recovered only 1 ppm copper sulfate from unfiltered Nile water 6 hours after addition of 20 ppm $CuSO_2$.

The question remains whether copper, adsorbed on bog soil, can be considered as a potential pollutant which may contaminate streams and ponds in small amounts. According to Lucas (*3*) organic soils held copper tenaciously in the zone of placement. The equivalent of 48 lb/acre of copper sulfate was found in the upper 8 inches of organic soil 5 years after the application of 50 lb/acre. Tobia and Hanna (*6*) obtained only 4.2 and 2.0 ppm copper in 1,000 ml of water leached through 30 g of soil (2.6% C) containing 24 and 30 ppm copper. They stated that organic matter and soil reaction rather than clay content correlated with the retention of copper by soil. Since organic matter controls the sorptive capacity of bog soil (*1*), it can be assumed that copper adsorbed on soil particles is released only in small quantities.

Acknowledgment

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LITERATURE CITED

(*1*) *Deubert, K. H. 1970.* Soil characteristics influencing dieldrin adsorption. Proc. of the Cornell 1970 Waste Manage. Conf. In press.

(*2*) *Deubert, K. H. and B. M. Zuckerman. 1969.* Distribution of dieldrin and DDT in cranberry bog soil. Pesticides Monit. J. 2:172-175.

(*3*) *Lucas, R. E. 1948.* Chemical and physical behavior of copper in organic soils. Soil Sci. 66:119-129.

(*4*) *Miller, C. W. 1966.* Dieldrin persistence in cranberry bogs. J. Econ. Entomol. 59:905-906.

(*5*) *Riemer, D. N. and S. J. Toth. 1967.* Behavior of copper sulfate in small ponds. Northeastern Weed Contr. Conf. 21:534-540.

(*6*) *Tobia, S. K. and A. S. Hanna. 1958.* Effect of copper sulfate added to irrigation water on copper status of Egyptian soils. I. Amount of copper retained by soils. Soil Sci. 85:302-306.

(*7*) *American Public Health Association, Inc. 1967.* Standard methods for the examination of water and waste water. New York, N.Y.

Pesticide Residues in Hale County, Texas, Before and After Ultra-Low Volume Aerial Application of Malathion [1]

G. O. Guerrant, L. E. Fetzer, Jr.,
and J. W. Miles

ABSTRACT

In ultra-low volume aerial sprayings of malathion for control of arthropod-borne encephalitis in 1967 in Hale County, Texas, the amount of malathion deposited was determined by measuring the amount found on exposed filter papers; the average concentration found was 1.5 mg/ft², or 65% of the applied dosage. The maximum concentration found in environmental waters was 0.5 ppm malathion, which decomposed with a half-life of 0.5 to 10 days, depending upon pH. Methods for collecting, transporting, and determining malathion residues are described.

The chlorinated hydrocarbon residues present in the spray area were monitored also and found to consist mainly of DDT and BHC isomers. Most of the sampled waters contained less than 1 ppb of the individual chlorinated pesticides.

Introduction

Malathion was used against *Culex tarsalis* mosquitoes in selected communities in Hale County in northwestern Texas from June through August 1967 to reduce the transmission of encephalitis virus to humans. This is an area of high incidence of human infection from July through September after the buildup of the mosquito population following spring rains and agricultural irrigation. To evaluate the effectiveness of ultra-low volume (ULV) aerial application of malathion in reducing vector mosquito populations and the occurence of human encephalitis, the towns of Plainview and Abernathy were selected for study, with Petersburg as a control. Malathion low-volume concentrate (95%) was applied at the rate of 3.0 fluid oz/acre by the U. S. Air Force Tactical Air Command, Special Aerial Spray Flight, 4500th Air Base Wing, Langley Air Force Base, Va. The application was made from a C-123 cargo aircraft flying at an altitude of 150 feet at an air speed of 150 mph. During the test local municipal agencies applied 3% BHC-5% DDT dust as an independent routine mosquito control measure. In addition, the usual crop protection pesticides were being used since the area is predominantly agricultural.

Mitchell *et al.* (*4,5*) have described the environmental features of Hale County and reported on the effect of malathion ULV sprayings on the mosquito populations and on western encephalitis virus activity. The primary objective of the study reported here was to measure the actual amount of malathion deposited and to determine the distribution and persistence of malathion in the treated areas. Other pesticides were also measured to obtain the total pesticide load in the environment before and after the malathion application. Water sources were selected for sampling since this represents an important route of introducing pesticides into higher vertebrates. To insure accuracy of data obtained, studies were also carried out to develop efficient methods for collecting, storing, and transporting samples. This included a study of the decomposition rate of malathion in field waters; laboratory data on the rate of hydrolysis at various pH values are presented.

Prespray samples for this study were taken on June 12, 1967; other samples were taken after the ULV sprayings on June 16, 21-22, July 26-27, and August 24,

[1] From the Technical Development Laboratories, Laboratory Division, National Communicable Disease Center, Health Services and Mental Health Administration, Public Health Service, U. S. Department of Health, Education, and Welfare, P. O. Box 2167, Savannah, Ga. 31402.

1967. Fig. 1 shows the corporate limits of the three municipalities sampled with sample sites numbered. These sites are identified and described in Table 1.

FIGURE 1.—*Sample sites within Plainview, Abernathy, and Petersburg, Tex.*

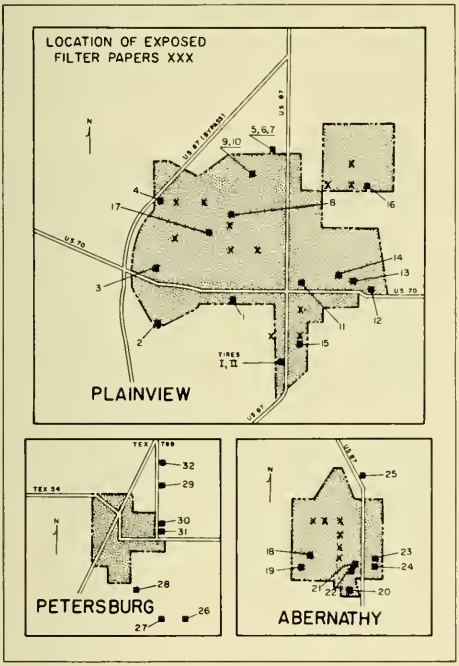


TABLE 1.—*Description of water sample collection sites shown on Fig. 1*

Site No.	Water source	pH
	PLAINVIEW	
1	Intermittent stream	7.2
2	Intermittent stream	7.4
3	Excavation pond	7.8
4	Borrow pit	8.0
5	Irrigation reservoir	9.5
6	Stock water tank	8.6
7	Stock water trough	8.6
8	Excavation pond	7.7
9	Stock water tank	7.7
10	Fishpond	8.1
11	Intermittent stream	7.6
12	Playa	7.9
13	Playa	6.8
14	Stock water tank	7.8
15	Intermittent stream	7.6
16	Stock water trough	7.6
17	Lake	7.4
I	Auto tire	8.2
II	Auto tire	8.0
	ABERNATHY	
18	Lake	7.4
19	Stock water tank	7.9
20	Stock water tank	8.5
21	Lake	7.6
22	Lake	7.4
23	Lake	7.6
24	Lake	7.7
25	Stock water tank	8.9
	PETERSBURG	
26	Playa	7.4
27	Playa	7.4
28	Stock water tank	
29	Stock water tank	8.2
30	Playa	7.6
31	Borrow ditch	7.4
32	Stock water tank	8.0

Measurement of Malathion Application Rate

Assessment of the mosquito kill and its effectiveness in reducing viral activity is dependent upon a knowledge of the dispersal and deposition of the malathion applications. The aerial application equipment and procedures used had been developed and tested in previous usage (*1,2*). The output of insecticide from the spray system was determined and adjusted by selection of appropriate orifice size and number to deliver at a known rate. This delivery rate was made compatible with the height and speed of the aircraft to give a coverage of 3.0 oz of malathion per acre. Spraying was done only during periods of optimum atmospheric conditions such as with wind currents below 10 mph and during periods of temperature inversions.

Prior to spraying, filter papers 15.0 cm in diameter were attached to plywood panels and distributed to prime locations within the area where they were exposed with surfaces parallel to the ground. Arrangement of the filter papers is sketched in Fig. 1; these were located along existing roads with the sampling pattern along lines parallel and also at right angles to the spraying flight pattern. Immediately after spraying, the papers were individually collected and stored in 6-oz prescription bottles which were shipped to Savannah, Ga., for analysis.

At the laboratory 50 ml of hexane was added to each bottle. The bottles were shaken and set aside overnight to extract the malathion for analysis by gas chromatography. A 1-μl sample was analyzed by using a Varian Aerograph Series 204b gas chromatograph equipped with a sodium thermionic detector. Separation was on a 5′ x ⅛″ SE-30 column on Chromosorb W, 60/80 mesh, DMCS, at 190 C. Concentrations were determined from peak area measurements.

To determine the effect of storage on the malathion-exposed filter papers, tests were devised to study decomposition under simulated field conditions. Malathion in hexane was added to a series of papers; the solvent was permitted to evaporate; and the dried papers were stored.

Samples were analyzed at selected times, and decomposition was found to be at the rate of 6% per day. The results showed that with the field samples, less decomposition would have occurred if solvent had been added to each sample when it was collected rather than when it arrived at the laboratory. Since this was not done, appropriate corrections were made for malathion decomposition during transportation to the laboratory.

Assuming aerial ULV application at the rate of 3 oz/acre, the theoretical concentration of malathion deposited was 2.39 mg/ft^2. The average concentration found following two aerial sprayings on June 16 and 21-22, 1967, was 1.5 mg/ft^2, or 65% of the application rate; the remaining 35% was presumed to have dissipated as a non-condensing vapor. This indicates good distribution in the test area. The data are presented in Table 2 and range from a low of 0.28 mg/ft^2 to a high of 4.39 mg/ft^2.

TABLE 2.—*Malathion found on filter papers exposed during aerial ULV spraying in Texas, June 1967*

Sample No.	Malathion (mg/ft^2)	
	June 16	June 21-22
PLAINVIEW		
A	1.14	
B	1.93	
C	1.47	
D	0.67	2.35
E	1.33	4.39
F	1.52	1.98
G	1.51	2.42
H		1.31
I		2.09
J		3.66
Overall mean	1.37	2.60
S.D.	0.39	1.06
ABERNATHY		
K	1.66	1.10
L	1.82	1.32
M	0.86	0.60
N	0.85	0.28
O		0.55
P	0.63	1.19
Q	1.93	1.98
Overall mean	1.29	1.00
S.D.	0.57	0.58

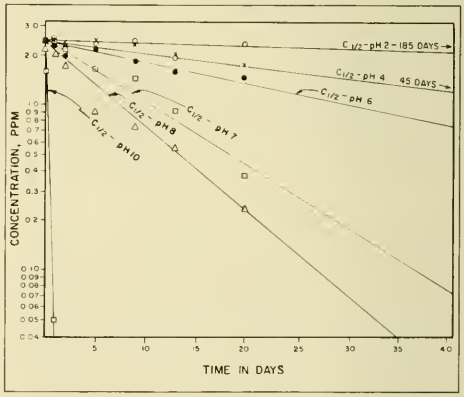
Decomposition of Malathion in Water

Malathion levels were lower than expected in water samples collected immediately after sprayings on June 16 and 21-22, 1967. This finding indicated that hydrolysis had occurred. To reduce malathion hydrolysis during sample shipment, samples taken in July and August were acidified to pH 4 by adding two drops of glacial acetic acid to each 8-oz sampling bottle at the time of collection.

The stability of malathion in water was studied further by determining the hydrolysis rate of known samples after storage at various pH values. Aqueous solutions containing 2.25 ppm malathion in Clark and Lubs buffer mixtures at pH values of 2, 4, 6, 7, 8, and 10 were prepared. Samples were analyzed immediately and at 1, 2, 5, 9, 13, and 20 days after treatment. Malathion was determined by hexane extraction with a vortex mixer and subsequent analysis on a MicroTek Model 220 gas chromatograph equipped with a phosphorus-specific flame photometric detector. Separation was on 3% OV-17 on 60/80 Chromosorb W, A.W., DMCS-treated, aluminum column, 2′ x ¼″, at 225 C, and a nitrogen carrier flow of 100 ml/minute.

Malathion concentrations found at the various times are shown in Fig. 2. The data show that malathion is stable at pH 2 with essentially 100% recovery at the end of the 20-day test period. At pH 4 and pH 6 after 20 days, the recovery of malathion was 72% and 63%, respectively. The decomposition rate increased further with increasing pH. At pH 7 and 8, only 16% and 11%, respectively, of the malathion remained after 20 days. At pH 10 malathion began to decompose almost immediately, and only 60% remained after 1 hour.

FIGURE 2.—*Decomposition of malathion in water with time at various pH values*



A logarithmic plot of concentration remaining versus time yields a straight line for each pH as shown, indicating that decomposition is a first-order reaction. Assuming decomposition follows the straight-line relationship shown, and interpolating or extrapolating for pH values of 2, 4, 6, 7, 8, and 10, malathion half-lifes found were 185, 45, 26, 8, 6, and 0.1 days respectively. These data are the decomposition rates determined at laboratory temperatures of 23 C and do not necessarily represent field and sample storage temperatures. Field water temperatures would be expected to be reasonably constant and to approximate average ambient temperatures; however, the temperature of surfaces might exceed ambient

temperatures in certain instances by 20 C. This temperature difference can also occur in confined storage during shipment and can result in a greater decomposition rate. At the pH values for field sites shown in Table 1 (6.8-9.5) malathion has a half-life of from 0.5 to 10 days at 23 C. At the highest pH values found in the field significant changes in concentration occurred within the first hour after application. Even the field samples with the lowest pH would be expected to undergo appreciable decomposition in 1 or 2 days. Data from repetitive samplings of field waters after spraying are consistent with this finding.

Results of these tests indicate that acidifying the July and August samples to pH 4 was an effective measure for reducing malathion hydrolysis. Because of the hydrolysis occurring in the June water samples, these results are not included in this paper.

Malathion in Field Waters

The concentrations of malathion in the water samples taken immediately after the spraying on July 26-27, 1967, are presented in Table 3. These samples were collected approximately 4 hours after spraying. The general procedure for collecting samples was to locate a pool of water which was completely exposed to the aerial spraying operation, usually a livestock watering tank, intermittent stream, excavation pond, or borrow pit. Each sample was taken in an 8-oz prescription bottle to which 2 drops of glacial acetic acid had been added. Although the water samples contained very little suspended matter, they were passed through a plug of pesticide-free glass wool prior to extraction.

TABLE 3.—*Malathion residues in waters in Hale County, Texas, 4 hours after spraying on July 26–27, 1967*

Site No.	Malathion (ppm)
PLAINVIEW	
1	.11
2	.018
3	.011
4	.026
5	.000
6	.002
7	.007
8	.018
9	.006
10	.010
11	.017
12	.020
13	.005
14	.007
15	.010
16	.010
17	.014
ABERNATHY	
18	.061
19	.025
20	.51
21	.025
22	.068
23	.082
24	.12
25	.06
PETERSBURG	
26	.000
27	.000
28	.000
29	.000
30	.000
31	.000
32	.000

The samples were analyzed for malathion by extracting 100 ml of water with three consecutive 25-ml portions of purified *n*-hexane. The *n*-hexane extracts were combined and concentrated to 5 ml for gas chromatographic analysis. A modified Barber-Colman Series 5000 gas chromatograph, a Hamilton injection block, and an Ionics tritium electron capture detector operating at 20v DC were employed. The separating column was 3′ x ⅛″ packed with 5% OV-17 on 100/120 mesh Anachrom SD. Temperatures were 195, 205, and 230 C for the column, detector, and injector, respectively. Nitrogen carrier gas flow was 30 ml/minute with 70 ml/minute detector purge. Under the conditions employed, the detector was sensitive only to malathion and two BHC isomers. This detector response appeared unusual, but repeated tests of the usual chlorinated hydrocarbons such as DDT, DDE, heptachlor, heptachlor epoxide, aldrin, and dieldrin, gave no response. This is the specific Lovelock design (*3*) of the electron capture detector. It was operated, however, with nitrogen carrier in the DC mode rather than with argon-methane carrier and pulsed electrical input.

The minimum detectable quantity of malathion was 0.0005 ppm. The hexane extraction method, as used for recovery of malathion, was verified by extracting known additions of malathion from water under the same conditions.

Only four samples contained malathion concentrations high enough (.082, .11, .12, and .51 ppm) for possible kill of mosquito larvae. The highest concentration (.51 ppm) was in a sample from a livestock watering tank in southeastern Abernathy. Samples collected in Petersburg, the control area, had no residues.

Water samples after the spraying of Plainview on August 24, 1967, were collected along with control samples from Petersburg at 4-, 24-, and 48-hour intervals. Results of analyses of these samples are tabulated in Table 4.

These samples were analyzed on a MicroTek gas chromatograph, Model MT-220, employing a phosphorus-sensitive flame photometric detector. The separating column consisted of 2.5% E301 plus 0.25% Epon 1001 on Chromosorb W, 100/120 mesh, 4′ x 3/16″ O.D. at a nitrogen flow of 100 ml/minute, at 160 C. The detection limit with the Flame photometric detector was 0.001 ppm.

TABLE 4.—*Malathion residues in waters in Hale County, Texas, after August 24, 1967, spraying*

Site No.	Malathion (ppm)		
	4 hours	24 hours	48 hours
Plainview			
2	0.15	<0.001	<0.001
5	0.015	<0.001	<0.001
7	0.021	0.006	0.001
8	0.50	<0.001	<0.001
11			<0.001
13	0.021	0.001	<0.001
14	0.037	<0.001	<0.001
16	0.029	<0.001	
I	0.059	0.027	<0.001
II	0.082	0.013	0.006
Paint carton	0.233		
Petersburg			
28	<0.001	<0.001	<0.001
30	<0.001	<0.001	<0.001
31	0.001	<0.001	0.001

Samples designated by Roman numerals (Table 4) were taken from automobile tires located in southern Plainview. The tires, with water added, had been placed at these sites for sampling to determine the effectiveness of the spray treatment upon such prime mosquito-breeding sites. The sample containing the highest concentration (0.50 ppm) was collected from an excavation pond 4 hours after spraying. This concentration, identical to the maximum found 4 hours after the July spraying, decreased to less than 0.001 ppm after 24 hours. The maximum concentration found after 24 hours (0.027 ppm) was from the No. I tire sample, and this amount decreased to less than 0.001 ppm after 48 hours. The maximum concentration found after 48 hours was 0.006 ppm and occurred in the No. II tire sample.

Although different gas chromatographs and conditions have been used for the analysis of malathion as reported in Tables 2, 3 and 4, the results are directly comparable.

Chlorinated Pesticides in Field Waters

As part of the overall investigation, water samples were also analyzed for chlorinated pesticides. The average amounts of pesticides found in the samples collected on June 12, 16, and 21-22 are tabulated in Table 5; analyses of August samples are presented in Table 6.

One hundred milliliters of each sample was extracted three successive times with 25-ml portions of petroleum ether. The extracts were combined and concentrated to 5-ml volume for analysis by gas chromatography. The following equipment and conditions were used: A MicroTek Model 2500R gas chromatograph with a 5% QF-1 column 6' x ¼" O.D. on 90/100 mesh Anakrom ABS (Analabs, Inc., Hamden, Conn.) at 181 C and 100 ml/minute nitrogen flow with a tritium electron capture detector in the DC mode; a modified Barber-Colman

TABLE 5.—*Chlorinated pesticides in waters in Hale County, Texas, June 1967*

Site No.	Residues in ppb [1]												
	BHC				*p,p'* DDT	*o,p'* DDT	*p,p'* DDE	*o,p'* DDE	*p,p'* DDD	Dieldrin	Aldrin	Heptachlor	Heptachlor epoxide
	α	β	γ	Δ									
1	1.	.3	.4	.2	.3	.3	.4	.4					
2	1.	1.	1.	.8	.7	.03	.2		.2	.02	.2	.09	
3					.2	.2	.2						
5					.2		.2	.1				.3	
6	.1	.1	.1		.4	.1	.1			.02			
7	.2		.1		1.	.5	.5		.4				
8	1.	.2	.9	.8	1.	.6	.4		.4	.05			
9	.1	.1	2.	.1	.8	.7	.3	.1	.4				
10	.3	.2	.5	.1	.4	.05	.06						
11	1.	1.	.3	.6		.5	.02		.9				.2
12	1.	3.	.5	.5	.3	.5	.2			.02	.2		
13	.6	.3	.7	.3	.7	.6	.5	.2	.5				
14	.1	.03	.02		.2	.1	.1		.1				
15	1.	.6	5.	.4	.3	.3	.2		.2			.2	
16	.4	.2	.01	.1	.4	.2	.1		.1				
17	3.	1.	1.	.9	.5	.2	.2	.2	.4				
18	.6	1.	.1	.1	.6	.2	.3		.2				
19	.02		.01		.6	.4	.3		.1				
20	.5	.5	1.		.8	.07	.6	.09	.07	.02	.4		
21	.2	.1	.3	.3	.6	.5	.1					2.	.5
22	.2	.3	.4	.3	.2		.3			.1	.2		.1
23	.3	2.	1.	1.	2.	.4	.3	.2	.3	.02	.2		.06
24	.4	.4	.3	.3	3.		.3	1.		.02	.2		
25	1.	.7	.6	.6	.6	.2	.2		.1				
26	.1		.1		.6	.3	.4						
27	.04	.02	.08	.02	.6	.2	2		.1	.02			
28					.5	.2	.2						
29	.01		.01	.01	.6	.2	1			.02			
30	1.	.6	.1	.3	.5	.1	.2			.02			
31	1.	.3	3.	.4	.1	.9	.6			.02			
32	.4	.2	2.		1.	.1	.6	.2	.4				
I	.1	.05	.09	.01	1.	.7	.1	1.5	.3			.3	.3
II	.3	.2	.2		.7	.6	.8		.1			.1	

[1] Averaged results of samples taken on June 12, 16, and 21-22, 1967.

TABLE 6.—*Chlorinated pesticides found in water, Hale County, Texas, August 1967*

Site No.	Residues in ppb [1]										
	BHC				*p,p'* DDT	*o,p'* DDT	*p,p'* DDE	*o,p'* DDE	*p,p'* DDD	Dieldrin	Heptachlor
	α	β	γ	Δ							
1	.1	.2	.1	.1	.1	.4		.1	.1		
2	.06	.2	.02	.02	.5	.3	.1		.01	.4	
5	.1	.2	.06	.06	.5	.05				.4	
7	.2	.3	.1	.1	.2	.2	.3		.1	.1	
8	.7	.2	3.		Present	2	.3		.1	.2	
13	.1	.1	.2	.4	Present	.5					
14	.1	.04	.04	.04	.4	2	.1		.1	.02	
16	.1	.1	.05	.05	Present	.05	.09		.03		
28	.04					.06	.03				.1
30					.3						
31	.4		.3	.3			.6			.02	

[1] Averaged results of samples taken at 4-, 24-, and 48-hour intervals.

Series 5000 gas chromatograph with a Hamilton injection port, an Ionics high-temperature nickel-63 electron capture detector, an Infotronics Model EA-1 electrometer, and a MicroTek Model No. 630410 variable pulse electron capture power supply. Separation was obtained on a 4′ x 4-mm I. D. glass column of 3% GC grade GE SE-30 on 100/120 mesh Chromosorb W, DMCS acid washed (Applied Science Laboratories, State College, Pa.), at 190 C and 60 ml/minute argon-methane carrier. The detector was operated in the pulsed mode at 30v, 1 microsecond pulse every 100 microseconds.

The limiting sensitivity varies somewhat for the different compounds and was approximately 0.01 ppb for *p,p'*-DDT. The extraction procedure was checked by use of water spiked with known quantities of DDT isomers.

The primary reason for using the two chromatographs with different columns, detectors, and operating conditions was to confirm the pesticides present; each result reported is the average of four determinations. There were few unidentified chromatographic peaks. Many samples contained an appreciable amount of elemental sulfur which interfered with the aldrin determination on the SE-30 column. The identification of pesticides present was based on relative retention times. Reference standards were prepared of the persistent chlorinated pesticides. In addition, the pesitcides used by local municipal agencies plus the crop protection pesticides applied by aerial applicators were included. The retention times and peak heights of these standards were used to determine the pesticides present in the field samples. All water samples contained residues of DDT and BHC and related isomers. The concentrations found in one-third of the samples were less than 1 ppb.

Summary

The malathion application rate by aerial ultra-low volume (ULV) spraying can be reliably measured by a chemical method involving the exposure of filter paper panels for subsequent extraction with hexane and analysis of the recovered malathion by gas chromatography. Malathion is subject to hydrolysis in water. The rate is pH-dependent, and field water samples must be acidified to reduce decomposition during transport to the laboratory. The concentration half-life varies from 8 days in neutral solution to 2½ hours in alkaline solution at pH 10.

The maximum malathion concentration found in the environmental waters of Hale County, Texas, after field ULV spraying was 0.5 ppm, and complete decomposition occurred in 1 day. Chemical data indicate that ULV spraying of 3 oz/acre of malathion produces negligible malathion contamination of the area waters.

The chlorinated pesticides present in the waters of the environment of Hale County, Texas, during ULV malathion spraying has been determined. All waters contained residues of DDT and BHC and related isomers. The concentraitons found in one-third of the samples were less than 1 ppb.

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The use of trade names and commercial sources is for identification purposes only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(1) *Kilpatrick, J. W. 1967.* Performance specifications for ultra-low volume aerial application of insecticides for mosquito control. Pest Contr. 35 (May):80-84.

(2) *Kilpatrick, J. W. and C. T. Adams. 1967.* Emergency measures employed in the control of St. Louis encephalitis epidemics in Dallas and Corpus Christi, Texas, 1966. Calif. Mosq. Contr. Ass. Proc. 35:53.

(3) *Lovelock, J. E. 1961.* Ionization methods for the analysis of gases and vapors. Anal. Chem. 33:162-178.

(4) *Mitchell, C. J., R. O. Hayes, Preston Holden, H. R. Hill, and T. B. Hughes, Jr. 1969.* Effects of ultra-low volume applications of malathion in Hale County, Texas. I. Western encephalitis virus activity in treated and untreated towns. J. Med. Ent. 6(2):155-162.

(5) *Mitchell, C. J., J. W. Kilpatrick, R. O. Hayes, and H. W. Curry. 1970.* Effects of ultra-low volume applications of malathion in Hale County, Texas. II. Mosquito populations in treated and untreated areas. J. Med. Ent. 7(1): 85-91.

PESTICIDES IN SOIL

DDT Moratorium in Arizona—Agricultural Residues After 1 Year [1]

G. W. Ware, B. J. Estesen, C. D. Jahn, and W. P. Cahill

ABSTRACT

Generally, the DDT moratorium in Arizona, begun in 1969, has been only moderately successful during its first year because of some apparent agricultural use of DDT in the three major irrigated areas. Alfalfa residues declined slightly, while beef fat residues remained the same. Both irrigated and desert soil residues also remained relatively stable showing little decline.

Introduction

Arizona's 1-year moratorium on the agricultural use of DDT was established by the Board of Pesticide Control in January 1969. At the same time they requested that the residue laboratory of the Department of Entomology, University of Arizona, monitor the primary irrigated areas for changes in residues of DDT and its related degradation products (DDTR). The reasons for such monitoring were (1) to determine whether violations occurred and (2) to measure the decline of environmental DDT residues following 20 consecutive years of agricultural use.

Because of our previous experience (*1*) we chose to sample growing alfalfa, as well as the upper 10 inches of soil in these alfalfa fields, on a quarterly basis and also beef fat from selected feed lots following the cyclic changes in beef feeding practices.

Sampling Methods

Alfalfa and soil samples were collected from the three major irrigated areas—the Salt River Valley around Phoenix, Pinal County, and the Yuma mesa and valley. Desert soil samples were also collected adjacent to and northwest and southeast of the three irrigated areas. Ten alfalfa fields and four desert soils were sampled from each of the three areas in January, May-June, September, and December of 1969. In addition, an earlier study (*1*) was continued on the 60-mile Maricopa County east-west transect known as Baseline Road, providing a reference standard and continuity to the moratorium monitoring back to 1966.

Alfalfa samples were hand-harvested 2 inches above the soil surface. Each sample consisted of 20 subsamples varying between 0.5 and 1.0 ft^2 in area, totaling approximately 3 lb of green alfalfa.

Each soil sample consisted of twenty 10-inch deep plugs, 1 inch in diameter, taken randomly with a standard soil sampler.

Beef fat samples, 50 to 100 g in size, were removed with a scalpel from the left kidney of carcasses after chilling 24 hours in the slaughterhouse.

Analytical Methods

Green alfalfa samples were extracted with solvent as previously described (*1*). Screened soil samples consisting of 25-g aliquots were extracted in a Soxhlet apparatus for 16 hours with an azeotropic solvent, hexane and acetone (14:59). After filtering, the extracts were washed, dried through sodium sulfate, and refrigerated.

A rapid, on-column extraction cleanup method for animal fat (*2*) was used for the beef fat samples. This method handles a maximum of 0.8 g of fat tissue in the single-step procedure and permits a large number of samples to be processed in a day.

[1] From the Department of Entomology, The University of Arizona, Tucson, Ariz. 85721.

An aliquot of alfalfa or soil extract was cleaned on a 4-inch colum of activated Florisil topped with ¼ inch of sodium sulfate. The extract was eluted with 250 ml of 6% diethyl ether in hexane, reduced in volume by evaporation, and adjusted to 5 ml in a glass-stoppered centrifuge tube for analysis by electron capture gas-liquid chromatography. Recovery standards and analytical reagent blanks were carried through the extraction and cleanup procedures for each day's analyses. Recoveries were consistently 90 to 100%; however, these corrections were not applied to the data presented. The minimum sensitivity of the analytical method was arbitrarily set at 0.02 ng for *p,p'*- and *o,p'*-DDT and DDE. The relative sensitivities were .001 ppm for alfalfa, .003 ppm for soil, and .060 ppm for beef fat, based on a minimum sample size and 6 μl extract injected into the chromatograph. Analytical confirmatory tests were conducted on a random basis. Because of interfering peaks from toxaphene encountered in the September and December alfalfa samplings, in the confirmatory tests all extracts were dehydrohalogenated after Florisil cleanup and measured only as *o,p'*- and *p,p'*-DDE according to the methods described by Cahill *et al.* (*3*).

Results and Discussion

The analytical results of alfalfa, soil, and beef fat samplings during the moratorium are presented in Tables 1-8, as total DDTR. Table 1, showing the continued Baseline Road study indicates a general reduction in alfalfa residues each year, with the exception of residues found in fields 2 and 11 in December 1969. The higher DDTR levels in this sampling indicate a probable agricultural use of DDT in that vicinity subsequent to September 8, the date of previous sampling.

Data on alfalfa residues from Maricopa County (Table 2) show two fields with high levels in December. These results are similar to those for Baseline Road, which is also in Maricopa County. In Pinal County, alfalfa from three fields sampled in September and one sampled in December had high residues (Table 3). In Yuma County, alfalfa from five fields sampled in September and one in December had high residues (Table 4). It seems apparent from these data that DDT was used for agricultural purposes in both Pinal and Yuma Counties prior to the September 6 sampling.

TABLE 1.—*DDTR residues in green alfalfa along Baseline Road, Maricopa County, Arizona 1967–69*

Field No.	Residues in PPM									
	1967				1968			1969		
	Mar.	*May*	*Aug.*	*Nov.*	*Feb.*	*Sept.*	*Dec.*	*Apr.*	*Sept.*	*Dec.*
2	.055	.018	—	.305	.094	.220	.045	.016	.038	.127
3	.019	.048	.283	—	—	—	.052	.020	.027	.025
4	.052	.021	.170	.165	.070	.120	.064	.027	.038	.051
5	.039	.025	—	.077	—	.060	.044	—	.020	.091
6	.019	.016	.277	.116	—	—	—	.023	.035	.060
8	.039	.028	.794	.455	—	—	.161	—	—	—
9	.045	.031	—	—	—	.076	—	.017	.034	.055
10	.063	.032	.350	.187	.110	.092	—	.029	.054	.095
11	.043	.017	.453	.283	.176	.580	.091	.029	.064	.189
12	.011	.012	.299	.114	—	.077	.042	.005	.025	.055
13	.072	.023	.606	—	—	—	.065	—	—	—
Average	.042	.025	.404	.213	.113	.175	.068	.021	.037	.083

TABLE 2.—*DDTR residues in green alfalfa during 1969 DDT moratorium, Maricopa County, Arizona*

Field No.	Residues in PPM			
	Jan.	May	Sept.	Dec.
1	.087	.021	.042	.075
2	.303	.025	.062	.049
3	.102	.021	.078	.059
4	.107	.020	.047	.043
5	.049	.012	.030	.067
6	.113	.027	.064	.122
7	.082	.033	.034	.052
8	.125	.084	.056	—
9	.085	.029	.044	.073
10	—	.011	—	.123
Average	.117	.028	.051	.074

TABLE 3.—*DDTR residues in green alfalfa during 1969 DDT moratorium, Pinal County, Arizona*

Field No.	Residues in PPM			
	Jan.	May	Sept.	Dec.
1	.047	.006	.042	.047
2	.047	.011	.031	.063
3	.142	.012	.187	—
4	.231	.051	.076	.095
5	.092	.006	.130	.024
6	.038	.004	.058	.012
7	.079	.011	.118	.052
8	.068	.007	.071	.029
9	.054	.014	.068	.027
10	.078	.011	.077	.171
Average	.088	.013	.086	.058

In Maricopa County, residues in soils from both alfalfa fields and desert sites showed a relatively stable picture, with the exception of fields 6 and 8 (Table 5). In Pinal and Yuma Counties also, residues in these soils remained constant throughout the year (Tables 6 and 7). The stability of these DDTR soil residues was unexpected since our soil insecticide field plots indicate a residual half-life of approximately 2 years for DDT under irrigated cultivation conditions and slightly longer for desert plots.

DDTR residues in beef fat, shown in Table 8, indicate no change from the pre-moratorium levels. The wide variation in residues between feed lots is a reflection of the feed types and geographic feed sources in Arizona.

TABLE 4.—*DDTR residues in green alfalfa during 1969 DDT moratorium, Yuma County, Arizona*

Field No.	Residues in PPM			
	Jan.	June	Sept.	Dec.
1	.047	.012	.373	.048
2	.039	.010	.098	.043
3	.049	.014	.256	.079
4	.057	.009	.093	.041
5	.057	.017	.545	.095
6	.044	.009	.317	.049
7	.059	.016	.241	.106
8	.036	.002	.045	.032
9	.021	.003	.056	.016
10	.046	.015	.074	.054
Average	.046	.011	.210	.056

TABLE 5.—*DDTR residues in soils during 1969 DDT moratorium, Maricopa County, Arizona*

Alfalfa Field No.	Residues in PPM			
	Jan.	May	Sept.	Dec.
1	0.54	0.34	0.57	0.53
2	1.54	2.22	1.88	2.04
3	0.59	[1] 1.33	1.60	1.81
4	0.74	0.71	0.68	0.64
5	0.44	0.23	0.35	0.26
6	3.92	3.70	3.41	5.05
7	1.22	1.22	1.22	1.43
8	4.08	4.34	[1] 4.54	5.21
9	2.41	2.14	2.32	2.35
10	[1] .34	0.48	0.26	0.30
Average	1.58	1.67	1.68	1.96
Desert Site No.				
1	0.13	0.07	0.41	0.39
2	0.35	0.18	0.60	0.26
3	0.67	0.16	0.11	0.21
4	[1] 2.51	1.14	3.50	2.90
Average	0.92	0.39	1.16	0.94

[1] No sample—this figure represents the mean of the values shown for samples collected in the other 3 months.

TABLE 6.—*DDTR residues in soils during 1969 DDT moratorium, Pinal County, Arizona*

Alfalfa Field No.	Residues in PPM			
	Jan.	May	Sept.	Dec.
1	4.64	2.79	4.08	3.47
2	1.48	1.66	1.79	2.07
3	2.89	[1] 2.89	3.06	2.72
4	2.35	2.75	2.12	2.30
5	0.33	0.74	0.50	0.54
6	0.12	0.13	0.14	0.11
7	2.68	2.17	2.75	2.62
8	0.14	0.13	0.17	0.12
9	1.06	0.90	1.07	1.21
10	1.16	1.46	1.28	1.42
Average	1.69	1.56	1.70	1.66
Desert Site No.				
1	0.15	0.05	0.14	0.14
2	0.32	0.10	0.42	0.18
3	0.05	0.06	0.14	0.14
4	1.06	0.67	1.14	1.26
Average	0.40	0.22	0.46	0.43

[1] No sample—this figure represents the mean of the values shown for samples collected in the other 3 months.

TABLE 7.—*DDTR residues in soils during 1969 DDT moratorium, Yuma County, Arizona*

Alfalfa Field No.	Residues in PPM			
	Jan.	June	Sept.	Dec.
1	0.16	0.17	0.16	0.10
2	0.60	0.62	0.64	0.64
3	1.72	1.79	1.52	1.67
4	1.25	1.37	1.42	1.19
5	0.91	1.09	0.67	1.20
6	1.29	1.13	1.28	1.11
7	1.85	1.47	1.51	1.65
8	0.07	0.07	0.06	0.08
9	0.00	0.02	0.01	0.00
10	0.31	0.24	0.23	0.21
Average	0.82	0.80	0.75	0.79
Desert Site No.				
1	0.38	0.27	0.30	0.27
2	0.07	0.07	0.03	0.05
3	0.06	0.04	0.03	0.04
4	0.01	0.02	0.00	0.02
Average	0.13	0.10	0.09	0.10

TABLE 8.—*DDTR residues in beef fat from selected Arizona feed lots*

Feed Lot No.[1]	Residues in PPM		
	Nov. 1968	May 1969	Dec. 1969
1	1.34	0.74	0.59
2	1.07	1.27	1.93
6	1.15	0.77	0.75
9	0.80	0.14	0.71
12	0.47	0.48	0.84
Average	0.97	0.68	0.96

[1] Average of five animals per feed lot.

Despite the apparent agricultural use of DDT in all three major irrigated areas, the DDT moratorium would be considered moderately successful. It appears that the most frequent use of DDT was in Yuma County, followed by Pinal and Maricopa Counties. Alfalfa fields and adjacent desert soil residues are higher in Maricopa and Pinal Counties than Yuma County by a factor of two. Residues in green alfalfa from three counties were generally lowered by the moratorium and in the same order of magnitude; residues in beef fat remained at the pre-moratorium levels.

This study is a contribution to Regional Project W-45, "Residues of Selected Pesticides—Their Nature, Distribution, and Persistence in Plants, Animals and the Physical Environment." University of Arizona Agricultural Experiment Station journal series No. 1601.

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(1) *Ware, G. W., B. J. Estesen and W. P. Cahill. 1968.* Pesticides in soil—an ecological study of DDT residues in Arizona soils and alfalfa. Pesticides Monit. J. 2(3): 129-132.

(2) *Cahill, W. P., B. J. Estesen and G. W. Ware. 1970.* A rapid on-column extraction-cleanup method for animal fat. Bull. Environ. Contamination Toxicol. (in press).

(3) *Cahill, W. P., B. J. Estesen and G. W. Ware. 1970.* Determination of DDT in the presence of toxaphene residues. Bull. Environ. Contamination Toxicol. (in press).

APPENDIX

Chemical Names of Compounds Mentioned in This Issue

ALDRIN	Not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
COPPER SULFATE	$CuSO_4 \cdot H_2O$
DDD (TDE) (including its isomers and dehydrochlorination products)	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; technical TDE contains some *o,p'*-isomer also
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
DDT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; technical DDT consists of a mixture of the *p,p'*-isomer and the *o,p'*-isomer (in a ratio of about 3 or 4 to 1)
DIELDRIN	Not less than 85% of 1,2,3,4,10,10-hexachloro(6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethano=naphthalene
AZINPHOSMETHYL	*0,0*-dimethyl *S*(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithionate
HEPTACHLOR	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
HEPTACHLOR EPOXIDE	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
MALATHION	diethyl mercaptosuccinate, *S*-ester with *0,0*-dimethyl phosphorodithioate
METHYL PARATHION	*0,0*-dimethyl *0*-*p*-nitrophenyl phosphorothioate
PARATHION	*0,0*-diethyl *0*-*p*-nitrophenyl phosphorothioate

The PESTICIDES MONITORING JOURNAL welcomes from all sources qualified data and interpretive information which contribute to the understanding and evaluation of pesticides and their residues in relation to man and his environment.

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- All material should be submitted in duplicate (original and one carbon) and sent by first-class mail in flat form—not folded or rolled.
- Manuscripts should be typed on 8½ x 11 inch paper with generous margins on all sides, and each page should end with a completed paragraph.
- All copy, including tables and references, should be double spaced, and all pages should be numbered. The first page of the manuscript must contain authors' full names listed under the title, with affiliations, and addresses footnoted below.
- Charts, illustrations, and tables, properly titled, should be appended at the end of the article with a notation in text to show where they should be inserted.
- Charts should be drawn so the numbers and texts will be legible when considerably reduced for publication. All drawings should be done in black ink on plain white paper.
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- The "number system" should be used for literature citations in the text. List references alphabetically, giving name of author/s/, year, full title of article, exact name of periodical, volume, and inclusive pages.

Pesticides ordinarily should be identified by common or generic names approved by national scientific societies. The first reference to a particular pesticide should be followed by the chemical or scientific name in parentheses—assigned in accordance with CHEMICAL ABSTRACTS nomenclature. Structural chemical formulas should be used when appropriate. Published data and information require prior approval by the Editorial Advisory Board; however, endorsement of published information by any specific Federal agency is not intended or to be implied. Authors of accepted manuscripts will receive edited typescripts for approval before type is set. After publication, senior authors will be provided with 100 reprints.

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The *Pesticides Monitoring Journal* is published quarterly under the auspices of the WORKING GROUP, Subcommittee on Pesticides, President's Cabinet Committee on the Environment, and its Panel on Pesticide Monitoring as a source of information on pesticide levels relative to man and his environment.

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CONTENTS

Volume 4 September 1970 Number 2

Toxaphene and DDT Residues in Ladino Clover Seed Screenings[1]

T. E. Archer

ABSTRACT

Ladino clover seed crop screenings field-contaminated with DDT and its analogs and toxaphene were analyzed for these compounds in a composite sample and in 13 separate fractions of the composite sample. The total residue for DDT and its analogs was 20.0 ppm and 21.1 ppm for toxaphene. The residues were excessively high for use of the plant material as animal feed. Two fractions, representing 29% of the composite sample, contained 74% of the DDT and 70% of the toxaphene. These fractions were ladino clover chaff which was 19% of the composite and contained 9.1 ppm DDT and 9.4 ppm toxaphene and soil which was 10% of the composite and contained 5.8 ppm DDT and 5.4 ppm toxaphene.

Introduction

Large quantities of pesticides have been applied to ladino clover and alfalfa seed crops during their production. The seed-cleaning processes after harvest result in such by-products as straw, chaff, weed, and other seeds which constitute a source of animal feed. These have been used either separately or in feedstuff mixtures although recommendations are usually against such use.

The quantitative measurement of combinations of Aramite, DDT, toxaphene, and endrin has previously been reported in specific animal feeds (*1*). Various workers have studied the analytical characteristics of toxaphene after treatment with sulfuric-fuming nitric acid mixtures during residue determinations (*6,7,8*). The decontamination of malathion residues from ladino clover seed screenings has also been reported (*2*). The persistence of methyl parathion residues on sunflower seeds was investigated (*5*); it was found that if allowable residues were set as low as 0.1 ppm, the minimum interval between methyl parathion treatments of 0.5 and 1.0 lb/acre and harvest would be 7 and 14 days, respectively.

The present investigations were undertaken to determine the levels of DDT and its analogs and toxaphene in a composite sample of field-contaminated ladino clover seed crop screenings and also in separate components. If the contaminated components are identified, procedures could possibly be developed to eliminate them and thus lower the pesticide levels in the seed screenings to make a more acceptable animal feed.

Methods and Materials

SAMPLING PROCEDURES

The commercial seed screenings (13.0% moisture) were obtained from a ladino clover seed crop which had been treated with DDT and toxaphene for pest control during field growth. An application of 1 lb/acre DDT plus 2 lb/acre toxaphene was made on May 28 and another of 1½ lb/acre DDT plus 3 lb/acre toxaphene on July 5.

[1] From the Department of Environmental Toxicology, University of California, Davis, Calif. 95616.

SAMPLE FRACTIONATION

A 100-g composite sample of the seed screenings was weighed and sieved by shaking over stacked screen sieves ranging from 16 to 100 mesh until the sample was separated into seven crude fractions, including that material which was collected on the bottom pan of the stacked screens. These 7 fractions were further separated with the aid of an illuminated circular magnifier (Luxo Magnifier, Luxo-Lamp Corp., Port Chester, N.Y.) into 13 individual samples for analysis. The fractions were identified and the percent weight of each fraction in relation to the total composite sample was calculated (Table 1). The individual fractions were then extracted and analyzed for DDT and its analogs and toxaphene.

SAMPLE EXTRACTION AND CLEANUP

In addition to the fractions mentioned above, 10 g of a composite sample and 10-g portions of separated fractions, in triplicate, were individually extracted by refluxing for 30 minutes with 100 ml of solvent consisting of 90 ml of benzene, 10 ml of ethyl alcohol, and 0.5 ml of 12N hydrochloric acid. The reflux extraction was performed 3 times, and the solvent was pooled and concentrated *in vacuo* at 50-60 C prior to cleanup.

The solvent extracts were cleaned up on Florisil (activated at 270 C for 3 hours). DDT and its related degradation products (DDTR) and toxaphene were eluted from the Florisil with 390 ml of 30% diethyl ether and 70% pentane, and recoveries exceeded 90%. The extracted plant material was treated with ethanolic potassium hydroxide and analyzed; the residues found were summed with those found in the solvent extracts previously treated with ethanol alkalki (*4*).

DETECTION AND DETERMINATION OF PESTICIDES

All chemicals used were reagent grade. The DDTR pesticides were recrystallized analytical standards, and the toxaphene analytical standard was obtained from Hercules Powder Co., Wilmington, Del.; the reagent grade solvents were redistilled shortly before use. Gas-liquid chromatography (GLC) and thin-layer chromatography (TLC) procedures were employed routinely, either separately or in combination. No decomposition of the toxaphene applied to the GLC column after treatment with ethanolic potassium hydroxide (*3*) was evident. No decomposition of the DDT was evident on the GLC column, and its retention time on the GLC and R_f value on the TLC were different from those of either DDD or DDE. The gas chromatograph was the Varian Aerograph Model 1200 equipped with an electron capture detector and a Leeds and Northrup Speedomax W 1-mv recorder with a chart speed of ½ inch per minute. Areas under the peaks were measured with a polar planimeter. The chromatographic column, an 8 ft. x 4 mm I.D. Pyrex glass coil packed with 60/80 mesh silylated Chromosorb W, acid washed, was coated with 5% Dow 710 silicone fluid and 5% SE-30 silicone gum rubber. Nitrogen carrier gas (50 psi, 20 cc/minute) and a column temperature of 220 C gave the best results and was used in these experiments. A practical method sensitivity was established at 0.01 ppm. Recoveries through the entire procedures of extraction, cleanup, and analysis exceeded 90%. All residue data are expressed on a dry-weight basis.

Thin-layer chromatography was employed for screening and in combination with GLC as an analytical method. Silica gel H (0.5 mm layer thickness) adsorbent, developing solvent (100% pentane), and the silver nitrate: 2-phenoxyethanol color test (*9*) as a dip solution were employed. For quantitative work, chromatogram areas containing the unknowns were extracted from the silica gel with pentane after comparing R_f values (0.41, DDE; 0.26, DDT; 0.14, DDD; and 0.10 to 0.20, toxaphene) with those of parallel standard pesticide tracers, and the extracts were analyzed by GLC. The toxaphene extracts from TLC were treated with ethanolic potassium hydroxide (*3*) prior to analysis by GLC for the purpose of increasing the analytical method sensitivity and to eliminate interference from DDTR on the GLC. Recoveries were within the limits previously described.

Results and Discussion

Table 1 shows the levels of DDTR and toxaphene found in the fractionated 100-g sample. Fraction A (Buckhorn) was the largest component (53.4% by weight) and contained relatively low levels of pesticide (0.67 ppm DDTR and 4.26 ppm toxaphene). Fractions B through E comprised approximately 41% of the total composite sample and contained 94% of the DDTR and 85% of the toxaphene. Fraction C (soil, 10.1% by weight) contained 29% of the DDTR and 26% of the toxaphene residues present in the composite sample. The remaining fractions (F through M) made up approximately 6% of the total composite sample; and although in some of these individual fractions the pesticide residues were high, their levels contributed very small amounts to the total levels in the composite sample.

Table 2 shows the residue levels of DDD, DDE, and DDT and their sums found in individual 10-g fractions after adjusting the levels according to the percentages by weight of each fraction in relation to a composite sample. Generally, in each fraction, the largest percentage of the DDTR residues was DDT followed by DDD and DDE. The total residues in fractions A through M were DDD, 1.89 ppm; DDE, 1.26 ppm; DDT, 13.60 ppm, with a sum of 20.00 ppm DDTR including 3.20 ppm found as DDTR in the extracted plant material.

TABLE 1.—*Levels of DDTR and toxaphene in fraction components of ladino clover seed crop screenings*

[All residues are reported on a dry-weight basis; ND = not detectable]

Fraction Identification	Code Letter	Type of Seed	% of Total Weight	Residues in PPM	
				DDTR	Toxaphene
Buckhorn (*Plantago lanceolata*)	A	Weed	53.4	0.67	4.26
Chaff—landino clover	B	Legume	19.3	47.08	48.00
Soil	C	—	10.1	57.28	53.00
Ladino clover seeds	D	Legume	5.9	47.28	27.40
Ryegrass (*Lolium spp.*)	E	Grass	5.6	19.42	28.30
Watergrass (*Echinochloa crusgalli*)	F	Grass	2.4	10.75	9.39
Fall dandelion (*Leontodon autumnalis*)	G	Weed	1.6	15.76	9.90
Knotweed (*Polygonum ariculare*)	H	Weed	0.6	2.94	1.65
Naked watergrass (*Echinochloa crusgalli*)	I	Grass	0.6	47.06	70.40
Oxtongue (*Picris eschioides*)	J	Weed	0.32	2.32	11.80
Bermudagrass (*Cynadon dactylan*)	K	Grass	0.13	31.36	0.77
Storkbill (*Eradium cicutarium*)	L	Weed	0.04	14.75	1.25
Miscellaneous—unidentified	M	—	0.01	29.40	22.50

TABLE 2.—*Levels of DDTR in individual 10-g fractions and the composite of these fractions. Levels are adjusted according to the percentage of each plant fraction in a composite sample (Table 1).*

[All residues are reported on a dry-weight basis; ND = not detectable]

Code Letter[1]	DDD (PPM)	% of Total Residue	DDE (PPM)	% of Total Residue	DDT (PPM)	% of Total Residue	DDTR (PPM)	DDTR in Extracted Screenings (PPM)	Sum Total DDTR (PPM)
A	ND	—	0.0121	9.5	0.1140	90.5	0.1261	0.2270	0.3531
B	1.1500	15.1	0.8220	10.8	5.6700	74.1	7.6420	1.4400	9.0820
C	0.3150	6.5	0.1670	3.4	4.3600	90.1	4.8420	0.9370	5.7790
D	0.2170	8.8	0.1800	7.3	2.0700	83.9	2.4670	0.3030	2.7700
E	ND	—	0.0150	1.7	0.8630	98.3	0.8780	0.2020	1.0800
F	0.0566	23.3	0.0200	8.2	0.1670	68.5	0.2436	0.0182	0.2618
G	0.0477	19.3	0.0243	10.0	0.1710	70.7	0.2430	0.0140	0.2570
H	0.0088	5.8	0.0004	2.7	0.0124	91.5	0.0216	0.0052	0.0268
I	0.0810	29.1	0.0123	4.4	0.1850	66.5	0.2783	0.0458	0.3241
J	0.0025	40.9	0.0005	8.6	0.0031	50.5	0.0061	0.0013	0.0074
K	0.0136	36.1	0.0074	19.5	0.0168	44.4	0.0378	0.0029	0.0407
L	0.0007	15.2	0.0019	41.2	0.0017	36.6	0.0043	0.0013	0.0056
M	0.0004	13.5	0.0006	21.2	0.0018	65.3	0.0028	ND	0.0028

[1] Letter codes and sample identification (Table 1).

TABLE 3.—*Levels of toxaphene in individual 10-g fractions and the composite of these fractions. Levels are adjusted according to the percentage of each plant fraction in a composite sample (Table 1).*

[All residues are reported on a dry-weight basis; ND = not detectable]

Code Letter [1]	Residues in PPM		
	Toxaphene	Toxaphene in Extracted Screenings	Sum Total Toxaphene
A	2.28	ND	2.28
B	9.37	ND	9.37
C	5.36	ND	5.36
D	1.61	ND	1.61
E	1.32	0.26	1.58
F	0.23	ND	0.23
G	0.16	ND	0.16
H	0.01	ND	0.01
I	0.42	ND	0.42
J	0.04	ND	0.04
K	ND	ND	ND
L	ND	ND	ND
M	ND	ND	ND
Total	20.80	0.26	21.06

[1] Letter codes and sample identification (Table 1).

Table 3 shows the residue levels of toxaphene found in individual fractions and adjusted according to the percentages by weight of each fraction in relation to a composite sample. Approximately 96% of the toxaphene residues were in fractions A through E with a total adjusted residue on the composite sample basis of 21.06 ppm.

Table 4 contains data for comparing the total residues of DDTR and toxaphene found in fractions A through M with the total residues of the pesticides obtained by direct analyses on a composite seed crop screenings sample. The level of DDTR residues obtained in the composite sample by direct analysis was 22.9 ppm as compared to 20.0 ppm in the adjusted total residue for the fractionated sample (Table 2). The toxaphene residue obtained in the composite sample by direct analysis was 20.4 ppm as compared to 21.1 ppm in the adjusted total residue for the fractionated sample (Table 2).

TABLE 4.—*Comparison of the levels of toxaphene and DDTR in ladino clover seed crop screenings as determined by direct analysis of a composite sample with calculated adjusted levels as determined from analyses on 13 fractions of a composite sample*

[All residues are reported on a dry-weight basis; ND = not detectable]

Sample	Residues in PPM								
	DDT	DDE	DDD	DDTR	DDTR in Extracted Seed Screenings	Sum Total DDTR	Toxaphene	Toxaphene in Extracted Seed Screenings	Sum Total Toxaphene
Composite ladino clover seed screenings	16.8	1.6	2.3	20.7	2.2	22.9	18.1	2.3	20.4
Fractionated sample of screenings reported as composite	13.6	1.3	1.9	16.8	3.2	20.0	20.8	0.3	21.1
Percent total residue recovered in fractionated screenings sample with respect to that residue found in the composite sample						87.3%			103.4%

If 2 fractions of the 13 (ladino clover chaff and soil) were eliminated from the composite sample, the remaining 71% of the sample would contain only 26% of the total DDTR and 30% of the toxaphene. The remaining fractions would be much more acceptable as animal feed. However, complete removal of all contaminants from the feed would be ideal, and investigations are currently in progress for developing procedures for practical removal of all contaminants from the feed by physical and chemical methods.

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See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(1) *Archer, T. E. 1968.* Quantitative measurement of combinations of aramite, DDT, toxaphene, and endrin in crop residues. Bull. Environ. Contamination Toxicol. 3:71.

(2) *Archer, T. E. 1969.* Decontamination of malathion from ladino clover seed screenings. J. Poultry Sci. 48:2075.

(3) *Archer, T. E. and D. G. Crosby. 1966.* Gas chromatography measurement of toxaphene in milk, fat, blood, and alfalfa hay. Bull. Environ. Contamination Toxicol. 1:70.

(4) *Crosby, D. G. and T. E. Archer. 1966.* A rapid analytical method for persistent pesticides in proteinaceous samples. Bull. Environ. Contamination Toxicol. 1:16.

(5) *Dorough, H. W., N. M. Randolph, and G. H. Wimbish. 1966.* Persistence of methyl parathion residues on sunflower seeds. Bull. Environ. Contamination Toxicol. 1:86.

(6) *Erro, F., A. Bevenue, and H. Beckman. 1967.* A method for the determination of toxaphene in the presence of DDT. Bull. Environ. Contamination Toxicol. 2:372.

(7) *Kawano, Y., A. Bevenue, H. Beckman, and F. Erro. 1969.* Studies on the effect of sulfuric-fuming nitric acid treatment on the analytical characteristics of toxaphene. J. Ass. Offic. Agr. Chem. 52:167.

(8) *Klein, A. K. and J. D. Link. 1970.* Elimination of interferences in the determination of toxaphene residues. J. Ass. Offic. Agr. Chem. 53:524.

(9) *Mitchell, L. C. 1958.* Separation and identification of chlorinated organic pesticides by paper chromatography. XI. A study of 114 pesticide chemicals: technical grades produced in 1957 and reference standards. J. Ass. Offic. Agr. Chem. 41:781.

Chlorinated Hydrocarbon Residues in the Milk Supply of Ontario, Canada[1]

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ABSTRACT

A province-wide survey of chlorinated hydrocarbon insecticide levels in the fluid milk of Ontario was carried out between November 1967 and June 1969. Composite samples were collected from bulk tankers, each representing an average of 16 producers. With a total collection of 1,651 samples, each of 27,000 producers in the Province was sampled at least once. DDT plus its metabolites and dieldrin were present in almost all milk fat tested; lindane was found in only 8% of the samples and heptachlor epoxide in 3%. Milk from 20 producers contained levels in butterfat which exceeded the administrative tolerances of 0.1 ppm for dieldrin and heptachlor epoxide and 1 ppm for lindane and DDT plus metabolites. Seventeen of these cases were dieldrin violations; three were DDT or DDD; and one was due to lindane. These high residues were traced to contaminated feed in seven cases and to improper spraying or use of contaminated spray in five cases; in four herds, the source of the insecticide could not be found. The disappearance of residues from butterfat, after removal of the source, was an exponential function of time. This disappearance was not clearly evident in herds where multiple sources of low-level contamination occurred. The average levels of chlorinated insecticide residues in fluid milk of the Province were 0.134 ppm DDT and its metabolites, 0.031 ppm dieldrin, 0.005 ppm lindane, and 0.001 ppm heptachlor epoxide.

Introduction

The Canadian province of Ontario ranks first in the Nation with respect to milk production and milk consumption. Approximately 6.7 billion pounds of milk are produced per annum with a farm value of about $260 million (*1*). This represents 930 lb per capita for the 7.15 million inhabitants (census of 1967). Exports of milk and dairy products amount to approximately $14 million.

Milk supplies have been monitored for chlorinated hydrocarbon insecticides in several countries. Clifford *et al.* (*3,4*) and Duggan (*5*) have reported levels in fluid milk and dairy products in the United States. Bro-Rasmussen *et al.* (*2*) have published residue levels in Danish milk and butter.

Since no comprehensive data on chlorinated hydrocarbon insecticide levels in milk were available for the province of Ontario, a monitoring survey was initiated to determine residue levels and to attempt to correlate these levels with the particular type of farming operation.

Ontario can be divided into southern and northern parts. The southern part, which represents only 13% of the Province, is largely devoted to agriculture; the northern section which is part of the Great Canadian Shield, has only a few small pockets of agriculture around the industrial areas. The great majority of the territory is covered by forests, lakes, rock outcrops, or tundra.

From an agricultural point of view, the southern part can be divided into southern, western, central, and eastern regions (Fig. 1), each differing in the type of agriculture practiced (Table 1). The area most intensively used for agriculture is in the southern region where high-value crops such as tobacco, corn, soybeans, fruit, and vegetables are produced. Some cash crops are grown close to Lake Huron in the western region, but the mainstay of agricultural production in this region is livestock and, in particular, beef production. The central region has some cash crop farming along Lakes Ontario and Simcoe, but the largest area is devoted to tourism, forestry, and mixed farming. The eastern

[1] From the Provincial Pesticide Residue Testing Laboratory, Ontario Department of Agriculture and Food, Guelph, Ontario, Canada.

FIGURE 1.—*Map of the Province of Ontario divided into five regions*

region has a potential for increased cash crop production, but the main pursuit is dairying and raising livestock. Northern Ontario has isolated pockets of mixed agriculture near the larger population centers.

As a consequence of the differing types of agricultural production from area to area and region to region, it is to be expected that differences will be found in the pattern of use of pesticides. In 1968, a survey was carried out by the Ontario Department of Health, under the Pesticides Act (*13*), to discover the use pattern of chlorinated hydrocarbon insecticides in agriculture. The results of this survey are shown in Table 1. Almost 90% of the DDT and cyclodienes used in the Province was applied in the southern region. Unfortunately, the amounts only account for that used in agriculture and do not include the widespread nonagricultural application for forest and parkland protection and control of nuisance insects. Such uses for DDT have been widespread in the central and northern regions.

A population of 926,000 dairy cows is distributed over the 5 regions on some 27,000 farms. Milk produced in the southern region is utilized mainly as fluid milk or for butter production. The western region represents the largest butter-producing area, while the central region represents the largest fluid milk-producing area. Milk from the eastern region is used primarily for the production of cheese and butter. Milk produced in northern Ontario is consumed mainly as domestic fluid milk (Table 2).

Sampling Procedure

The milk monitoring program was initiated by the Ontario Department of Agriculture and Food in November 1967 and completed in June 1969. During this period, a total of 1,651 composite milk samples, representative of each of the 5 agricultural regions, was collected and analyzed. The survey was intended to include samples from all producers in Ontario who marketed fluid milk. Since the large number of producers made individual sampling impractical, 1-quart composite samples, representing 8,000 to 20,000 lb of

TABLE 1.—*Type of cropping, distribution of livestock and use of insecticides in the regions of Ontario (1968)*

	Southern	Western	Central	Eastern	Northern	Total
Total land area (acres x 10^6)	5.2	6.5	10.2	7.3	191	220.2
Total no. of farms (x 10^3)	36.7	31.7	17.0	18.7	5.8	109.9
Crop distribution:			PERCENT			Acreage (x 10^6)
Cereal	28	40	14	14	4	2.7
Legumes	85	14	0.5	0.5	0	0.4
Corn	64	20	8	8	0	1.3
Hay	16	31	17	26	10	3.4
Fruit	76	11	12	1	0	0.02
Vegetables	52	24	16	5	3	0.11
Livestock distribution:			PERCENT			Numbers (x 10^6)
Cattle	22	40	15	18	5	3.2
Swine	32	49	12	6	1	2.0
Sheep	19	35	20	15	11	0.26
Insecticide use: [1]			PERCENT			Pounds (x 10^3)
DDT + DDD	89.3	5.3	3.3	2.1	<0.1	302
Aldrin + Dieldrin	89.8	9.2	1.0	—	—	32

[1] Data obtained by request from the Ontario Department of Health, Toronto.

TABLE 2.—*Population of dairy cows and dairy herd replacements and the annual production and utilization of milk in Ontario*

Region of Ontario	Numbers (x 10^3)		Quantities produced (lb x 10^3)			
	Dairy cows	Replacements	Butter	Cheese	Fluid milk	Manufactured
Southern	207	58	287,894	55,754	480,570	110,542
Western	281	72	1,243,163	81,437	202,219	50,046
Central	138	40	173,087	199,237	801,008	286,025
Eastern	258	74	546,234	584,888	237,637	92,101
Northern	42	9	42,675	26,289	154,146	15,878
Ontario (Total)	926	253	2,293,053	947,605	1,875,580	554,592

fluid milk from 5 to 20 producers along a truck route, were collected from bulk milk carriers at the time of delivery at their respective dairies or processors.

With this sampling procedure, it was possible to reduce the number of samples required for analysis without sacrificing geographical identity. The 1-quart milk samples were systematically collected by personnel of the Milk Commission, Ontario Department of Agriculture and Food, and delivered promptly to the Provincial Pesticide Residue Testing Laboratory under refrigerated conditions.

Analytical Procedure

All samples were processed and the butterfat removed immediately upon receipt at the laboratory. The milk was allowed to warm to room temperature, and after a thorough shaking, the butterfat was separated according to the procedure described by Moubry *et al.* (*10*). Approximately 100 ml of milk was transferred to a 200-ml volumetric flask and, with constant mixing, the flask was filled to the neck with a detergent reagent consisting of 50 g sodium tetraphosphate plus 24 ml of Triton X-100 per liter of water. This mixture was placed in a water bath at 95 C until the clear butterfat layer separated into the neck of the flask. The butterfat was transferred into vials and held under refrigeration for subsequent analysis.

Chlorinated hydrocarbon insecticides were isolated from the butterfat by the one-step Florisil column method of Langlois *et al.* (*9*) with some minor modifications. Florisil, 60/100 mesh, activated commercially at 1200 F, was reheated at 135 C for a minimum of 24 hours. Upon cooling to room temperature, the adsorbent was partially deactivated by the addition of water at the rate of 5 ml per 100 g Florisil. It was then held in an air-tight container, and allowed to equilibrate during a 12-hour tumbling period. Cleanup was carried out in 25 mm x 300 mm Pyrex columns fitted with Teflon stopcocks and 350-ml glass reservoirs. The eluting solution consisted of a 1:4 mixture of dichloromethane:hexane (v/v). All solvents employed were reagent grade chemicals which had been redistilled.

Butterfat was fluidized by placing in a warm oven; 1.00 g was transferred with a dropping tube to 25 g of conditioned Florisil and mixed thoroughly until a free-flowing powder was obtained. Twenty-five grams of deactivated Florisil was poured into a chromatographic column to form the bottom half of the cleanup system. This was prewashed with 50 ml of a 1:1 mixture of dichloromethane and hexane. The butterfat-Florisil mixture was then introduced to form the top layer. The column was eluted with 300 ml of the 1:4 dichloromethane:hexane elution mixture at a percolation rate of approximately 5 ml/minute. Eluates were concentrated just to dryness with rotary vacuum at 45 C; the residue was redissolved in 5.0 ml of hexane and transferred to

a glass-stoppered tube for subsequent gas chromatographic analysis.

A Varian Aerograph Model 1200 gas-liquid chromatograph, equipped with a 250 mc tritium electron capture detector, was used for all quantitative assays. Operating parameters were as follows:

Column:	5′ x ⅛″ Pyrex, packed with 4% SE-30 + 6% QF-1 on Chromosorb W, preconditioned 72 hours at 225 C
Temperature:	Column 175 C Detector 200 C Injector 225 C
Carrier gas:	Nitrogen at 40 ml/minute

Injection volumes were kept constant at 5μl for both sample solutions and comparison standards. Where necessary, sample solutions were diluted until chromatographic responses were within the linear range of the detector. Since all analyses were carried out under isothermal and isobaric conditions, peak heights alone were used for quantitation.

Qualitative residue confirmations in milk samples exhibiting abnormal quantitative and/or qualitative characterstics were accomplished by thin-layer chromatography and by chemical conversion procedures (7).

Recoveries of chlorinated hydrocarbons by the analytical procedure were checked regularly by fortification directly into the milk prior to butterfat separation. Averaged recoveries were as follows: lindane, 91%; heptachlor epoxide, 94%; dieldrin, 92%; *p,p′*-DDE, 94%; *p,p′*-DDD, 90%; and *p,p′*-DDT, 93%. The data presented in this report do not include recovery corrections.

Where the composite sample contained residues of 0.1 ppm dieldrin or heptochlor epoxide or 1.00 ppm DDT plus metabolites or lindane, the milk from each producer making up the composite sample was collected for analysis. When a producer had been singled out as producing milk that exceeded the above mentioned levels, an immediate investigation was undertaken on his farm to find the source of contamination. This involved sampling feed, litter, water, medicines, and sprays for analysis. When the source of contamination was established, corrective measures were introduced, and a regular check was kept on the milk supply to ensure a decline in the residual levels.

Results

THE PROVINCIAL MILK SURVEY

A total of 1,651 bulk tankers representing 286 dairies, creameries, and cheese factories located in 214 towns and cities of Ontario were sampled and analyzed. Each sample represented an average of 16.3 producers or 10,900 lb of milk. The average total residue of chlorinated hydrocarbons in the butterfat was 0.171 ppm, consisting of 0.134 ppm DDT and its metabolites, 0.031 ppm dieldrin, 0.005 ppm lindane, and 0.001 ppm heptachlor epoxide (Table 3). This total residue was slightly lower than the level of 0.227 ppm reported by Duggan (5) for milk sampled between 1963 and 1966 in the United States. The level for DDT and its metabolites was identical in both surveys at 0.134 ppm; levels of dieldrin, lindane, and heptachlor epoxide averaged two-thirds, one-half, and one-tenth, respectively, of the amounts reported in the U.S. survey.

Separation of the Ontario data on a regional basis revealed that the highest average level of DDT and metabolites occurred in the southern region (Table 3) where the major use of DDT in agriculture is practiced (Table 1). In this region, DDT levels in butterfat ranged from 0.120 ppm in milk produced in counties where general farming practices were followed and increased up to 0.328 ppm in counties of intensive cash crop production.

The counties with the highest use pattern of DDT in 1968 tended to have higher residues in the milk, but several exceptions were noted. DDT residues in the central region varied from 0.056 ppm in counties of little agricultural activity to 0.215 ppm in the counties of intensive fruit and vegetable production. Most counties between these two extremes fell into the general order of their use pattern. In the eastern and western regions, where general mixed agriculture is pursued, the total DDT residue was slightly over 0.1 ppm with little significance in the differences between counties. The ranges in levels were 0.084 to 0.137 ppm in the eastern region and 0.058 to 0.148 ppm in the western region. The northern region of the Province exhibited the lowest residue levels of DDT, ranging from 0.018 to 0.087 ppm in butterfat.

Few milk samples in the Province were free of DDT and its metabolites (Table 4). Only 0.6% of samples from the eastern region contained nondetectable residues of *p,p′*-DDE; in all other regions, 100% of the samples contained measurable amounts. Levels of *p,p′*-DDD were nondetectable in 0.3, 1.0, and 7.1% of the milk samples from the central, eastern, and northern regions, respectively, and *p,p′*-DDT was nondetectable in 0.3, 0.8, and 1.4% of the samples from the same three regions.

There were no detectable lindane residues in milk samples from the northern region. The average levels of lindane in the central, eastern, and southern regions were between .001-.002 ppm in the butterfat; however, levels were nondetectable in over 96% of all samples from these regions (Tables 3 and 4). Only 9 of 46 counties in these three regions produced milk that contained detectable amounts of lindane, and in no

TABLE 3.—*Residues of chlorinated hydrocarbons found in Ontario-produced milk (November 1967-June 1969)*

Region of Ontario	Number of samples	Average level of chlorinated hydrocarbons in butterfat of milk (ppm)						
		p,p'-DDE	*p,p'*-DDD	*p,p'*-DDT	Total DDT	Lindane	Dieldrin	Heptachlor epoxide
Central	387	.075	.034	.037	.146	.001	.030	ND
Eastern	489	.046	.023	.032	.101	.001	.022	ND
Northern	70	.023	.017	.022	.062	ND	.024	ND
Southern	372	.115	.037	.041	.193	.002	.043	<.001
Western	333	.057	.023	.034	.114	.017	.037	.003
Ontario (Total)	1651	.070	.029	.035	.134	.005	.031	.001

TABLE 4.—*Frequency distribution of insecticide residues in butterfat of milk in Ontario (November 1967-June 1969)*

Insecticide	Residue levels (Ranges in ppm)	Frequency by region of Ontario (%)					
		Central	Eastern	Northern	Southern	Western	Ontario Total
p,p'-DDE	.00—.09 [1]	79.6	98.8	100	59.7	94.0	84.6
	.10—.19	18.3	1.2	—	30.4	5.7	12.6
	.20—.29	2.1	—	—	6.7	0.3	2.1
	.30—.39	—	—	—	2.7	—	0.6
	.40+	—	—	—	0.5	—	0.1
p,p'-DDD	.00—.09 [1]	96.4	99.2	97.1	98.4	99.1	98.2
	.10—.19	2.6	0.8	2.9	1.0	0.9	1.4
	.20—.29	0.0	—	—	0.2	—	0.1
	.30—.39	0.3	—	—	0.2	—	0.1
	.40+	0.7	—	—	0.2	—	0.2
p,p'-DDT	.00—.09 [1]	97.4	97.4	97.1	96.5	97.9	97.3
	.10—.19	2.6	2.2	2.9	2.7	1.8	2.3
	.20—.29	—	0.2	—	0.5	—	0.2
	.30—.39	—	—	—	0.3	0.3	0.1
	.40+	—	0.2	—	—	—	0.1
Dieldrin	.00—.04 [1]	92.0	98.6	97.1	66.9	84.7	87.0
	.05—.09	7.2	1.2	2.9	31.2	13.8	12.0
	.10—.20	0.8	0.2	—	1.6	1.2	0.8
	.20+	—	—	—	0.3	0.3	0.2
Heptachlor epoxide	.00	100	100	100	96.5	89.2	97.0
	.01—.09	—	—	—	3.5	10.5	2.9
	.10+	—	—	—	—	0.3	0.1
Lindane	.00	96.6	99.4	100	96.5	71.2	92.4
	.01—.09	3.4	0.4	—	3.2	23.7	6.4
	.10+	—	0.2	—	0.3	5.1	1.2

[1] Nondetectable residue levels: *p,p'*-DDE—0.6% in eastern region; *p,p'*-DDD—0.3, 1.0, and 7.1% in central, eastern, and northern regions; *p,p'*-DDT—0.3, 0.8 and 1.4% in central, eastern, and northern regions; and dieldrin—0.3 and 0.6% in central and eastern regions.

case was the residue level higher than 0.002 ppm in the butterfat. In the western region, only 5 of the 10 counties produced milk with detectable amounts of lindane, and levels ranged from 0.007 to 0.045 ppm. Lindane residues were believed to be caused by three main sources: (*1*) the use of lindane vaporizers in milk rooms, (*2*) contamination of animal diets with treated seed, and (*3*) direct application to dairy herds for insect control.

The highest average dieldrin levels (0.043 ppm) were recorded from milk samples from the southern region where the majority of aldrin and dieldrin is used in agriculture. A range of 0.026 to 0.070 ppm was present in the 12 counties of this region; those counties with the highest levels were intensively involved in tobacco, fruit, and vegetable production. Residue levels of dieldrin in the western region varied from 0.021 to 0.055 ppm. In the central, eastern, and northern regions, dieldrin levels in butterfat ranged from 0.019 to 0.033, 0.018 to 0.035, and 0.015 to 0.040 ppm, respectively. In samples from the central and eastern regions, 0.3% and 0.6%, respectively, contained nondetectable dieldrin levels. In the central, eastern, and northern regions, respectively, 99.2, 99.8, and 100% of fat samples tested showed dieldrin levels below 0.10 ppm. Administrative tolerances of 0.10 ppm in butterfat were exceeded by 1.9% of the samples from the southern region, 1.5% from the western region, 0.8% from the central region, and 0.2% from the eastern region.

Milk produced in the southern and western regions contained only low amounts of heptachlor epoxide (Table 3). In the southern region, the average level was below 0.001 ppm, and milk originating from only two of 12 counties had detectable residues. These counties bordered on three counties in the western region where heptachlor had been used for turnip production and/or seed treatment of cereal grain. Both cull turnips and treated seed were suspected as having been used as feed

for dairy cattle causing the appearance of heptachlor epoxide in the milk. The use of treated grain for feed is a violation of the Feeds Act and Regulations (6) and the use of cull turnips is not recommended. In the southern and western regions, 97 and 89%, respectively, of the samples had nondetectable levels of heptachlor epoxide. Only 0.3% of samples in the western region contained residues that were at the 0.1 ppm level or greater, i.e., levels above the permitted administrative tolerances. Heptachlor epoxide did not appear in milk fat from the central, eastern, or northern regions.

HERDS WITH HIGH RESIDUES

Where a composite sample contained residues in the fat above 1.0 ppm DDT or lindane, or 0.1 ppm dieldrin or heptachlor epoxide, samples of milk from each producer contributing to the composite were collected for analysis. From 17 bulk tankers rechecked, a total of 20 individual producers were found delivering milk with residues above these levels. In five cases, the residues were between 0.10 and 0.20 ppm dieldrin. In these cases, the milk was resampled at 2-week intervals; and if the dieldrin residue was found to be declining, no further action was taken, and the source of contamination was not determined. There were 12 producers with butterfat containing between 0.33 and 3.17 ppm dieldrin, one producer with 1.72 ppm lindane, and three producers with 3.36 to 17.7 ppm DDT and metabolites (Tables 5, 6, and 7). In each of these cases, the producers were visited, and a total of 258 samples of feed, litter, water, spray, etc., were collected and analyzed. In addition, 354 milk samples, including individual cow samples and composite herd samples, were collected for analysis.

In seven cases, the residues were the result of ingestion of contaminated feed or litter. In five cases, cattle had been sprayed with an insecticide not registered for that purpose or one that was contaminated with a persistent chlorinated hydrocarbon insecticide. In four cases, the source was no longer present and could not be determined, and four additional cases were not investigated. (Table 8).

Studies with dairy herds which exhibited residues above the tolerance levels for total DDT and dieldrin indicated that the rate of decline of these insecticides was dependent upon the original level and generally followed a decay curve pattern. The length of time required for a decline to reach acceptable levels varied according to the initial level, the effectiveness of removing the source, and the insecticide causing the problem. The time

TABLE 5.—*Distribution of dieldrin in Herds 1 and 2 at time of discovery and the decline of these residues*

Dieldrin in milk fat (Ranges in ppm)	Herd 1		Herd 2	
	No. of cows	Average ppm	No. of cows	Average ppm
.00— .49	12	0.14	14	0.34
.50— .99	2	0.71	11	0.73
1.00—1.49	10	1.27	6	1.17
1.50—1.99	8	1.70	2	1.67
2.00 & over	7	3.43		

Days after discovery	Dieldrin (ppm)			
	Seven highest cows—Herd 1			Herd 2
	Back fat	Milk fat	Hair	Milk fat
0	—	3.43	—	0.95
9	—	—	—	1.04
20—26	—	2.30	—	0.75
47—53	—	1.34	0.15	—
71—75	2.58	0.94	0.05	0.53
96—99	—	0.68	0.05	0.37
124—133	0.60	0.41	0.02	0.23
140—145	—	0.24	—	0.31
165	—	0.12	—	—
184	—	—	—	0.16
206—218	0.16	0.09	—	—
Biological half-life (days)	34	37	—	58

TABLE 6.—*Decline of dieldrin residues in milk after discovery and removal of source*

Days after discovery	Dieldrin residues (ppm) in herds (No.)							
	4	5	6	7	8	9	10	11
0	0.58	0.94	1.09	0.44	0.78	2.20	0.66	1.60
15	—	0.90	1.08	0.35	—	—	—	—
20	—	—	—	—	—	—	—	2.17
29	1.58	0.89	1.02	0.28	—	—	—	—
38	—	0.52	1.04	0.21	0.46	0.98	0.30	1.63
50	0.80	0.49	1.09	—	—	—	—	—
65	0.40	—	—	—	—	—	—	1.52
78	—	0.53	1.12	—	—	—	—	0.75
85	0.33	0.47	—	—	0.17	0.69	—	—
103	—	—	1.26	—	—	0.39	0.23	—
126	—	—	0.77	—	0.13	0.49	—	—
145	—	0.52	0.85	0.10	0.10	0.09	0.49	—
163	—	0.45	0.37	—	—	—	—	—
187	—	—	—	—	—	—	—	0.19
200	—	0.40	0.29	—	—	—	0.23	—
230	0.11	0.53	0.37	—	—	—	—	0.10
275	—	0.16	0.27	—	—	—	—	—
308	—	—	—	—	—	—	0.05	—
322	—	0.22	0.18	—	—	—	—	—
364	—	0.23	—	—	—	—	—	—
Biological half-life (days)	50	—	—	38	38	30	—	40

TABLE 7.—*Decline in DDT and metabolites in Herds 12, 13, and 14*

Days after discovery	Content in butterfat (ppm)			
	p,p'-DDE	*p,p'*-DDD	*p,p'*-DDT	Total DDT
HERD 12				
0	6.20	10.10	1.40	17.7
8	4.90	5.90	1.70	12.5
13	4.70	5.21	1.50	11.4
21	4.50	5.00	1.50	11.0
29	4.51	3.98	1.29	9.78
41	3.26	3.05	1.03	9.34
61	3.90	3.35	1.25	8.50
77	6.04	4.28	1.23	11.55
90	5.63	3.45	1.33	10.41
105	5.79	2.60	0.95	9.34
125	5.65	1.60	0.93	8.18
140	3.12	0.87	0.48	4.47
216	3.29	0.28	0.47	4.04
246	4.27	0.24	0.54	5.08
300	1.84	0.05	0.28	2.17
425	1.07	0.06	0.18	1.31
482	0.23	0.02	0.04	0.29
530	0.09	0.06	0.07	0.22
HERD 13				
0	0.66	0.85	4.29	5.80
43	0.41	0.15	1.98	2.54
155	0.22	0.06	0.57	0.85
HERD 14 [1]				
0	0.11	2.71	0.54	3.36
24	0.34	0.34	0.40	1.08
41	0.17	0.44	0.48	1.09
41 (3 heifers only)	0.36	1.41	1.51	3.28
63	0.14	0.23	0.41	0.98
78	0.13	0.10	0.34	0.57

[1] This producer was a can shipper, and it is conceivable that only one can was sampled.

TABLE 8.—*Sources of contamination in dairy herds with high residues of persistent insecticides*

Method of contamination	Source of contamination	Number of herds	Contaminating insecticide
By ingestion	Treated seed grains in feed	1	Aldrin, dieldrin
	Feed grains stored in contaminated bins	1	Aldrin
	Hay and feed grain low level contamination	1	Dieldrin
	Hay	2	Dieldrin
	Sweet corn silage	1	DDT, metabolites
	Sawdust litter	1	Dieldrin
By dusting or spraying	Contaminated spray (Ciodrin, Vapona)	2	Aldrin, dieldrin
	Contaminated wettable powder used as dust (Rotenone)	1	DDT
	Improper use	2	Lindane, DDT
Unknown	No source found	4	Dieldrin
	Not investigated	4	Dieldrin

required was usually in excess of 200 days (Tables 5, 6, and 7). High lindane residues (>1.0 ppm) dropped rapidly to acceptable levels in about 2 weeks.

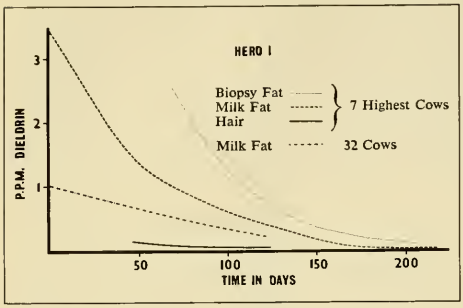
Herds with High Dieldrin

Herd 1. The composite milk sampled from the bulk tanker prior to delivery at the dairy revealed a dieldrin content of 0.48 ppm. This high dieldrin residue was traced back to one producer who was contributing milk with a level of 3.17 ppm dieldrin in the butterfat. Analysis of various samples in an attempt to locate the source of contamination revealed that dieldrin levels were only .005 ppm in soil, .003 to .004 ppm in hay and grass .0001 ppm in drinking water, and nondetectable in corn, cereal, litter, and feed additives. It was concluded that none of these sources were responsible for the extremely high dieldrin level present.

The milk from each cow of the herd was analyzed (Table 5), and this revealed a wide range of levels that failed to resolve the possible means of contamination. Twelve animals with the lowest residues, averaging 0.14 ppm dieldrin, were mostly heifers that had only recently entered the herd. Hamburger made from calves born to some of the contaminated animals contained 2.2 ppm dieldrin on a whole-tissue basis. The seven animals with the highest dieldrin levels were removed from the herd for study.

Milk, hair, and fat biopsy were analyzed from these seven animals, and the loss of dieldrin was found to be an exponential function of time (Table 5 and Fig. 2). The decline in milk fat from 3.5 to 0.1 ppm took almost 200 days on a diet with nondetectable or insignificant levels of dieldrin. Two animals were dry at day 96, and

FIGURE 2.—*Decline in dieldrin content of hair, milk fat, and fat biopsies taken from Herd 1 at varying intervals following discovery*



one of these calved at day 218. The level of dieldrin at the last milking of one was 0.57 ppm, and the first test after calving was 0.56 ppm. During this time period, the residue in the milk of the other five cows had declined from 0.68 to 0.09 ppm. The average level of the 32 cows remaining on the farm declined slowly from 0.94 to 0.22 ppm in 120 days. Analysis of hair from the seven test animals showed a steady decline from 0.15 to 0.02 ppm in 80 days. Fat biopsies were taken on three separate occasions and showed the level of dieldrin in the body fat to be considerably higher than that present in the milk fat.

Herd 2. Investigation of a composite bulk tanker sample containing 0.15 ppm dieldrin in butterfat located the source with one herd contributing a level of 0.95 ppm. Analysis of the feed revealed either trace levels (.004 ppm) or nondetectable levels of dieldrin in all except one concentrate which contained 0.01 ppm. Samples of wood shavings used for litter were found to contain dieldrin at levels from 2 to 15 ppm. This herd had been kept on a restricted diet; and, as a result, wood shavings were being consumed. These wood shavings were traced back to teak timbers which had originally been grown and treated in Columbia, South America, and shipped to Ontario. Table 5 shows the average levels after analysis of individual herd cows. The level of dieldrin in the butterfat rose until the wood shavings were removed as litter, and then a decline to 0.16 ppm occurred over a period of 184 days.

Herd 3. An excessive level (0.35 ppm) was found in milk from only one producer; this level declined to 0.05 ppm in 46 days, and no investigation for a source was undertaken.

Herd 4. A large bulk tanker was found to have a total cyclodiene level of 0.19 ppm. Upon investigation, milk samples from five producers were found to contain levels between 0.10 and 0.20 ppm, one at 0.33 ppm, and one at 0.58 ppm. After further investigation, no source of contamination could be found for the first six. In the case of the producer with the highest level, it was found that seed grain which had been treated with aldrin and heptachlor was being used in the herd's diet. The concentrate feed contained 2.6 ppm heptachlor, 1.91 ppm aldrin, and 0.50 ppm chlordane; and over a 30-day feeding period, the milk fat residues of dieldrin rose from 0.58 to 1.58 ppm. When the contaminated feed was removed from the diet, the dieldrin level dropped from 1.58 to .11 ppm in 201 days (Table 6).

Herds 5 and 6. The composite sample contained 0.16 ppm dieldrin, and two producers were found with milk containing 0.94 ppm and 1.09 ppm, respectively. Initial investigation failed to locate a source of dieldrin, and the residue in the butterfat remained unchanged. Further investigation on both farms revealed sources of hay and feed, previously overlooked, that were contaminated. On the first farm, hay and oats which contained 0.05 and 0.004 ppm dieldrin, respectively, were being fed to the herd. Following the removal of these feeds, the level dropped from 0.89 to 0.49 ppm in 21 days and then leveled out for the next 200 days (Table 6). Further tests revealed low levels on pasture grasses ranging from 0.01 to 0.03 ppm on a dry-weight basis which were growing in soil that contained levels from 0.003 to 0.06 ppm. A further decline to 0.23 ppm occurred after clipping these pastures. On the second farm, the source was found to be hay that contained 0.06 ppm dieldrin and 0.07 ppm aldrin. This hay was removed, and hay that contained 0.003 ppm was substituted. On this latter hay, the milk residue decreased from 1.26 to 0.18 ppm in 219 days. Pasture grasses were found to contain 0.005 ppm on a dry-weight basis while soil contained 0.002 ppm. It was concluded that general contamination of many feeds on these two farms made it difficult to remove the source and obtain a more rapid disappearance of the residues.

Herd 7. The composite milk sample from the bulk tanker contained 0.10 ppm dieldrin in the butterfat, and the producer's milk fat contained 0.44 ppm dieldrin. The investigation revealed that an insecticide spray, used to control flies, was contaminated with 780 ppm of aldrin plus dieldrin, 214 ppm of DDT, and 118 ppm of endrin. Analysis of a similar batch of spray from the manufacturer failed to show contamination at the factory level. After removal of the spray, the residue declined from 0.44 ppm to 0.10 ppm in 145 days (Table 6).

Herds 8 and 9. A composite sample containing 0.37 ppm dieldrin in the butterfat was found to be caused by two producers with milk fat residue levels of 0.78 and 2.2 ppm of dieldrin. After investigation, it was discovered that the dieldrin in Herd 8 came from spraying the herd with two organophosphorus insecticides that were contaminated with 15 ppm aldrin. When this spraying was discontinued, the residue level dropped from 0.78 to 0.10 ppm in 145 days (Table 6).

Herd 9, when originally studied, revealed a dieldrin residue level of 2.20 ppm in butterfat. Over a period of 103 days, this level dropped to 0.39 ppm. No source of dieldrin contamination could be located until the level was noted to rise to 0.49 ppm, when it was discovered that this herd had been placed on new pasture which contained 0.004 ppm dieldrin (dry-weight basis). After the herd was removed from this pasture, the butterfat residue levels declined to 0.09 ppm within 3 weeks (Table 6).

Herd 10. The composite butterfat sample contained 0.16 ppm dieldrin, and the individual producer was delivering milk with 0.66 ppm in the butterfat. The investigation showed that hay, produced in 1967 and 1968, was contaminated with 0.01 ppm dieldrin; feed grains were contaminated with 0.01 and 0.02 ppm; and dried beet pulp had 0.01 ppm. The combination of all three sources was considered responsible for contributing to the dieldrin residue. On removal of these feeds, the residue in the milk fat declined from 0.66 to 0.23 ppm in 103 days (Table 6).

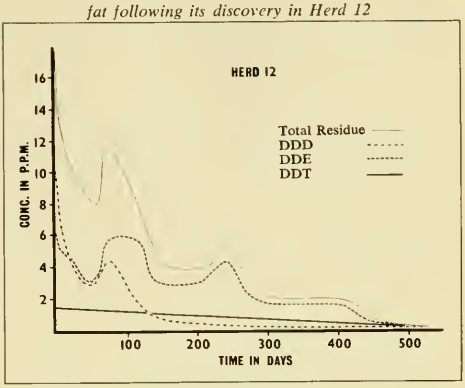
Herd 11. The bulk tanker contained butterfat with a residue of 0.14 ppm dieldrin, and the producer was found to be delivering milk with 1.60 ppm in the butterfat. Wheat and oat grains were found to be contaminated with 0.5 to 0.6 ppm aldrin and 0.01 to 0.04 ppm dieldrin, respectively. The grains had been stored in wooden granary bins that had been treated with aldrin. Hexane washings of the bins resulted in solutions containing 26 ppm aldrin. It was concluded that the grain became contaminated as the result of volatilization of aldrin from the wood. When the feeding of this grain was discontinued, the residue in the butterfat declined from 1.60 to 0.10 ppm in 230 days (Table 6).

Herds with High DDT and Metabolites

Herd 12. This herd was discovered after a bulk tanker was found with a total DDT level of 6.78 ppm. The investigation showed milk fat with 17.7 ppm at the farm level. Soil on the farm contained only 0.01 ppm; concentrates and feeds contained from a trace to 0.01 ppm; and silage had only 0.03 ppm. Oat straw had the highest residue with 0.11 ppm. At a later date, a total source of DDD and DDT of 40 ppm was located on sweet corn silage. After removal of this silage from the feed, the level dropped from 17.7 to 0.22 ppm in 530 days (Table 7).

The rise in the residue at 77 days was associated with a number of heifers and cows that calved. Sampling three of these animals at random revealed an average residue level of 21.3 ppm DDT plus metabolites in their milk fat. Following this rise, the decline in residue continued at an exponential rate (Table 7 and Fig. 3).

FIGURE 3.—*Changes in DDT and its metabolites in milk fat following its discovery in Herd 12*



Herd 13. A bulk tanker was located with 2.30 ppm, and the producer was found to be delivering milk with 5.80 ppm DDT and metabolites. An investigation revealed that the herd had been sprayed with technical DDT. The total DDT level declined from 5.80 to 0.85 ppm in 155 days while the respective drop in *p,p'*-DDT was from 4.29 to 0.57 ppm (Table 7).

Herd 14. A producer was discovered delivering milk with 3.36 ppm DDT and metabolites in the butterfat. The investigation uncovered a rotenone wettable powder that contained 6.4 ppm DDT and DDD. This wettable powder had been used to dust a group of recently purchased heifers that were infested with lice. Among a total milking herd of 17 animals, only 3 had been treated with the contaminated powder, and the contribution from these 3 was sufficient to raise the residue level of DDT and its metabolites to an average level of 3.36 ppm. This level declined to 0.57 ppm over a period of 78 days.

Herd with High Lindane

Herd 8. In addition to a high dieldrin residue in the milk, a lindane level of 1.72 ppm was also present. This lindane residue was found to originate from spray treatment for lice control one day before sampling. The lindane residue dropped to 0.12 ppm in 41 days and 0.03 ppm in 84 days.

Discussion

DIELDRIN

The analytical data indicated that dieldrin residue levels in butterfat from milk produced in agricultural areas where aldrin and/or dieldrin had been used extensively for insect control were only approximately twice as high as dieldrin residue levels in areas where little or

none of these cyclodienes were used. This would suggest that the cyclodienes have considerable mobility in the environment. The fact that aldrin was used almost to the exclusion of dieldrin would further suggest the high environmental mobility of aldrin. It is reported that aldrin is moderately volatile (vapor pressure 6 x 10^{-6} mm at 25 C) and hence could move into the atmosphere to explain the wide and fairly even distribution of dieldrin residues in the butterfat of milk in the Province (*8*). The use of animal feed produced in other areas could partially explain this rather uniform background level of dieldrin but could not fully explain dieldrin presence in areas where dairy farms produced most of their own feed. As was expected, only dieldrin and no aldrin was detected in butterfat samples.

The pattern of decline of high dieldrin residues in adipose tissue was similar to that reported by Moubry *et al.* (*11,12*). In the seven highest cows in Herd 1, the analyses of back fat samples indicated that dieldrin levels were considerably higher than those found in the butterfat, and the exponential disappearance of dieldrin was found to be slightly more rapid in the back fat. A biological half-life was calculated at 34 and 37 days, respectively, for back fat and milk fat (Table 5). In seven herds where sources of dieldrin were located and removed, biological half-lives ranged from 30 to 50 days (Tables 5 and 6). This wide range could reflect either the effectiveness of removal of the dieldrin source or the general background dietary level. The declines of residues in Herds 3, 5, 6 and 10 were complicated by the presence of multiple sources that were not identified or removed simultaneously.

Of the 11 herds investigated, 7 were located in the southern region, 3 in the western region, and 1 in the central region. This frequency closely followed the use pattern of aldrin and dieldrin in these respective regions (Table 1). In only one case was the use of aldrin by a producer connected to the presence of high dieldrin levels in milk. In all other cases, the farmer was unaware of the presence of aldrin or dieldrin on the farm. In four of these instances, the pesticide was present in feed either as the result of drift or treated seed grain mixed with the feed. In one case, the dieldrin was present in wood-shaving litter and in two cases as the result of contamination of insect spray formulations.

As a result of the general distribution and persistence of dieldrin in milk, whether from the use or misuse of aldrin and dieldrin, the Department of Health of Ontario in 1969 introduced a complete ban on the sale of these insecticides under the Pesticide Act, 1969 (*13*).

DDE, DDD, AND DDT

The largest agricultural use of DDT (89%) is confined to the southern region of the Province. However, the residue levels of DDT and its metabolites in milk from this area were only three times higher than the levels in the northern region where less than 0.1% of this insecticide is used in agriculture. This suggests possible mobility in the environment; but, more probably, the residues in the northern region may result from the use of DDT for nonagricultural purposes, i.e., recreation and parklands, industrial expansion, and commercial forestry operations. It should be noted that, although the amount of DDT used for agricultural purposes was 10 times that of aldrin and dieldrin combined, the number of herds which violated tolerance levels for DDT was only 3 as compared to 17 for dieldrin; however, the residue tolerance of dieldrin is only one-tenth that of DDT and metabolites. Two of these herds were located in the southern region and one in the western region. In two of these cases, high DDT residues resulted from direct application of DDT to dairy cows (in one instance as a contaminant in an approved insecticide). The third case resulted from a feeding diet which contained corn silage with DDT plus metabolite levels of 40 ppm.

The decline of DDT levels in Herd 12 initially followed a normal pattern and then exhibited a sudden rise. This was attributed to the introduction of milk from 10 freshly calved cows that were secreting much higher residues in their milk than the rest of the herd. If the beginning of decline is taken from this latter point, then the biological half-lives of DDE, DDD, DDT and total were calculated at 76, 37, 98 and 76 days, respectively. In Herd 13, which had been subjected to a DDT spray, these respective half-lives were 66, 47, 39 and 65 days; and in Herd 14, they were 85, 14, 52 and 63 days. The elimination of DDE appeared to take the longest time and that of DDD the shortest. Although disappearance rates of the DDT varied widely from one herd to another, the disappearance of the DDT plus its metabolites was remarkably similar with half-lives ranging from 63 to 76 days in the three herds. The half-life for DDT and its metabolites was approximately twice that found for dieldrin.

LINDANE

Approximately 8% of the milk samples analyzed contained detectable levels of lindane, and since these samples were localized to only a few areas, the lindane source was comparatively easy to locate. High lindane residues resulted primarily from use of treated seed grains as feed and by direct application for insect control. Neither of these practices is permitted in dairy husbandry, and both are readily remedied by education and extension programs or by the threat of litigation. Only one herd was located (in the southern region) which exhibited levels of lindane residues at high enough concentration to study the half-life which was calculated to be about 14 days.

HEPTACHLOR EPOXIDE

Heptachlor epoxide was detected in only 3% of the milk samples. These findings were confined to areas where heptachlor is or had been widely used for seed treatment and/or the cultivation of turnips. Since only small quantities of heptachlor have been used in Ontario agriculture, no problems of high residues were encountered.

Conclusion

The residues of chlorinated hydrocarbon insecticides in Ontario-produced milk were at levels similar to or lower than those reported in surveys from other parts of the world (*2,3,4,5*). The low incidence of excessive residues (20 per 27,000) indicate that the Ontario farmer practices insect control with caution. In only 2 cases were high residues caused by improper use of insecticides; the remaining 18 problems arose from the introduction of contaminants from outside the particular farm, i.e., purchase of contaminated feed or contamination of insecticide formulations.

Lindane residues disappeared rapidly from contaminated animals with a biological half-life of approximately 2 weeks. Dieldrin and DDT and its metabolites exhibited lower rates with half-lives of approximately 6 weeks and 10 weeks, respectively.

Background residue levels of DDT in milk could only partially be related to the use of DDT in agriculture and might be partially influenced also by nonagricultural applications of the insecticide. The general appearance of dieldrin residue over all areas of the Province suggests a high environmental mobility for aldrin and/or dieldrin. Lindane and heptachlor epoxide residues resulted primarily from localized use.

The use of DDT in Ontario has recently been severely restricted, and a total ban on aldrin, dieldrin, and heptachlor became effective in 1969. These restrictions should be reflected shortly in a change in the general background residue levels in Ontario-produced milk.

Acknowledgments

The authors wish to thank Mr. G. A. McCague, Chairman of the Milk Commission, Ontario Department of Agriculture and Food, Toronto, and the members of his branch who participated in the collection of milk samples across the Province. A special thanks goes to Mr. E. H. Smith, Milk Commission, for coordinating and organizing the sample collection. Appreciation is expressed to Mrs. G. E. Bowers, Provincial Pesticide Residue Testing Laboratory, Ontario Department of Agriculture and Food, who confirmed residues by thin-layer chromatography and to Dr. D. J. Ecobichon, University of Dalhousie, Halifax, Nova Scotia, for collection and analysis of the fat biopsies from Herd 1.

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(*1*) *Ontario Department of Agriculture and Food. 1968.* Agricultural statistics for Ontario. Publ. 20. Parliament Buildings, Toronto.

(*2*) *Bro-Rasmussen, F., Sv. Dalgaard-Mikkelsen, Th Jakobsen, Sv. O. Koch, F. Rodin, E. Uhl, and K. Voldum-Clausen. 1968.* Examinations of Danish milk and butter for contaminating organochlorine insecticides. Residue Rev. 23:55-69.

(*3*) *Clifford, P. A. 1957.* Pesticide residues in fluid market milk. Public Health Rep. 72:729.

(*4*) *Clifford, P. A., J. L. Bassen, and P. A. Mills. 1959.* Chlorinated organic pesticide residues in fluid milk. Public Health Rep. 74:1109.

(*5*) *Duggan, R. E. 1967.* Residues in food and feed. Pesticides Monit. J. 1:2-8.

(*6*) Feed Act and Feed Regulations. 1960. Chapter 14, established by PC 1967-1072. Queen's Printer and Controller of Stationery, Ottawa, 1967.

(*7*) *Hamence, J. H., P. S. Hall, and D. J. Caverly. 1965.* The identification and determination of chlorinated pesticide residues. Analyst 90:649.

(*8*) *Harris, C. R., and E. P. Lichtenstein. 1961.* Factors affecting the volatilization of insecticidal residues from soil. J. Econ. Entomol. 54:1038-1045.

(*9*) *Langlois, B. E., A. R. Stemp, and B. J. Liska. 1964.* Analysis of animal food products for chlorinated insecticides. J. Milk and Food Technol. 27:202.

(*10*) *Moubry, J. R., G. R. Myrdal, and H. P. Jensen. 1967.* Screening method for the detection of chlorinated hydrocarbon pesticide residues in fat of milk, cheese and butter. J. A.O.A.C. 50:885.

(*11*) *Moubry, R. J., G. R. Myrdal, and W. E. Lyle. 1968.* Investigation to determine the respective residue amounts of DDT and its analogues in the milk and back fat of selected dairy animals. Pesticides Monit. J. 2:47-50.

(*12*) *Moubry, R. J., G. R. Myrdal, and A. Sturges. 1968.* Rate of decline of chlorinated hydrocarbon pesticides in dairy milk. Pesticides Monit. J. 2:72-79.

(*13*) The Pesticides Act, 1967. Statutes of Ontario. Chapter 74, and Ontario Regulation 445/67. Printed and published by Frank Fogg, Queen's Printer, Toronto, 1969.

Residues of Chlorinated Hydrocarbons in Soybean Seed and Surface Soils From Selected Counties of South Carolina[1]

W. R. McCaskill,[2] B. H. Phillips, Jr.,[2] and C. A. Thomas[3]

ABSTRACT

A residue study of soybeans grown on soil with a history of cotton production was initiated in 1967 to determine the chlorinated hydrocarbon residue levels in soybeans and if a relationship existed between the concentration of chlorinated hydrocarbons in soybeans and soils. Soybean and soil (0-6 inch depth) samples were collected in three counties from each of four selected soil regions. In addition, soybean samples were collected from nine buying stations within the test areas. The residue levels of chlorinated hydrocarbons in soybeans varied from 0.007–0.156 μg/g (ppm) and in the soil from 0.000–3.582 μg/g. There was no significant correlation between amounts of chlorinated hydrocarbons in soybeans and residues in soil. Soybeans from the nine buying stations contained a slightly lower average concentration of chlorinated hydrocarbons than the soybeans from the test areas. Approximately 40% of the soybean samples contained detectable levels of aldrin, heptachlor, or chlordane. The study indicated that residues of DDT probably would not exceed present tolerance levels provided current pesticide recommendations are followed.

Introduction

Insects affecting cotton production are usually controlled by one or more of the chlorinated hydrocarbons. In recent years large acreages of land formerly planted to cotton have been converted to soybeans.

Bruce *et al.* (*2*) grew soybeans, peanuts, oats, corn, and barley on soil that had been treated with varying rates of aldrin and heptachlor up to 20 lb/acre. The concentration of pesticides in plants varied directly with the level of pesticides in the soil, but seed with a high oil content, such as soybeans and peanuts, contained the highest levels of pesticides.

Morgan *et al.* (*4*) found that 2 lb/acre each of aldrin, heptachlor, chlordane, and endrin applied as granules to separate plots resulted in relatively high residues of aldrin and heptachlor in both the shell and nuts of peanuts and of chlordane and endrin in the nuts.

Bruce and Decker (*1*) reported that the concentration of chlorinated hydrocarbons in the soil was from 10 to 15 times larger than the residue concentration in soybean seed. However, the residue levels in both soil and soybeans decreased with time.

In 1967, West Germany lowered the tolerances on chlorinated hydrocarbons allowed in soybeans. As soybeans grown on land formerly used for cotton might contain excessive levels of chlorinated hydrocarbons, a survey was initiated to determine the residue levels in soybeans from four areas of South Carolina and to determine if there was a correlation between the levels of chlorinated hydrocarbons in the soil and the soybeans.

Sampling Procedures

Soybean and soil samples were collected from the Piedmont (Anderson, Greenville, and Spartanburg counties), Sandhills (Aiken, Lexington, and Richland counties), the Pee Dee section (Darlington, Marlboro, and Lee counties), and Savannah River Valley section

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(Allendale, Barnwell, and Hampton counties) of the Coastal Plain. The county agents contacted farmers that had been following the recommended insecticide program for cotton and selected fields planted to soybeans that had a long history of cotton production. Soybeans were harvested, and soil samples (0-6 inch depth) were taken from a 12′ x 12′ section of each field. Each county agent collected soybean and soil samples from 10 fields (a total of 120 soybean and soil samples). In addition, 29 soybean samples were collected from 9 buying stations within the test areas.

Analytical Procedures

Each sample (soybeans and soil) was mixed thoroughly. The soybeans were ground in a Servall Omni-Mixer. Soil samples were air dried and screened through a 2-mm sieve.

EXTRACTION

Samples were extracted by a modification of the procedure outlined by Van Middelem and Moye (*3*). Each sample—50 g of soybeans or 25 g of soil—was shaken for 1 hour on a wrist-arm shaker with 100 ml of a 1:1 hexane-acetone mixture. The extracting solution was decanted through a filter into a 500-ml separatory funnel. The sample was shaken for another hour with an additional 100 ml of 1:1 hexane-acetone mixture, and the filtrate was added to the original extracting mixture. The acetone was removed from the mixture with three washings (100 ml each) of distilled water.

Soils

The hexane was evaporated to dryness and the residue transferred to a 100-ml bottle with 25 ml of nanograde hexane.

Soybeans

The pesticides were extracted from the hexane with four 25-ml portions of hexane-saturated acetonitrile. The acetonitrile was evaporated to dryness, and the residue was dissolved in hexane and transferred to a column of 20 g of activated Florisil (containing 5% H_2O). The pesticides were eluted from the column and separated from the remaining oils with 80 ml of a 6% ether-94% hexane mixture. The extract was evaporated to dryness and the residue transferred to a 100-ml bottle with 25 ml of nanograde hexane.

Analysis

Five microliters of each sample was injected into a MicroTek 220 gas chromatograph and concentration of DDE, *o,p′*-DDT, *p,p′*-DDT, lindane, heptachlor, aldrin, and chlordane determined with an electron capture detector with a Ni_{63} source. Retention times were cross-checked on 6′ x ¼″ stainless-steel columns containing 5% SE-30, 5% DC-200, and 5% QF-1.

Average recoveries of pesticides were as follows:

Insecticide	Average Percent Recovery	
	Soil	Soybeans
Aldrin	50.5	21.4
DDE	38.0	40.4
o,p′-DDT	60.5	39.9
p,p′-DDT	81.0	60.4
Heptachlor	54.0	28.0
Lindane	53.5	58.0

Recovery values were obtained by adding pesticide standards to soil and soybeans at the following rates: 0.5 ng each of aldrin, heptachlor, and lindane; 1.25 ng of DDE; 1.14 ng of *o,p′*-DDT; and 3.86 ng of *p,p′*-DDT. Since heptachlor epoxide and dieldrin were not detected, some of the samples containing heptachlor and aldrin were analyzed by a GLC using a different column system (1.5% OV-17/1.95% QF-1) from that reported. The peaks from the two column systems were identical, and no peaks for heptachlor epoxide and dieldrin were found on either system. Florisil that had been deactivated with 5% H_2O did not retain heptachlor epoxide and dieldrin.

Recovery factors were not applied to the reported values (Tables 1, 2, and 3).

Results and Discussion

The concentration of chlorinated hydrocarbons in soybeans from the 120 fields varied from 0.007 μg/g to 0.156 μg/g (Table 1). Even the sample with the highest concentration of chlorinated hydrocarbons did not exceed established tolerance levels for DDT.

The concentration of pesticides in both soybeans and soils (0-6 inch depth) varied with the soil region. The soybeans from the Piedmont contained the lowest average concentration of chlorinated hydrocarbons while the soybeans from the two areas of the Coastal Plain region had the highest concentrations (Table 2). Conversely, the soils of the Piedmont contained the highest levels of pesticides while the lowest concentrations were in the soils of the Sandhill region.

The surface soils contained from 8 (Savannah River Valley and Sandhills) to 26 (Piedmont) times more extractable chlorinated hydrocarbons than the soybeans. The difference between the concentration of pesticides in the soil and the uptake by soybeans appears closely related to soil texture and percent organic matter. Piedmont surface soils contain more clay and are higher

in aluminum and iron oxides than the soils of the other regions. Soils of the Pee Dee area contain more organic matter than the soils from the Savannah River Valley while the soils of the Sandhill region are coarse-textured sands.

There was no significant correlation between the amounts of chlorinated hydrocarbons in the topsoil and the residue levels in the soybeans. Although difference in soil retention was a contributing factor, the poor correlation was probably due to cultural practices, such as deep plowing and subsoiling, which mechanically moved a portion of the pesticide residues into the root zone below the 6-inch depth.

The soybean samples collected from nine buying stations contained a slightly lower average concentration of chlorinated hydrocarbons (Table 3) than the average of the soybeans from the survey fields in the same counties. The soybeans from the fields selected in the survey appear to be representative, in regard to pesticide residues, of the soybeans from the area that were moving into commercial channels.

Sixty-three of the 149 soybean samples contained detectable levels of aldrin and/or heptachlor, and 1 sample contained detectable levels of chlordane (0.001 μg/g). These are chlorinated hydrocarbons for which a zero or no-tolerance has been established. Forty-six samples of soybeans contained aldrin (0.001-0.008 μg/g, mean of 0.002 μg/g), and 21 soybean samples contained heptachlor (0.001-0.020 μg/g, mean of 0.0035 μg/g). However, only one sample from the Piedmont region (farms and buying stations) had detectable levels of these three chlorinated hydrocarbons. None of the soybean samples containing DDT exceeded established tolerance levels.

This survey indicates that there is little danger of DDT concentrations in soybeans grown on soils formerly planted to cotton exceeding the present tolerances if current pesticide recommendations for soybeans are followed. However, soybeans grown on soils with a history of aldrin and heptachlor application probably will contain detectable levels of these chlorinated hydrocarbons.

TABLE 1.—*Pesticide residue data for soils and soybeans from four regions of South Carolina (residue levels shown for individual compounds are those that were found in fields containing the highest and lowest total amount of chlorinated hydrocarbons)*

Soil Region	County	Residues in μg/g													
		Total Chlorinated Hydrocarbons		Breakdown by Individual Compounds											
				DDE		*o,p'*-DDT		*p,p'*-DDT		Lindane		Heptachlor		Aldrin	
		High Field	Low Field	High Field	Low Field	High Field	Low Field	High Field	Low Field	High Field	Low Field	High Field	Low Field	High Field	Low Field
SOILS															
Piedmont	Anderson	2.392	.074	.504	.026	.356	.017	1.480	.024	.052	.007	—	—	—	—
	Spartanburg	2.355	.019	.490	.019	.339	—	1.510	—	.016	—	—	—	—	—
	Greenville	1.152	.032	.270	.008	.142	.007	.730	.017	.010	—	—	—	—	—
Sandhills	Richland	1.428	.103	.386	.039	.150	.017	.856	.047	.036	—	—	—	—	—
	Aiken	1.327	.052	.322	.027	.199	.010	.806	.014	—	.001	—	—	—	—
	Lexington	.614	—	.290	—	.054	—	.270	—	—	—	—	—	—	—
Pee Dee	Darlington	1.241	.354	.170	.058	.215	.052	.856	.244	—	—	—	—	—	—
	Marlboro	1.699	—	.378	—	.325	—	.996	—	—	—	—	—	—	—
	Lee	1.243	.166	.270	.035	.139	.017	.810	.114	.024	—	—	—	—	—
Savannah River Valley	Hampton	3.582	.006	.540	—	.522	—	2.520	—	—	.006	—	—	—	—
	Allendale	1.239	.036	.300	.014	.143	—	.790	.022	—	—	—	—	.006	—
	Barnwell	1.668	—	.290	—	.298	—	1.080	—	—	—	—	—	—	—
SOYBEANS															
Piedmont	Anderson	.068	.019	.065	.015	—	—	—	—	.003	.004	—	—	—	—
	Spartanburg	.068	.008	.046	.006	.011	.002	.011	—	—	—	—	—	—	—
	Greenville	.036	.007	.010	.007	.014	—	.012	—	—	—	—	—	—	—
Sandhills	Richland	.116	.030	.011	.010	.005	.003	.040	.006	.060	.011	—	—	—	—
	Aiken	.067	.015	.031	.001	.017	.009	.016	—	.003	.005	—	—	—	—
	Lexington	.061	.022	.010	.012	.022	.001	.026	.003	.003	.006	—	—	—	.001
Pee Dee	Darlington	.156	.026	.009	.010	.018	.007	.129	.008	—	.001	—	—	—	—
	Marlboro	.108	.021	.041	.019	.035	—	.028	—	.002	.002	.002	—	—	—
	Lee	.095	.015	.010	.010	.008	.001	.012	—	.063	.002	—	—	.002	.002
Savannah River Valley	Hampton	.143	.020	.024	.005	.061	.007	.056	.008	—	—	—	—	.002	—
	Allendale	.146	.022	.062	.018	.033	.001	.044	—	.002	.001	.002	.002	.003	—
	Barnwell	.083	.026	.008	.013	.021	.004	.034	.007	.020	.002	—	—	—	—

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(1) *Bruce, W. N. and G. C. Decker. 1966.* Insecticide residues in soybeans grown in soil containing various concentrations of aldrin, dieldrin, heptachlor, and heptachlor-epoxide. J. Agr. Food Chem. 14:395-398.

(2) *Bruce, W. N., G. C. Decker, and Jean G. Wilson. 1966.* The relationship of the levels of insecticide contamination of crop seeds to their fat content and soil concentration of aldrin, heptachlor and their epoxides. J. Econ. Entomol. 59:179-81.

(3) *Van Middelem, C. H. and H. A. Moyle. 1966.* "Initial Results on Florida Preliminary Soils." Memo to S-58 cooperators.

(4) *Morgan, L. W., D. B. Leuck, E. W. Beck, and D. W. Woodham. 1967.* Residues of aldrin, chlordane, endrin, and heptachlor in peanuts grown in treated soil. J. Econ. Entomol. 60:1289-91.

TABLE 2.—*Average total residues of chlorinated hydrocarbons in surface soils and soybeans from four regions of South Carolina*

Soil Region	County	Residues in μg/g [1]							
		DDE	*o,p'*-DDT	*p,p'*-DDT	Lindane	Heptachlor	Aldrin	Chlordane	Total
SOILS									
Piedmont	Anderson	.418	.179	.682	.026	—	—	—	1.305
	Spartanburg	.191	.106	.433	.008	—	—	—	0.737
	Greenville	.112	.029	.178	.007	—	—	—	0.326
	Average	.244	.105	.431	.014	—	—	—	0.789
Sandhills	Richland	.102	.061	.209	.003	—	—	.212	0.586
	Aiken	.118	.065	.278	.002	—	.001	—	0.463
	Lexington	.053	.018	.062	—	—	—	—	0.133
	Average	.090	.048	.180	.002	—	—	.078	0.398
Pee Dee	Darlington	.147	.155	.472	—	—	—	—	0.774
	Marlboro	.139	.132	.379	.008	—	—	—	0.659
	Lee	.144	.074	.378	.002	—	—	—	0.598
	Average	.144	.122	.411	.004	—	—	—	0.681
Savannah River Valley	Hampton	.205	.110	.509	.009	—	—	—	0.833
	Allendale	.108	.049	.214	.002	—	—	—	0.374
	Barnwell	.066	.051	.234	.002	—	—	.033	0.386
	Average	.127	.069	.315	.004	—	—	.010	0.525
SOYBEANS									
Piedmont	Anderson	.018	.004	.006	.002	—	—	—	0.030
	Spartanburg	.016	.007	.010	.001	—	—	—	0.034
	Greenville	.010	.006	.009	.002	—	—	—	0.027
	Average	.015	.006	.008	.002	—	—	—	0.031
Sandhills	Richland	.011	.010	.019	.020	—	—	—	0.060
	Aiken	.016	.011	.006	.005	—	—	—	0.038
	Lexington	.011	.005	.015	.007	—	.001	—	0.039
	Average	.013	.009	.014	.011	—	—	—	0.047
Pee Dee	Darlington	.011	.008	.027	.012	—	.001	—	0.059
	Marlboro	.026	.020	.014	.004	.003	.001	—	0.068
	Lee	.011	.009	.019	.009	—	.002	—	0.050
	Average	.016	.012	.020	.008	.001	.001	—	0.058
Savannah River Valley	Hampton	.017	.021	.018	—	—	.002	—	0.058
	Allendale	.025	.016	.016	.002	.002	—	—	0.061
	Barnwell	.015	.011	.016	.005	—	—	.001	0.048
	Average	.019	.016	.017	.002	.001	.001	—	0.056

[1] County values are the means of 10 locations.

TABLE 3.—*Comparison of the average levels of chlorinated hydrocarbons in soybeans from survey fields to levels in soybeans collected from buying stations*

Soil Region	County	Residues in μg/g [1]							
		DDE	*o,p'*-DDT	*p,p'*-DDT	Lindane	Heptachlor	Aldrin	Chlordane	Total
				FIELDS					
Piedmont	Anderson	.018	.004	.006	.002	—	—	—	.030
Sandhills	Aiken	.016	.011	.006	.005	—	—	—	.038
	Lexington	.011	.005	.015	.007	—	.001	—	.039
Pee Dee	Darlington	.011	.008	.027	.012	—	.001	—	.059
	Marlboro	.026	.020	.014	.004	.003	.001	—	.068
	Lee	.011	.009	.019	.009	—	.002	—	.050
Savannah River Valley	Hampton	.017	.021	.018	—	—	.002	—	.058
	Allendale	.025	.016	.016	.002	.002	—	—	.061
	Barnwell	.015	.011	.016	.005	—	—	.001	.048
Average		.017	.012	.015	.005	.001	.001	—	.051
				BUYING STATIONS					
Piedmont	Anderson	.019	.003	—	.003	—	—	—	.024
Sandhills	Aiken	.021	.004	.009	.002	—	—	—	.036
	Lexington	.010	.003	.009	.003	—	.001	—	.026
Pee Dee	Darlington	.011	.011	.027	.002	—	.002	—	.053
	Marlboro	.015	.032	.018	.007	.005	.001	—	.078
	Lee	.013	.014	.019	.004	—	.001	—	.051
Savannah River Valley	Hampton	.016	.020	.024	.002	—	.003	—	.065
	Allendale	.008	.004	.015	.001	—	—	—	.028
	Barnwell	.011	.008	.017	.003	—	—	—	.039
Average		.014	.012	.017	.003	.001	.001	—	.047

[1] County values are the means of 10 locations.

PESTICIDES IN PEOPLE

Serum Organochlorine Pesticide Levels in People in Southern Idaho[1]

Michael Watson, W. W. Benson, and Joe Gabica

ABSTRACT

In a study of 1,000 serum samples from people in southern Idaho, p,p'-DDE was found in 99.8% of all individuals, with a mean concentration of 22.0 parts per billion (ppb). Samples were selected with no consideration of sex, race, age, or prior medical history at the time of collection. Pesticide levels within the group differed somewhat from those of similar demographic studies; this most likely may be attributed to regional differences in pesticide usage, the relatively large number of persons sampled, and possibly by the method of testing.

Introduction

Canyon County, Idaho, is one of 15 areas in the United States currently participating in a Community Studies Research Program to determine the effects of pesticides on human health. In-depth investigation of the total environment is being conducted to determine the type and quantity of pesticide exposure.

The study reported here was undertaken during the period 1967-1968 in order to establish a base line of serum pesticide levels in a sample group of Idahoans.

Serum, rather than whole blood, was chosen for this study because of its known higher pesticide content (*2,5*). Although little is currently known concerning the relationship of serum pesticide levels to the more extensive "body burden" of adipose tissues, it appears that some proportionality may exist (*4*).

Sampling Procedures

Blood samples from 1,000 Idahoans were obtained at the Medical Center at Nampa, Idaho. For the sake of simplicity, first-time visitors to the Center for any reason at all who were willing to participate in this study were chosen. Selection was based wholly upon availability, and variables such as age, sex, race, and prior medical history were not considered. Although such a sampling method would by no means result in an unbiased representative cross-section of Idahoans, it was nonetheless contended that useful preliminary data for the southern Idaho region could be obtained in this manner.

Laboratory Procedures

EXTRACTION

Whole bloods were centrifuged immediately to separate the serums. The serums were then frozen and taken to the Idaho State Health Laboratory in Boise for subsequent extraction and analysis for organochlorine pesticides.

Serum extraction was carried out by a revised method of Dale and Cueto, as recommended by the Primate Research Branch Laboratory in Perrine, Fla. (*2*). Two ml of serum were placed in a 15-ml centrifuge tube; 6 ml of nanograde hexane was added; and the mixture was agitated for 3 minutes on a Vortex mixer. Following centrifugation at 2,000 rpm for 10 minutes, the hexane layer was transferred into a 50-ml concentrator tube by means of a disposable pipette. This extraction procedure was repeated three times. Emulsions that occasionally formed were broken by the addition of small amounts of acetonitrile. The three combined hexane fractions were then concentrated with a two-ball Snyder column on a

[1] From the Idaho Community Studies on Pesticides, Idaho Department of Health, Statehouse, Boise, Idaho, 83707.

steam bath to a volume of 500 μl. Five-microliter portions were then injected into a MicroTek 220 gas chromatograph equipped with a tritium-foil electron capture detector.

ANALYSIS

The operating parameters for gas chromatographic analysis were as follows:

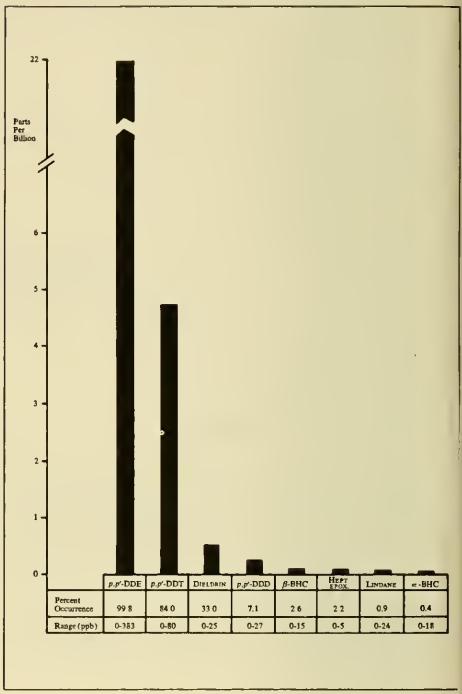
Columns:	1.5%, OV-17; 1.95%, QF-1 on Chromosorb W, DMCS, H.P., 100/120 mesh	
	4%, SE-30; 6%, QF-1 on Chromosorb W, DMCS, H.P., 100/120 mesh	
Temperatures:	Column	200 C
	Injection chamber	225 C
	Detector	210 C
Column air flow:	SE-30/QF-1	100 ml/minute
	OV-17/QF-1	70 ml/minute

All qualitative retention times were based on the retention time of aldrin. Quantitation of pesticide residues was based on relative peak heights. Recovery, which ranged from 80%-100% was based on the addition of a known amount of aldrin to each sample prior to extraction. (Although spiking the sample in this manner is not indicative of 100% recovery of the organically bound pesticides, it does provide an index of efficiency for the methodology used.)

Results and Discussion

Eight different pesticide residues were present at detectable levels in the serums of the study group. In decreasing order of detection frequency, these were: *p,p'*-DDE, *p,p'*-DDT, dieldrin, *p,p'*-DDD, β-BHC, heptachlor epoxide, lindane, and α-BHC. The mean concentrations, ranges, and percent occurrences of these residues are shown in Fig. 1. Of the total serums analyzed, 99.8% contained *p,p'*-DDE at a mean concentration of 22.0 ppb; *p,p'*-DDT occurred in 84% of the serums and averaged 4.7 ppb. Dieldrin, found in 33% of the samples, averaged 0.5 ppb. Only 7.1% of the serums contained *p,p'*-DDD, the average serum level being 0.24 ppb. Fewer than 2.6% of the individuals sampled contained any of the four remaining residues, the means of which were all less than 0.07 ppb (Fig. 1). In a similar study by Davies *et al.* (*4*) of pesticide levels in whole bood of 68 Florida Caucasians, *p,p'*-DDE was found at a mean concentration of 8 ppb, and a range of 2-19 ppb was reported. These values are considerably lower than those found in the Idaho residents, but some of this variation is probably due to their use of whole blood rather than serum or plasma since the extraction methods in both studies were essentially the same.

FIGURE 1.—*Mean levels of organochlorine pesticides in 1,000 serum samples*



Dale *et al.* (*3*) on the other hand, found plasma *p,p'*-DDE levels in a randomly chosen group of 20 persons of unspecified race in Georgia averaging 19.6 ppb and ranging from 3.9 to 41.6 ppb. They also reported an average of 17 ppb *p,p'*-DDT with a range from 2.4 to 49.0 ppb and dieldrin at a mean of 1.9 ppb and a range of 1.2 to 6.3 ppb.

Our findings for mean levels of serum *p,p'*-DDE tend to approximate Dale's results, despite his use of a different method of extraction; however, the range for *p,p'*-DDE in the Idaho group was much greater than those reported by either Dale (*2*) or Davies *et al.* (*4*). Dale's mean levels for *p,p'*-DDT and dieldrin were greater than those found in Idaho residents, but the Idaho ranges were again much higher. Such variation seems most likely due to the relatively small numbers of individuals sampled in the investigations cited, as well as differing geographical and ecological conditions such as the relative predominance of agriculture in Southern Idaho.

Analysis of the three most commonly occurring residues as a function of sex of the person sampled showed several apparent differences (Fig. 2). Males had a higher

FIGURE 2.—*Sex differences in mean serum levels of p,p'-DDE, p,p'-DDT, and dieldrin*

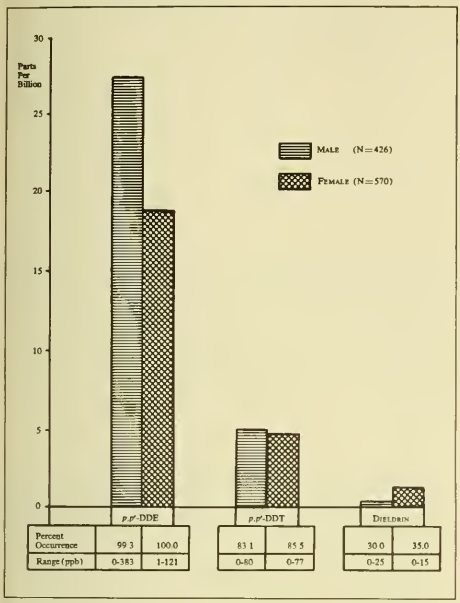
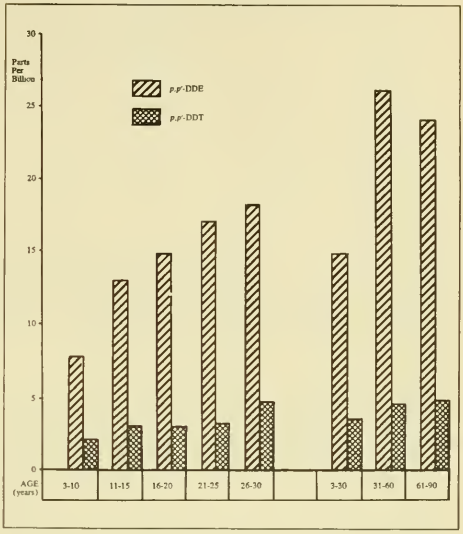


FIGURE 3.—*Age differences in mean serum levels of p,p'-DDE and p,p'-DDT*



mean serum level of *p,p'*-DDE (27.3 ppb) than females (18.8 ppb). Mean serum concentrations of *p,p'*-DDT, however, were similar in both sexes (4.9 ppb in males versus 4.6 ppb in females). Dieldrin, on the other hand, was more concentrated in women (1.3 ppb) than in men (0.3 ppb) as shown in Fig. 2. Dale reported a similar trend for *p,p'*-DDE, but males in his study had more *p,p'*-DDT and dieldrin than did females. Davies, using 23 men and 45 women, found *p,p'*-DDE levels in whole blood nearly equal for both sexes (8.3 and 7.9 ppb for men and women, respectively), but these mean values are both considerably less than those found in Idaho residents. Again, the use of so few individuals in the comparative studies makes meaningful correlation difficult. However, overall differences between the sexes in pesticide levels are probably influenced to a significant degree by the greater complexity of female hormonal mechanisms, differences in body fat deposition, etc.

Serum pesticide levels also varied considerably among Idaho residents as a function of age (Fig. 3 and Table 1). The age of the individual was recorded for 782 serum samples, and the highest mean *p,p'*-DDE level (26 ppb) was found in persons 31-60 years of age. The 61- to 90-year-old individuals averaged a slightly lower level (24 ppb) while serums of persons in the 3- to 30-year age group averaged only 14.8 ppb. Lower *p,p'*-DDE levels in younger persons are even more apparent when the 3- to 30-year-old individuals are treated as a separate subgroup. Persons from 3 to 10 years of age averaged only 7.9 ppb, but this value increased steadily and dramatically to a high mean of 18.2 ppb in the 26- to 30-year-old subgroup. Mean concentrations, ranges, and percent occurrences of both *p,p'*-DDE and *p,p'*-DDT throughout individuals from 3 to 30 years of age are shown in Table 1.

In comparing *p,p'*-DDE blood levels among persons of different age groups, Davies found that individuals 1-7 years of age had a mean of 8.4 ppb and a range of 2 to 17 ppb. Levels in those 18 years and older averaged 9.0 ppb and ranged from <1.0 to 55 ppb. Our results for *p,p'*-DDE levels in very young persons (3 to 10 years) compare favorably with these findings, but persons older than age 10 showed higher means and ranges at all age levels, as well as a much more dramatic increase at each successive age group between age 11 and 30 (Fig. 3 and Table 1). Idahoans age 31-60 had considerably more *p,p'*-DDE than did those under age 30. Since the onset of widespread DDT usage was not until the early 1940's (*1*), many persons in the 3-30 age group lack comparable exposure time, thus supporting this finding. Idahoans in the 61- to 90-year category had slightly lower mean *p,p'*-DDE levels and ranges than those aged 31-60. While this difference is slight, it could possibly be attributed to dietary changes among older persons, as well as to their increased likelihood of undergoing chemotherapy, since persons currently taking drugs were not eliminated from this survey. The ability of

TABLE 1.—*Percent occurrence, means, and ranges of* p,p'-*DDE and* p,p'-*DDT in serum of different age groups*

Age (years)	N	*p,p'*-DDE			*p,p'*-DDT		
		Percent occurrence	Mean (ppb)	Range (ppb)	Percent occurrence	Mean (ppb)	Range (ppb)
3-10	3	100.0	7.9	5.6- 10.9	66.6	2.1	0- 4.1
11-15	22	100.0	13.0	5.9- 28.8	81.8	3.0	0-20.3
16-20	96	99.0	14.9	0 - 47.1	76.0	3.0	0-15.5
21-25	94	100.0	17.1	1.3- 62.4	89.4	3.2	0-14.7
26-30	59	100.0	18.2	1.0- 89.8	83.1	4.7	0-29.8
Total (3-30)	274	99.6	14.8	0 - 89.8	82.5	3.5	0-29.8
31-60	230	100.0	26.0	10.0-102.0	89.6	4.5	0-42.0
61-90	278	100.0	24.0	1.0-133.0	85.3	4.8	0-29.0

certain drugs, e.g., phenobarbital, to induce hepatic microsomal enzyme systems responsible for the metabolism of various organochlorine pesticides (*6*) should certainly not be discounted as a possible contributing factor.

Unlike *p,p'*-DDE, *p,p'*-DDT mean levels did not show such dramatic differences with respect to age. Several trends are nonetheless apparent. As with *p,p'*-DDE, *p,p'*-DDT residue averages were lowest (3.5 ppb) in the 3-to 30-year age group (Fig. 3 and Table 1). Highest mean *p,p'*-DDT levels (4.8 ppb) were found in persons 61-90 years of age, while individuals of 31-60 years had a slightly lower level (4.5 ppb). Within the 3- to 30-year age group, those from 3-10 years of age had the lowest mean level of *p,p'*-DDT (2.1 ppb); the 11- to 20-year-olds had an average level of 3.0 ppb; and average levels of *p,p'*-DDT in those 21-25 years of age reached 3.2 ppb. This mean then increased to 4.7 ppb in persons aged 26-30. Since *p,p'*-DDT is metabolized in the body to *p,p'*-DDE, such a comparative lack of clear-cut *p,p'*-DDT increase in older persons would be expected—the more accurate index of exposure being *p,p'*-DDE.

Conclusions

As was expected, the Idaho residents sampled in this study do not differ basically from persons in other areas in the accumulation of serum organochlorine pesticide residues. Certain pesticide level variations noted among Idahoans when compared to other study groups are most likely due to regional differences in pesticide usage and to the relatively large number of persons sampled. Sex differences noted in pesticide levels are probably influenced by hormonal mechanisms, as was concluded in similar studies. In persons under 30 years of age, mean levels of *p,p'*-DDE and, to a lesser degree, *p,p'*-DDT seem to be increasing. From this, one could assume that as age increases, pesticide retention would reach a plateau similar to the levels found in older persons.

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See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(*1*) *Chichester, C. O., ed., 1965.* Research in pesticides. Academic Press, New York.
(*2*) *Dale, W. E., A. Curley, and C. Cueto. 1966.* Hexane extractable chlorinated insecticides in human blood. Life Sci. 5:47-54.
(*3*) *Dale, W. E., A. Curley, and W. J. Hayes, Jr. 1967.* Determination of chlorinated insecticides in human blood. Ind. Med. Surg. 36(4):275-280.
(*4*) *Davies, J. E., W. F. Edmundson, A. Maceo, A. Barquet, and J. Cassady. 1969.* An epidemiologic application of the study of DDE levels in whole blood. Amer. J. Public Health 59(3):435-441.
(*5*) *Edmundson, W. F., J. E. Davies, G. A. Nachman, and R. L. Roeth. 1969.* *P,p'*-DDT and *p,p'*-DDE in blood samples of occupationally exposed workers. Public Health Rep. 84(1):53-58.
(*6*) *Williams, C. H. 1969.* Drug-pesticide interactions. FDA Papers 3(7):14-17.

RESIDUES IN FISH, WILDLIFE AND ESTUARIES

Significance of DDT Residues From the Estuary Near Penascola, Fla.[1]

David J. Hansen and Alfred J. Wilson, Jr.

ABSTRACT

Pesticide residues in fishes from the estuary near Pensacola, Fla., monitored from April 1964 to November 1965, are compared with residues in fishes exposed to DDT in the laboratory. DDT in fish exposed to 0.1 ppb p,p'*-DDT for 5 weeks failed to increase after the second week, when maximum concentrations reached 38,000 times that in the test water. Loss of DDT from these fish was slow, 78%-87% in 8 weeks. The amounts of DDD or DDE in fish did not increase either during or after exposure.*

Residues in fish from the estuary rarely exceeded 0.1 ppm except in those collected from the lower estuary in the summer and fall when the amount of DDT and its metabolites reached 1.3 ppm. Fish from the lower estuary had more DDT and less DDD and DDE than fish from the upper estuary. The DDT content in lower estuarine fish increased in July, August, and September. One source of DDT was a county-sponsored spray program centered in the lower estuary in July and August.

Introduction

Both fish and shellfish accumulate pesticides from the water. Fish build up residues gradually and store them mainly in the fat. In shellfish, however, these chemicals are more generally distributed in the tissues and are concentrated continually in proportion to the level of environmental pollution. For example, oysters exposed to concentrations of DDT as low as 0.1 ppb in the surrounding water may concentrate up to 7 ppm in their tissues in about a month (*1*). This biological magnification is indicative of the extent to which trace pollutants in the environment may be concentrated and enter the food web within a relatively short time posing a threat to the reproduction, survival, or marketability of fish or shellfish.

In this study we determined the concentrations of organochlorine pesticide residues in several species of fish collected throughout the year at different locations in the estuary near Pensacola, Fla., and the rates of storage and retention of DDT in fish in the laboratory.

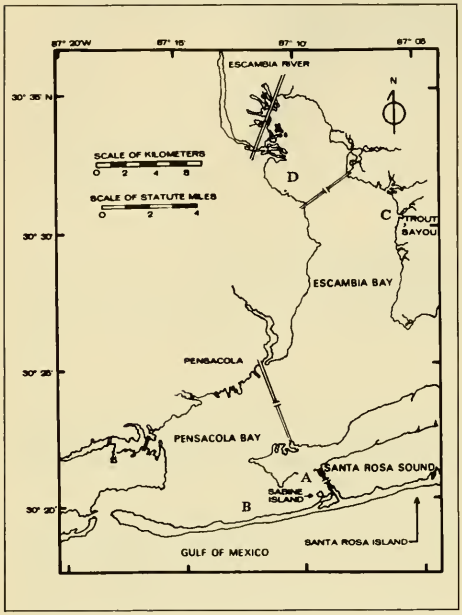
Methods and Procedures

Fish were collected with a 5-meter otter trawl of 12-mm bar mesh at four locations within the estuary (Fig. 1) at about monthly intervals from April 1964 to November 1965. Pinfish, *Lagodon rhomboides,* were collected in the lower estuary (locations A and B) and Atlantic croaker, *Micropogon undulatus,* were caught in the upper estuary (locations C and D). An additional nonpelagic species—either pigfish, *Orthopristis chrysopterus;* silver perch, *Bairdiella chrysura;* or spot, *Leiostomus xanthurus*—was also captured at each location. In order to assure a representative sample, when possible, a composite sample of at least 10 fish of each age group, species, and location was collected and frozen prior to pesticide analysis. This was deemed necessary because of the large variations between individuals. For example, residues of DDT and its metabolites in seven individual yearling pinfish collected from location A in August 1964 ranged from 0.5 to 13.7 ppm (mg/kg, wet weight) and from 0.4 to 1.1 ppm in nine fish caught in September 1965.

Although dieldrin, endrin, or BHC was found in some samples at concentrations up to 0.02 ppm, DDT and its metabolites were the predominant pesticides.

[1] Contribution No. 99, Bureau of Commercial Fisheries Center for Estuarine and Menhaden Research, Pesticide Field Station, Gulf Breeze, Fla. 32561.

FIGURE 1.—*Collection sites in the estuary near Pensacola, Fla., 1964-1965*



We investigated the rates at which fish remove DDT from water under controlled conditions in the laboratory to aid in understanding results obtained in the field. Initially, 100 pinfish or 50 croakers, average standard length 25 and 50 mm, respectively, were used in each treatment and "control" group. Each species was exposed to *p,p'*-DDT at 1.0 ppb (μg/liter) for 2 weeks or 0.1 ppb for 5 weeks, according to the flowing water bioassay technique described by Lowe (*2*). Fish exposed to 0.1 ppb DDT were placed in pesticide-free water for 8 additional weeks to establish flushing rates. All test fish were fed daily on ground fish from this estuary with the naturally occurring DDT content previously determined. At selected intervals, five individuals were removed from each group and frozen prior to pesticide analysis.

Fish from the field and laboratory were analyzed for residues by the same procedures. Determination of pesticide residues was made on pooled samples of fish which had been thawed, homogenized, and mixed well prior to analysis. A 30-g aliquot was taken from the ground composite, mixed with anhydrous sodium sulfate in a blender, and extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and transferred to 250-ml separatory funnels. The extracts were diluted to 25 ml with petroleum ether and partitioned with two 50-ml portions of acetonitrile previously saturated with petroleum ether. The acetonitrile was evaporated just to dryness and the residue eluted from a Florisil column (*3*). The sample was then identified and quantitated by electron capture gas chromatography. Three columns of different polarity (DC-200, QF-1, and mixed DC-200 and QF-1) were used to confirm identification. Operating parameters on Varian Aerograph 610D gas chromatographs were as follows:

Columns:	5′ x ⅛″ O.D., Pyrex glass, packed with 3% DC-200, 5% QF-1, and a 1:1 ratio of 3% DC-200 and QF-1, all on 80/100 mesh Gas Chrom Q
Temperature:	Detector 210 C Injector 210 C Oven 190 C
Carrier Gas:	Prepurified nitrogen at a flow rate of 40 ml/minute.

A few samples were analyzed using thin-layer chromatography. The lower limit of detectability was 0.01 ppm. Laboratory tests conducted during the sampling period gave the following recovery rates: *p,p'*-DDE, 80%-85%; *p,p'*-DDD (TDE), 92%-95%; *p,p'*-DDT, 91%-95%. Data in this report do not include a correction factor for percent recovery. Polychlorinated biphenyl (PCB) compounds were not detected in these samples.

Results

FIELD STUDIES

Fish from the lower estuary contained the highest pesticide residues in the late summer and fall, up to 1.3 ppm DDT and metabolites; whereas, residues in fish from the upper estuary varied monthly but were generally less than 0.1 ppm (Table 1). The ratio of DDT to its metabolites was greatest in fish from the lower estuary, particularly those from collection site A, in July, August, and September. In October and November the relative amounts of DDD and DDE in fish from the lower estuary increased. In fish from the upper estuary there was no pronounced seasonal shift in the total pesticide content nor in the amount of DDT, which was usually less than found in fish from the lower estuary. Butler (*4*) monitored pollution in this estuary and identified a similar increase in DDT residues in plankton, mussels, and oysters in the summer and fall in the lower estuary. The main source of the pesticide was, therefore, near the lower estuary rather than the rivers entering the upper estuary. The results of our investigations led to the discovery of a county-

sponsored program that used DDT to control the larvae of the dogfly, *Stomoxys calcitrans*, a biting insect. During July and August, DDT spraying was evidently concentrated near populated areas at the lower estuary.

Fish from the lower estuary in their second year of life (age group I) contained more DDT and its metabolites than fish in their first year of life (age group 0). Pesticide residues in the older fish were highest in 93% of the 41 samples, where both age groups were caught in the same month ($\chi^2 = 29.88$, df:1). The average pesticide content (DDT + DDD + DDE) in ppm of fish of different age groups was as follows:

	Age Group	
	0	I
Sampling location A		
Pinfish	0.17	0.48
Pigfish	0.11	0.48
Sampling location B		
Pinfish	0.12	0.25
Pigfish	0.05	0.19

TABLE 1.—*Pesticide content (mg/kg, wet weight—whole fish) in five species of fish from the estuary near Pensacola, Fla. 1964-1965*

Species	Month Collected	Location	Age Group [1]	DDT	DDD	DDE	DDT and Met.	No. of Fish in Composite Sample
			1964					
Pinfish	May	A	O	.03	.01	.02	.06	15
			I	.03	.06	.03	.12	6
		B	O	.03	.03	.02	.08	40
			I	.06	.06	.03	.15	6
Atlantic croaker		C	O	.06	.06	.09	.21	32
		D	O	.01	.02	.01	.04	10
Spot			O	.01	.01	.02	.04	20
Pinfish	June	A	O	.01	.01	.01	.03	10
			I	.16	.19	.05	.40	10
Pigfish			O	.01	.01	.01	.03	9
			I	.06	.15	.06	.27	6
Silver perch			O	.01	<.01	.01	.02	14
			I	.05	.05	.08	.18	10
Spot			O	.01	.01	.01	.03	10
Pinfish		B	O	.01	.01	.01	.03	10
			I	.06	.06	.06	.18	10
Atlantic croaker		C	O	.01	.01	.01	.03	8
Spot			O	<.01	<.01	<.01	<.01	12
Atlantic croaker		D	O	<.01	<.01	<.01	<.01	8
Spot			O	<.01	<.01	<.01	<.01	6
Pinfish	July	A	O	.06	.02	.01	.09	10
			I	.62	.37	.12	1.11	10
Pigfish			O	.13	.02	.03	.18	10
Silver perch			O	.17	.05	.12	.34	10
			I	.63	.02	.13	.78	10
Spot			O	.03	.01	.01	.05	10
Pinfish		B	O	.06	.08	.02	.16	10
			I	.12	.09	.06	.27	5
Pigfish			O	.03	.03	.06	.12	10
			I	.12	.08	.11	.31	3
Silver perch			O	.03	.02	.02	.07	10
			I	.02	.02	.02	.06	10
Atlantic croaker		C	O	.01	.06	.06	.13	10
Spot			O	.01	.01	.01	.03	10
Atlantic croaker		D	O	.02	.02	.03	.07	10
Spot			O	.01	.01	.02	.04	10
Pinfish	Aug.	A	O	.38	.13	.03	.54	10
			I	.54	.27	.08	.89	10
Pigfish			O	.11	.02	.03	.16	10
			I	.57	.42	.08	1.07	10
Pinfish		B	O	.14	.08	.03	.25	10
			I	.10	.10	.04	.24	5
Silver perch			O	.05	.03	.04	.12	10
			I	.10	.07	.04	.21	10
Atlantic croaker		C	O	.02	.05	.03	.10	10
Spot			O	.02	.01	.02	.05	10
Atlantic croaker		D	O	.02	.05	.05	.12	10
Spot			O	.01	.01	.01	.03	10

TABLE 1.—*Pesticide content (mg/kg, wet weight—whole fish) in five species of fish from the estuary near Pensacola, Fla. 1964-1965*—Continued

Species	Month Collected	Location	Age Group [1]	DDT	DDD	DDE	DDT and Met.	No. of Fish in Composite Sample
			1964—Continued					
Pinfish	Sept.	A	O	.26	.18	.03	.47	10
			I	.58	.25	.09	.92	8
Pigfish			O	.08	.05	.03	.16	10
			I	.26	.43	.09	.78	2
Pinfish		B	O	.25	.18	.04	.47	10
			I	.31	.25	.09	.65	4
Silver perch			O	.10	.05	.02	.17	10
Atlantic croaker		C	O	.03	.04	.04	.11	6
Spot			O	.02	.02	.01	.05	10
Atlantic croaker		D	O	.02	.04	.03	.09	10
Spot			O	.01	.02	.02	.05	10
Pinfish	Oct.	A	O	.19	.16	.05	.40	91
Pigfish			O	.14	.14	.06	.34	10
			I	.16	.26	.08	.50	1
Pinfish		B	O	.15	.09	.03	.27	10
Pigfish			O	.11	.08	.05	.24	10
Spot		C	O	.03	<.01	.05	.08	10
Spot		D	O	.02	.01	.04	.07	10
Pinfish	Nov.	A	O	.35	.47	.12	.94	10
Silver perch			O	.07	.09	.07	.23	10
Pinfish		B	O	.40	.27	.11	.78	4
Silver perch			O	.14	.14	.10	.38	5
Atlantic croaker		D	O	.02	.02	.03	.07	31
Spot			O	.01	.03	.07	.11	10
			1965					
Atlantic croaker	Jan.	D	O	.02	.01	.03	.06	45
Spot	Feb.	A	O	.15	.12	.12	.39	10
Spot		C	O	.04	<.01	.04	.08	20
Atlantic croaker		D	O	.03	<.01	.04	.07	10
Spot	March	A	O	.05	.10	.02	.17	9
Spot		B	O	.09	.09	.07	.25	10
Atlantic croaker		C	O	.04	<.01	.04	.08	10
Spot			O	.02	<.01	.04	.06	10
Pinfish	April	A	O	.06	.06	.03	.15	20
			I	.04	.05	.02	.11	10
Spot			O	.02	.02	.02	.06	10
			I	.03	.05	.02	.10	10
Pinfish		B	O	.03	.02	.02	.07	20
			I	.07	.11	.04	.22	9
Spot			O	.01	.01	.01	.03	9
			I	.02	.04	.02	.08	3
Atlantic croaker		C	O	.01	.04	.02	.07	10
Spot			O	.01	.01	.02	.04	10
Atlantic croaker		D	O	.02	.02	.02	.06	10
Spot			O	.03	.03	.02	.08	10
Pinfish	May	A	O	.02	.01	.01	.04	10
			I	.06	.05	.03	.14	10
Pigfish			O	.01	.01	.01	.03	10
			I	.11	.22	.07	.40	10
Pinfish		B	O	.01	.01	.01	.03	10
			I	.07	.07	.04	.18	10
Pigfish			O	.01	.01	.01	.03	10
			I	.06	.08	.04	.18	10
Atlantic croaker		C	O	.01	.02	.02	.05	10
Spot			O	.02	.02	.03	.07	10
Atlantic croaker		D	O	.02	.02	.01	.05	10
Spot			O	.01	.02	.01	.04	10

TABLE 1.—*Pesticide content (mg/kg, wet weight—whole fish) in five species of fish from the estuary near Pensacola, Fla. 1964-1965*—Continued

Species	Month Collected	Location	Age Group [1]	DDT	DDD	DDE	DDT and Met.	No. of Fish in Composite Sample
			1965—Continued					
Pinfish	June	A	O	.02	.01	.01	.04	10
			I	.07	.09	.03	.19	10
Pigfish			O	.03	.01	.01	.05	10
			I	.10	.14	.04	.28	10
Pinfish		B	O	.01	.01	<.01	.02	10
			1	.07	.06	.03	.16	10
Pigfish			O	.01	.01	.01	.03	10
			I	.06	.09	.04	.19	10
Atlantic croaker		C	O	.01	.01	.01	.03	10
Spot			O	.01	.01	.01	.03	10
Atlantic croaker		D	O	.01	.01	.01	.03	10
Spot			O	.01	.02	.01	.04	10
Pinfish	July	A	O	.01	.01	<.01	.02	10
			1	.09	.06	.04	.19	10
Pigfish			O	.02	.01	.01	.04	10
			I	.10	.14	.05	.29	10
Pinfish		B	O	.01	<.01	<.01	.01	10
			I	.07	.06	.03	.16	10
Pigfish			O	.01	<.01	<.01	.01	10
			I	.02	.04	.02	.08	10
Atlantic croaker		C	O	.01	.02	.01	.04	10
Spot			O	.02	.03	.02	.07	10
Atlantic croaker		D	O	.01	.03	.01	.05	10
Spot			O	.02	.04	.02	.08	10
Pinfish	Aug.	A	I	.15	.13	.05	.33	10
Pigfish			1	.16	.18	.07	.41	10
Pinfish		B	I	.09	.07	.03	.19	10
Pigfish			I	.10	.12	.05	.27	6
Atlantic croaker		C	O	<.01	<.01	<.01	<.01	10
Spot			O	<.01	<.01	.02	.02	10
Atlantic croaker		D	O	.01	.02	.01	.04	10
Spot			O	.01	.02	.02	.05	10
Pinfish	Sept.	A	O	.18	.05	.02	.25	10
			I	.13	.13	.04	.30	3
Pigfish			O	.02	.04	.04	.10	9
			I	.06	.12	.04	.22	5
Pinfish		B	O	.03	.02	.01	.06	10
			I	.14	.14	.05	.33	10
Pigfish			O	.02	.01	<.01	.03	10
			I	.06	.09	.03	.18	2
Spot		C	O	.02	.04	.02	.08	10
Pinfish	Oct.	A	O	.07	.08	.03	.18	10
			I	.32	.48	.11	.91	10
Pigfish			O	.16	.16	.08	.40	10
Pinfish		B	O	.09	.04	.01	.14	10
Pigfish			O	.02	.05	.02	.09	10
			I	.08	.10	.04	.22	3
Pinfish	Nov.	A	O	.39	.33	.18	.90	10
Silver perch		B	O	.43	.51	.32	1.26	7

[1] O = Fish in their first year of life.
I = Fish in their second year of life.

TABLE 2.—*Levels of DDT, DDD, and DDE in ppm wet weight in fish food, control fish, and fish exposed to 0.1 and 1.0 ppb p,p'-DDT (each sample consisted of at least five fish)*

Species	Concentration in Test Water (ppb)	Exposure Time															After flushing 8 weeks		
		Start			Day 3			Week 1			Week 2			Week 3-5 (Weekly average)					
		DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE	DDT	DDD	DDE
Food		.08	.07	.02	.08	.07	.02	.08	.07	.02	.09	.08	.05	.10	.08	.06	.08	.09	.05
Pinfish	Control	.01	<.01	.01	.06	.04	.03	.06	.04	.03	.17	.13	.12	.20	.15	.14	.15	.16	.10
Atlantic croaker	Control	.01	.01	.01	.08	.06	.05	.09	.07	.07	—	—	—	.13	.10	.09	.23	.17	.17
Pinfish	0.1	.01	<.01	.01	.57	.05	.04	.85	.07	.06	3.8	.11	.09	3.3	.26	.17	.42	.15	.08
Atlantic croaker	0.1	.01	.01	.01	.33	.07	.07	.80	.08	.07	1.1	.08	.07	1.4	.11	.12	.31	.23	.16
Pinfish	1.0	.01	<.01	.01	2.8	.08	.06	6.9	.17	.07	10.6	.28	.14	—	—	—	[1]6.3	.52	.25
Atlantic croaker	1.0	.01	.01	.01	2.3	.06	.05	6.9	.13	.12	12.0	.09	.08	[2]10.0	.22	.21	—	—	—

[1] Residue after 4 weeks of flushing.
[2] Residue after 3 weeks of exposure.

LABORATORY STUDIES

Results of laboratory studies on uptake and retention of DDT in water by pinfish and Atlantic croakers showed that residues in fish exposed to 0.1 ppb DDT reached a maximum in 2 weeks and remained nearly constant until the exposure ended 3 weeks later (Table 2). Residues in these fish, depending on species and exposure, represent a maximum concentration of DDT from 10,000 to 38,000 times that in the test water. Contrary to what might be expected, there was no increase in the amounts of DDD or DDE in test fish as compared to control fish. We have no explanation for this phenomenon. The concentration of DDT and metabolites in control fish increased from 0.02 to 0.57 ppm in response to the DDT content of their food.

DDT was lost slowly from fish previously exposed to 0.1 ppb (Table 2). After 8 weeks in pesticide-free water, the loss of DDT from pinfish was 87% and from Atlantic croakers 78%. The pesticide content at that time was similar to that in control fish. This loss was not accompanied by an increase in the concentration of DDD or DDE. After 4 weeks in pesticide-free water, pinfish, previously exposed to 1.0 ppb DDT for 2 weeks, had lost 41% of the accumulated DDT.

These tests further the understanding of our field study. In laboratory tests, DDT was rapidly stored without increase in DDD or DDE while fish from the estuary usually had as much DDD and DDE as DDT. This indicates that fish from this estuary obtained the pesticide after it had been metabolized and passed through the food web.

Discussion

DDT residues in different species of fish can be compared and used to identify the source of a pollutant, providing the rate of uptake of DDT by each species is known and the fish remain in one location long enough for their DDT content to reflect the magnitude of contamination in that particular area. Pinfish and Atlantic croakers contained similar residues when eating the same food or after exposure to 1 ppb DDT; however, when these two species were exposed to 0.1 ppb DDT, pinfish stored 2.4 times as much DDT as croakers. This difference in storage traits must be considered when comparing pesticide contamination in different areas represented by different species. Benthic fishes, like the five species used in this study, usually remain in one location while pelagic fishes do not. Pesticide residues in benthic fishes would, therefore, be better indicators of contamination in a particular area than the residues in pelagic fishes.

The migrations of both pelagic and benthic fishes transport DDT in and out of the estuary. Atlantic croakers and pinfish migrate to the Gulf of Mexico in the late fall and rarely return to this estuary (*5*). Butler (*6*) stated that this removed each year about ½ lb of DDT from this estuary.

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(*1*) *Butler, Philip A. 1966.* The problem of pesticides in estuaries. Trans. Amer. Fish. Soc. Spec. Publ. No. 3:110-115.

(*2*) *Lowe, J. I. 1964.* Chronic exposure of spot, *Leiostomus xanthurus,* to sublethal concentrations of toxaphene in seawater. Trans. Amer. Fish. Soc. 93(4):396-399.

(*3*) *Mills, Paul A., J. H. Onley, and R. A. Gaither. 1963.* Rapid method for chlorinated pesticide residues in nonfatty foods. J. Ass. Offic. Agr. Chem. 46(2):186-191.

(*4*) *Butler, Philip A.* 1968. Fixation of DDT in estuaries. Trans. 31st N. Amer. Wildlife and Natur. Resources Conf. p. 184-189.

(*5*) *Hansen, David J. 1970.* Food, growth, migration, reproduction, and abundance of pinfish, *Lagodon rhomboides,* and Atlantic croaker. *Micropogon undulatus,* near Pensacola, Florida. 1963-1965. U. S. Fish Wildlife Serv., Fish. Bull. 68(1):135-146.

(*6*) *Butler, Philip A. 1968.* Pesticides in the estuary. Proc. Marsh and Estuary Manage. Symp. p. 120-124.

Chlorinated Hydrocarbon Pesticides in Representative Fishes of Southern Arizona[1]

Donald W. Johnson[2] and Sam Lew[3]

ABSTRACT

Chlorinated hydrocarbon residues are reported for representative fishes of the lower Colorado River Basin. While most residues were in the ppb range, DDT and metabolites to 187.5 ppm were found. Toxaphene was a common contaminant at levels as great as 172.9 ppm. Dieldrin was also present in significant concentrations. DDE was the principal residue found. These findings should prove of value in appraising the effect of the 1969 ban on the use of DDT in Arizona. They appear sufficient to warrant concern for fish and fish-consuming populations.

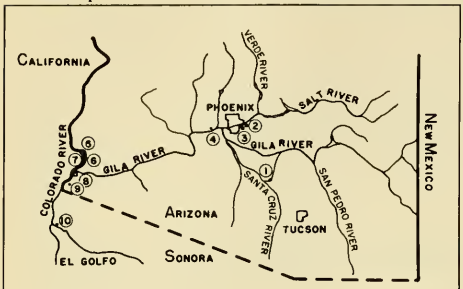
Introduction

A 1965 estimate of cotton land in southern Arizona was 345,000 acres. Pesticide application for each acre averaged 7.59 lb of toxaphene, 1.35-4.00 lb of DDT, 1.20 lb of endrin, 0.58-1.02 lb of endosulfan and undeterminable quantities of aldrin, dieldrin, BHC, heptachlor, and chlordane. DDT residue levels in starlings from the Phoenix area, reflecting DDT contamination in southern Arizona, are the Nation's highest—20 ppm (*1*); the Gila River appears to be the most DDT-burdened stream of 20 sampled in the Western United States (*2*). Fish kills nationwide for 1967 were up 21% from 1966, and insecticides were involved in 16% of the kills for which reports were submitted to the Federal Water Quality Administration. No cause was designated for Arizona kills, and eight States including the cotton-growing State of Mississippi submitted no report (*3*). DDT use in Arizona for 1967 increased by a factor of five from 1965. In 1967, three times as much toxaphene was applied as in 1965. From 1967 to 1968, a period marked by legislative opposition to DDT, use of less-studied chlorinated hydrocarbons increased (Strobane and Telone 8 and 7 times, respectively); the use of the organic phosphate parathion increased 11 times (*4*). Although this may provide some relief for ectotherms from acute toxicity, organic phosphates are generally more toxic to endotherms (*5*). A fluctuating pattern of pesticide use producing continuous change in residue levels further compounds the difficult task of determining the significance of sublethal residues in fish tissues to both fish and human populations (*5*).

The residues reported here represent reference data from a preliminary survey of some chlorinated pesticides found in representative fishes of the lower Colorado River Basin. The carp (*Cyprinus carpio*) is probably the most abundant and widely distributed fish in southern Arizona. Channel catfish (*Ictalurus punctatus*) and green sunfish (*Lepomis cyanellus*) are also abundant and occur throughout the Lower Colorado River Basin. Threadfin shad (*Dorosoma petenense*) is a common forage species heavily utilized by carnivorous species including the channel catfish and green sunfish. Tilapia (*Tilapia mossambica*) is an herbivore that has been established in the lower Colorado and the irrigation canals of the Buckeye and Yuma area where it contributes to the sport fishery. The Sonoran (*Catostomus insignis*) and Gila (*Pantosteus clarki*) suckers are bottom feeders and abundant in Phoenix area canals. The last fish for which data are presented is the striped mullet (*Mugil cephalus*). An amphidromous bottom feeder characterized by an exceptionally high fat content, it is found in the Gulf of California and the waters of the Colorado River and canals below Imperial Dam. Collection sites and dates are shown in Fig. 1.

[1] Work completed at Zoology Department, Arizona State University, Tempe, Ariz. 85281.
[2] Present adress, University of California (Berkeley), Bodega Marine Laboratories, Bodega Bay, Calif. 94923.
[3] Pesticide Laboratory, Arizona State Health Department, Phoenix, Ariz. 85007.

FIGURE 1.—*Southern Arizona showing origin of pesticide residues presented—collection sites and dates are listed*



1. Picacho Reservoir (8-28-66)
2. Mesa Canal (11-14-66)
3. Tempe Canal (1-21-67)
4. Buckeye Canal (12-10-65)
5. Martinez Lake (7-24-66)
6. Mitry Lake (8-10-66)
7. Imperial Dam (12-6-68)
8. Yuma Canal (3-16-66)
9. Summerton Canal (9-25-66)
10. El Golfo de Santa Clara (6-7-69)

Materials and Methods

Fishes were collected by gill net or seine. All tissues were frozen prior to extraction for analysis by gas chromatography. Extraction and chromatographic analysis were completed at the Arizona State Health Laboratory in Phoenix by standard methods (*6*). Five-gram samples were used for muscle, liver, and low-fat organs. Half-gram samples of fatty tissue were extracted. Elution for endrin and dieldrin extraction was not completed for all tissues analyzed. The Aerograph Pestilyzer Model 680 gas chromatograph with ⅛″ x 5′ glass columns was used. The nonpolar column was packed with 2% Dow 11 on Chromosorb G, the polar column with 2% QF-1 on Chromosorb G. Nitrogen at 20 psi was the carrier gas at an oven temperature of 180 C. Toxaphene was identified by the location of the "*y*" peak and quantified through its height. Standard peak height curves of *v*, *w*, *x*, and *y* were obtained by injecting a series of toxaphene standards from 0.5-30 ng. The *v*, *s*, and *x* peak heights for an unknown are extrapolated from the standard graphs based on "*y*" peak height. Differences from the extrapolated *v*, *w*, and *x* values indicate the quantity of DDE, TDE, and DDT, respectively. The most abundant of the DDT compounds was *p,p′*-DDE, and there was no significant interference in determinations by polychlorinated biphenyls (PCB's). Risebrough, Reiche, and Olcott have recently published a similar conclusion (*7*). Confusion of toxaphene and PCB's is considered unlikely. During the period of the study use of PCB's in Arizona was unreported, while DDT and toxaphene represented 41% and 40%, respectively, of all agricultural chlorinated hydrocarbons applied. High-residue specimens in agricultural areas reflected the relative presence of DDT compounds and toxaphene as environmental contaminants. Toxaphene chromatograms produced recognizable characteristic peaks on a mountain-like profile. PCB chromatograms do not exhibit this profile and when compared to toxaphene were distinctive. Sensitivity levels were based on check samples which were spiked with DDT, TDE, and DDE at two levels, 0.2 ppm and 0.02 ppm. Recoveries averaged 88%, 85%, and 91% for the higher level and 88%, 86%, and 84% for the lower level. Values reported here were not corrected after recoveries were calculated. Recoveries for dieldrin and toxaphene were not determined. The only confirmation technique used was comparing the retention time of the unknown/known ratios from polar (QF-1) and nonpolar (QV-1) columns.

Results and Discussion

Residue data are shown by species and tissue in Table 1. The following conclusions can be made from these data:

1. In 1966, it was not uncommon for fishes in southern Arizona to far exceed the interim guideline for DDT and its metabolites of 5 ppm established for fish in 1969 by the FDA (*8*).
2. The 5-ppm level of DDT contamination was exceeded in the edible flesh by a factor as great as 23 in carp, 8 in channel catfish, 4 in tilapia, 2 in suckers, and 37 in the striped mullet.
3. In addition to DDT, toxaphene and dieldrin were present in some fish in extremely high concentrations together with trace amounts of endrin.
4. Most DDT either enters the tissue as DDE or is rapidly metabolized to that form.
5. DDT and its metabolites are found concentrated in liver tissue.
6. Concentration may be closely correlated to fat content of the tissues.

Feeding habits and age may explain the variation in residue levels between species within a body of water. Local agricultural practices can explain seasonal and geographic variation. The limitation of small sample sizes and the absence of seasonal collections and residue analyses from all species present at each site make interpretation highly speculative. The data, however, have provided a degree of support for the above conclusions. In addition, they provide reference values for comparison with those which will be reported subsequent to the current Arizona ban on the application of DDT. A primary goal of the National Pesticide Monitoring Program is the location of possible problem areas where

concentration levels justify concern. The only surveillance station in the Southwest is located above Imperial Dam on the Colorado River (*8*). Agricultural waste water from Arizona's cropland enters the system below this station. The 1966 data on pesticide residues in fish resulting from agricultural contamination of southern Arizona waters are sufficient to warrant concern for both fish and fish-consuming populations. A comprehensive surveillance program should not overlook this source of environmental contamination (*5*).

Acknowledgments

The staff of the Arizona State Health Laboratory provided analytical support and full cooperation in carrying out this study. Arizona Game and Fish personnel cooperated in the collection of fish as did E. McClendon and S. Silver.

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(*1*) *Martin, W. E. 1969.* Organochlorine insecticide residues in starlings. Pesticides Monit. J. 3(2):102-114.

(*2*) *Manigold, D. B. and J. A. Schulze. 1969.* Pesticides in selected western streams—a progress report. Pesticides Monit. J. 3(2):124-135.

(*3*) *Federal Water Pollution Control Administration. 1968.* Pollution caused fish kills—1967. Fed. Water Pollut. Contr. Admin. Eighth Annu. Rep. CWA-7, p. 16.

(*4*) *Roan, C. C., D P. Morgan, C. H. Kreader, and L. Moore. 1969.* Pesticide use in Arizona as shown by sales. Progr. Agr. Ariz. 21:14-15.

(*5*) *Johnson, D. W. 1968.* Pesticides and fishes—a review of selected literature. Trans. Amer. Fish. Soc. 97:398-424.

(*6*) *Food and Drug Administration. 1968.* Pesticide analytical manual, U. S. Dep. Health, Educ. and Welfare. Vol. 1, Sect. 211.13A, 211.15, 302.1, 302.13b, 302.14, 302.15, 302.4a, 302.4e, and 311.01-311.01j.

(*7*) *Risebrough, R. W., P. Reiche, and H. S. Olcott. 1969.* Current progress in the determination of the polychlorinated biphenyls. Bull. Environ. Contamination Toxicol. 4:192-201.

(*8*) *Henderson, C., W. L. Johnson, and A. Inglis. 1969.* Organochlorine insecticide residues in fish (National Pesticide Monitoring Program). Pesticides Monit. J. 3(3):145-171.

TABLE 1.—*Pesticide residues in fish, by species and tissue*

Specimen	Tissue	Standard Length (cm)	Residues in ppm (Wet Weight) [1]					Collection Site
			DDE	DDD	DDT	Dieldrin	Toxaphene	
Cyprinus carpio								
M-2 [2]	"muscle"		90.0	16.9	8.5	2.1		8
M-2	red muscle		0.15	0.06	0.03			5
P-4	red muscle	16	0.30	0.08	0.16	ND		1
P-5	red muscle	16	0.22	0.10	0.15			1
M-2	white muscle		0.01	0.01	0.01			5
P-5	white muscle	16	0.05	0.02	0.05			1
P-4	white muscle	16	0.02	0.01	0.02	ND		1
M-1	scraped skin		0.22	0.07	0.03			5
M-0	scraped skin		0.13	0.09	0.03			5
P-5	scraped skin	16	0.33	0.15	0.22			1
P-4	scraped skin	16	0.28	0.07	0.21	0.01		1
— [2]	fat		48.0	15.8	13.0	1.14	50.0	4
— [2]	fat		153.0	24.8	7.2	0.5		8
M-0	fat		1.15	0.70	0.18			5
M-3	blood		0.03	0.01	0.01	0.002		5
M-7	blood		ND	0.01	0.01	0.002		5
P-1 to P-6	blood	15-21	0.03	0.01	0.02	0.002		1
P-5	gills	16	0.17	0.08	0.07		0.45	1
P-4	gills	16	0.22	0.09	0.12		0.42	1
P-5	kidney	16	0.69	0.28	0.48			1
P-4	kidney	16	0.48	0.12	0.54			1
M-0	liver		0.20	0.11	0.08			5
P-5	liver	16	1.25	0.83	0.38			1
P-4	liver	16	1.22	0.48	0.18			1
M-0	ovaries		0.02	0.01	0.01			5
M-1	ovaries		0.02	0.01	0.01			5
P-5	ovaries	16	0.07	0.05	0.06			1
P-4	ovaries	16	0.11	0.04	0.05	ND		1
M-2	intestine contents		ND	ND	0.002	0.008		5

TABLE 1.—*Pesticide residues in fish, by species and tissue*—Continued

Specimen	Tissue	Standard Length (cm)	Residues in ppm (Wet Weight) [1]					Collection Site
			DDE	DDD	DDT	Dieldrin	Toxaphene	
Channel catfish *Ictalurus punctatus*								
— [2]	"muscle"		18.4	6.8	13.7	0.06	6.8	8
P-11	red muscle	23	1.87	0.60	0.66		0.55	1
P-11	white muscle	23	0.04	0.02	0.03		ND	1
M-8	white muscle		0.17	0.05	0.04			5
M-9	white muscle		0.27	0.05	0.06			5
S-3 [3]	skin and muscle	14.5	1.54	0.03	0.13			9
P-11	scraped skin	23	1.00	0.28	0.33		1.32	1
M-9	scraped skin		0.51	0.13	0.23			5
— [2]	fat		38.0	14.0	25.0	0.6	8.2	8
P-11	fat	23	34.78	10.42	11.16		11.38	1
M-9	fat		7.23	0.96	1.84			5
P-11,12	blood	26, 28	0.10	0.05	0.04	0.003		
P-11	gills	23	0.48	0.17	0.23	0.35		1
P-11	kidney	24	0.81	0.32	0.32			1
M-8	kidney		0.30	0.12	0.13			5
M-9	kidney		0.19	0.04	0.06		ND	5
— [2]	liver		19.8	8.4	9.0	1.2		8
P-11	liver	24	0.24	0.11	0.05			1
M-9	liver		0.09	0.03	0.02			5
M-8	intestine		0.06	0.02	0.02		ND	5
P-11	intestine contents	24	0.07	0.01	0.02		ND	1
S-3 [3]	viscera	14.5	0.63	0.02	0.05			8
Green sunfish *Lepomis cyanellus*								
P-34	white muscle	13	0.08	0.01	0.04			1
P-35	white muscle	14	0.06	0.01	0.03			1
P-34	scraped skin	13	0.45	0.02	0.10			1
P-35	scraped skin	14	0.15	0.02	0.06			1
P-31	gills	13	0.26	0.03	0.07			1
P-35	gills	14	0.11	0.02	0.10			1
P-34	liver	13	3.86	0.68	0.15			1
P-35	liver	14	3.07	1.03	0.39			1
P-35	ovaries	14	6.59	1.41	2.46			1
P-34	testes	13	1.56	0.12	0.44			1
P-34	caecae	13	1.20	0.13	0.09			1
P-35	caecae	14	1.10	0.34	0.17			1
Threadfin shad *Dorosoma petenensis*								
P-29	whole fish	12	0.58	0.30	0.95		1.05	1
M-13	whole fish		0.04	0.02	0.03			5
M-14	whole fish		0.03	0.02	0.04			5
P-33	skin and muscle	11	0.82	0.66	1.67		1.73	1
P-31	gills	11	1.4	0.58	2.5		4.75	1
P-31	ovary	11	0.12	0.07	0.23		0.70	1
P-31	viscera (w/o gills or ovary)	11	0.86	0.61	0.91		1.71	1
Tilapia *Tilapia mossambica*								
— [2]	"muscle"		8.8	6.6	4.5	1.0		8
S-1 [3]	red muscle	15	0.01	ND	ND			9
— [3]	white muscle	15	ND	ND	ND			9
S-1 [3]	scraped skin	15	0.01	ND	0.01			9
S-2 [3]	skin and muscle	15	0.01	0.03	0.03			9
S-1 [3]	gills	15	0.45	0.05	0.06			9
— [2]	liver		10.3	9.7	3.2			8
S-1 [3]	viscera (w/o gills)	15	0.02	ND	ND			9
S-2 [3]	viscera (w/o gills)	15	0.07	0.02	0.03			9

TABLE 1.—*Pesticide residues in fish, by species and tissue*—Continued

Specimen	Tissue	Standard Length (cm)	Residues in ppm (Wet Weight) [1]					Collection Site
			DDE	DDD	DDT	Dieldrin	Toxaphene	
Sonoran sucker *Catastomus insignis*								
MC-1	skin and muscle	16	0.42	ND	0.21	ND	ND	2
MC-2	skin and muscle	16	0.33	0.05	0.18	ND	0.25	2
TC-7	skin and muscle	10	1.89	0.30	0.98	ND	2.19	3
TC-8	skin and muscle	10	5.89	0.69	2.89	ND	5.78	3
TC-9	skin and muscle	13	1.28	0.13	0.68		1.60	3
TC-1	skin and muscle	13	2.10	0.23	1.18	ND	2.63	3
TC-2	skin and muscle	12	1.70	0.23	1.18	ND	4.00	3
TC-3	skin and muscle	11	0.80	0.15	0.40	ND	1.10	3
TC-1-3	kidneys	11-13	1.99	0.30	1.65	ND	4.23	3
MC-1	viscera	16	4.75	1.25	1.25	trace	2.75	2
MC-2	viscera	16	6.00	2.00	2.75	ND	4.50	2
TC-7	viscera	10	9.65	1.02	5.59	0.007	13.71	3
TC-8	viscera	10	15.22	2.17	11.24	trace	15.95	3
TC-9	viscera	13	11.33	1.33	4.89		9.11	3
TC-1	viscera	13	12.00	1.38	7.25	ND	13.75	3
TC-2	viscera	12	22.50	3.25	17.75	trace	27.50	3
TC-3	viscera	11	30.42	0.84	15.00	trace	172.92	3
Gila sucker *Pantosteus clarki*								
TC-6	whole fish	6.5	7.25	1.50	6.75	trace	25.00	3
TC-4	skin and muscle	10.5	0.71	0.09	0.49	ND	0.71	3
TC-5	skin and muscle	9.5	1.75	0.36	1.49	ND	4.91	3
TC-4	viscera	10.5	26.15	2.75	10.55	0.01	25.24	3
TC-5	viscera	9.5	16.15	3.13	16.93	trace	42.94	3
Striped mullet *Mugil cephalus*								
—	"muscle" [4]	18	0.005	0.005	0.105			10
—	"muscle"	18	0.005	0.005	0.005			10
—	"muscle"	18	0.005	0.005	0.005			10
—	"muscle"	18	0.005	ND	ND			10
—	"muscle"	18	0.005	ND	ND			10
—	"muscle"	18	0.005	ND	ND			10
—	"muscle"	33	0.140	0.095	0.200			7
—	"muscle"	35	0.195	0.145	0.440			7
—	"muscle"	39	0.235	0.130	0.075			7
—	"muscle"	38	0.265	0.100	0.200			7
—	"muscle"	36	0.135	0.100	0.145			7
—	"muscle"	36	0.185	0.100	0.240			7
— [3]	red muscle	42	45.00	92.50	50.00			6
— [3]	white muscle	42	23.00	50.00	28.50			6
— [3]	skin and muscle	42	3.50	6.75	3.75			6
— [3]	scraped skin	42	0.10	0.18	0.11			6
— [3]	adipose eyelid	42	0.04	0.08	0.05			6

[1] ND = not detected; blank = data not obtainable.
[2] Collected by Arizona Game and Fish personnel.
[3] Collected by E. McClendon.
[4] Fillet including both red and white muscle.

Pesticide Residues in Channel Catfish From Nebraska[1]

N. P. Stucky

ABSTRACT

Channel catfish (Ictalurus punctatus) *were collected in all of the major watersheds in Nebraska during the summer of 1964. Individual fat samples and composite blood samples obtained from these fish were analyzed to determine the concentrations of residues of DDT and its metabolites* (o,p'-*DDT,* p,p'-*DDT,* p,p'-*DDD, and DDE) and dieldrin. A total of 178 fish, collected from 18 sites, were analyzed. As expected, the fat samples contained higher concentrations of the pesticides than did the blood samples. DDT residues were found in all fat samples, and average levels from 10 fish sampled at each site ranged from a low of 2.2 ppm in the sandhills region to a high of 92.2 ppm in Salt Creek below Lincoln, Nebr. Average dieldrin residues in fat samples ranged from 0.1 to 6.7 ppm. All values are expressed as ppm (μg/g) of residue detected in each sample of fat. The composite blood samples were found to contain DDT residue concentrations ranging from a low of less than 0.01 ppm to a high of 0.16 ppm. Dieldrin residue concentrations ranged from a low of less than 0.01 ppm to a high of 0.07 ppm.*

Introduction

In 1964, the Research Division of the Nebraska Game and Parks Commission initiated a statewide exploratory study to determine the extent of environmental contamination by pesticides. The primary objective of this investigation was to determine the concentrations of DDT (including its isomers and metabolites) and dieldrin residues in Nebraska watersheds using the channel catfish, *Ictalurus punctatus,* as an indicator species. In 1968, Lyman *et al* (*7*) used fish to demonstrate the presence of DDT in an aquatic environment. Anderson and Everhard (*1*) conducted similar studies relating to DDT in fish, and Weiss (*11*) discussed the use of fish as indicator organisms to determine the extent of environmental contamination by pesticides.

The channel catfish was chosen as the species to be analyzed primarily because of its ubiquitous occurrence, omnivorous food habits, and value as a sport and food fish.

During the summer of 1964, fat samples and composite blood samples were obtained from channel catfish in all major watersheds throughout the State. Analyses for DDT and dieldrin were performed in our laboratory. Data were evaluated to determine the relative concentrations of pesticide residues in channel catfish within each watershed.

Due to the mobility of channel catfish as reported by Welker (*12*) and Muncy (*9*), the levels expressed in this study should be interpreted as quantitative values representing the amount of DDT and dieldrin contamination of fish at the collection site but not throughout entire watersheds. However, results of these analyses can indicate areas in the State where pesticide concentrations exceed the maximum allowable level established by the Food and Drug Administration and therefore warrant additional study.

Sampling Procedures

Eighteen collection sites were selected for study (Fig. 1), representing all of the major drainage systems in Nebraska. Topography and land use in the watersheds were the primary considerations in selection of the collection sites. With respect to these physical factors, homogeneity of the watersheds above the collection sites was sought as much as possible.

[1] From the Research Division, Nebraska Game and Parks Commission, Lincoln, Nebr. 68509.

Samples were collected during a 2-week period in the summer of 1964. Ten channel catfish were collected at each site by means of either a back-pack shocker or rotenone. An exception to this was the site on the Middle Loup River from which only eight fish were collected after several days of sampling. To eliminate the possibility of introducing additional variables, an effort was made to obtain fish ranging from 25 cm to 35 cm in total length. At several sites however, it was necessary to deviate from this range in order to obtain a sample of 10 fish.

In the field a sample of visceral fat was obtained from each fish, and one or more composite blood samples were obtained for each collection site. Blood samples were obtained by removing the caudal fin with scissors and allowing blood to drain into a vial. Blood and fat samples were then placed in a cooler where they were held until freezing.

Analytical Procedures

Both the quantitative and qualitative analyses were carried out using an Aerograph Model 204, dual column, electron capture gas chromatograph. The following instrument parameters were used:

Columns:

For dieldrin and DDE—Metal, ⅛″ x 5′ containing 4½′ of 4% SE-30/6% QF-1 and 6″ of 3% OV-17 on 60/80 Chromosorb W, regular solid support

For o,p′-DDT, p,p′-DDT, and p,p′-DDD—Glass, ⅛″ x 5′ containing 11% OV-17/QF-1 on 80/100 Gas Chrom Q, DMCS treated solid support

Temperatures:

Column	185 C
Detector	195 C
Injector	230 C

Carrier Gas Flow Rate (Nitrogen):

For dieldrin and DDE column—75 and 35 ml/minute for fat and blood, respectively

For o,p′-DDT, p,p′-DDT and p,p′-DDD Column—42 and 35 ml/minute for fat and blood, respectively

The method employed in the extraction of pesticides was rapid and convenient for an exploratory study of this nature; however, it is not recommended for a study where extreme accuracy is of paramount importance. The single distribution method was first applied to the extraction of pesticides in 1965 by Beroza and Bowman (2). The solvent system used was as follows:

Lower phase: 70% DMF (dimethylformamide)
Upper phase: isooctane

Each fat sample was carefully weighed to within 0.0001 g, attempting to maintain a range within 0.1-0.3 g. The sample was then placed in 5 ml of 70% DMF and subjected to 10 minutes of ultrasonic vibration. Five ml of isooctane was added, and this mixture was shaken vigorously for 2 minutes. Equilibration between the two phases was accomplished by either centrifugation or allowing the mixture to stand for a period of 2 to 24 hours. Aliquots (1-6 μl) of the upper phase were then qualitatively and quantitatively analyzed for pesticides by injection into the columns described above. Each sample was analyzed by this method, and a separate injection for each of the five standards (pesticide samples of a known concentration) followed every sample.

The minimum level to be recorded quantitatively was set at 0.01 ppm for dieldrin, DDE, *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDD. Recovery effectiveness ranged from 76% to 99%. The results expressed in this study were corrected accordingly.

Results and Discussion

The exploratory nature of this study warranted expression of the results only as average concentrations present at each collection site. Data were not subjected to statistical treatment such as the calculation of standard error because the mobility of channel catfish makes it unreasonable to assume that all fish at a given location should have the same residue concentration. As pointed out by Buhler *et al.* (*4*), residue concentrations may also be a function of size of fish. To demonstrate extremes in concentrations found at each collection site, ranges are included in the data presented for fat samples. Because blood samples were comprised of up to five fish, ranges are not given.

FAT

DDT

The residue concentrations of DDT and its isomers and metabolites found in the fat of channel catfish in Nebraska watersheds are presented in Table 1 and Fig. 1. The values represent averages for the 10 fish collected from each sampling site. While samples were analyzed for the residual concentration of each specific isomer and metabolite of DDT and are expressed as such, the most significant value is the "total DDT" (Table 1) because, as pointed out by Spencer (*10*), the ratio of DDT to its metabolites changes in accordance with the length of storage time prior to analysis. The samples collected in this study were analyzed over a 1-year period.

Residues ranged from a low of 2.16 ppm (Niobrara River) in the sandhills region, to a high of 92.16 ppm in Salt Creek below Lincoln, Nebr.

A followup study to locate the main source of DDT pollution is presently being done on Salt Creek where the concentration was found to be 92.2 ppm. Laboratory analyses showed these fish to be comprised of approximately 9% fat. Therefore, by extrapolation, these fish contained approximately 10.3 ppm DDT, on a whole-fish basis, well above the maximum allowable level of 5.0 ppm established by the Food and Drug Administration.

The results of this investigation are supported by a study by Henderson *et al.* (5). Three samples comprised of a total of 15 channel catfish, collected from the Missouri River at Nebraska City, were analyzed for residues of DDT and its metabolites. Concentrations ranged from 0.21 to 2.03 ppm on a whole-fish basis. This compares to the range of 0.15 to 2.20 (1.67 - 24.46 ppm on a fat basis) found in fish from the Missouri River in this study.

Dieldrin

Dieldrin residues were found in fat samples from channel catfish collected from all 18 Nebraska watersheds. Residual concentrations found in the various watersheds are presented in Table 2 and Fig. 2. Values shown are averages of the 10 fish collected at each site. Residues ranged from a low of 0.08 ppm in the Middle Loup River to a high of 6.71 ppm in the Missouri River.

FIGURE 1.—*Residues of DDT (including its isomers and metabolites) in fat of channel catfish from Nebraska watersheds (values expressed to nearest 0.1 ppm)*

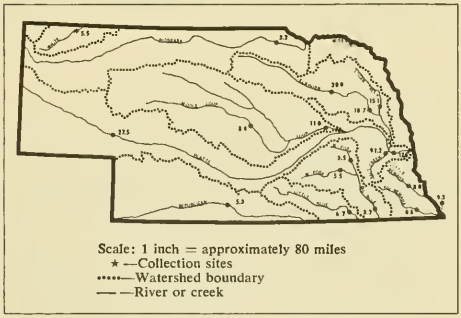


FIGURE 2.—*Residues of dieldrin in fat of channel catfish from Nebraska watersheds (values expressed to nearest 0.1 ppm)*

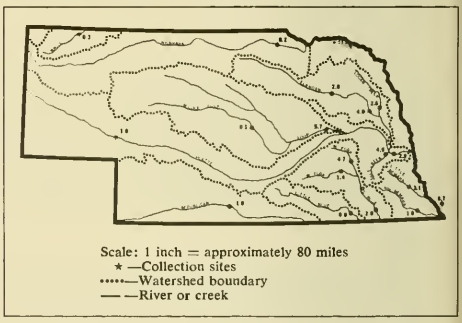


TABLE 1.—*Concentration of DDT in fat samples from 10 channel catfish collected at each site*

Watershed	Average Residue Levels in PPM					Range of total DDT and metabolites (10 fish) (ppm)
	DDE	*o,p'*-DDT	*p,p'*-DDT	*p,p'*-DDD	Total DDT	
Missouri River	3.54	0.13	3.17	2.41	9.25	1.67- 24.46
Niobrara River	1.01	0.03	0.80	0.32	2.16	0.72- 2.99
White River	0.92	0.03	3.81	0.75	5.51	2.19- 8.62
Platte River						
Lower	2.84	0.86	8.57	5.28	17.55	8.58- 35.86
Upper	17.89	0.24	5.06	4.35	27.54	5.86- 87.17
Salt Creek	10.57	4.50	39.85	37.24	92.16	13.61-258.68
Loup River	3.81	0.62	3.54	3.59	11.56	0.28- 26.59
Middle Loup River	4.52	0.01	2.66	1.25	8.44	2.80- 28.66
Elkhorn River						
Lower	5.13	3.11	5.23	5.24	18 71	2.71- 38.79
Upper	10.29	2.24	4.29	4.03	20.85	4.75- 56.86
Logan Creek	4.40	0.05	2.65	8.04	15.14	9.60- 23.22
Little Nemaha River	2.44	0.41	5.25	1.74	9.84	3.74- 31.24
Big Nemaha River	4.38	0.06	2.42	1.14	8.00	3.64- 35.50
Big Blue River	1.56	0.12	1.43	0.63	3.74	1.73- 10.44
West Fork Big Blue River	2.33	0.03	1.54	1.64	5.54	2.87- 10.83
North Fork Big Blue River	1.68	0.02	1.28	0.51	3.49	1.28- 6.91
Little Blue River	1.60	0.14	4.04	0.87	6.65	1.93- 21.02
Republican River	1.39	0.14	2.63	1.17	5.33	1.17- 16.91

The dieldrin residue concentration found by Henderson *et al.* (*5*) in channel catfish from the Missouri River ranged from 0.04 to 0.18 ppm on a whole-fish basis. The concentrations found in this study ranged from 0.32 to 1.43 ppm (2.88-12.86 on a fat basis).

BLOOD

As would be expected, the chlorinated hydrocarbon pesticide residue concentrations were considerably lower in blood samples than in fat. Results of the analyses of composite blood samples from each watershed (with the exception of the Middle Loup River and the Elkhorn River, upper site, from which no samples were obtained) are presented in Table 3. The composite blood samples were found to contain average DDT residue concentrations ranging from a trace (<0.01 ppm) to a high of 0.16 ppm. Dieldrin residue concentrations ranged from a trace to a high of 0.07 ppm.

TABLE 2.—*Concentration of dieldrin in fat samples from 10 channel catfish collected at each site*

Watershed	Dieldrin Residues in PPM	
	Average	Range
Missouri River	6.71	2.88-12.86
Niobrara River	0.16	<0.01- 0.72
White River	0.26	0.08- 0.53
Platte River		
Lower	2.88	1.36- 4.79
Upper	1.78	0.69- 5.58
Salt Creek	4.52	2.58- 7.35
Loup River	5.68	1.87- 9.55
Middle Loup River	0.08	<0.01- 0.27
Elkhorn River		
Lower	3.98	0.74-11.25
Upper	2.79	0.52- 5.76
Logan Creek	2.62	1.95- 3.10
Little Nemaha River	3.08	2.08- 4.13
Big Nemaha River	0.99	0.31- 1.82
Big Blue River	2.55	1.32- 3.97
West Fork Big Blue River	1.42	0.29- 2.19
North Fork Big Blue River	4.72	0.69- 7.64
Little Blue River	0.63	0.34- 1.17
Republican River	0.98	0.33- 2.03

TABLE 3.—*Concentration of pesticides in blood samples from channel catfish*

[T = Trace, <0.01 ppm]

Watershed	Residues in PPM	
	Total DDT	Dieldrin
Missouri River	0.15	0.07
Niobrara River	T	T
White River	0.01	T
Platte River		
Lower	0.05	0.04
Upper	0.13	0.01
Salt Creek	0.12	0.02
Loup River	0.05	0.03
Middle Loup River	—	—
Elkhorn River		
Lower	0.06	0.03
Upper	—	—
Logan Creek	0.16	0.07
Little Nemaha River	0.07	0.03
Big Nemaha River	0.06	0.02
Big Blue River	0.01	0.02
West Fork Big Blue River	0.12	0.01
North Fork Big Blue River	0.03	0.02
Little Blue River	0.12	0.01
Republican River	0.02	0.01

NOTE: Samples were comprised of blood from 1-5 fish.

A review of literature indicated that blood samples are not generally used in pesticides monitoring work. Bridges *et al.* (*3*) found that blood samples from black bullheads, *Ictalurus melas*, contained a high of 2.7 ppm DDT and metabolites. Samples were obtained 13 months after the farm pond in which the fish were held had been treated with 0.02 ppm DDT. Witt *et al.* (*13*) found that a good correlation existed between DDT in blood and the amount in adipose tissue. Studies of the Mississippi River fish kills by Mount *et al.* (*8*) indicate that acute toxicity, resulting from endrin, can be diagnosed from blood concentrations, which are independent of time of exposure and water concentration. Johnson (*6*) suggests that for monitoring work, analyses of blood samples may be more practical than other techniques more commonly employed.

Acknowledgments

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This is a contribution of Federal Aid in Fish Restoration, Project F-4-R, Nebraska.

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(*1*) *Anderson, R. B. and W. H. Everhart. 1966.* Concentrations of DDT in landlocked salmon *(Salmo salar)* at Sebago Lake, Maine. Trans. Amer. Fish. Soc. 95(2): 160-164.

(*2*) *Beroza, M. and M. C. Bowman. 1965.* Identification of pesticides at nanogram level by extraction p-values. Anal. Chem. 37(2):291-292.

(*3*) *Bridges, W. R., J. B. Kallman, and K. A. Andrews. 1963.* Persistence of DDT and its metabolites in a farm pond. Trans. Amer. Fish. Soc. 92(4):421-427.

(*4*) *Buhler, D. R., E. M. Rasmusson, and E. W. Shanks. 1969.* Chronic oral DDT toxicity in juvenile coho and chinook salmon. Toxicol. Appl. Pharamacol. 14(3):535-555.

(*5*) *Henderson, Croswell, L. Wendell Johnson, and Anthony Inglis. 1969.* Organochlorine insecticide residues in fish (National Pesticide Monitoring Program). Pesticides Monit. J. 3(3):145-172.

(*6*) *Johnson, Donald W. 1968.* Pesticides and fishes—A review of selected literature. Trans. Amer. Fish. Soc. 97(4):398-424.

(*7*) *Lyman, Lee D., William A. Tompkins, and James A. McCann. 1968.* Massachusetts pesticide monitoring study. Pesticides Monit. J. 2(3):109-122.

(*8*) *Mount, D. I., L. W. Vigor, and M. L. Schafer. 1966.* Endrin: use of concentration in blood to diagnose acute toxicity to fish. Science 152:1388-1390.

(*9*) *Muncy, R. J. 1958.* Movements of channel catfish in Des Moines River, Boone County, Iowa. Iowa State Coll. J. Sci. 23:563-571.

(*10*) *Spencer, Donald A. 1967.* Problems in monitoring DDT and its metabolites in the environment. Pesticides Monit. J. 1(2):54-57.

(*11*) *Weiss, Charles M. 1965.* Use of fish to detect organic insecticides in water. J. Water Pollut. Contr. Fed. 37: 647-658.

(*12*) *Welker, Bill. 1967.* Movements of marked channel catfish in the Little Sioux River, Iowa. Trans. Amer. Fish. Soc. 96(3):351-353.

(*13*) *Witt, J. M., W. H. Brown, G. I. Shaw, L. S. Maynard, L. M. Sullivan, F. M. Whiting, and J. W. Stull. 1966.* Rate of transfer of DDT from the blood compartment. Bull. Environ. Contamination Toxicol. 1:187-197.

PESTICIDES IN SOIL

"Apparent" Organochlorine Insecticide Contents of Soils Sampled in 1910[1]

B. E. Frazier, G. Chesters, and G. B. Lee

ABSTRACT

A total of 34 soil samples taken in Wisconsin between 1909 and 1911 were extracted for organochlorine insecticides and analyzed by gas chromatography on 3 columns—a mixed QF-1/IV-17 column; a mixed QF-1/DC-200 column; and a diethylene glycol succinate column. The samples had been stored continuously since collection in tightly sealed glass jars and presumably were free of insecticide contamination. Of the 34 samples 32 showed some "apparent" insecticide residues on at least one of the columns. Because peaks corresponding to particular organochlorine insecticides on chromatograms from one column did not recur on other columns, it was concluded that the peaks arose from co-extracted indigenous soil components. Peaks corresponding to heptachlor epoxide on the QF-1/OV-17 column and to aldrin on the QF-1/DC-200 column provided greatest interference in chromatographic determination.

Introduction

Multicolumn gas chromatography has been used successfully to determine qualitatively and quantitatively the content of organochlorine insecticides in soils; however, the method has not been tested extensively using soil samples known to be free of insecticides. The chromatogram of one uncontaminated soil revealed small interfering peaks, suggesting that concentrated soil extracts may show "apparent" insecticides where none exist (*1*). In another investigation, five uncontaminated soil samples gave gas chromatographic responses to γ-BHC, aldrin, and endrin (*2*). One response which was apparently caused by γ-BHC was in fact caused by sulfur. By use of multicolumn gas chromatography, aldrin-like compounds found in plant materials proved to be interfering compounds (*3*). Extraction and analytical procedures suitable for organochlorine insecticide determinations in soils must be able to disprove "apparent" residues when used on soils known to be free of insecticide residues.

In the routine analysis of soil samples for insecticides it is advantageous if methods can be designed to eliminate cleanup. Partitioning and Florisil cleanup steps are used commonly, but contamination from indigenous soil components may still be present (*2*), and a confirmatory analysis is necessary.

This investigation on insecticide-free samples was designed to determine the extent of interference with insecticide determination arising from indigenous soil components. Insecticide-free soils were obtained from a collection of 34 soil samples collected between 1909 and 1911 and stored in tightly stoppered glass jars since that time.

Methods and Materials

The soils (Table 1) consisted of samples with a wide range of textural class; their organic matter content ranged from 1.0% to 7.9%. An unpublished report on the soil samples indicates that organic matter was determined by a chromic acid wet oxidation method, but details of the procedure are not available. The textural class was obtained by observation. Although the description of the methodology is inadequate, it is believed that these data are as reliable as data which might be obtained presently on 60-year-old samples.

The organochlorine insecticides—γ-BHC, heptachlor and its epoxide, aldrin, dieldrin, endrin, *p,p'*-DDD, *p,p'*-DDT, and *p,p'*-methoxychlor—used as standards were those described earlier (*4*).

[1] From the Department of Soil Science, University of Wisconsin, Madison, Wis. 53706.

TABLE 1.—*Properties of soil samples used*

Sample No.	Soil Series	Texture	Percent Organic Matter
1	Ontonagon	clay	3.4
2	Miami	silt loam	2.2
3	Fayette	silt loam	1.4
4	Miami	silt loam	2.4
5	Dubuque	silt loam	1.8
6	Plainfield	sand	1.1
7	Tama	silt loam	4.1
8	Tama	silt loam	7.9
9	Morley	silt loam	2.7
10	Plano	silt loam	5.2
11	Oshkosh	silt loam	2.6
12	Plano	silt loam	5.7
13	Ontonagon	clay	3.0
14	Oshkosh	clay loam	1.7
15	Miami	silt loam	2.3
16	Goodman	silt loam	3.3
17	Fox	silt loam	3.7
18	Fox	silt loam	1.5
19	Warsaw	silt loam	5.3
20	Casco	sandy loam	2.3
21	Plainfield	sand	1.8
22	Plano	silt loam	6.2
23	Oshkosh	silt loam	2.1
24	Warsaw	sandy loam	2.9
25	Huntsville	silt loam	7.6
26	Miami	silt loam	1.5
27	Plainfield	sand	1.3
28	Tama	silt loam	4.3
29	Oshkosh	clay loam	3.9
30	Fayette	silt loam	4.4
31	Plainfield	sand	1.7
32	Hixton	fine sandy loam	1.5
33	Lomira	silt loam	2.3
34	Hochheim	gravelly loam	2.1

A Packard Model 7620 gas-liquid chromatograph was used for analysis of organochlorine insecticides. Gas chromatographic conditions were: carrier gas, N_2 with flow rate of 125 ml/minute; ^{3}H-foil electron-capture detector at 210 C, 50 volts; column temperature, 190 C; inlet temperature, 235 C; outlet temperature, 225 C.

The three types of columns were: (1) 2 parts 10% QF-1, 1 part 3% OV-17 on 60/80 mesh Gas Chrom Q (2 meters x 4 mm I.D.); (2) 1 part 17% QF-1, 1 part 11% DC-200 on 60/80 mesh Gas Chrom Q (2 meters x 4 mm I.D.); and (3) 10% diethelene glycol succinate (DGS) on 60/80 mesh Gas Chrom Q (1 meter x 4 mm I.D.). The instrument incorporates the use of glass columns and on-column injection to avoid sample degradation resulting from contact of the sample with metal surfaces.

Air-dried soil from the surface 20 cm was extracted in quantities of 100 g or 50 g (when a limited amount was available) with 200 ml of a 41:59 Skelly B:acetone azeotropic mixture using a Soxhlet technique in all glass apparatus (5). Concentration of the extract to 25 ml was achieved by forced air evaporation at 40 C. For samples 1-9 inclusive a fivefold dilution of the 25 ml concentrated extract was required for quantitative gas chromatography. The concentrated extracts were not subjected to any method of cleanup.

Results and Discussion

Using three-column gas chromatography most of the soil extracts gave peaks corresponding to organochlorine insecticides. On the QF-1/OV-17 column, 32 of 34 samples apparently showed measurable quantities of "heptachlor epoxide," and 24 samples contained "heptachlor"; peaks comparable to γ-BHC, aldrin, and dieldrin were found in a few samples (Table 2). On the QF-1/DC-200 column 20 of 34 samples apparently contained measurable quantities of "aldrin" with slight interference from "γ-BHC" and "dieldrin." A low degree of confusion between organochlorine insecticides and indigenous soil components was found on the DGS column; small amounts of "γ-BHC" and "heptachlor epoxide" would have been reported if this column had been used exclusively. No indigenous soil components which would interfere with determination of endrin, *p,p′*-DDD, *p,p′*-DDT, or *p,p′*-methoxychlor were found on any of the columns. Major interferences (>100 ppb) were found for "heptachlor epoxide" in soil samples 1-8 inclusive on the QF-1/OV-17 column and for "aldrin" in samples 1, 2, 5, and 6 on the QF-1/DC-200 column.

Chromatograms of the Skelly B:acetone extract of sample 9 on each of the three columns are shown in Fig. 1, 2, and 3. This sample was chosen because it was qualitatively similar to the other soil samples while showing a moderate amount of interference with in-

TABLE 2.—*"Apparent" organochlorine insecticide contents of air-dried soils sampled in 1910*

Sample No.	"Apparent" Insecticide Contents in PPB on Columns									
	QF-1/OV-17					QF-1/DC-200			DGS	
	Heptachlor Epoxide	Heptachlor	γ-BHC	Aldrin	Dieldrin	γ-BHC	Aldrin	Dieldrin	γ-BHC	Heptachlor Epoxide
1	823	3	3	0	0	0	141	0	0	0
2	807	3	2	0	0	0	294	0	4	0
3	742	1	0	0	0	0	13	0	0	0
4	696	5	2	0	0	0	63	0	9	0
5	564	20	9	5	0	0	164	0	9	0
6	378	5	6	0	0	0	100	0	3	0
7	188	1	1	0	0	0	60	0	0	0
8	156	0	2	0	0	0	26	0	0	0
9	51	2	0	0	0	0	43	0	2	0
10	49	3	2	0	0	0	8	0	0	2
11	43	5	2	1	0	0	0	0	0	0
12	40	5	2	0	0	0	0	0	0	3
13	33	8	0	2	0	0	12	0	2	2
14	28	6	0	0	0	0	8	0	3	0
15	25	8	2	2	0	0	0	0	3	0
16	20	0	0	0	0	0	41	0	0	0
17	19	4	0	0	0	0	4	0	0	0
18	14	3	0	0	0	0	4	0	2	0
19	9	1	1	0	0	0	20	0	0	0
20	8	2	0	0	0	0	0	0	0	0
21	7	8	0	9	0	0	0	40	4	6
22	7	2	0	0	0	0	0	0	0	0
23	7	1	0	0	0	0	6	0	0	4
24	4	0	2	0	0	6	5	0	0	0
25	4	1	0	0	0	0	0	0	0	0
26	4	4	0	0	3	0	0	0	2	1
27	3	2	0	0	0	0	0	0	0	0
28	3	0	0	0	0	0	0	0	0	0
29	2	0	0	0	0	0	0	0	0	0
30	2	0	0	0	0	0	0	0	0	0
31	2	0	0	0	0	0	3	0	0	0
32	1	0	2	0	0	0	3	0	0	0
33	0	0	0	0	0	0	0	0	3	0
34	0	0	0	0	0	0	0	0	2	0

secticide determination (Table 2). Extensive contamination by "heptachlor epoxide" and slight contamination by "γ-BHC" and "heptachlor" are indicated on the QF-1/OV-17 column (Fig. 1). "Aldrin" is the apparent contaminant found on the QF-1/DC-200 column (Fig. 2) while a small amount of "γ-BHC" was found on the DGS column (Fig. 3). Interfering peaks were classed as those which had a retention time (R_t) equal to R_t ±30 seconds of that of the standard insecticide. With a larger discrepancy in R_t-value, it is believed that the insecticide would be resolved satisfactorily from the indigenous soil contaminant.

From examination of Table 2, it can be seen that the "apparent" insecticide residues in the soil arise from indigenous compounds which display chromatographic characteristics similar to a particular organochlorine insecticide. However, the characteristic is unique to one of the columns and not displayed on either of the other two columns. The use of a combination of any two of the three columns described would reveal satisfactorily any chromatographic discrepancies arising from co-extraction of naturally occurring soil components. Six of the samples indicated small amounts of "heptachlor epoxide" on the DGS column which might be considered confirmatory for the "heptachlor epoxide" found on the QF-1/OV-17 column. However, no peaks corresponding to heptachlor epoxide were found on the QF-1/DC-200 column, and it is not believed that this discrepancy would lead to confusion if only two columns were used since the quantities of "heptachlor epoxide" on DGS are extremely small; the highest amount was 6 ppb for sample 21.

In Table 1 the samples are arranged in decreasing order of "apparent" insecticide contamination based on the "heptachlor epoxide" peak shown on the QF-1/OV-17 column. No relationship was found between "apparent" insecticidal contamination (Table 2) and the soil properties described in Table 1.

On each of the three columns a large peak was found which displayed an R_t-value greater than that for *p,p'*-methoxychlor on the QF-1/OV-17 and QF-1/DC-200 columns which are relatively nonpolar. However, on the relatively polar DGS column, the peak had a short R_t-value (comparable to that of dieldrin and endrin on the DGS column) which would interfere with insecticide determination (Fig. 3). This peak was found to result from the forced air evaporation of the soil extracts using Tygon tubing to blow air over the extracts. Small amounts of Tygon must have been dissolved by the Skelly B:acetone solvent during this procedure since Skelly B was capable of extracting the material from Tygon in less than 10 seconds (Fig. 3).

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See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(1) *Bowman, M. C., H. C. Young, and W. F. Barthel. 1965.* Minimal concentrations of aldrin, dieldrin, and heptachlor in soil for control of White-Fringed Beetles as determined by parallel gas chromatographic and biological assays. J. Econ. Entomol. 58:896-902.

(2) *De Vries, D. M., R. D. Collins, and R. T. Rossi. 1968.* What is a residue—or analytical artifacts? Symposium on the science and technology of residual insecticides in food production with special reference to aldrin and dieldrin. Shell Oil Company.

(3) *Goodwin, E. S., R. Goulden, and J. G. Reynolds. 1961.* Rapid identification and determination of residues of chlorinated pesticides in crops by gas-liquid chromatography. Analyst 86:697-709.

(4) *Pionke, H. B., J. G. Konrad, G. Chesters, and D. E. Armstrong. 1968.* Extraction of organochlorine and organophosphate insecticides from lake waters. Analyst 93:363-367.

(5) *Pionke, H. B., G. Chesters, and D. E. Armstrong. 1968.* Extraction of chlorinated hydrocarbon insecticides from soils. Agron. J. 60:289-292.

FIGURE 1.—*Gas chromatograms of organochlorine insecticide standards and the Skelly B: acetone extract of soil sample 9 on a QF-1/OV-17 column*

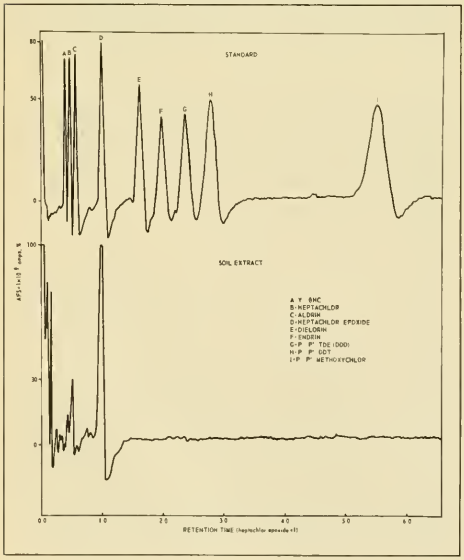


FIGURE 2.—*Gas chromatograms of organochlorine insecticide standards and the Skelly B: acetone extract of soil sample 9 on a QF-1/DC-200*

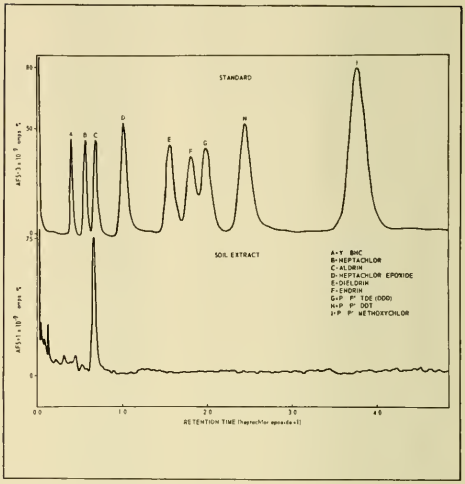
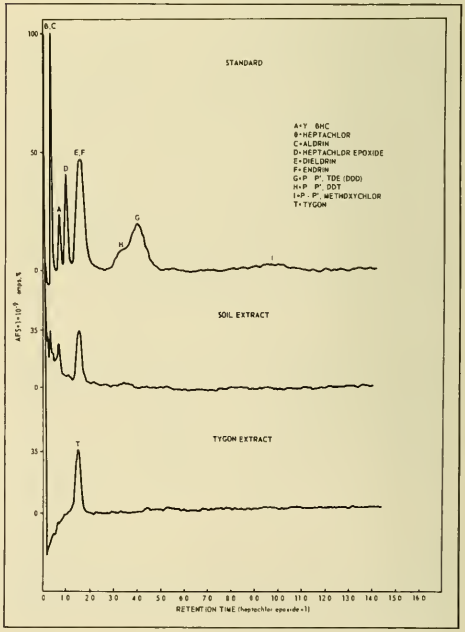


FIGURE 3.—*Gas chromatograms of organochlorine insecticide standards and the Skelly B: acetone extract of soil sample 9 and Tygon tubing on a DGS column*



PESTICIDES IN WATER

Pesticides in Surface Waters of the United States— a 5-Year Summary, 1964-68[1]

James J. Lichtenberg, James W. Eichelberger, Ronald C. Dressman, and James E. Longbottom

ABSTRACT

This report summarizes the results of five annual synoptic surveys (1964-68) for chlorinated hydrocarbon pesticides in surface waters of the United States. The results showed widespread occurrence of these compounds. The number of occurrences reached a peak in 1966 and then declined sharply in 1967 and 1968. Dieldrin and DDT and its congeners DDE and DDD were the compounds most frequently detected throughout the 5-year period. The maximum concentrations found have not exceeded permissible limits as they relate to human intake directly from a domestic water supply. However, they have often exceeded the environmental limit of 0.050 μg/liter recommended by the Federal Committee on Water Quality Criteria.

Introduction

Since September 1964, the Federal Water Quality Administration has conducted annual synoptic surveys for chlorinated hydrocarbon pesticides in surface waters (*1,2,3*). In September 1967 the fourth such survey was conducted, and in June 1968, the first spring survey was made. This surveillance activity has been a part of a continuing program for determining refractory organic substances in surface waters. The purpose is to provide information on present levels and trends of pesticides in waters to permit pollution control authorities to assess the degree of hazard and, if necessary, to provide the required control.

Through 1967 the surveys were conducted in September when streamflows are minimal. The 1968 survey was conducted in June in an effort to obtain comparative data during runoff periods after pesticide application.

Previous reports (*2,3*) have compared synoptic grab sample data with data obtained by the carbon adsorption method (CAM). Generally good agreement was noted between the two types of samples, and no further comparisons are reported here.

Samples were collected through the cooperative efforts of Federal, State, local, and private agencies at approximately 100 sampling stations. These stations are located mainly on interstate and international boundary waters at locations ranging from water treatment plant intakes to sites near mouths of rivers as they discharge to tidal waters.

This report summarizes the data obtained throughout the 5 surveys with emphasis on the 1967 and 1968 surveys. The number of samples analyzed for these surveys was 110 and 114, respectively. A total of 529 samples were analyzed for the 5 surveys.

Methods

The basic procedures for determination of chlorinated hydrocarbon pesticides are detailed in U.S. Department of the Interior Publication WP-22 (*4*) and in the "FWPCA Method for Chlorinated Hydrocarbon Pesticides in Water and Wastewater" (5). The samples were collected in 1-quart glass bottles (two per sample) equipped with screw caps fitted with Teflon liners. The samples were subjected to liquid-liquid extraction with 15% ethyl ether in hexane, dried over anhydrous sodium sulfate and concentrated to approximately 5 ml in a Kuderna-Danish evaporator. The extracts were carefully evaporated to 0.5 ml in a warm water bath, and up to 10 μl was injected into an electron capture gas chromatograph. If no response was obtained, the extracts were further concentrated to a maximum of

[1] From the Analytical Quality Control Laboratory, Federal Water Quality Administration, U.S. Department of the Interior, 1014 Broadway, Cincinnati, Ohio 45202.

0.2 ml and again injected into the chromatograph. Those samples producing a response were then subjected to thin-layer chromatographic separation. The eluates from the thin layer were concentrated as before and again injected into the chromatograph.

The results were confirmed by multiple gas chromatographic analyses of the thin-layer eluates using the following conditions:

(1) A Perkin-Elmer Model 880 equipped with a parallel plate electron capture detector, an aluminum column 6′ x ¼″ O.D. packed with Gas-Chrom Q (60/80 mesh) coated with 5% QF-1 and 3% Dow-200, and a nitrogen carrier flow of 100 ml/minute. Temperatures were: injection port—250 C, column oven—185 C, and detector—205 C.

(2) A MicroTek Model 179 equipped with a Ni^{63} detector, an aluminum column 6′ x ¼″ O.D. packed with Gas Chrom Q (60/80 mesh) coated with 5% OV-17, and a nitrogen carrier flow of 100 ml/minute. Temperatures were: injection port—250 C, column oven—205 C, and detector—360 C.

(3) A MicroTek Model 179 equipped with a flame photometric detector, a glass column 4′ x 4 mm I.D. packed with Gas Chrom Q (60/80 mesh) coated with 2% Reoplex-400, and a carrier flow of 75 ml/minute. Temperatures were: injection port—185 C, column oven—185 C, and detector—160 C.

When the quantity of pesticide in the sample permitted, further confirmation was obtained using a microcoulometric gas chromatograph under conditions similar to (2) above; in which case, a lack of response for thiophosphate pesticides was considered as supporting evidence for their presence. Similarly, lack of response to the flame photometric detector supported the identification of the organochlorine pesticides.

Recovery data were obtained by dosing distilled water samples with the pesticides and carrying them through the entire analytical procedure. Recovery of organochlorine pesticides ranged from 65%-97% when samples were dosed at levels of 25-100 ng/liter. Recoveries of thiophosphate pesticides ranged from 40%-75% at dosages of 50-500 ng/liter. Every tenth sample was run in duplicate. Where results differed, the higher value was reported.

The methods are specific for dieldrin, endrin, DDT, DDE, DDD, aldrin, heptachlor, heptachlor epoxide, lindane, BHC, γ-chlordane, and technical chlordane. In addition, the use of the flame photometric detector provided specificity for many organophosphorus pesticides. For the 1967 and 1968 surveys, samples were also analyzed for methyl parathion, parathion, fenthion, ethion, malathion, and carbophenothion.

The practical lower limit of detectability for the chlorinated pesticides is 0.001 to 0.002 μg/liter, except for technical chlordane which has a limit of 0.005 μg/liter. Toxaphene can be detected, if it is present, at levels of the order of 1 μg/liter. The detection limits for the phosphorus compounds are 0.010 to 0.025 μg/liter. All results are reported without correction for recovery efficiencies. Thus, the reported concentrations represent minimum values, the actual value being equal to or greater than the reported value.

Results and Discussion

The results of the 1967 and 1968 surveys are listed in Tables 1 and 2. Table 3 lists the total number of samples and positive pesticide occurrences for each of the five surveys. The data show that the total occurrences peaked in 1966 and fell off significantly in 1967 and 1968. Fig. 1 summarizes the percent occurrences of 10 pesticides for the 5 surveys. It shows that the occurrences decreased sharply after 1966 for all pesticides except BHC, which showed only a slight decline. It also shows that the 1966 peak in total occurrences (Table 3) is largely due to the increase in DDD occurrences. The spring survey showed a slight increase in dieldrin and DDT.

Fig. 2 shows the geographical occurrence of dieldrin, the DDT group, and BHC for 1967 and 1968. Table 4 summarizes the occurrences (1964-68) by FWQA region. In 1966, the number of occurrences peaked in the South Central Region and in all regions east of the Mississippi. The Missouri Basin Region showed a gradual decline from 1964-1966, then a very sharp drop in 1967 and 1968. In the Southwest and Northwest Regions the occurrences fluctuated from 1964-1966 and then fell off to virtually nothing in 1967 and 1968. Throughout the 5 surveys dieldrin dominated the pesticide occurrences in all regions and in total occurrences with 199 positive results. DDT was second in overall occurrences with 86. DDT and its congeners DDE and DDD as a group accounted for 183 occurrences, Aldrin and chlordane were low with just two and five occurrences, respectively. Consistent geographical relationships among the various pesticides are difficult to identify; however, the overall occurrences show that dieldrin was slightly predominant in all regions east of the Mississippi and the DDT group, considered as one, was predominant in regions west of the Mississippi.

Since 1966, BHC has been detected in 10 of 12 samples from the main stem of the Ohio River. This consistent occurrence was verified by the results of the analyses of monthly CAM samples performed in this laboratory. The synoptic surveys and additional investigations by this laboratory produced only one positive result for BHC in eight major tributaries to the Ohio. That one was at Pittsburgh on the Allegheny River in September 1966. Twenty-three other BHC occurrences were widely scattered throughout the Country.

Endrin was found in over 30% of the samples in 1964; the reduction of endrin occurrences to zero in 1968 is particularly significant in light of its association with major fish kills in the Lower Mississippi prior to 1964.

Heptachlor was found in 14% of the samples in 1965 and in less than 1% thereafter. Heptachlor epoxide was found in approximately 14% of the samples in 1965 and 1966 and dropped to zero thereafter.

The 10 locations at which the highest levels of each pesticide were observed for each survey are listed in Table 5. Individual locations varied considerably. However, 2 stations on the Savannah River, at North Augusta, S. C. and Port Wentworth, Ga., were in the top 10 locations for dieldrin occurrences for all 5 surveys. Other rivers and locations that were consistently in the top 10 are the Merrimack, Schuylkill, Connecticut, Delaware, Potomac, Lower Ohio, Lower Mississippi, Missouri (at Kansas City), Rio Grande, and Red River (North).

The highest level of each pesticide found is listed in Table 6 along with water quality criteria for public water supplies and farmstead uses (*6*) and suggested maximum reasonable stream allowance (*7*). While the maximum concentrations have not exceeded permissible limits as they relate to human intake directly from a domestic water supply, they have in some cases exceeded or come quite close to the maximum reasonable allowance suggested by Ettinger and Mount (*7*). Because of the biological concentration factor, these levels are considered hazardous in waters from which fish are harvested for human consumption. In addition, because of their toxicity to fish, the Federal Committee on Water Quality Criteria recommends that environmental levels of these substances not be permitted to rise above 0.050 μg/liter (*6*).

Of the 84 stations where samples were collected in all 5 surveys, 12 had at least 1 positive occurrence in each survey. These are listed in Table 7. All but one of these are east of the Mississippi River. In addition, 16 widely spread locations had at least 1 positive occurrence in 4 of the 5 surveys.

Since pesticides are so common in surface waters, it is of interest to note those locations at which they are absent or occur infrequently. Table 8 lists the Stations that fall in this category. Locations in the west and northwest dominate this group.

Spring runoff after pesticide application was expected to cause an increase in the number of occurrences and in concentration levels in agricultural areas. Such an increase was not evident from the data obtained. This may be, in part, due to the wet spring experienced in much of the Country in 1968 which delayed planting and subsequent pesticide application in many areas. As a result, our collection period may have been too early to catch an increased pesticide load.

FIGURE 1.—*Percent occurrence of ten chlorinated hydrocarbon pesticides, 1964-68*

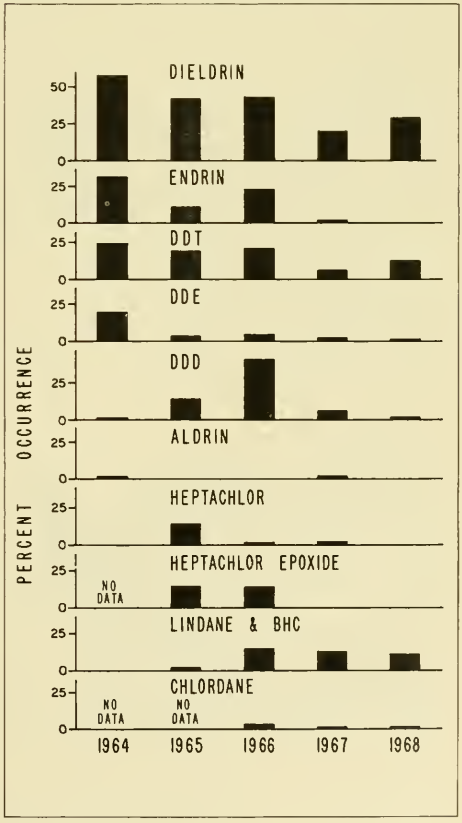


FIGURE 2.—*Occurrence of chlorinated hydrocarbon pesticides in surface waters, synoptic surveys of 1967 and 1968. (●—present; o—not detected)*

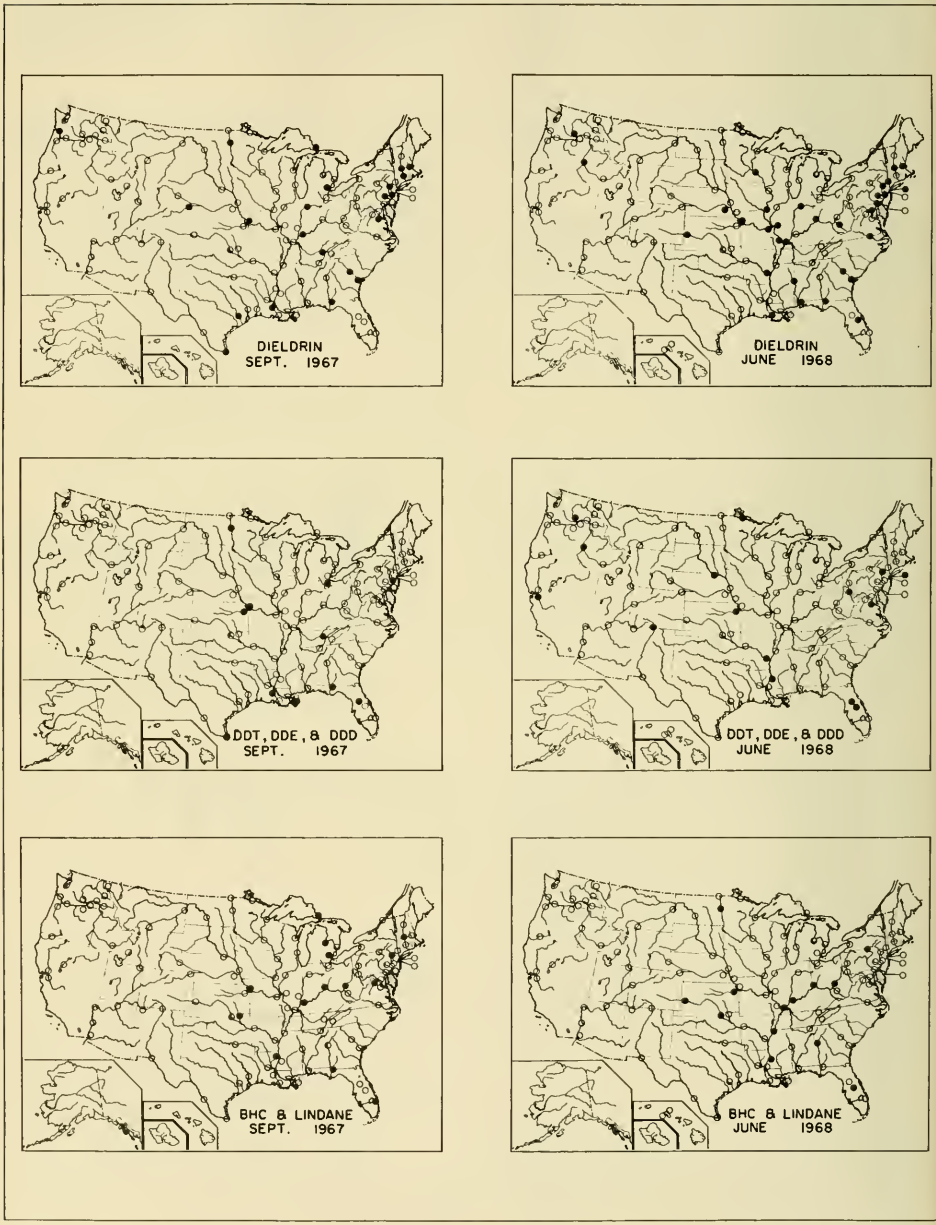


TABLE 1.—*Results of synoptic survey for pesticides in surface waters, September 1967*

Location	Concentration in μg/liter [1]						
	Dieldrin	Endrin	DDT	DDE	DDD	Lindane	BHC
NORTHEAST REGION							
Connecticut River							
Enfield Dam, Conn.	.005	—	—	—	—	—	—
Northfield, Mass.	.017	—	—	—	—	.002	—
Wilder, Vt.	—	—	—	—	—	—	—
Schuylkill River							
Philadelphia, Pa.	.044	—	—	—	—	—	—
Hudson River							
Poughkeepsie, N.Y.	—	—	—	—	—	—	—
Narrows, N.Y.	—	—	—	—	—	—	—
Merrimack River							
Lowell, Mass.	.066	—	—	—	—	—	—
Delaware River							
Trenton, N.J.	.010	—	.017	—	.036	—	—
Martins Creek, Pa.	.013	—	—	—	—	.002	—
Raritan River							
Perth Amboy, N.J.	—	—	—	—	—	—	—
Delaware Bay							
a	—	—	—	—	—	—	—
b	—	—	—	—	—	—	—
MIDDLE ATLANTIC REGION							
Potomac River							
Great Falls, Md.	—	—	—	—	—	—	—
Washington, D.C.	.025	—	—	—	—	—	—
Shenandoah River							
Berryville, Va.	—	—	—	—	—	—	.002
Susquehanna River							
Conowingo, Md.	—	—	—	—	—	—	—
Sayre, Pa.	—	—	—	—	—	—	—
Roanoke, River							
John H. Kerr Dam, Va.	—	—	—	—	—	—	—
Neuse River							
Raleigh, N.C.	—	—	—	—	—	—	—
SOUTHEAST REGION							
Apalachicola River							
Chattahoochee, Fla.	.015	—	—	—	.053	.003	—
Beauclair River							
Lake Apopka, Fla.	—	—	.316	.050	.231	—	—
Escambia River							
Century, Fla.	—	—	—	—	—	—	—
Oklahawa River							
Orlando, Fla.	—	—	—	—	—	—	—
W. Palm Beach Canal							
W. Palm Beach, Fla.	—	—	—	—	—	.003	—
Chattahoochee River							
Lanett, Ala.	—	—	—	—	—	P	—
Savannah River							
Port Wentworth, Ga.	.039	—	—	—	—	—	—
North Augusta, S.C.	.087	—	—	—	—	—	—
Clinch River							
Kingston, Tenn.	.004	—	—	—	.032	—	—
Tennessee River							
Bridgeport, Ala.	—	—	—	—	—	—	—
Lenoir City, Tenn.	—	—	—	—	—	—	—
Tombigbee River							
Columbus, Miss.	—	—	—	—	P	—	—
OHIO BASIN REGION							
Allegheny River							
Pittsburgh, Pa.	—	—	—	—	—	—	—
Kanawha River							
Winfield Dam, W. Va.	—	—	—	—	—	P	—
Monongahela River							
Pittsburgh, Pa.	—	—	—	—	—	—	—
Ohio River							
Cairo, Ill.	—	—	—	—	—	—	—
Evansville, Ind.	.020	—	—	—	—	—	.008
Cincinnati, Ohio	—	—	—	—	—	—	.013
Above Addison, Ohio	—	—	—	—	—	—	006
Wabash River							
Lafayette, Ind.	.009	—	—	—	—	—	—
New Harmony, Ind.	—	—	—	—	—	—	—

TABLE 1.—*Results of synoptic survey for pesticides in surface waters, September 1967*—Continued

Location	Concentration in µg/liter [1]						
	Dieldrin	Endrin	DDT	DDE	DDD	Lindane	BHC
GREAT LAKES REGION							
St. Lawrence River Massena, N.Y.	—	—	—	—	—	—	P
Lake Erie Buffalo, N.Y.	P	—	—	—	—	—	—
Detroit River Detroit, Mich.	.014	—	—	—	—	—	.002
St. Clair River Port Huron, Mich.	—	—	—	—	—	—	—
St. Mary's River Sault Ste. Marie, Mich.	.004	—	—	—	—	.003	—
Saginaw River Bay City, Mich.	P	—	—	—	—	—	.007
Lake Superior Duluth, Minn.	—	—	—	—	—	—	—
Lake Michigan Milwaukee, Wis.	—	—	—	—	—	—	—
Maumee River Toledo, Ohio	—	.086	—	—	.270	—	—
Illinois River Peoria, Ill.	—	—	—	—	—	—	—
Mississippi River Cape Girardeau, Mo.	—	—	—	—	—	—	—
E. St. Louis, Ill.	—	—	—	—	—	—	—
Burlington, Iowa	—	—	—	—	—	—	—
Dubuque, Iowa	—	—	—	—	—	—	—
St. Paul, Minn.	—	—	—	—	—	—	—
Fox River Green Bay, Wis.	—	—	—	—	—	—	—
MISSOURI BASIN REGION							
Missouri River St. Louis, Mo.	—	—	—	—	—	—	—
Kansas City, Kans.	.012	—	.066	—	—	.010	—
Omaha, Nebr.	—	—	—	—	—	—	—
Yankton, S.Dak.	—	—	—	—	—	—	—
Bismarck, N.Dak.	—	—	—	—	—	—	—
North Platte River Henry, Nebr.	—	—	—	—	—	—	—
Platte River Plattsmouth, Nebr.	—	—	—	—	—	—	—
South Platte River Julesburg, Colo.	.024	—	—	—	—	—	—
Yellowstone River Sidney, Mont.	—	—	—	—	—	—	—
Rainy River Baudette, Minn.	—	—	—	—	—	—	—
Red River (North) Grand Forks, N.Dak.	.087	—	.054	—	—	—	—
Emerson, Manitoba	P	—	—	—	—	—	—
Kansas River Lawrence, Kans.	—	.133	—	—	.840	—	—
Big Horn River Hardin, Mont.	—	—	—	—	—	—	—
SOUTH CENTRAL REGION							
Atchafalaya River Morgan City, La.	—	—	—	—	—	—	—
Arkansas River Pendleton Ferry, Ark.	—	—	—	—	—	P	—
Fort Smith, Ark.	—	—	—	—	—	—	—
Ponca City, Okla.	—	—	—	—	—	—	—
Coolidge, Kans.	—	—	—	—	—	—	—
Brazos River Arcola, Tex.	.024	—	—	—	P	—	—

TABLE 1.—*Results of synoptic survey for pesticides in surface waters, September 1967*—Continued

Location	Concentration in μg/liter [1]						
	Dieldrin	Endrin	DDT	DDE	DDD	Lindane	BHC
SOUTH CENTRAL REGION—Continued							
Mississippi River							
New Orleans, La.	—	—	.019	—	—	—	—
Vicksburg, Miss.	—	—	—	—	—	—	—
Delta, La.	—	—	—	—	—	.024	—
West Memphis, Ark.	—	—	—	—	—	—	—
New Roads, La.	.008	—	—	—	.015	—	—
Red River (South)							
Alexandria, La.	—	—	—	—	—	—	—
Denison, Tex.	—	—	—	—	—	—	—
Rio Grande River							
Brownsville, Tex.	.002	—	.018	.022	—	—	—
El Paso, Tex.	—	—	—	—	—	—	—
Alamosa, Colo.	—	—	—	—	—	—	—
Verdigris River							
Nowata, Okla.	—	—	—	—	—	.009	—
Trinity River							
Houston, Tex.	—	—	—	—	—	—	—
SOUTHWEST REGION							
Bear River							
Preston, Idabo	—	—	—	—	—	—	—
Colorado River							
Yuma, Ariz.	—	—	—	—	—	—	—
Parker Dam, Calif.	—	—	—	—	—	—	—
Boulder City, Nev.	—	—	—	—	—	—	—
Page, Ariz.	—	—	—	—	—	—	—
Green River							
Dutch John, Utah	—	—	—	—	—	—	—
Klamath River							
Keno, Oreg.	—	—	—	—	—	—	—
Sacramento River							
Greens Landing, Calif.	—	—	—	—	—	—	—
San Joaquin River							
Vernalis, Calif.	—	—	—	—	—	—	—
San Juan River							
Shiprock, N. Mex.	—	—	—	—	—	—	—
Truckee River							
Farad, Calif.	—	—	—	—	—	—	—
NORTHWEST REGION							
Clearwater River							
Lewiston, Idaho	—	—	—	—	—	—	—
Columbia River							
Clatskanie, Oreg.	.018	—	—	—	—	—	—
Bonneville Dam, Oreg.	—	—	—	—	—	—	—
McNary Dam, Oreg.	—	—	—	—	—	—	—
Pasco, Wash.	—	—	—	—	—	—	—
Pend Oreille River							
Albeni Falls, Idaho	—	—	—	—	—	—	—
Snake River							
Wawawai, Wash.	—	—	—	—	—	—	—
American Falls, Idaho	—	—	—	—	—	—	—
Spokane River							
Post Falls, Idaho	—	—	—	—	—	—	—
Willamette River							
Portland, Oreg.	—	—	—	—	—	—	—
Yakima River							
Richland, Wash.	—	—	—	—	—	—	—

[1] The Lanett, Ala. sample contained .036 μg/liter of chlordane (tech). The Nowata, Okla. sample contained .002 μg/liter of aldrin and .003 μg/liter of heptachlor. The Wawawai, Wash. sample contained .050 μg/liter of parathion and .380 μg/liter of ethion. All other samples gave negative results for aldrin, heptachlor, heptachlor epoxide, parathion, methyl parathion, fenthion, ethion, malathion, and carbophenothion.

NOTE: — = not detected.

P = presumptive. Data are reported as presumptive in instances where the results of chromatography were highly indicative but did not meet all requirements for positive identification and quantification.

TABLE 2.—*Results of synoptic survey for pesticides in surface waters, June 1968*

Location	Concentration in μg/liter [1]						
	Dieldrin	Endrin	DDT	DDE	DDD	Lindane	BHC
NORTHEAST REGION							
Connecticut River							
Enfield Dam, Conn.	—	—	—	—	—	—	—
Northfield, Mass.	.022	—	—	—	—	—	—
Wilder, Vt.	—	—	—	—	—	—	—
Schuylkill River							
Philadelphia, Pa.	.027	—	—	—	—	—	—
Hudson River							
Poughkeepsie, N.Y.	.013	—	—	—	—	—	—
Narrows, N.Y.	.004	—	.030	—	—	—	—
Merrimack River							
Lowell, Mass.	.012	—	—	—	—	—	—
Delaware River							
Trenton, N.J.	.007	—	—	—	—	—	—
Martins Creek, Pa.	.007	—	.015	—	—	—	—
Raritan River							
Perth Amboy, N.J.	—	—	—	—	—	—	—
Delaware Bay	—	—	—	—	—	—	—
MIDDLE ATLANTIC REGION							
Potomac River							
Great Falls, Md.	.007	—	—	—	—	—	—
Washington, D.C.	—	—	.033	—	—	—	—
Shenandoah River							
Berryville, Va.	—	—	—	—	—	—	—
Susquehanna River							
Conowingo, Md.	.007	—	—	—	—	—	—
Sayre, Pa.	—	—	—	—	—	—	.009
Roanoke, River							
John H. Kerr Dam, Va.	.010	—	—	—	—	—	—
Neuse River							
Raleigh, N.C.	—	—	—	—	—	—	—
SOUTHEAST REGION							
Apalachicola River							
Chattahoochee, Fla.	.027	—	—	—	—	—	—
Beauclair River							
Lake Apopka, Fla.	—	—	.220	.041	.156	—	—
Escambia River							
Century, Fla.	.006	—	—	—	—	—	—
Oklahawa River							
Orlando, Fla.	.004	—	.005	—	—	—	.015
W. Palm Beach Canal							
W. Palm Beach, Fla.	—	—	—	—	—	—	—
Chattahoochee River							
Lanett, Ala.	—	—	—	—	—	—	.025
Savannah River							
Port Wentworth, Ga.	.039	—	—	—	—	—	—
North Augusta, S.C.	.059	—	—	—	—	—	—
Tennessee River							
Bridgeport, Ala.	—	—	—	—	—	—	—
Lenoir City, Tenn.	—	—	—	—	—	—	—
Oak Ridge, Tenn.	—	—	—	—	—	—	—
Tombigbee River							
Columbus, Miss.	.407	—	—	—	—	—	—
OHIO BASIN REGION							
Allegheny River							
Pittsburgh, Pa.	—	—	—	—	—	—	—
Kanawha River							
Winfield, W.Va.	.154	—	—	—	—	—	—
Monongahela River							
Pittsburgh, Pa.	—	—	.051	—	—	—	—
Ohio River							
Cairo, Ill.	.005	—	—	—	—	—	.020
Evansville, Ind.	—	—	—	—	—	—	.055
Cincinnati, Ohio	.014	—	—	—	—	—	.028
Above Addison, Ohio	—	—	—	—	—	—	.112
Wabash River							
Lafayette, Ind.	.005	—	—	—	—	—	—

TABLE 2.—*Results of synoptic survey for pesticides in surface waters, June 1968*—Continued

Location	Concentration in μg/liter [1]						
	Dieldrin	Endrin	DDT	DDE	DDD	Lindane	BHC
GREAT LAKES REGION							
St. Lawrence River							
Massena, N.Y.	—	—	—	—	—	—	—
Lake Erie							
Buffalo, N.Y.	—	—	—	—	—	—	—
Detroit River							
Detroit, Mich.	—	—	—	—	—	—	—
Grand River							
Grand Haven, Mich.	—	—	—	—	—	—	—
St. Clair River							
Port Huron, Mich.	—	—	—	—	—	—	—
St. Mary's River							
Sault Ste. Marie, Mich.	—	—	—	—	—	—	—
Saginaw River							
Bay City, Mich.	—	—	—	—	—	—	—
Lake Superior							
Duluth, Minn.	—	—	—	—	—	—	—
Lake Michigan							
Milwaukee, Wis.	—	—	—	—	—	—	—
Maumee River							
Toledo, Ohio	—	—	—	—	—	—	—
Illinois River							
Peoria, Ill.	—	—	—	—	—	—	—
Mississippi River							
Cape Girardeau, Mo.	.014	—	—	—	—	—	—
E. St. Louis, Ill.	.011	—	—	—	—	—	—
Burlington, Iowa	.010	—	—	—	—	—	—
Dubuque, Iowa	—	—	—	—	—	—	—
St. Paul, Minn.	.011	—	—	—	—	—	—
Fox River							
Green Bay, Wis.	—	—	—	—	—	—	—
MISSOURI BASIN REGION							
Missouri River							
St. Louis, Mo.	.010	—	—	—	—	—	-
Kansas City, Kans.	.009	—	—	—	—	—	—
Omaha, Nebr.	—	—	—	—	—	—	—
Yankton, S. Dak.	—	—	.053	—	—	—	—
Bismarck, N. Dak.	—	—	—	—	—	—	—
St. Joseph, Mo.	—	—	—	—	—	—	—
North Platte River							
Henry, Nebr.	—	—	—	—	—	—	—
Platte River							
Plattsmouth, Nebr.	.005	—	—	—	—	—	—
South Platte River							
Julesburg, Colo.	—	—	—	—	—	—	—
Yellowstone River							
Sidney, Mont.	—	—	—	—	—	—	—
Rainy River							
Baudette, Minn.	—	—	.037	—	—	—	—
Red River (North)							
Grand Forks, N.Dak.	—	—	—	—	—	—	.027
Emerson, Manitoba	—	—	—	—	—	—	—
Kansas River							
Lawrence, Kans.	—	—	.008	—	—	.003	—
Big Horn River							
Hardin, Mont.	—	—	—	—	—	—	—
SOUTH CENTRAL REGION							
Atchafalaya River							
Morgan City, La.	.005	—	—	—	—	—	—
Arkansas River							
Pendleton Ferry, Ark.	.005	—	.037	—	—	—	—
Fort Smith, Ark.	—	—	—	—	—	—	—
Ponca City, Okla.	—	—	—	—	—	—	.013
Coolidge, Kans.	.009	—	—	—	—	—	.025
Brazos River							
Arcola, Tex.	—	—	—	—	—	—	—
Mississippi River							
New Orleans, La.	—	—	—	—	—	—	—
Vicksburg, Miss.	—	—	.109	—	—	.004	—
West Memphis, Ark.	—	—	—	—	—	—	.005
St. Francisville, La.	—	—	—	—	—	—	—

TABLE 2.—*Results of synoptic survey for pesticides in surface waters, June 1968*—Continued

Location	Concentration in µg/liter [1]						
	Dieldrin	Endrin	DDT	DDE	DDD	Lindane	BHC
SOUTH CENTRAL REGION—Continued							
Red River (South)							
Alexandria, La.	—	—	—	—	—	—	—
Denison, Tex.	—	—	—	—	—	—	—
Rio Grande River							
Brownsville, Tex.	—	—	—	—	—	—	—
El Paso, Tex.	—	—	—	—	—	—	—
Alamosa, Colo.	—	—	.029	—	—	—	—
Verdigris River							
Nowata, Okla.	—	—	—	—	—	—	—
Trinity River							
Houston, Tex.	—	—	—	—	—	—	—
SOUTHWEST REGION							
Bear River							
Preston, Idaho	—	—	—	—	—	—	—
Colorado River							
Yuma, Ariz.	—	—	—	—	—	—	—
Parker Dam, Calif.	—	—	—	—	—	—	—
Boulder City, Nev.	—	—	—	—	—	—	—
Page, Ariz.	—	—	—	—	—	—	—
Loma, Colo.	—	—	—	—	—	—	—
Green River							
Dutch John, Utah	—	—	—	—	—	—	—
Klamath River							
Keno, Oreg.	—	—	—	—	—	—	—
Sacramento River							
Green's Landing, Calif.	—	—	—	—	—	—	—
San Joaquin River							
Vernalis, Calif.	—	—	.030	—	—	—	—
San Juan River							
Shiprock, N. Mex.	—	—	—	—	—	—	—
Truckee River							
Farad, Calif.	—	—	—	—	—	—	—
Kiikii Stream							
Oahu, Hawaii	—	—	—	—	—	—	—
Waikele Stream							
Oahu, Hawaii	—	—	—	—	—	—	—
NORTHWEST REGION							
Clearwater River							
Lewiston, Idaho	—	—	—	—	—	—	—
Columbia River							
Clatskanie, Oreg.	—	—	—	—	—	—	—
Bonneville Dam, Oreg.	—	—	—	—	—	—	—
McNary Dam, Oreg.	—	—	—	—	—	—	—
Pasco, Wash.	—	—	—	—	—	—	—
Pend Oreille River							
Albeni Falls, Idaho	—	—	—	—	—	—	—
Snake River							
Wawawai, Wash.	—	—	—	—	—	—	—
Payette, Idaho	.004	—	.015	—	—	—	—
American Falls, Idaho	—	—	—	—	—	—	—
Spokane River							
Post Falls, Idaho	—	—	—	—	—	—	—
Willamette River							
Portland, Oreg.	—	—	—	—	—	—	—
Yakima River							
Richland, Wash.	.006	—	.017	—	—	—	—

[1] The Lanett, Ala. sample contained .169 µg/liter of chlorane (tech). All samples gave negative results for aldrin, heptachlor, heptachlor epoxide, parathion, methyl parathion, fenthion, ethion, malathion, and carbophenothion.

NOTE: — = not detected.

TABLE 3.—*Total number of chlorinated pesticide occurrences*

Year	Number of Samples Collected	Number of Samples With Positive Occurrences	Total Number of Positive Occurrences
1964	97	73	130
1965	99	56	120
1966	109	80	177
1967	110	34	56
1968	114	48	63
Total	529	291	546

TABLE 4.—*Pesticide occurrences by FWQA Region, 1964-68*

Pesticide	Northeast	Middle Atlantic	Southeast	Ohio Basin	Great Lakes Basin	Missouri Basin	South Central	Southwest	Northwest	Total
Dieldrin	31	14	28	20	22	25	34	13	12	199
Endrin	4	4	9	2	7	13	19	5	4	67
DDT	6	4	10	9	2	18	18	10	9	86
DDE	2	1	3	1	4	6	4	5	3	29
DDD	10	6	10	4	10	10	10	4	4	68
Aldrin	0	0	0	0	0	0	1	1	0	2
Heptachlor	1	0	1	2	3	4	3	2	0	16
Heptachlor epoxide	2	2	3	3	7	6	3	2	1	29
Lindane	2	0	2	0	1	2	3	0	0	10
BHC	2	2	3	12	4	3	7	2	0	35
Chlordane	0	1	3	0	0	0	0	1	0	5
Total	60	34	72	53	60	87	102	45	33	546
No. of Samples	53	32	50	41	76	70	86	65	56	529

TABLE 5.—*Top 10 locations at which highest levels were observed*

Location	µg/liter	Location	µg/liter	Location	µg/liter	Location	µg/liter	Location	µg/liter
1964		1965		1966		1967		1968	
DIELDRIN									
Savannah River N. Augusta, S. C.	0.118	Tombigbee River Columbus, Miss.	0.100	Merrimack River Lowell, Mass.	0.167	Savannah River N. Augusta, S. C.	0.087	Tombigbee River Columbus, Miss.	0.407
Merrimack River Lowell, Mass.	0.071	Merrimack River Lowell, Mass.	0.068	Savannah River N. Augusta, S. C.	0.110	Red River (North) Grand Forks, N. Dak.	0.087	Kanawha River Winfield Dam, W. Va.	0.154
Potomac River Great Falls, Md.	0.040	Savannah River N. Augusta, S. C.	0.051	Savannah River Port Wentworth, Ga.	0.048	Merrimack River Lowell, Mass.	0.066	Savannah River N. Augusta, S. C.	0.059
Schuylkill River Philadelphia, Pa.	0.032	Kanawha River Winfield Dam, W. Va.	0.045	Susquehanna River Conowingo, Md.	0.031	Schuylkill River Philadelphia, Pa.	0.044	Savannah River Port Wentworth, Ga.	0.039
Rio Grande El Paso, Tex.	0.032	Rio Grande River Alamosa, Colo.	0.029	Delaware Bay	0.025	Savannah River Port Wentworth, Ga.	0.039	Schuylkill River Philadelphia, Pa.	0.027
Platte River Plattsmouth, Nebr.	0.023	Tennessee River Lenoir City, Tenn.	0.028	Connecticut River Northfield, Mass.	0.017	Potomac River Washington, D. C.	0.025	Apalachicola River Chattahoochee, Fla.	0.027
Connecticut River Northfield, Mass.	0.022	Ohio River Cairo, Ill.	0.028	Connecticut River Endfield Dam, Conn.	0.016	South Platte River Julesburg, Colo.	0.024	Connecticut River Northfield, Mass.	0.022
Savannah River Port Wentworth, Ga.	0.020	Mississippi River Dubuque, Iowa	0.024	Schuylkill River Philadelphia, Pa.	0.015	Brazos River Arcola, Tex.	0.024	Ohio River Cincinnati, Ohio	0.014
Mississippi River Vicksburg, Miss.	0.017	Missouri River Kansas City, Kans.	0.023	Chattahoochee River Lanett, Ala.	0.015	Ohio River Evansville, Ind.	0.020	Mississippi River Cape Girardeau, Md.	0.014
Mississippi River New Roads, La.	0.016	Savannah River Port Wentworth, Ga.	0.022	Kanawha River Winfield Dan, W. Va.	0.015	Columbia River Clatskanie, Oreg.	0.018	Hudson River Poughkeepsie, N. Y.	0.013
ENDRIN									
Potomac River Great Falls, Md.	0.094	Mississippi River West Memphis, Ark.	0.116	Hudson River Narrows, N. Y.	0.069	Kansas River Lawrence, Kans.	0.133	NONE	
Rio Grande River El Paso, Tex.	0.067	Atchafalaya River Morgan City, La.	0.019	South Platte River Julesburg, Colo.	0.063	Maumee River Toledo, Ohio	0.086		
Big Horn River Hardin, Mont.	0.026	Delaware River Trenton, N. J.	0.018	Savannah River Port Wentworth, Ga.	0.031				
Mississippi River Vicksburg, Miss.	0.025	Tombigbee River Columbus, Miss.	0.015	St. Joseph River Benton Harbor, Mich.	0.029				
Connecticut River Northfield, Mass.	0.025	Clinch River Kingston, Tenn.	0.015	Lake Superior Duluth, Minn.	0.022				
Red River (North) Grand Forks, N. Dak.	0.023	Rio Grande River Alamosa, Colo.	0.014	Savannah River N. Augusta, S. C.	0.022				
Mississippi River New Roads, La.	0.023	Monongahela River Pittsburgh, Pa.	0.014	Bear River Preston, Idabo	0.019				
Yellowstone River Sidney, Mont.	0.021	Tennessee River Lenoir City, Tenn.	0.009	Clearwater River Lewiston, Idaho	0.015				
Columbia River Clatskanie, Oreg.	0.019	Red River (North) Grand Forks, N. Dak.	0.009	Connecticut River Northfield, Mass.	0.014				
Atchafalaya River Morgan City, La.	0.018	Mississippi River Delta, La.	0.008	Mississippi River Delta, La.	0.014				
DDT									
Maumee River Toledo, Ohio	0.087	Rio Grande River Alamosa, Colo.	0.149	Brazos River Arcola, Tex.	0.123	Beauclair River Lake Apopka, Fla.	0.316	Beauclair River Lake Apopka, Fla.	0.220
Red River (North) Grand Forks, N. Dak.	0.072	San Juan River Shiprock, N. Mex.	0.125	Rio Grande River El Paso, Tex.	0.046	Missouri River Kansas City, Kans.	0.066	Mississippi River Vicksburg, Miss.	0.109
San Joaquin River Vernalis, Calif.	0.066	Colorado River Page, Ariz.	0.058	Mississippi River Vicksburg, Miss.	0.044	Red River (North) Grand Forks, N. Dak.	0.054	Missouri River Yankton, S. Dak.	0.053

TABLE 3.—*Top 10 locations at which highest levels were observed*—Continued

Location	μg/liter	Location	μg/liter	Location	μg/liter	Location	μg/liter	Location	μg/liter
1964		1965		1966		1967		1968	
DDT—Continued									
Atchafalaya River Morgan City, La.	0.047	Platte River Plattsmouth, Nebr.	0.039	Arkansas River Fort Smith, Ark.	0.042	Mississippi River New Orleans, La.	0.019	Monongahela River Pittsburgh, Pa.	0.051
Mississippi River Vicksburg, Miss.	0.041	Spokane River Post Falls Dam, Idaho	0.037	Potomac River Great Falls, Md.	0.038	Rio Grande River Brownsville, Tex.	0.018	Rainy River Baudette, Minn.	0.037
Bear River Preston, Idaho	0.034	Red River (North) Grand Forks, N. Dak.	0.034	Mississippi River Delta, La.	0.031	Delaware River Trenton, N. J.	0.017	Arkansas River Pendleton Ferry, Ark.	0.037
Columbia River Clatskanie, Oreg.	0.034	Ohio River Cairo, Ill.	0.023	Missouri River Kansas City, Kans.	0.029			Potomac River Washington, D. C.	0.033
Red River (South) Alexandria, La.	0.031	South Platte River Julesburg, Colo.	0.023	Delaware River Trenton, N. J.	0.028			Hudson River Narrows, N. Y.	0.030
Willamette River Portland, Oreg.	0.029	Mississippi River Delta, La.	0.019	Lake Superior Duluth, Minn.	0.026			San Joaquin River Vernalis, Calif.	0.030
Apalachicola River Chattahoochee, Fla.	0.027	Mississippi River Vicksburg, Miss.	0.017	Snake River American Falls, Idaho	0.025			Rio Grande River Alamosa, Colo.	0.029
DDE									
Maumee River Toledo, Ohio	0.015	San Juan River Shiprock, N. Mex.	0.009	Brazos River Arcola, Tex.	0.004	Beauclair River Lake Apopka, Fla.	0.050	Beauclair River Lake Apopka, Fla.	0.041
Bear River Preston, Idaho	0.011	Detroit River Detroit, Mich.	0.008	San Joaquin River Vernalis, Calif.	0.003	Rio Grande River Brownsville, Tex.	0.022		
Mississippi River St. Paul, Minn.	0.011	Yellowstone River Sidney, Mont.	0.002	St. Lawrence River Messena, N. Y.	0.002				
South Platte River Julesburg, Colo.	0.009	Platte River Plattsmouth, Nebr.	P	Columbia River Clatskanie,Oreg.	0.001				
Delaware River Martins Creek, Pa.	0.008	Rainy River Baudette, Minn.	P	Arkansas River Pendleton Ferry, Ark.	P				
Mississippi River West Memphis, Ark.	0.007			Red River (South) Alexandria, La.	P				
Columbia River Clatskanie, Oreg.	0.005			Rio Grande River El Paso, Tex.	P				
San Joaquin River Vernalis, Calif.	0.005			Lake Superior Duluth, Minn.	P				
Snake River Payette, Idaho	0.005			Hudson River Poughkeepsie, N. Y.	P				
Seven Stations	0.004			Hudson River Narrows, N. Y.	P				
DDD									
Shenandoah River Berryville, Va.	0.083	Rio Grande River Brownsville, Tex.	0.026	Connecticut River Endfield Dam, Conn.	0.013	Kansas River Lawrence, Kans.	0.840	Beauclair River Lake Apopka, Fla.	0.156
All others	<0.075	Delaware River Trenton, N. J.	0.018	Rio Grande River Brownsville, Tex.	0.013	Maumee River Toledo, Ohio	0.270		
		Willamette River Portland, Oreg.	0.013	St. Joseph River Benton Harbor, Mich.	0.013	Beauclair River Lake Apopka, Fla.	0.231		
		Missouri River Kansas City, Kans.	0.011	Raritan River Perth Amboy, N. J.	0.012	Apalachicola River Chattahoochee, Fla.	0.053		
		St. Lawrence River Messena, N. Y.	0.010	Detroit River Grosse Isle, Mich.	0.012	Delaware River Trenton, N. J.	0.036		
		Platte River Plattsmouth, Nebr.	0.010	Potomac River Great Falls, Md.	0.012	Clinch River Kingston, Tenn.	0.032		

TABLE 5.—*Top 10 locations at which highest levels were observed*—Continued

Location	μg/liter	Location	μg/liter	Location	μg/liter	Location	μg/liter	Location	μg/liter
1964		1965		1966		1967		1968	
				DDD—Continued					
		Waikele Stream Oahu, Hawaii	0.008	Arkansas River Pendleton Ferry, Ark.	0.012	Mississippi River New Roads, La.	0.015		
		Red River (South) Alexandria, La.	0.008	Chattahoochee River Lanett, Ala.	0.011	Tombigbee River Columbus, Miss.	P		
		Merrimack River Lowell, Mass.	0.007	Atchafalaya River Morgan City, La.	0.010	Brazos River Arcola, Tex.	P		
		Potomac River Washington, D. C.	0.007	Missouri River Kansas City, Kans.	0.010				
				BHC					
Delaware River Martins Creek, Pa.	P	Red River (North) Grand Forks, N. Dak.	0.004	Ohio River Cincinnati, Ohio	0.056	Ohio River Cincinnati, Ohio	0.013	Ohio River Addison, Ohio	0.112
Mississippi River West Memphis, Ark.	P	Ohio River Cairo, Ill.	0.002	Hudson River Narrows, N. Y.	0.034	Ohio River Evansville, Ind.	0.008	Ohio River Evansville, Ind.	0.055
All others	<0.025	Verdigris River Nowata, Okla.	P	Ohio River Addison, Ohio	0.026	Saginaw River Bay City, Mich.	0.007	Ohio River Cincinnati, Ohio	0.028
		Connecticut River Endfield Dam, Conn.	P	Rio Grande River El Paso, Tex.	0.023	Ohio River Addison, Ohio	0.006	Red River (North) Grand Forks, N. Dak.	0.027
		Monongahela River Pittsburgh, Pa.	P	South Platte River Julesburg, Colo.	0.022	Shenandoah River Berryville, Va.	0.002	Chattahoochee River Lanett, Ala.	0.025
				Trinity River Livingston, Tex.	0.013	Detroit River Detroit, Mich.	0.002	Arkansas River Coolidge, Kans.	0.025
				Allegheny River Pittsburgh, Pa.	0.013	St. Lawrence River Messena, N. Y.	P	Ohio River Cairo, Ill.	0.020
				Mississippi River St. Paul, Minn.	0.012			Oklawaha River Orlando, Fla.	0.015
				Mississippi River Vicksburg, Miss.	0.010			Arkansas River Ponca City, Okla.	0.013
				San Joaquin River Vernalis, Calif.	0.008			Susquehanna River Sayre, Pa.	0.009
				Chattahoochee River Lanett, Ala.	0.008				
				Arkansas River Ponca City, Okla.	0.008				

P = presumptive. Data are reported as presumptive in instances where the results of chromatography were highly indicative but did not meet all requirements for positive identification and quantification.

TABLE 6.—*Maximum pesticide concentration found vs. permissible water supply criteria and reasonable stream allowance*
[blanks = criteria not given]
μG/LITER

PESTICIDE	PERMISSIBLE CRITERIA [1]	DESIRABLE CRITERIA [1]	MAXIMUM REASONABLE STREAM ALLOWANCE [1]	MAXIMUM CONCENTRATION FOUND
Dieldrin	17	absent	0.25	0.407
Endrin	1	do	0.1	0.133
DDT	42	do	0.5	0.316
DDE				0.050
DDD				0.840
Heptachlor	18	absent	1.0	0.048
Heptachlor epoxide	18	do	1.0	0.067
Aldrin	17	do	0.25	0.085
Lindane (BHC)	56	do	5.0	0.112
Chlordane	3	do	0.25	0.169
Methoxychlor	35	do	20.0	(3)
Toxaphene	5	do	2.5	(4)
Organophosphates plus Carbamates	100	do		0.380
Herbicides: 2,4-D plus 2,4,5-T and 2,4,5-TP	100	do		(3)
Phenols	1	do		(3)

[1] From the "Report of the Committee on Water Quality Criteria" (*6*)
[2] Suggested by Ettinger and Mount (*7*)
[3] Not determined
[4] Not detected

TABLE 7.—*Locations with high frequency of pesticide occurrence (at least one pesticide found in each survey)*

RIVER	LOCATION
Merrimack	Lowell, Mass.
Delaware	Trenton, N. J.
Delaware	Martins Creek, Pa.
Schuylkill	Philadelphia, Pa.
Potomac	Great Falls, Md.
Apalachicola	Chattahoochee, Fla.
Chattahoochee	Lanett, Ala.
Savannah	Port Wentworth, Ga.
Savannah	North Augusta, S. C.
Ohio	Evansville, Ind.
Ohio	Cincinnati, Ohio
Kansas	Lawrence, Kans.

TABLE 8.—*Locations with low frequency of pesticide occurrence*

RIVER	LOCATION	SURVEYS	OCCURRENCES
Connecticut	Wilder, Vt.	5	1
Raritan	Perth Amboy, N. J.	3	1
Lake Erie	Buffalo, N. Y.	5	1
St. Clair	Port Huron, Mich.	4	0
Rainy	International Falls, Minn.	3	0
Colorado	Parker Dam, Ariz.-Calif.	5	0
Colorado	Boulder City, Nev.	5	1
Truckee	Farad, Calif.-Nev.	5	0
Green	Dutch John, Utah	5	0
Snake	American Falls, Utah	3	1
Pend Oreille	Albeni Falls, Idaho	5	0
Klamath	Keno, Oreg.	5	1
Columbia	McNary Dam, Oreg.	5	0
Columbia	Pasco, Wash.	5	1
Columbia	Bonneville, Oreg.	3	1

Summary and Conclusions

The occurrences of chlorinated hydrocarbon pesticides continue to be widespread. However, after reaching a peak in 1966, the total number of occurrences throughout the Country dropped sharply in 1967 and 1968. This trend is consistent with production and usage reports of the U.S. Department of Agriculture (*8*) and the U.S. Department of the Interior (*9*) which show a trend toward decreased use of the persistent chlorinated hydrocarbon compounds and an increase in the use of organophosphorus and carbamate compounds. The absence of a corresponding increase in the occurrences of organophosphates may be due to their relatively rapid hydrolysis rate in water and the method of analysis which was not designed specifically for this class of compounds.

The data reported here and the grab sample and CAM sample data reported earlier (*1,2,3*) represent pesticide levels and trends in the major interstate waterways sampled. They do not, necessarily, reflect the conditions existing in all sub-basins or areas of heavy pesticide use, such as irrigation districts. For example, in extensive surveillance operations conducted by FWQA in the Lower Colorado River area during the summers of 1967 and 1968, the occurrences were frequent and the levels generally higher for both chlorinated and organophosphorus pesticides *unpublished data.*

Dieldrin continued to dominate the pesticide occurrences, although the total number of occurrences had dropped significantly.

BHC has been found consistently in the main stem of the Ohio River since 1966. The source or sources of this material have not yet been determined.

The pesticide concentrations found were 1/10 to 1/500 of the permissible levels for water supplies given in Water Quality Criteria (*6*). However, in some instances the concentrations found have exceeded the suggested maximum reasonable stream allowance (*7*), as well as the environmental limit recommended by the Committee on Water Quality Criteria (*6*).

Future surveys should be conducted to determine if the decreasing trend of chlorinated hydrocarbon pesticides occurrences is continuing. The methods of analysis should include procedures specifically designed to determine organophosphorus compounds. A greatly expanded sampling program would be necessary to determine seasonal variations in pesticide occurrences. This could best be done on a regional basis.

See Appendix for chemical names of compounds mentioned in this paper.

Acknowledgments

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LITERATURE CITED

(1) *Weaver, L., C. G. Gunnerson, A. W. Breidenbach, and J. J. Lichtenberg. 1965.* Chlorinated hydrocarbon pesticides in major U. S. river basins, a synoptic view. Public Health Rep. 80:481-493.

(2) *Breidenbach, A. W., C. G. Gunnerson, F. K. Kawahara, J. J. Lichtenberg, and R. S. Green. 1967.* Chlorinated hydrocarbon pesticides in major river basins 1957-65. Public Health Rep. 82:139-156.

(3) *Green, R. S., C. G. Gunnerson, and J. J. Lichtenberg. 1967.* Pesticides in our national waters. *In* agriculture and the quality of our environment, Amer. Ass. for the Advance of Sci. Publ. 85, p. 137-156.

(4) *Breidenbach, A. W., J. J. Lichtenberg, C. F. Henkey, D. J. Smith, J. W. Eichelberger, and H. Stierli. 1966.* The identification and measurement of chlorinated hydrocarbon pesticides in surface waters. U. S. Dep. of the Interior. Publ. WP-22.

(5) *U. S. Department of the Interior, Federal Water Pollution Control Administration. 1969.* FWPCA method for chlorinated hydrocarbon pesticides in water and wastewater.

(6) *U. S. Department of the Interior, Federal Water Pollution Control Administration. 1968.* Water quality criteria—report of the National Technical Advisory Committee to the Secretary of the Interior. p. 20, 37, and 116.

(7) *Ettinger, M. B. and D. I. Mount. 1967.* A wild fish should be safe to eat. Environ. Sci. Technol. 1:203-205.

(8) *U. S. Department of Agriculture, Agricultural Stabilization and Conservation Service. 1968.* The pesticide review—1968. ASCS-155. p. 38-43.

(9) *U. S. Department of the Interior, Federal Water Pollution Control Administration. Central Pacific Basins Project. 1967.* Effect of San Joaquin master drain on San Francisco Bay and Delta. p. 40-41.

APPENDIX

Chemical Names of Compounds Mentioned in This Issue

ALDRIN	Not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
ARAMITE®	2-(*p-tert*-butylphenoxy)-1-methylethel 2-chloroethyl sulfite
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
CARBOPHENOTHION	*S*-[(*p*-chlorophenylthio)methyl] *0,0*-diethyl phosphorodithioate
CHLORDANE	1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
DDD (TDE)	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; technical DDD contains some *o,p'*-isomer also.
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
DDT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; technical DDT consists of a mixture of the *p,p'*-isomer and the *o,p'*-isomer (in a ratio of about 3 or 4 to 1)
DIELDRIN	Not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethano=naphthalene
ENDOSULFAN	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide
ENDRIN	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
ETHION	*0,0,0',0'*-tetraethyl *S,S'*-methylene bisphosphorodithioate
FENTHION	*0,0*-dimethyl *0*-[4-(methylthio)-*m*-tolyl] phosphorothioate
HEPTACHLOR	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
HEPTACHLOR EPOXIDE	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
LINDANE	1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer
MALATHION	diethyl mercaptosuccinate, *S*-ester with *0,0*-dimethyl phosphorodithioate
METHOXYCHLOR	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane
METHYL PARATHION	*0,0*-dimethyl *0*-*p*-nitrophenyl phosphorothioate
PARATHION	*0,0*-diethyl *0*-*p*-nitrophenyl phosphorothioate
TOXAPHENE	chlorinated camphene containing 67% to 69% chlorine
2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4,5-TP	2-(2,4,5-trichlorophenoxy)propionic acid

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The Pesticide Monitoring Panel consists of representatives of the Agricultural Research Service, Consumer and Marketing Service, Federal Extension Service, Forest Service, Department of Defense, Fish and Wildlife Service, Geological Survey, Federal Water Quality Administration, Food and Drug Administration, Environmental Health Service, Department of Defense, National Science Foundation, and Tennessee Valley Authority.

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Pesticide monitoring activities of the Federal Government, particularly in those agencies represented on the Pesticide Monitoring Panel which participate in operation of the national pesticides monitoring network, are expected to be principal sources of data and interpretive articles. However, pertinent data *in summarized form*, together with interpretive discussions, are invited from both Federal and non-Federal sources, including those associated with State and community monitoring programs, universities, hospitals, and nongovernmental research institutions, both domestic and foreign. Results of studies in which monitoring data play a major or minor role or serve as support for research investigation also are welcome; however, the *Journal* is not intended as a primary medium for the publication of basic research. Manuscripts received for publication are reviewed by an Editorial Advisory Board established by the Monitoring Panel. Authors are given the benefit of review comments prior to publication.

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RESIDUES IN FOOD AND FEED

P. F. Corneliussen[1]

Pesticide Residues in Total Diet Samples (V)

ABSTRACT

Pesticide residue levels detected in ready-to-eat foods remained at relatively low levels during the fifth year of the Total Diet Study in its present form. Samples were collected from 30 markets in 24 different cities. Population of cities ranged from less than 50,000 to 1,000,000 or more. Averages and ranges of pesticides commonly found are reported for the period June 1968-April 1969 by region and food class. Pesticides found infrequently also are reported for this period by region and food class. Data showing loss of residues through cooking and processing of food are presented. Results of recovery studies with various classes of pesticides are also presented.

The study of pesticide residues in ready-to-eat foods, conducted by the Food and Drug Administration from June 1964 through April 1968 has been described in earlier reports (*2,3,4,9*). This report covers the period June 1968 through April 1969. Tabular data are included comparable to that reported for the previous years.

No changes were made in the sampling and compositing procedures described in the "Food and Feed Section" of the initial issue of the *Pesticides Monitoring Journal* (*6*). Earlier reports (*2,3,4,9*) discuss data collected from June 1964 through April 1965, June 1965 through April 1966, June 1966 through April 1967, and June 1967 through April 1968, respectively. On the basis of these data, average daily pesticide intake from the diet was calculated and reported elsewhere (*5,7*). Dietary intake for this reporting period will be discussed in a future publication. During the 4-year period, June 1964 through April 1968, the residues of most pesticide chemicals present in a high consumption well-balanced diet have been below and in most cases substantially below the limits established for acceptable daily intakes by the World Health Organization and the United Nations Committees (*8*) and in no case above the safe levels anticipated when legal tolerances were established for food.

Samples were collected from 30 markets in 24 different cities. Population of cities ranged from less than 50,000 to 1,000,000 or more, with average sampling from the 250,000-500,000 bracket. The samples were analyzed for the presence of chlorinated hydrocarbons, organic phosphates, chlorophenoxy acids, bromides, arsenic, amitrole, carbaryl (Sevin®), cadmium, and dithiocarbamate residues.

Quantitative values reported for both chlorinated and organic phosphorus compounds were obtained by either electron capture or thermionic gas-liquid chromatography. Confirmation was made by thin layer chromatography and/or microcoulometric gas-liquid chromatography. This procedure determines chlorinated compounds at a sensitivity of 0.003 ppm (heptachlor epoxide) and organic phosphorus compounds at 0.05 ppm (parathion). The analytical sensitivity for both of

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these classes of compounds varies with the individual compound being measured. Each composite was also tested for chlorophenoxy acids and esters at a sensitivity of 0.02 ppm; for amitrole at a sensitivity of 0.05 ppm; for dithiocarbamates, calculated as zineb, at a sensitivity of 0.2 ppm; for carbaryl at a sensitivity of 0.2 ppm; for bromides at a sensitivity of 0.5 ppm; and for arsenic as As_2O_3 at a sensitivity of 0.1 ppm.

Methods used in these studies are described in the FDA *Pesticide Analytical Manual*, Vol. I and II (*1*). No correction was made for recovery studies which were performed continuously. Some finite residues are reported which are below the stated sensitivity levels. For example, values as low as 0.001 ppm for chlorinated pesticides are reported although the quantitative sensitivity level is 0.003 ppm. Such values are not quantitative but are more useful than a "trace" reporting for estimating dietary intake.

Each composite was examined for cadmium during this period. Although cadmium residues do not result from pesticide usage, increased awareness of this potential hazard warranted inclusion in this study. All reported results were obtained through the use of either polarography or atomic absorption. The procedure is sensitive to 0.01 ppm cadmium (*Gajan, R. J., FDA DHEW personal communication, 1967*).

The vegetable and fruit composites from five market baskets in each of the geographical areas were examined for residues of chlorinated hydrocarbon, organophosphorus, and chlorophenoxy acid pesticides both before and after preparation of the food items by dieticians. Table 3 presents comparative data for residues which were found six or more times in a given food group through the current year. The data indicate a loss in residue when food is prepared for eating through peeling, stripping outer leaves, cooking, etc. In most cases, the water used to cook the fruit or vegetables was discarded. This study did not determine the relative significance of any of the individual processing operations in reducing the residue content of table-ready food. This report, except for Table 3, presents data only on prepared composites in accordance with past reports.

Results

The total of 30 market baskets examined (360 food class composites) was unchanged from previous reporting periods. A total of 1336 residues were detected during the current reporting period. While this appears to be a marked increase over the 1088 reported for the previous period, the increase in the total number of residues was due to an increase in the number of cadmium residues reported. Discounting cadmium reportings for both periods, 1092 residues were detected during the current period, and 1045 were detected in the previous period.

The increase in cadmium reportings this period is due to the use of either atomic absorption or polarography for analysis without the requirement of the previous period for confirmation by both techniques. Collaborative work and continuous recovery studies have not shown significant differences between these techniques down to the 0.01 ppm level for cadmium.

There has been no significant change in the levels, frequency, or types of residues from those in the past. Thirty-four different residues were found in the samples during the current period. The frequency of the residues is given in Table 1. The most common residues, maximum levels of those residues, and residues reported less frequently are discussed below for each class.

DAIRY PRODUCTS: Ten chlorinated organic pesticides were found in varying combinations in 26 of the 30 composites. The most common, and their maximum values on a fat basis were: DDE (0.280 ppm); DDT (0.129 ppm); dieldrin (0.073 ppm); heptachlor epoxide (0.045 ppm); TDE (0.088 ppm); and BHC (0.03 ppm). Also present were PCP, diazinon, lindane, MCP, and arsenic (As_2O_3). Bromides were found (0.5 ppm to 13 ppm) in 21 of the 30 composites. Cadmium was detected in 10 composites (0.09 ppm maximum).

MEAT, FISH, AND POULTRY: Thirteen chlorinated pesticides were found in 29 of the 30 composites in varying combinations. Most common were DDT, DDE, TDE, dieldrin, BHC, heptachlor epoxide, and lindane with maximum values of 0.73 ppm, 0.470 ppm, 0.25 ppm, 0.170 ppm, 0.130 ppm, 0.082 ppm, and 0.03 ppm respectively, all on a fat basis. Also present were heptachlor, PCP, aldrin, diazinon, endrin, toxaphene, MCP, and malathion. Arsenic (As_2O_3) was detected 15 times (0.1 ppm to 1.0 ppm). Bromides were detected in 18 of the 30 composites (1.0 to 28 ppm). Cadmium was detected in 21 composites (maximum of 0.06 ppm).

GRAIN AND CEREAL PRODUCTS: A total of 13 chlorinated organic pesticides were found in 26 of the 30 composites in varying combinations. Most common were DDT, lindane, dieldrin, DDE, and TDE, with respective maximum values of 0.024 ppm, 0.009 ppm, 0.069 ppm, 0.004 ppm, and 0.015 ppm. Thirteen composites contained malathion, with a maximum value of 0.073 ppm. Also present were aldrin, diazinon, heptachlor epoxide, Perthane®, PCP, methyl parathion, TCNB, heptachlor, methoxychlor, ronnel, and BHC. Arsenic (As_2O_3) was found in seven composites (0.1 ppm to 0.2 ppm). Bromides were detected in 27 of the 30 composites (1.0 ppm to 47 ppm). Cadmium was detected in 27 composites (maximum 0.08 ppm).

POTATOES: Ten chlorinated organic pesticides were found in 20 of the 30 composites. The most common pesticides found were dieldrin, DDT, DDE, and endrin, with maximum respective values of 0.006 ppm, 0.02 ppm, 0.004 ppm, and 0.009 ppm. Also detected were heptachlor epoxide, chlordane, lindane, TCNB, endosulfan sulfate, diazinon, and TDE. Arsenic (As_2O_3) was detected in three composites, each at 0.1 ppm. Bromides were found in 21 of 30 composites (0.5 ppm to 33 ppm). Cadmium was found in 26 composites (0.02 ppm to 0.13 ppm).

LEAFY VEGETABLES: A total of 14 chlorinated organic pesticides were found in 25 of 30 composites. Most commonly found were DDT, DDE, TDE, and endosulfan with maximum respective levels of 0.044 ppm, 0.032 ppm, 0.012 ppm, and 0.042 ppm. Five composites contained parathion with a maximum value of 0.09 ppm. Also detected were dieldrin, lindane, BHC, Dacthal®, heptachlor epoxide, Perthane®, diazinon, methyl parathion, endrin, chlorbenside, toxaphene, disulfoton, and 2,4-D. Arsenic (As_2O_3) was detected four times each at 0.1 ppm. Bromides were found 21 times (1.0 ppm to 15 ppm). Cadmium was found 27 times (0.01 ppm to 0.23 ppm).

LEGUME VEGETABLES: A total of eight chlorinated organic pesticides were found in 15 composites. DDT, DDE, and TDE were found most frequently, with maximum values of 0.086 ppm, 0.006 ppm, and 0.022 ppm, respectively, one or more of these three was found in 15 of the 30 composites. Also detected were dieldrin, lindane, parathion, carbaryl, toxaphene, Dacthal®, and TCNB. Bromides were detected in 17 composites (1.0 to 32 ppm). Arsenic (As_2O_3) was detected three times, each at 0.1 ppm. Cadmium was detected 16 times (0.01 ppm to 0.03 ppm).

ROOT VEGETABLES: Seven chlorinated organic pesticides were found in 16 of 30 composites. DDE was found 13 times at a maximum of 0.027 ppm, and DDT was found 12 times at a maximum of 0.026 ppm. Also found were TDE, dieldrin, aldrin, heptachlor, and toxaphene. Bromides were detected in 18 of 30 composites (0.5 ppm to 12 ppm). Arsenic (As_2O_3) was detected three times, each at 0.1 ppm, and cadmium 24 times (0.01 ppm to 0.08 ppm).

GARDEN FRUITS: A total of 11 chlorinated organic pesticides were detected in 26 of 30 composites. Most common were DDT, DDE, TDE, dieldrin, lindane, and toxaphene at respective maximum levels of 0.140 ppm, 0.006 ppm, 0.100 ppm, 0.028 ppm, 0.004 ppm, and 0.230 ppm. Five composites contained parathion at a maximum level of 0.033 ppm. Also found were endosulfan, heptachlor epoxide, PCP, malathion, endrin, diazinon, and Dacthal®. Bromides were found in 17 of 30 composites (1.0 ppm to 44 ppm). Arsenic (As_2O_3) was found four times, each at 0.1 ppm and cadmium 25 times (0.01 ppm to 0.07 ppm).

FRUITS: Nine chlorinated organic pesticides were found in 25 of 30 composites. DDT, DDE, TDE, and dicofol were found most frequently at maximum respective levels of 0.072 ppm, 0.005 ppm, 0.033 ppm, and 0.19 ppm. Ethion was found in 6 of 30 composites at a maximum 0.265 ppm level. Also found were heptachlor epoxide, endosulfan, lindane, dieldrin, carbaryl, ovex, and malathion. Bromides were found in 17 of 30 composites (0.5 to 84 ppm). Arsenic (As_2O_3) was found 5 times, each at 0.1 ppm and cadmium 15 times (0.01 ppm to 0.38 ppm).

OILS, FATS, AND SHORTENING: A total of 8 chlorinated organic pesticides were found in 14 of 30 composites. Most common were DDT, DDE, TDE, BHC, and dieldrin at maximum respective levels of 0.018 ppm, 0.031 ppm, 0.022 ppm, 0.041 ppm, and 0.025 ppm. Also found were lindane, heptachlor epoxide, diazinon, PCP, and parathion. Five composites contained malathion; one of these levels was unusually high, at 2.99 ppm, but the laboratory could not determine the source of the residue because of an inadequate supply of the individual food components. Bromides were found in 22 of 30 composites (1.0 ppm to 70 ppm). Arsenic (As_2O_3) was found twice, each at 0.1 ppm. Cadmium was found in 27 composites (0.01 ppm to 0.13 ppm).

SUGARS AND ADJUNCTS: A total of 7 chlorinated organic pesticides were found in 10 of 30 composites. DDT was found in 8 of 30 composites (maximum 0.141 ppm), DDE in 6 composites (maximum 0.002 ppm), and lindane in 5 composites (maximum 0.011 ppm). Also detected were TDE, dieldrin, PCP, and MCP. Bromides were found in 19 of 30 composites (1.0 ppm to 58 ppm). Arsenic (As_2O_3) was found in five composites, each at 0.1 ppm. Cadmium was found in 18 of 30 composites (0.01 ppm to 0.07 ppm).

BEVERAGES: DDT was found once at a trace level. Bromides were found in 14 of 30 composites (1.0 ppm to 8.0 ppm). Arsenic (As_2O_3) was found in three composites, each at 0.1 ppm, and cadmium was found in eight composites (0.01 ppm to 0.04 ppm).

Bromide reportings include naturally occurring bromides as well as residues from pesticide treatment. Of the 360 composites, 232 contained bromides above the sensitivity level of 0.5 ppm. This incidence is 64.4% as compared with 76.9%, 83.1%, and 76.8% for 1967-1968, 1966-1967, and 1965-1966, respectively. A total of 8.6% of

the residues exceeded 25 ppm compared with 5.8%, 4.2% and 3.8% for the three respective earlier periods.

The data obtained for the fifth year of the study are reported in detail in Table 2a, where findings are arranged by food class and region. Similar information is given in Table 2b for pesticides found infrequently (less than five detections per commodity class). The data are reported in the same format used for earlier periods (*2,4,9*) for comparison. Trace amounts, <0.001 ppm, are not included in the averages. Where no average value is given, the results on the individual composites are shown.

In these tabulations, as in the earlier reports, the bromide and arsenic values are reported on an "as is" basis for three food classes: Dairy Products (I); Meat, Fish, and Poultry (III); and Oils, Fats, and Shortening (X), even though the earlier tabulations (*4*) indicated a "fat basis." Cadmium results are also reported on an "as is" basis in all cases.

Discussion

The presence of chlorinated organic residues was confirmed in 233 of the 360 composites examined (64.7%) for this class of chemicals. Corresponding percentages for previous years were 65.6% for 1967-1968, 62.3% for 1966-1967, and 53.8% for 1965-1966. Organic phosphorus compounds were found in 59 composites. The three previous reporting periods showed 26, 25, and 27 detections of organic phosphorus residues, respectively.

Chlorophenoxy acids and PCP were found 14 times during the current year, with 10 of the 14 being PCP; 7 chlorophenoxy acid residues were found in 1967-1968, 8 were found in 1966-1967, and 13 were found in 1965-1966.

Carbaryl was detected in three composites. No carbaryl was found in the previous period while there were four occurrences in the 1966-1967 period.

No dithiocarbamates or amitrole was detected during the current period. Unprepared fruits and vegetables were examined for dithiocarbamates before compositing in order to prevent decomposition by hydrolysis. Previous report periods showed zero to four dithio-carbamate findings (calculated as zineb). Amitrole has never been found in any total diet composites.

Recovery studies were conducted through the entire year with all classes of pesticides in various food groups. Table 4 gives recovery data for seven of the more commonly occurring organochlorine pesticides, as well as data for representative pesticides in the other residue categories.

It should be pointed out that each recovery experiment consisted of a single determination for the blank and a single determination for the spiked sample. Generally, these determinations were made simultaneously and often the spiking level was much less than the blank level. In other cases, not enough recovery experiments were performed to be statistically significant. Such recovery data are not reported. Many recovery experiments were performed for each class of compounds, but Table 4 presents only those which fit these criteria.

Based on the recovery data it is apparent that residue reportings may vary considerably from the "true" value; however, results thus far are useful in appraising the national residue picture. At low fortification levels, recoveries from zero to 200% may be encountered. Accuracy is expected to increase with higher fortification levels.

See Appendix for chemical names of compounds not included in Table 1.

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LITERATURE CITED

(*1*) *Barry, H. C., J. G. Hundley, L. Y. Johnson. 1963. (Revised 1964, 1965, 1968).* Pesticide Analytical Manual, Vol. I and II, Food and Drug Admin., U. S. Dep. of Health, Educ., and Welfare.

(*2*) *Corneliussen, P. E. 1969.* Pesticide residues in total diet samples (IV) Pesticides Monit. J. 2(4):140-152.

(*3*) *Duggan, R. E., H. C. Barry, and L. Y. Johnson. 1966.* Pesticide residues in total diet samples. Science 151: 101-104.

(*4*) *Duggan, R. E., H. C. Barry, and L. Y. Johnson. 1967.* Pesticide residues in total diet samples (II). Pesticides Monit. J. 1(2):2-12.

(*5*) *Duggan, R. E. and G. Q. Lipscomb. 1969.* Dietary intake of pesticide chemicals in the United States (II), June 1966-April 1968. Pesticides Monit. J. 2(4):153-162.

(*6*) *Duggan, R. E. and F. J. McFarland. 1967.* Assessments include raw food and feed commodities, market basket items prepared for consumption, meat samples taken at slaughter. Pesticides Monit. J. 1(1):1-5.

(*7*) *Duggan, R. E. and J. R. Weatherwax. 1967.* Dietary intake of pesticide chemicals. Science 157:1006-1010.

(*8*) *Food and Agriculture Organization and World Health Organization. 1967.* Evaluation of some pesticide residues in food. Report of a joint meeting of the FAO Working Party and the WHO Expert Committee on Pesticide Residues, 1966. PL:CP/15; WHO/Food Add./67.32.

(*9*) *Martin, R. J. and R. E. Duggan. 1968.* Pesticide residues in total diet samples (III). Pesticides Monit. J. 1(4):11-20.

TABLE 1.—*Number of composites where pesticide residues were found and ranges in the amounts (June 1968-April 1969)*

Pesticide	No. of Composites with Residues	No. of Positive Composites with Residues below Sensitivity Level [1]	Ranges At and Above Sensitivity Level (PPM)
CADMIUM	244	0	0.01-0.38
BROMIDES	232	0	0.5-84
DDT 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane	176	13	0.003-0.73
DDE 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene	142	49	0.003-0.47
TDE 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane	101	24	0.003-0.25
DIELDRIN not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene	91	24	0.003-0.17
ARSENIC (As_2O_3)	57	0	0.1-1.0
LINDANE 1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer	48	30	0.003-0.03
HEPTACHLOR EPOXIDE 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan	44	8	0.003-0.082
BHC 1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers	38	7	0.003-0.13
MALATHION diethyl mercaptosuccinate, *S*-ester with *0,0*-dimethyl phosphorodithioate	21	13	0.054-2.99
ENDOSULFAN 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide	19	1	0.003-0.033
DIAZINON *0,0*-diethyl *0*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate	14	14	
DICOFOL (KELTHANE®) 4,4'-dichloro-a-(trichloromethyl) benzhydrol	13	0	0.004-0.19
TOXAPHENE chlorinated camphene containing 67% to 69% chlorine	13	1	0.022-0.33
ENDRIN 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-*endo*-5,8-dimethanonaphthalene	12	5	0.003-0.019
PARATHION *0,0*-diethyl *0*-*p*-nitrophenyl phosphorothioate	12	12	
PCP pentachlorophenol	10	5	0.02-0.04
ETHION *0,0,0',0'*-tetraethyl *S,S'*-methylene bisphosphorodithioate	6	5	0.265
DACTHAL® 2,3,5,6-tetrachloroterephthalic acid dimethyl ester	6	5	0.032
HEPTACHLOR 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	6	2	0.003-0.014
ALDRIN not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene	5	4	0.004
METHYL PARATHION *0,0*-dimethyl *0*-*p*-nitrophenyl phosphorothioate	5	5	
PERTHANE® 1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane	4	0	0.01-0.528
TCNB 1,2,4,5-tetrachloro-3-nitrobenzene	3	2	0.007
MCP 4-chloro-2-methyl-phenoxyacetic acid	3	1	0.04-0.047
CARBARYL 1-naphthyl methylcarbamate	3	2	0.3
CHLORDANE 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	2	0	0.026-0.043
CHLORBENSIDE *p*-chlorobenzyl *p*-chlorophenyl sulfide	1	0	0.029
METHOXYCHLOR 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl) ethane	1	1	
RONNEL *0,0*-dimethyl *0*-2,4,5-trichlorophenyl phosphorothioate	1	1	
2,4-D 2,4-dichlorophenoxyacetic acid	1	1	
DISULFOTON *0,0*-dimethyl *S*-2-(ethylthio) ethyl phosphorodithioate	1	1	
OVEX *p*-chlorophenyl *p*-chlorobenzenesulfonate	1	0	0.003

[1] Pesticide chemicals capable of being detected by the specified analytical methodology may be confirmed qualitatively but are not quantifiable when they are present at concentrations below the sensitivity level.

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1968-April 1969)*

[T = Trace <0.001 PPM]

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
	I. Dairy Products (8-13% Fat) [1] Residues In Parts Per Million—Fat Basis				
DDT					
Average	0.027	0.036	0.030	0.004	0.019
Positive Composites					
Number	3	4	4	4	5
Range	0.014-0.129	0.015-0.113	0.03-0.074	0.004-0.007	0.014-0.034
DDE					
Average	0.014	0.018	0.190	0.005	0.015
Positive Composites					
Number	3	5	5	4	6
Range	0.021-0.037	0.015-0.034	0.14-0.280	0.005-0.010	0.007-0.020
TDE					
Average	0.020		0.019	0.004	0.006
Positive Composites					
Number	3	1	4	4	4
Range	0.01-0.088	T	0.01-0.042	0.004-0.007	0.008-0.012
DIELDRIN					
Average		0.052	0.021		0.020
Positive Composites					
Number	0	6	4	1	6
Range		0.028-0.073	0.019-0.059	0.004	0.010-0.039
HEPTACHLOR EPOXIDE					
Average	0.011	0.034		0.002	0.013
Positive Composites					
Number	3	6	0	2	6
Range	0.015-0.030	0.025-0.045		0.004-0.005	0.007-0.024
BHC					
Average		0.010	0.014		0.007
Positive Composites					
Number	1	4	4	0	5
Range	0.017	0.008-0.020	0.01-0.03		0.005-0.013
TOTAL BROMIDES					
Average	1.0	3.0	2.0	1.5	3.5
Positive Composites					
Number	5	3	4	5	4
Range	1.0-2.0	1.0-13	2.0-3.0	0.5-5.0	3.0-7.0
CADMIUM					
Average	0.02		0.01	<0.01	<0.01
Positive Composites					
Number	3	0	3	2	2
Range	0.01-0.09		0.01-0.02	0.01	0.01-0.02
	II. Meat, Fish, and Poultry (17-23% Fat) [1] Residues In Parts Per Million—Fat Basis				
DDT					
Average	0.287	0.055	0.092	0.020	0.052
Positive Composites					
Number	5	6	6	4	6
Range	0.145-0.73	0.029-0.083	0.016-0.147	0.014-0.055	0.030-0.076
DDE					
Average	0.171	0.039	0.240	0.019	0.033
Positive Composites					
Number	5	6	6	4	6
Range	0.099-0.337	0.016-0.088	0.020-0.470	0.014-0.049	0.018-0.049
TDE					
Average	0.076	0.045	0.043	0.015	0.034
Positive Composites					
Number	3	4	6	2	6
Range	0.076-0.22	T-0.25	0.011-0.079	0.034-0.056	0.010-0.076
DIELDRIN					
Average	0.030	0.026	0.030		0.015
Positive Composites					
Number	2	5	6	1	6
Range	0.129-0.170	0.011-0.078	0.006-0.077	0.005	0.009-0.029

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1968-April 1969)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
II. Meat, Fish, and Poultry (17-23% Fat) [1]—Continued Residues In Parts Per Million—Fat Basis					
HEPTACHLOR EPOXIDE					
Average	0.024	0.017			0.013
Positive Composites					
Number	3	6	0	0	6
Range	0.027-0.082	0.008-0.036			0.005-0.021
BHC					
Average		0.028	0.008		0.003
Positive Composites					
Number	1	6	4	0	5
Range	0.053	T-0.130	T-0.02		T-0.009
LINDANE					
Average	0.003		0.007		0.004
Positive Composites					
Number	2	0	2	0	4
Range	T-0.016		0.014-0.03		0.003-0.014
ARSENIC (As_2O_3)					
Average	0.2		0.1	0.4	<0.1
Positive Composites					
Number	4	0	4	4	3
Range	0.2-0.4		0.1-0.4	0.2-1.0	0.1
TOTAL BROMIDES					
Average	6.0	5.5	2.0	3.5	5.5
Positive Composites					
Number	5	3	2	3	5
Range	2.0-17	1.0-28	4.0-7.0	4.0-9.0	2.0-14
CADMIUM					
Average	0.02	0.01	0.01	0.02	0.01
Positive Composites					
Number	4	4	4	5	4
Range	0.01-0.06	0.01-0.02	0.01-0.03	0.02-0.04	0.01
III. Grain and Cereal [1] Residues In Parts Per Million					
DDT					
Average	0.009	0.004	0.006	0.002	0.005
Positive Composites					
Number	5	5	4	3	5
Range	0.007-0.024	T-0.010	0.003-0.016	0.003-0.005	0.002-0.011
DDE					
Average	0.001	<0.001			0.001
Positive Composites					
Number	3	4	1	0	5
Range	T-0.004	T-0.002	0.001		T-0.003
TDE					
Average	0.003		<0.001		0.001
Positive Composites					
Number	2	1	2	0	3
Range	0.003-0.015	T	0.001-0.002		0.001-0.005
DIELDRIN					
Average	0.012		0.003		0.004
Positive Composites					
Number	3	1	3	1	5
Range	T-0.069	T	0.005-0.006	0.02	0.003-0.006
LINDANE					
Average	0.003	0.001	0.001	0.001	0.002
Positive Composites					
Number	3	4	3	2	5
Range	T-0.009	T-0.002	0.002	0.002-0.003	0.001-0.007
MALATHION					
Average	0.013	0.041	0.006		0.026
Positive Composites					
Number	2	4	3	0	4
Range	0.036-0.041	0.043-0.073	0.005-0.02		0.014-0.060

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1968-April 1969)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
		III. Grain and Cereal [1]—Continued Residues In Parts Per Million			
ARSENIC (As_2O_3)					
Average	0.1				<0.1
Positive Composites					
Number	4	0	1	0	2
Range	0.1		0.1		0.1-0.2
TOTAL BROMIDES					
Average	12	30	6.0	25	14
Positive Composites					
Number	6	6	4	6	5
Range	7.0-17	13-47	4.0-12	15-36	1.0-43
CADMIUM					
Average	0.04	0.03	0.04	0.03	0.02
Positive Composites					
Number	5	6	5	6	5
Range	0.02-0.08	0.02-0.04	0.03-0.06	0.02-0.05	0.02-0.04
		IV. Potatoes [1] Residues In Parts Per Million			
DDT					
Average	0.002			0.006	
Positive Composites					
Number	5	1	1	2	1
Range	T-0.006	T	0.001	0.019-0.02	T
DDE					
Average	<0.001		<0.001		
Positive Composites					
Number	2	1	2	1	0
Range	T-0.003	T	T-0.001	0.004	
DIELDRIN					
Average	0.001	0.002	0.002		
Positive Composites					
Number	2	3	5	0	1
Range	0.001-0.004	0.004-0.006	0.001-0.005		T
ENDRIN					
Average			0.004		0.004
Positive Composites					
Number	1	0	5	0	5
Range	T		0.002-0.009		0.002-0.009
TOTAL BROMIDES					
Average	3.5	8.5	3.5	10	3.5
Positive Composites					
Number	5	4	4	4	4
Range	0.5-8	3.0-33	3.0-9.0	4.0-32	3.0-9.0
CADMIUM					
Average	0.04	0.04	0.04	0.05	0.04
Positive Composites					
Number	6	5	5	5	5
Range	0.02-0.09	0.03-0.07	0.03-0.06	0.02-0.13	0.03-0.06
		V. Leafy Vegetables [1] Residues In Parts Per Million			
DDT					
Average	0.008	0.005	0.022	0.003	0.014
Positive Composites					
Number	5	3	5	3	5
Range	T-0.022	0.003-0.023	0.009-0.044	0.003-0.010	0.007-0.031
DDE					
Average			0.005		0.008
Positive Composites					
Number	1	1	3	1	3
Range	T	0.007	0.004-0.020	0.020	0.007-0.032

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1968-April 1969)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
		V. Leafy Vegetables [1]—Continued Residues In Parts Per Million			
ENDOSULFAN (TOTAL)					
Average	0.010				0.016
Positive Composites					
Number	2	1	0	1	4
Range	0.018-0.042	0.003		T	0.014-0.033
TDE					
Average			0.002		0.001
Positive Composites					
Number	0	0	3	0	2
Range			T-0.012		0.003-0.004
PARATHION					
Average			0.002		
Positive Composites					
Number	0	1	2	1	1
Range		0.007	0.003-0.007	0.09	0.002
TOTAL BROMIDES					
Average	3.0	5.0	1.5	6.0	2.5
Positive Composites					
Number	5	3	4	6	3
Range	1.0-9.0	2.0-15	2.0-3.0	1.0-10	2.0-7.0
CADMIUM					
Average	0.06	0.08	0.04	0.04	0.04
Positive Composites					
Number	6	6	6	5	4
Range	0.02-0.10	0.01-0.23	0.02-0.06	0.02-0.12	0.03-0.10
		VI. Legume Vegetables [1] Residues In Parts Per Million			
DDT					
Average	0.001	T	0.024		0.015
Positive Composites					
Number	2	2	3	0	2
Range	0.002-0.004	T	0.020-0.071		0.005-0.086
DDE					
Average	T		0.002		0.001
Positive Composites					
Number	3	1	2	0	3
Range	T	T	0.005-0.006		T-0.005
DE					
Average			0.003		0.003
Positive Composites					
Number	1	0	2	0	4
Range	0.022		0.008-0.010		T-0.011
TOTAL BROMIDES					
Average	2.5	6.0	1.0	2.0	5.5
Positive Composites					
Number	5	2	2	3	5
Range	1.0-7.0	5.0-32	1.0-6.0	3.0-5.0	2.0-10
CADMIUM					
Average	0.01	<0.01	0.01	0.01	
Positive Composites					
Number	5	2	4	4	1
Range	0.01-0.03	0.01-0.02	0.01	0.01-0.02	0.01
		VII. Root Vegetables [1] Residues In Parts Per Million			
DDT					
Average	0.001	0.004	0.002		0.007
Positive Composites					
Number	2	3	3	1	3
Range	T-0.005	0.003-0.014	T-0.009	0.003	0.004-0.026

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1968-April 1969)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
	VII. Root Vegetables [1]—Continued Residues In Parts Per Million				
DDE					
Average	0.001	0.001	0.006		0.007
Positive Composites					
Number	1	3	5	1	3
Range	0.008	T-0.008	T-0.015	0.001	0.001-0.027
TOTAL BROMIDES					
Average	3.0	2.0	1.5	4.0	3.0
Positive Composites					
Number	4	3	3	4	4
Range	1.0-10	0.5-12	2.0-5.0	3.0-12	1.0-7.0
CADMIUM					
Average	0.03	0.02	0.03	0.02	0.01
Positive Composites					
Number	5	4	5	5	5
Range	0.02-0.05	0.02-0.04	0.01-0.08	0.01-0.06	0.01-0.03
	VIII. Garden Fruits [1] Residues In Parts Per Million				
DDT					
Average	0.003	0.032	0.048	0.020	0.037
Positive Composites					
Number	2	6	6	2	4
Range	0.004-0.015	0.008-0.066	0.007-0.140	0.021-0.102	0.013-0.100
DDE					
Average		0.002	0.002		0.002
Positive Composites					
Number	1	3	5	0	4
Range	T	0.002-0.005	0.001-0.006		0.001-0.005
TDE					
Average		0.026	0.004	0.006	0.012
Positive Composites					
Number	1	4	3	3	6
Range	0.010	0.016-0.100	0.003-0.017	0.004-0.025	0.005-0.016
DIELDRIN					
Average	0.006	0.001	0.002		
Positive Composites					
Number	2	3	4	1	1
Range	0.006-0.028	T-0.005	0.001-0.003	0.002	0.006
LINDANE					
Average			0.001		T
Positive Composites					
Number	1	1	2	0	2
Range	T	0.003	0.002-0.004		T
TOXAPHENE					
Average			0.056		0.038
Positive Composites					
Number	0	0	4	0	2
Range			0.03-0.230		0.077-0.150
PARATHION					
Average			<0.001		0.010
Positive Composites					
Number	0	0	2	0	3
Range			T-0.002		0.007-0.033
TOTAL BROMIDES					
Average	2.0	8.5	1.0	6.0	2.5
Positive Composites					
Number	4	3	3	3	4
Range	1.0-6.0	2.0-44	1.0-4.0	1.0-19	2.0-5.0
CADMIUM					
Average	0.03	0.01	0.02	0.02	0.01
Positive Composites					
Number	6	3	5	6	5
Range	0.01-0.07	0.02-0.03	0.02-0.04	0.01-0.04	0.01-0.02

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1968-April 1969)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
IX. Fruits [1] Residues In Parts Per Million					
DDT					
Average	0.010	0.010	0.014		0.003
Positive Composites					
Number	5	4	4	1	4
Range	0.006-0.021	0.003-0.032	0.003-0.072	0.004	0.003-0.005
DDE					
Average	0.001	0.001	0.002		<0.001
Positive Composites					
Number	4	2	4	1	3
Range	T-0.004	0.003	0.001-0.005	0.003	0.001
TDE					
Average	0.003	0.007	0.007	0.004	0.002
Positive Composites					
Number	2	2	2	2	3
Range	0.007-0.011	0.009-0.033	0.009-0.033	0.001-0.025	0.001-0.005
DICOFOL					
Average		0.052	0.083		0.005
Positive Composites					
Number	1	3	6	0	3
Range	0.058	0 019-0.148	0.007-0.19		0.004-0.017
ETHION					
Average		0.007			0.047
Positive Composites					
Number	1	2	1	0	2
Range	0.006	0.010-0.033	0.008		0.017-0.265
TOTAL BROMIDES					
Average	2.0	0.5	2.5	19	2.5
Positive Composites					
Number	5	2	3	4	3
Range	0.5-5.0	1.0-3.0	2.0-10	0.5-84	2.0-10
ARSENIC (As_2O_3)					
Average	<0.1		<0.1		
Positive Composites					
Number	2	0	2	0	1
Range	0.1		0.1		0.1
CADMIUM					
Average	0.01	<0.01	0.02	0.01	0.06
Positive Composites					
Number	4	3	2	4	2
Range	0.01-0.02	0.01	0.01-0.1	0.01-0.02	0.01-0.38
X. Oils, Fats, and Shortening (83-88% Fat) [1] Residues In Parts Per Million—Fat Basis					
DDT					
Average		0.004	0.001		0.005
Positive Composites					
Number	0	3	2	1	2
Range		0.007-0.009	T-0.004	0.003	0.011-0.018
DDE					
Average		0.003			0.004
Positive Composites					
Number	1	3	1	0	4
Range	0.031	0.002-0.008	0.005		0.004-0.008
TDE					
Average		0.001	0.002		0.007
Positive Composites					
Number	1	3	2	0	4
Range	T	T-0.004	T-0.010		0.004-0.022

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1968-April 1969)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
	X. Oils, Fats, and Shortening (83-88% Fat) [1]—Continued Residues In Parts Per Million—Fat Basis				
DIELDRIN					
Average	0.007	T			
Positive Composites					
Number	2	2	1	0	1
Range	0.016-0.025	T-0.002	0.008		0.005
BHC					
Average		0.003	0.004		
Positive Composites					
Number	0	2	2	0	1
Range		T-0.017	0.004-0.02		0.041
MALATHION					
Average	0.507				0.008
Positive Composites					
Number	2	0	0	0	3
Range	0.054-2.99				0.002-0.025
TOTAL BROMIDES					
Average	5.5	8.5	4.0	14	15
Positive Composites					
Number	6	5	3	3	5
Range	1.0-9.0	4.0-19	4.0-11	4.0-70	3.0-36
CADMIUM					
Average	0.02	0.02	0.04	0.02	0.04
Positive Composites					
Number	6	5	5	5	6
Range	0.02-0.03	0.02-0.03	0.02-0.11	0.02-0.04	0.01-0.13
	XI. Sugars and Adjuncts [1] Residues In Parts Per Million				
DDT					
Average	0.024		0.001		0.002
Positive Composites					
Number	2	0	2	1	3
Range	T-0.141		0.003	0.004	0.002-0.004
DDE					
Average	T		0.001		<0.001
Positive Composites					
Number	2	0	2	0	2
Range	T		0.002		0.001-0.002
LINDANE					
Average	0.002				<0.001
Positive Composites					
Number	2	0	1	0	2
Range	T-0.011		0.002		0.001
ARSENIC (As_2O_3)					
Average	<0.1				
Positive Composites					
Number	3	0	0	1	1
Range	0.1			0.1	0.1
TOTAL BROMIDES					
Average	3.5	2.5	3.0	4.5	23
Positive Composites					
Number	4	3	3	4	5
Range	3.0-8.0	3.0-9.0	3.0-8.0	1.0-18	4.0-58
CADMIUM					
Average	0.01	0.01	0.02	0.01	0.01
Positive Composites					
Number	4	3	5	3	3
Range	0.01-0.03	0.02-0.03	0.01-0.07	0.01-0.03	0.01-0.02

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1968-April 1969)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
XII. Beverages [1] Residues In Parts Per Million					
TOTAL BROMIDES					
Average	0.5	2.0	1.0	2.0	1.5
Positive Composites					
Number	2	3	2	3	4
Range	2.0	1.0-8.0	2.0-4.0	2.0-5.0	1.0-6.0
CADMIUM					
Average	<0.01			0.01	
Positive Composites					
Number	2	1	1	3	1
Range	0.01	0.01	0.01	0.01-0.04	0.01

Six composite samples examined at each of five sampling sites: Boston, Kansas City, Los Angeles, Baltimore, and Minneapolis.
NOTE: Bromide, cadmium, and arsenic values are reported on an "as is" basis (no drying or isolation of fat) for Dairy Products; Meat, Fish, and Poultry; and Oils, Fats, and Shortening.

TABLE 2b.—*Pesticides found infrequently—by food class and region (June 1968-April 1969)*

Pesticide	District	No. Composites	Amount
I. (a) Dairy Products (8-13% Fat) [1] Residues In Parts Per Million—Fat Basis			
PCP	Boston	2	0.005, 0.013
	Los Angeles	1	0.010
Lindane	Boston	1	T
	Los Angeles	1	0.02
Arsenic (As_2O_3)	Boston	1	0.1
	Minneapolis	1	0.1
Heptachlor	Minneapolis	1	0.008
Diazinon	Minneapolis	1	0.008
MCP	Boston	1	0.047
II. (a) Meat, Fish, and Poultry (17-23% Fat) [1] Residues In Parts Per Million—Fat Basis			
Heptachlor	Boston	1	0.014
	Minneapolis	1	0.004
PCP	Boston	1	0.04
	Los Angeles	1	0.02
Malathion	Boston	1	0.054
Aldrin	Boston	1	0.004
Diazinon	Minneapolis	1	0.011
Endrin	Los Angeles	1	0.019
Toxaphene	Los Angeles	1	0.19
MCP	Baltimore	1	0.04

Pesticide	District	No. Composites	Amount
III. (a) Grain and Cereal [1] Residues In Parts Per Million			
Aldrin	Boston	2	T
Diazinon	Los Angeles	1	T
	Minneapolis	2	0.002, 0.003
Heptachlor epoxide	Kansas City	1	T
	Minneapolis	1	0.001
Perthane®	Minneapolis	2	0.010, 0.038
PCP	Boston	1	0.03
	Los Angeles	1	0.020
Methyl parathion	Boston	1	0.033
Ronnel	Kansas City	1	T
Heptachlor	Minneapolis	1	T
Methoxychlor	Kansas City	1	T
TCNB	Boston	1	T
BHC	Los Angeles	1	T
IV. (a) Potatoes [1] Residues In Parts Per Million			
Heptachlor epoxide	Boston	2	T, 0.009
	Kansas City	1	0.010
	Los Angeles	1	0.004
Arsenic (As_2O_3)	Boston	1	0.1
	Los Angeles	1	0.1
	Minneapolis	1	0.1
Chlordane	Kansas City	1	0.043
	Los Angeles	1	0.026

TABLE 2b.—*Pesticides found infrequently—by food class and region (June 1968-April 1969)—Continued*

IV. (a) Potatoes [1]—Continued
Residues In Parts Per Million

Pesticide	District	No. Composites	Amount
Lindane	Los Angeles	2	T
Endosulfan sulfate	Minneapolis	2	0.004, 0.011
Diazinon	Los Angeles	1	T
	Minneapolis	1	T
TCNB	Boston	1	0.007
TDE	Minneapolis	1	0.001

V. (a) Leafy Vegetables [1]
Residues In Parts Per Million

Pesticide	District	No. Composites	Amount
Dieldrin	Kansas City	1	T
	Los Angeles	1	0.001
	Minneapolis	2	0.001, 0.003
Methyl parathion	Boston	1	0.008
	Los Angeles	1	T
	Minneapolis	2	0.001, 0.025
Lindane	Boston	1	T
	Kansas City	1	0.007
	Minneapolis	1	0.002
Endrin	Boston	1	0.017
	Minneapolis	2	0.002
Toxaphene	Los Angeles	3	T, 0.022, 0.33
Arsenic (As_2O_3)	Boston	2	0.1
	Los Angeles	1	0.1
	Minneapolis	1	0.1
Dacthal®	Kansas City	1	0.032
	Minneapolis	2	T, 0.004
Diazinon	Los Angeles	1	T
	Minneapolis	2	0.003, 0.004
BHC	Kansas City	1	0.004
	Minneapolis	1	0.001
Perthane®	Minneapolis	2	0.142, 0.528
Heptachlor epoxide	Minneapolis	1	T
Chlorbenside	Minneapolis	1	0.029
Disulfoton	Boston	1	0.002
2,4-D	Boston	1	0.012

VI. (a) Legume Vegetables [1]
Residues In Parts Per Million

Pesticide	District	No. Composites	Amount
Arsenic (As_2O_3)	Boston	2	0.1
	Los Angeles	1	0.1
Toxaphene	Los Angeles	2	0.04, 0.052
Carbaryl	Baltimore	1	T
	Minneapolis	1	T
Dieldrin	Boston	1	0.006
Lindane	Kansas City	1	T
Parathion	Kansas City	1	0.035
Dacthal®	Los Angeles	1	0.005
TCNB	Boston	1	T

VII. (a) Root Vegetables [1]
Residues In Parts Per Million

Pesticide	District	No. Composites	Amount
Dieldrin	Boston	1	T
	Kansas City	2	T, 0.007
Arsenic (As_2O_3)	Boston	2	0.1
	Minneapolis	1	0.1
TDE	Boston	1	0.001
	Los Angeles	1	T
Heptachlor	Minneapolis	2	0.002, 0.003
Aldrin	Minneapolis	1	0.001
Toxaphene	Los Angeles	1	0.036

VIII. (a) Garden Fruits [1]
Residues In Parts Per Million

Pesticide	District	No. Composites	Amount
Arsenic (As_2O_3)	Boston	3	0.1
	Los Angeles	1	0.1
Endosulfan	Kansas City	1	0.002
	Los Angeles	2	0.001, 0.007
	Minneapolis	1	T
Endrin	Los Angeles	2	T, 0.002
Diazinon	Los Angeles	1	T
	Minneapolis	1	0.004
Dacthal®	Minneapolis	2	T, 0.001
Heptachlor epoxide	Minneapolis	1	T
PCP	Los Angeles	1	T
Malathion	Los Angeles	1	T

IX. (a) Fruits
Residues In Parts Per Million

Pesticide	District	No. Composites	Amount
Dieldrin	Boston	1	T
	Kansas City	1	T
	Minneapolis	1	0.002
Heptachlor epoxide	Minneapolis	2	T, 0.001
Endosulfan	Minneapolis	2	0.002, 0.010
Lindane	Los Angeles	1	T
Malathion	Minneapolis	1	0.004
Ovex	Minneapolis	1	0.003
Carbaryl	Minneapolis	1	0.3

X. (a) Oils, Fats, and Shortening (83-88% Fat) [1]
Residues In Parts Per Million—Fat Basis

Pesticide	District	No. Composites	Amount
Lindane	Kansas City	1	0.002
	Los Angeles	2	0.002, 0.006
Heptachlor epoxide	Boston	1	T
	Kansas City	1	0.012
Diazinon	Minneapolis	2	0.002, 0.011
Arsenic (As_2O_3)	Boston	1	0.1
	Minneapolis	1	0.1
Parathion	Los Angeles	1	T
PCP	Los Angeles	1	T

TABLE 2b.—*Pesticides found infrequently—by food class and region (June 1968-April 1969)—Continued*

Pesticide	District	No. Composites	Amount
XI. (a) Sugars and Adjuncts[1] Residues In Parts Per Million			
TDE	Los Angeles	2	0.001
	Minneapolis	1	0.003
Dieldrin	Boston	1	T
	Los Angeles	1	0.003
MCP	Los Angeles	1	0.010
PCP	Los Angeles	1	0.040
XII. (a) Beverages Residues In Parts Per Million			
Arsenic (As_2O_3)	Boston	2	0.1
	Baltimore	1	0.1
DDT	Boston	1	T

[1] Six composite samples examined at each of five sampling sites: Boston, Kansas City, Los Angeles, Baltimore, and Minneapolis.

NOTE: Bromide and arsenic values are reported on an "as is" basis (no drying or isolation of fat) for Dairy Products; Meats, Fish, and Poultry; and Oils, Fats, and Shortening.

TABLE 3—*Comparison of residues before and after processing by dietician (average PPM levels for residues found six or more times per food group)*

Pesticide	Potatoes		Leafy Vegetables		Legume Vegetables		Root Vegetables		Garden Fruits		Fruits		Average Retention of Residues (%)
	IV		V		VI		VII		VIII		IX		Residues After Prep'n. / Residues Before Prep'n. × 100
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	
DDT	0.011	0.002	0.023	0.010	0.027	0.010	0.041	0.002	0.043	0.030	0.011	0.008	40
DDE	0.001	T	0.003	0.004	0.001	0.001	0.023	0.002	0.001	0.001	0.001	0.001	32
TDE	—	—	—	—	0.001	0.001	—	—	0.009	0.007	0.001	0.004	109
Dieldrin	0.001	0.001	—	—	—	—	—	—	0.002	0.002	—	—	100
Lindane	—	—	—	—	—	—	—	—	0.001	T	—	—	Not Calculable
Endosulfan (total)	—	—	0.011	0.006	—	—	—	—	—	—	—	—	55
Kelthane®	—	—	—	—	—	—	—	—	—	—	0.045	0.034	76
Toxaphene	—	—	—	—	—	—	—	—	0.043	0.021	—	—	49
Endrin	0.002	0.001	—	—	—	—	—	—	—	—	—	—	50
Ethion	—	—	—	—	—	—	—	—	—	—	0.024	0.014	58

TABLE 4.—*Recovery studies, June 1968-April 1969*
[( ) = Averages]

Pesticide	Type of Food Composite	Spike Level—PPM	Blank Level—PPM	Total Recovered—PPM	Number of Recovery Experiments
Heptachlor epoxide	0.000	Fatty	0.005	0.004-0.005	2
	Fatty	0.030	0.005	0.025	1
	Non-Fatty	0.003	0.000-0.001 (T)	0.002-0.003	6
	Non-Fatty	0.010	0.000	0.005-0.010 (0.008)	6
DDT	Fatty	0.050	0.000-0.023 (0.010)	0.041-0.069 (0.050)	6
	Non-Fatty	0.003	0.000-0.002 (0.001)	0.003-0.004 (0.004)	4
	Non-Fatty	0.010	0.000-0.007 (0.003)	0.005-0.017 (0.015)	6
	Non-Fatty	0.030	0.000	0.012	1
TDE	Fatty	0.010	0.000	0.009-0.010	2
	Non-Fatty	0.003	0.000	0.003-0.005	4
	Non-Fatty	0.050	0.000-0.016 (0.009)	0.052-0.076 (0.067)	5

TABLE 4.—*Recovery studies, June 1968-April 1969—Continued*

Pesticide	Type of Food Composite	Spike Level—PPM	Blank Level—PPM	Total Recovered—PPM	Number of Recovery Experiments
DDE	Fatty	0.050	0.007	0.030	1
	Non-Fatty	0.003	0.000-0.002 (T)	0.003-0.005 (0.004)	5
	Non-Fatty	0.010	0.000-0.006 (0.001)	0.009-0.020 (0.013)	7
	Non-Fatty	0.100	0.000	0.089-0.115 (0.101)	6
Dieldrin	Fatty	0.010	0.000	0.006-0.015	2
	Non-Fatty	0.003	0.000	0.003-0.004	7
	Non-Fatty	0.050	0.000-0.006 (0.001)	0.020-0.058 (0.049)	7
Aldrin	Fatty	0.003	0.000	0.002	2
	Non-Fatty	0.010	0.000-0.002 (0.001)	0.009-0.013 (0.010)	5
	Non-Fatty	0.050	0.000	0.026-0.059 (0.048)	6
Endrin	Fatty	0.010	0.000	0.008	1
	Fatty	0.030	0.000	0.023	1
	Non-Fatty	0.003	0.000	T-0.006 (0.003)	5
	Non-Fatty	0.010	0.000	0.004-0.015 (0.011)	7
	Non-Fatty	0.030	0.000	0.017	1
Malathion	Fatty	0.05	0-0.015 (0.008)	0.04-0.06 (0.05)	2
	Non-Fatty	0.05	0-0.057 (0.024)	0-0.087 (0.066)	6
	Non-Fatty	0.1	0	0.061-0.112 (0.087)	13
Parathion	Non-Fatty	0.05	0-0.008 (0.003)	0.014-0.058 (0.044)	12
	Non-Fatty	0.1	0	0.076-0.160 (0.102)	6
	Non-Fatty	1.0	0	0.88-1.01 (0.95)	3
Ethion	Non-Fatty	0.05	0	0.015-0.070 (0.046)	12
	Non-Fatty	0.1	0	0.023-0.110 (0.081)	12
2,4-DB	Fatty	0.02	0	T-0.033 (0.015)	4
	Fatty	0.05	0	0.02-0.06 (0.04)	3
	Non-Fatty	0.02	0	0.02 (0.02)	2
	Non-Fatty	0.05	0	0.04-0.06 (0.05)	3
	Non-Fatty	1.0	0	0.69-1.26 (0.96)	4
2,4,5-TP	Fatty	0.02	0	0-0.01 (0.003)	4
	Non-Fatty	0.5	0	0.18-0.68 (0.48)	9
PCP	Fatty	0.02	0	0-0.007 (0.003)	6
	Non-Fatty	0.05	0	0-0.01 (0.003)	6
	Non-Fatty	0.1	0	0-0.02 (0.003)	6
Carbaryl	Non-Fatty	0.2	0	0.15-0.20 (0.18)	26
	Non-Fatty	1.0	0	0.50-1.1 (0.88)	23
Amitrole	Non-Fatty	0.05	0	0.02-0.10 (0.05)	23
	Non-Fatty	0.1	0	0.05-0.14 (0.08)	22

TABLE 4.—*Recovery studies, June 1968-April 1969—Continued*

Pesticide	Type of Food Composite	Spike Level— PPM	Blank Level— PPM	Total Recovered— PPM	Number of Recovery Experiments
rsenic (As_2O_3)	Fatty	0.1	0-0.4 (0.16)	0.1-0.56 (0.25)	6
	Fatty	0.5	0-0.3 (0.06)	0.06-0.8 (0.40)	13
	Non-Fatty	0.1	0-0.1 (T)	0.05-0.25 (0.11)	12
	Non-Fatty	0.5	0-0.1 (T)	0.1-0.56 (0.43)	12
	Non-Fatty	1.0	0-0.1 (T)	0.5-1.0 (0.82)	12
romides	Fatty	5.0	0-5.0 (1.2)	1.0-11.5 (5.9)	13
	Fatty	50	1.5-6.0 (3.2)	15-46 (35.2)	3
	Non-Fatty	5.0	0-2.7 (1.1)	2.9-9.4 (6.0)	6
	Non-Fatty	50	0-43 (8.7)	12.5-82.0 (50.8)	15
admium (Polarography and A.A.)	Fatty	0.1	0-0.09 (0.02)	0-0.21 (0.11)	20
	Fatty	0.05	0-0.02 (0.01)	0.03-0.08 (0.05)	6
	Non-Fatty	0.01	0-0.01 (T)	0-0.02 (0.01)	5
	Non-Fatty	0.1	0-0.03 (0.01)	0.10-0.13 (0.11)	4
ineb	Non-Fatty	5.0	0	1-13 (3.5)	30
	Non-Fatty	1.0	0	0.4-0.9 (0.74)	6

Monitoring DDT Residues on Forage Plants Following a Forest Insect Control Program

Gerald S. Strickler and Paul J. Edgerton[1]

ABSTRACT

The amount of DDT reaching understory vegetation grazed by cattle, deer, and elk, and the DDT residue levels in herbage samples of a sedge, lupine, and sagebrush were determined for one prespray and three postspray sampling dates up to 1 year following aerial application of DDT for forest insect control. The DDT was applied at the start of the livestock grazing period.

Ground level DDT dosage ranged form 3-78% of the designated ¾-lb/acre rate. Average prespray DDT residue was 0.54, 11.43, and 3.53 ppm for elk sedge, lupine, and sagebrush, respectively. Corresponding postspray averages were 74.04, 87.08, and 61.02 ppm immediately after application; 13.66, 13.55, and 6.62 ppm for 4 months; and 2.07, 0.41, and 1.22 ppm 12 months after application. Residues in species plot samples from the first two postspray dates were significantly related to ground level dosage. Cycling of DDT was not indicated; the greater elk sedge residue 1 year after spraying was attributed to differences in sampling. Associated DDT residues in cattle and big game are briefly discussed.

Introduction

DDT, at the rate of ¾ of a pound in 1 gallon of fuel oil per acre, was applied to 66,000 acres of forest of ponderosa pine and Douglas-fir north of Burns, Oreg., in June 1965, to control an outbreak of Douglas-fir tussock moth, *Hemerocampa pseudotsugata* McD. Since a late spring application of DDT was necessary to control the moth, forage plants grazed by livestock, deer, and elk were exposed to DDT spray residues at the beginning of the summer grazing season. We, therefore, initiated a study to measure the amount of DDT reaching understory vegetation following helicopter application and to monitor DDT residues on three forage plants for one prespray and three postspray sampling dates. The latter three sampling dates were immediately after spray application, approximately 4 months after spraying when summer livestock grazing was terminated, and 12 months after spraying just prior to livestock grazing in the succeeding year.

Crouch and Perkins (*2*) graphically presented a summary of the DDT residues obtained in this study in a surveillance report of the insect control project. The purpose of this paper is to make data available on canopy cover, spray deposition rate, and subsequent residue content of plants grazed by livestock, deer, and elk.

Sampling Procedure

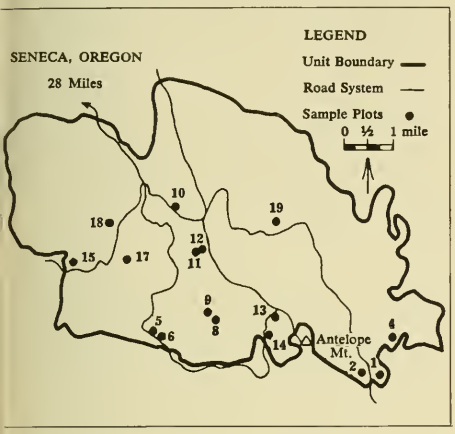
The plants selected for herbage residue analysis were elk sedge (*Carex geyeri* Boott), tailcup lupine (*Lupinus caudatus* Kell.), and big sagebrush *Artemisia tridentata* Nutt.). Sixteen ½-acre plots were located in the 23,000-acre Antelope Mountain unit of the spray project (Fig. 1). The Antelope Mountain acreage encompassed part of a larger allotment grazed by cattle. Because elk sedge and lupine are highly desirable forages for cattle, plots containing these species were fenced to prevent cattle grazing. All plots were accessible to deer and elk.

Within elk sedge and lupine plots, 20- to 50-g (dry weight) subsamples of herbage were clipped from each of 20 randomly located points. On sagebrush plots similar subsamples were obtained from the crowns of 21 shrubs. The area around each sampling point and shrub center was divided into quarters. Clipping was confined to one quarter at each point or shrub during each of four sampling dates:

Prespray	May 26-June 9, 1965
First postspray	June 21-27, 1965
Second postspray	Oct. 10-11, 1965
Third postspray	June 28-29, 1966

[1] Pacific Northwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, La Grande, Oreg.

FIGURE 1.—*Diagram of the Antelope Mountain unit and location of sampling plots. (Insufficient forage prevented sampling of plots 3, 7, 16, 20, and 21.)*



Size of the area clipped within each quarter varied with the distribution of the 20 to 50 g of herbage required for the sample.

Herbage samples consisted of leaf and twig growth of big sagebrush, all current growth of tailcup lupine, and both current and older (2-3 years) green leaves of elk sedge. Subsample clippings were composited, yielding approximately 1 to 2 lb of dry herbage per plot for DDT analyses.

To prevent possible transfer of DDT between species samples, clean plastic gloves were worn during clipping, and shears and weight scales were rinsed thoroughly with fresh acetone before each species sample was clipped. Care was taken not to contaminate samples with ground surface litter. The plastic sample bags were held off the ground by wire holders.

Composited samples were refrigerated on the day clipped, stored at approximately 0 F, and shipped frozen to the Agricultural Research Service Laboratory in Yakima, Wash., for DDT residue analysis. All samples were kept frozen until processed for analysis.

Just before spraying, nine oil-sensitive dye cards (*10*) were placed in a grid pattern within each ½-acre sample plot. The cards were supported by wire holders (*4*) approximately 1.5 feet above ground. Cards were not placed within 1.5 feet of a tree bole or under sagebrush crowns. Cards were collected after spraying, and the amount of spray (gal/acre) reaching the card level was estimated (*3,5*). Percentage cover of tree canopy was measured with a densiometer (*9*) at each dye-card location to determine if canopy cover differences were associated with differences in spray deposition.

Residue Analysis

Elk sedge samples were chopped and mixed in a reel-type cutting machine. Samples of sagebrush and lupine were ground and mixed in a Toledo meat grinder.

Small subsamples from each of the above species plot samples were composited and oven-dried to determine moisture content at the time of extraction.

For each species two subsamples per plot, each weighing 25, 50, or 100 g net, depending on species and sampling date, were either tumbled for 1 hour or steeped overnight and then tumbled for 1 hour in measured amounts of *n*-hexane, filtered through cotton plugs into sample bottles, and refrigerated until analyzed.

Aliquots of the extracts were shaken for 6 minutes with 4 g of a mixture of Florisil (60/100 mesh), Magnesol, and anhydrous sodium sulfate. The glassware was rinsed with a total of 75 ml of *n*-hexane which was combined with the filtrates. The filtrates were reduced in a warm water bath with an airstream to a volume of about 5 ml and transferred to test tubes. For those samples analyzed by an adaptation of a colorimetric method (*8*), the solvent was completely removed. For those analyzed by a Warner-Chilcott gas chromatograph, the filtrates were brought to dryness and the residues dissolved in a measured amount of *n*-hexane and refrigerated.

Prespray, first postspray, and second postspray sedge and lupine samples were analyzed colorimetrically. The residue figures from the first and second postspray samples were reduced by the amount of "apparent" DDT in the corresponding prespray samples.

The second postspray sagebrush samples and third postspray samples of all species were analyzed by gas chromatography and were not reduced by the "apparent" DDT in prespray samples. One untreated sagebrush sample collected near the laboratory when the second postspray samples were analyzed did not show any interference of plant waxes with the gas chromatograph method, so no reductions were made. The third postspray residue values were reduced by values obtained from a complete reagent blank. Identity of pesticides was confirmed by determination of extraction p-values (*1*).

The gas chromatograph employed a 183-cm coiled glass column 3.8 mm i.d. packed with 10% DC 200 on Gas Chrom Q. The electron capture detector employed Strontium 90. Operating temperatures were: column-233 C, injector-250 C, detector-256 C, and the outlet-277 C.

The nitrogen carrier gas flow rate was 160/minute, and nitrogen scavenger gas flow was 40 ml/minute at 2.82 kg/cm^2 (40 psi).

Standard DDT solutions were added to each species sample solution for each sappling period, and the percent recovery was determined. Average recovery efficiencies for the combined DDT isomers and DDE in each species ranged from 87-101% for the colorimetric method and 78-85% for the gas chromatograph method. All residue values were corrected to 100% recovery and to a dry-weight basis.

Results

Tree canopy cover, DDT deposition rate, and mean DDT residue* levels in the herbage samples from the 16 plots are presented in Table 1.

Prespray lupine, sagebrush, and elk sedge samples contained 11.43, 3.53, and 0.54 ppm DDT residues, respectively. Residues in sagebrush and lupine were unexpectedly high and indicated previous DDT application; however, an examination of management history of the study area did not reveal any definite sources of contamination.

There was no significant relationship between average canopy cover and average DDT deposition rate estimated from the dye cards. Variation in climatic conditions during the 2-week operation and in amounts of spray delivered by the helicopters resulted in different amounts of spray reaching the understory regardless of canopy cover differences. On the other hand, postspray plot sample residues were directly related to the average deposition rate. This relationship was significant ($P \leq .05$) for all but the third postspray elk sedge and sagebrush samples.

Lupine, elk sedge, and sagebrush herbage had average DDT residue levels of 87.08, 74.04, and 61.02 ppm, respectively, immediately after spraying and 13.55, 13.66, and 6.62 ppm 4 months after spraying. Except for plot 8, the pattern of decreasing DDT levels was essentially the same for all sample plots. Cattle had broken into plot 8 following the first postspray sampling, but because of the abundance of elk sedge the light grazing which occurred did not prevent subsequent sampling. Since the second postspray sample from plot 8 was the only sample increasing in residue, we assume that the cattle contaminated the herbage, after having previously grazed in vegetation with high DDT residue. A similar incident, but with heavier grazing of herbage, precluded sampling of both elk sedge and lupine in plot 13.

One year after spraying, average residue levels decreased to 0.41, 1.22, and 2.07 ppm for lupine, sagebrush, and elk sedge, respectively. For lupine and sagebrush, these averages were lower than prespray averages, but elk sedge residue was about four times higher.

Analysis of paired lupine and elk sedge samples clipped from the same plot showed that their DDT residue levels were significantly different ($P \leq .05$) only for prespray and third postspray samples. But, whereas lupine samples had the greater residue content before spraying, elk sedge residue content surpassed that in lupine 1 year after spraying.

Discussion

In this study, DDT residues in understory plants reached a high level immediately following aerial spray, decreased rapidly the following 4 months, and were less than, or similar to, prespray levels 1 year after spraying. These findings are comparable to those reported for other forest insect control projects (*6,7*).

The origin of the high DDT residues in the prespray samples is conjectural. Clarification might have been obtained if nonsprayed control samples from areas adjacent to the plots had been available for comparison at each date.

The low residues 1 year after spraying indicate that cycling of DDT did not occur. It should be remembered, however, that this assumption is based on (1) a comparison with surprisingly high prespray DDT residues in lupine and sagebrush and (2) our belief that the greater residue content in elk sedge compared with that in lupine and sagebrush 1 year after spraying was primarily due to differences in sampling. All clipped samples of lupine, which had the lowest residue content 1 year after spraying, were new growth. Clipped elk sedge samples, which had the highest residue content, necessarily contained a large percentage of 2- and 3-year-old green leaves which had been sprayed. Residue content of sagebrush was intermediate to that of lupine and elk sedge, and it was noted that in the process of stripping leaves from twigs some leaves and inflorescences of the previous season were included in its samples. Thus, differences in residue content 1 year after spraying were most likely an artifact of sampling; higher residues were measured in samples containing more herbage which was present during spray application.

We selected these species not only to provide information on plants of different growth form but also because they supply forage for cattle, deer, and elk. It should be noted that residue content of the first postspray samples of the three species tended to be more similar on a fresh-weight basis (field sample), especially for samples clipped from the same or adjacent plots. We, therefore,

* The term DDT residue is used in this paper with no distinction between deposited material and that resulting from decomposition or weathering processes or between materials within or on the plant.

suspect that all understory plants available to grazing animals had DDT residue contents similar to those sampled. It was also apparent from the residues remaining in the second postspray samples of these species that grazing animals were consuming forage with high DDT residue content during the 4-month grazing season.

Companion studies showed that DDT residues in adipose tissue of cattle, deer, and elk also increased after spraying and subsequently declined, with average residues declining below the 7 ppm legal tolerance 120 days (deer) and 150 days (cattle) after spraying (*2*). However, these residue values are considered low since both big game and cattle had access to and undoubtedly

TABLE 1.—*Plot canopy cover, spray deposition rate at ground level, and DDT residues on elk sedge, lupine, and sagebrush*

Sample Plot Number	Average Canopy Cover [1] (Percent)	Average Deposition Rate [1] (Gal/acre)	DDT Residue in PPM [2]			
			Prespray June 1965	First Postspray June 1965	Second Postspray Oct. 1965	Third Postspray June 1966
ELK SEDGE						
2	76	0.21	0.68	62.50	16.90	1.86
5	63	0.40	1.71	123.40	15.00	2.79
6	46	0.36	([3])	35.60	8.20	3.45
8	60	0.03	0.55	10.00	13.20	1.16
10	46	0.15	0.35	33.60	5.80	0.68
11	50	0.73	0.32	278.70	25.40	1.83
13	66	0.22	0.55	32.10	([4])	1.14
14	68	0.46	1.39	54.60	14.20	2.43
15	51	0.31	([3])	86.60	13.90	3.32
18	55	0.27	([3])	40.00	7.80	1.20
19	48	0.57	0.35	57.40	16.20	2.88
Plot average	57	0.34	0.54	74.04	13.66	2.07
Herbage moisture content (percent)			69	59	48	51
TAILCUP LUPINE						
6	46	0.36	9.92	69.00	11.40	0.35
10	46	0.15	9.92	56.20	3.40	0.32
11	50	0.73	29.00	218.10	18.70	0.56
13	66	0.22	([4])	62.20	([4])	([4])
15	51	0.31	5.00	43.60	16.60	0.30
18	55	0.27	3.33	65.70	12.40	0.42
19	48	0.57	([4])	94.80	18.80	0.51
Plot average	52	0.37	11.43	87.08	13.55	0.41
Herbage moisture content (percent)			88	79	10	78
BIG SAGEBRUSH						
1	46	0.24	2.98	45.10	6.20	1.68
4	33	0.38	4.48	37.80	6.00	1.06
9	18	0.17	2.98	27.30	4.10	0.64
12	23	0.78	1.98	156.20	13.20	2.11
17	26	0.19	5.23	38.70	3.60	0.60
Plot average	29	0.35	3.53	61.02	6.62	1.22
Herbage moisture content (percent)			56	67	48	68

[1] Average of nine values per sample plot.

[2] Each value is the average of two analyses per herbage sample based on dry weight and 100% recovery. The colorimetric method used gives the sum of the two isomers of DDT and the metabolite DDE. The lower limit of detectability was 0.04 ppm for combined DDT isomers and DDE. The gas chromatograph method, used for the second postspray sagebrush samples and all species for the third postspray samples, gives the sum of DDE, *o,p'*-DDT + TDE, and *p,p'*-DDT. The lower limits of detectability are 0.002, 0.005, and 0.04 ppm, respectively. Postspray values are corrected for "apparent" DDT found in prespray samples or in a complete reagent blank (third postspray samples).

[3] None detected.

[4] Not sampled because of insufficient herbage or accidental grazing by cattle.

grazed on untreated forage. The period and amount of grazing on the sprayed area, by big game particularly, is unknown. Nevertheless, in our study cattle contained average DDT residues well below the legal tolerance level 1 year after spraying and were grazing major forage plants no more contaminated with DDT than was measured before insect control operations.

See Appendix for chemical names of compounds mentioned in this paper.

This paper reports research involving pesticides. It does not contain recommendations for their use nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

Mention of trade names or commercial materials is for the convenience of the reader and does not constitute any preferential endorsement by the U.S. Department of Agriculture over similar products available.

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LITERATURE CITED

(1) *Beroza, Morton, and M. C. Bowman. 1965.* Identification of pesticides at nanogram level by extraction p-values. Anal. Chem. 37:291-292.

(2) *Crouch, Glenn L., and Randall F. Perkins. 1968.* Surveillance report—1965 Burns project, Douglas-fir tussock moth control. USDA Forest Serv. R-6 Rep. 20 p.

(3) *Davis, J. M., and K. R. Elliott. 1954.* Standards for estimating surplus spray deposits on oil-sensitive cards. Forest Serv., unnumbered pamphlet. U.S. Dep. of Agr. 7 p.

(4) *Maksymiuk, Bohdan. 1959.* Improved holders for spray deposit assessment cards. J. Econ. Entomol. 52:1029-1030.

(5) ———. *1963.* How to estimate the atomization of oil-base aerial sprays by the D-max method. USDA Forest Serv. Res. Note WO-1, 6 p.

(6) *Mussehl, Thomas W., and Robert B. Finley, Jr. 1967.* Residues of DDT in forest grouse following spruce budworm spraying. J. Wildlife Manage. 31:270-287.

(7) *Pillmore, Richard E., and Robert B. Finley, Jr. 1963.* Residues in game animals resulting from forest and range insecticide applications. Trans. N. Amer. Wildlife Conf. 28:409-422.

(8) *Stiff, Henry A., Jr., and Julia C. Castillo. 1945.* A colorimetric method for the micro-determination of 2, 2, bis (*p*-chlorophenyl) 1, 1, 1 trichlorethane (DDT). Science 101(2626):440-443.

(9) *Strickler, Gerald S. 1959.* Use of the densiometer to estimate density of forest canopy on permanent sample plots. USDA Forest Serv. Pacific Northwest Forest & Range Exp. Sta. Res. Note 180, 5 p.

(10) *White, H. W. 1959.* How to make oil-sensitive cards for estimating airplane spray deposit. Forest Serv. Forest Insect Lab., Beltsville, Md. U.S. Dep. of Agr. 3 p.

Residues in Sorghum Treated With the Isooctyl Ester of 2,4-D [1]

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ABSTRACT

An isooctyl ester formulation of 2,4-D was applied to grain sorghum at acid equivalent rates of 1.25 or 2.50 lb/acre. The growth stage of the sorghum at the time of treatment varied from preemergence to the dough stage. Residues of 2,4-D in the grain and forage of sorghum were determined by gas chromatography with a sensitivity level of approximately 0.2 ppm. No residues of 2,4-D were detected in the grain; residues in the forage ranged from less than 0.2 ppm to 5.25 ppm. The time interval between application of the herbicide and harvesting of the forage was the most critical factor in determining the residues. More 2,4-D remained in sorghum forage grown in Missouri than in forage grown in Oklahoma.

Introduction

Low volatile esters of 2,4-D have been used to control broadleaved weeds in grain sorghum [*sorghum bicolor* (L.) Moench] since the late 1940s. Registration of 2,4-D was obtained on the basis of no 2,4-D residue in the crop at harvest time. Since that time governmental agencies have required that finite tolerances be established for all herbicides used in the production of field crops. This report describes a chromatographic procedure for detecting 2,4-D in sorghum forage and grain to a sensitivity of 0.2 ppm and summarizes the results of analyses of sorghum treated at various stages of growth in the field.

Procedures for the determination of 2,4-D esters in plants have been published by Crafts (*1*) using radioactive tagging, Morre and Rogers (*5*) using three bioassays, Szabo (*6*) using paper chromatography, Yip and Ney (*7*) using microcoulometric gas chromatography, and Hagin and Linscott (*2*) using electron capture gas chromatography. These studies indicate that the ester is hydrolyzed upon absorption by the plant and subsequently moved in the plant as the free acid. Klingman *et al.* (*3*) found that about 75% of the 2,4-D applied to forage as the 2-ethylhexyl ester had been hydrolyzed to the free acid within ½ hour after application.

Morgan and Hall (*4*) studied the metabolism of 2,4-D acid by cotton and grain sorghum and found no 2,4-D in the grain. Either the 2,4-D was not translocated to the seed or it was destroyed or otherwise immobilized before reaching the developing fruit. Yip and Ney (*7*) used an alkaline hydrolysis of milk and an acid hydrolysis of forage to release possible bound forms of 2,4-D. The hydrolysis made it possible to analyze for the acid, ester, and bound forms of 2,4-D in a single sample.

Treatment and Sampling Procedures

MISSOURI

At Columbia, Mo., grain sorghum (variety AKS-614) was planted at the rate of 4 lb/acre approximately 1¼ inches deep on July 17, 1969. The soil was a Mexico silt loam which had been spring plowed and had received 500 lb/acre of 20-10-10 bulk fertilizer. The experimental design was a randomized complete block with four replications. Each plot consisted of two 40-inch rows, 32 feet long.

The preemergent treatment and treatment at the 15- to 20-inch stage were applied with a plot sprayer mounted on a small garden tractor; the 30-inch stage

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[1] Contribution from the Agricultural Experiment Stations of Missouri, Journal Series No. 6012, approved 6-24-70; Oklahoma, Journal Series No. 2028, approved 6-9-70; and Texas, Journal Series No. 8572, approved 6-2-70.

[2] Department of Soil and Crop Sciences, College of Agriculture, Texas A&M University, College Station, Texas 77843.

[3] University of Missouri, Columbia, Mo.

[4] Oklahoma State University, Stillwater, Okla.

was treated with an oiling rig. Both types of equipment applied 40 gal/acre at 40 psi. The application dates were July 17, August 11, and August 27, respectively. The rainfall for July was 5.01 inches; August, 2.40 inches; and September, 6.82 inches.

Even though the grain was immature, the plots were harvested on October 17, 1969, before the killing frost date. The heads and the stalks were separated, placed in plastic bags, and frozen. The samples were kept in frozen storage until shipment at which time they were packed in dry ice and shipped to Texas for analysis.

OKLAHOMA

OK612 grain sorghum was planted in 40-inch rows on a Norge loam soil near Perkins in northcentral Oklahoma. Planting was done with a commercial planter on June 5, 1969. The plots were three rows wide and 30 feet long in four replications. The treatments were made with a tractor-sprayer. The herbicide was applied in 30 gallons of water per acre at 30 psi. The treatment on June 10 was made to a dry soil when the air temperature was 70 F and the wind speed was 3 mph. There was 0.75 inches of rain on June 11. Treatment at the 10-inch stage was made on June 21 when the air temperature was 80 F and the wind speed was 6 mph. Soil moisture was adequate and the sorghum was growing vigorously. There was 0.8 inches of rain on June 24. On July 28 the sorghum plants were flowering when treated; the air temperature was 80 F; there was no wind; and soil moisture was adequate. Treatment of plants in the dough stage was made on August 19; there was no wind; the air temperature was 90 F; and soil moisture was adequate. The sorghum was separated into grain or forage samples, frozen, and shipped to Texas for analysis.

Analytical Procedures

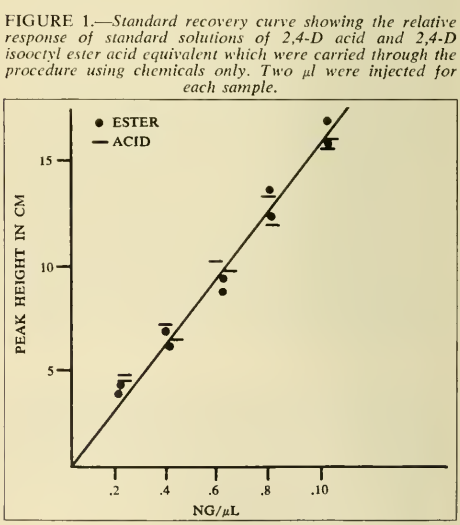
Forage samples were chopped into picees 1 cm long or smaller and thoroughly mixed before taking a 25-g portion for analysis. Most of the grain was in the dough stage and required no grinding prior to blending. The 25-g samples of both forage and grain were heated to a boil in 400-ml beakers containing 25 ml of 0.05 N HCl. After cooling, each sample was transferred to a blending cup containing 100-500 ml of 2-propanol and blended in a Waring blendor for 10 minutes. The homogenate was filtered through glass wool in a Buchner funnel. The blending cup was rinsed twice with 25 ml of 2-propanol and the rinses added to the residue in the Buchner funnel. The filtrate was returned to the beaker and 100 ml of 1 N KOH solution added. This mixture was evaporated on a warm hot plate to a volume of approximately 150 ml. The samples were kept under a fume hood overnight at 30 C. This hydrolysis converted all of the ester of 2,4-D to the potassium salt and possibly released bound 2,4-D from the plant filtrate. The basic solution was washed three times with petroleum ether. The aqueous phase was kept and the ether phase discarded. A number of these discards were analyzed to confirm that no ester of 2,4-D was in the ether. The sample was acidified by dropwise addition of concentrated HCl and extracted three times with 100 ml of petroleum ether. After evaporating the ether to dryness, the residue was methylated by heating the sample with 10 ml of 12.5% BF_3-methanol solution until BF_3 fumes were released. The methyl ester of 2.4-D was then extracted into 10 ml of hexane.

Samples were analyzed on a Barber Coleman Model 5360 electron capture gas chromatograph equipped with a radium 226 detector. A 6-foot spiral column packed with 10% DC 200 on 100/200 mesh gas chrom-Q was used. Injector, column, and detector temperatures were 260 C, 200 C and 240 C, respectively. The carrier gas was nitrogen at a flow rate of 75 ml/min. Peak height of known concentrations of 2,4-D methyl ester were compared to the unknowns to determine the amount of herbicide present.

Results and Discussion

The alkaline hydrolysis of the isooctyl ester of 2,4-D to 2,4-D acid was essentially complete since equivalent weights of ester and acid yielded equal amounts of the methyl ester of 2,4-D (Fig. 1). There was a linear relationship between the peak height on the chromatogram and the concentration of 2,4-D over a range of 0 to 1.0 μg/ml. A direct transesterification of the isooctyl ester of 2.4-D to the methyl ester of 2,4-D with BF_3-methanol is possible, but hydrolysis of the isooctyl

FIGURE 1.—*Standard recovery curve showing the relative response of standard solutions of 2,4-D acid and 2,4-D isooctyl ester acid equivalent which were carried through the procedure using chemicals only. Two μl were injected for each sample.*



ester to the acid prior to methylation facilitates the removal of impurities from the sample. When an alkaline hydrolysis was used, the percent recovery of 2,4-D ester from either sorghum grain or forage was approximately 86%. The detection limits of the procedure are approximately 0.2 ppm of 2,4-D when 25-g sorghum samples are used. If it is desirable to detect lower concentrations of 2,4-D, larger samples must be taken. However, if larger samples are taken, larger quantities of solvents are required for the extraction, or the percentage of recovery may be reduced. Also, background peaks are more troublesome when larger samples are taken.

In Oklahoma, sorghum 10 to 12 inches tall treated with 1.25 lb/acre of the isooctyl ester of 2,4-D contained 1.06 ppm 2 days after application and 0.90 ppm 1 week after application. Forage samples taken 4 weeks after application or later contained no detectable residues (<0.2 ppm). Likewise, grain harvested from the treated plots contained no detectable residues (Table 1).

TABLE 1.—*Residues of 2,4-D in sorghum forage and grain sprayed on June 21, 1969, with 2,4-D isooctyl ester at an acid equivalent rate of 1.25 lb/acre when sorghum was 10- to 12-inches tall (Perkins, Okla.)*

Date Sampled	Residues in PPM	
	Forage	Grain
June 23, 1969	1.06	—
June 28, 1969	0.90	—
July 19, 1969	<.2	—
Aug. 20, 1969	<.2	<.2

The residues in sorghum forage and grain at harvest following preemergent application with 1.25 lb/acre of 2,4-D and treatment when the sorghum was 10 inches tall, when the sorghum was flowering, and when the sorghum heads were in the dough stage, are shown in Table 2. Application made when the sorghum was flowering or in the dough stage resulted in detectable residues of 2,4-D in the sorghum forage at harvest. No residues of 2,4-D were detected in the grain at any time, indicating that sorghum does not readily incorporate 2,4-D into the grain even if the herbicide is applied when the grain is being formed.

TABLE 2.—*Residues of 2,4-D in sorghum forage and grain sprayed at several growth stages with 2,4-D isooctyl ester at an acid equivalent rate of 1.25 lb/acre, and harvested September 19, 1969 (Perkins, Okla.)*

Treatment		Residues at Harvest (PPM)	
Date	Stage	Forage	Grain
June 10, 1969	Preemergence	<.2	<.2
June 21, 1969	10 inches	<.2	<.2
July 28, 1969	Flowering	0.36	<.2
Aug. 19, 1969	Dough	3.16	<.2
Check		<.2	<.2

Table 3 shows data from sorghum grown in Missouri and treated at different stages of growth with various rates of 2,4-D. As with the Oklahoma samples, no 2,4-D residues were detected in the grain at anytime; however, 2,4-D persisted longer in the sorghum forage from Missouri than in the forage from Oklahoma. For example, Missouri sorghum 15 to 20 inches tall, treated with 1.25 lb/acre of 2,4-D, and harvested about 2 months later, contained 1.19 ppm. Oklahoma sorghum treated at a similar rate when the sorghum was flowering, and harvested about 2 months later, contained only 0.36 ppm. Likewise, sorghum in Missouri treated 6 weeks prior to harvest with 2.50 lb/acre of 2,4-D contained almost twice the residue found in Oklahoma sorghum treated 4 weeks prior to harvest with 1.25 lb/acre of 2,4-D. The hot, dry weather conditions in Oklahoma apparently increased the degradation of 2,4-D.

TABLE 3.—*Residues of 2,4-D in sorghum forage and grain sprayed with 2,4-D isooctyl ester at various rates and stages of growth, and harvested October 17, 1969 (University of Missouri, Columbia, Mo.)*

Date	Treatment Stage	Acid Equivalent	Residues at Harvest (PPM)	
		Rate (lb/acre)	Forage	Grain
July 17, 1969	Preemergence	2	<.2	<.2
Aug. 11, 1969	15-20 inches	1.25	1.19	<.2
Aug. 11, 1969	15-20 inches	2.50	1.84	<.2
Aug. 27, 1969	30 inches (flowering)	2.50	5.25	<.2
Check		0	<.2	<.2

See Appendix for chemical name of 2,4-D.

Acknowledgment

The authors thank the Thompson-Hayward Chemical Company for providing the chemical used in the study.

LITERATURE CITED

(1) *Crafts, A. S. 1960.* Evidence for hydrolysis of esters of 2,4-D during absorption by plants. Weeds 8:19.

(2) *Hagin, R. D. and D. L. Linscott. 1965.* Determination of 4-(2,4-dichlorophenoxy)-butyric acid (2,4-DB) and 2,4-dichlorophenoxyacetic acid in forage plants. J. Agr. Food Chem. 13:123.

(3) *Klingman, D. L., C. H. Gordon, G. Yip, and H. P. Burchfield. 1966.* Residues in the forage and in milk from cows grazing forage treated with esters of 2,4-D. Weeds 14:164.

(4) *Morgan, P. W. and W. C. Hall. 1963.* Metabolism of 2,4-D by cotton and grain sorghum. Weeds 11:130.

(5) *Morré, D. J. and B. J. Rogers. 1960.* The fate of long chain esters of 2,4-D in plants. Weeds 8:436.

(6) *Szabo, S. S. 1963.* The hydrolysis of 2,4-D esters by bean and corn plants. Weeds 11:292.

(7) *Yip, G. and R. E. Ney, Jr. 1966.* Analysis of 2,4-D residues in milk and forage. Weeds 14:167.

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Organochlorine Pesticides in Nursing Fur Seal Pups

Raymond E. Anas[1] and Alfred J. Wilson, Jr.[2]

ABSTRACT

Samples of muscle, brain, liver, blubber, and ingested milk from five nursing fur seal pups, Callorhinus ursinus, *were analyzed for organochlorine pesticides and polychlorinated biphenyl compounds (PCB's). Of 25 samples of tissue and ingested milk, 25 contained DDE; 19, DDD; 22, DDT; and 9 contained dieldrin. All of the samples of ingested milk and tissues, with the exception of brain tissue, contained trace amounts of PCB's. Concentrations of DDE, DDD, and DDT in the liver and brain of 4-month-old nursing pups tended to be higher than those found in 4-month fetuses studied previously.*

Pesticides are known to occur in seals in Antarctica (*5*, *9*), Scotland (*6*), and Canada (*6*). Adult and fetal northern fur seals, *Callorhinus ursinus,* collected on the Pribilof Islands, Alaska, in 1968 and off the Washington coast in 1969 also had detectable amounts of pesticides (*2*).

This report records the amounts of organochlorine pesticides found in the tissues and ingested milk of nursing northern fur seal pups collected on the Pribilof Islands in 1969. The pups were about 4 months old and had nursed approximately 12 times, but we do not know whether they had previously fed on marine organisms.

In a study by Abegglen *et al.* (*1*), four pups collected from October 6-27, 1961, on St. Paul Island were found to contain remains of either fish or amphipods. Of 20 pups sampled on November 14 and 15, 1966, on St. Paul Island, 9 contained milk. Of these nine, one pup showed positive evidence of having fed on marine organisms, and five additional pups may have fed on marine organisms (*personal communication, H. Kajimura and M. C. Keyes, Marine Mammal Biological Laboratory, Seattle, Wash.*). The amounts of pesticides accumulated from foraging is not known. However, since seal milk contains about 50% fat by weight (*3*), it is a natural reservoir for pesticides. The seal pup, therefore, cannot avoid accumulating pesticides each time it nurses if the mothers milk contains pesticides. Knowledge of pesticides in seal milk and in tissues of nursing pups is important, because mortality of fur seal pups might be associated with accumulation of pesticides.

Sampling and Analytical Procedures

Samples of muscle, brain, liver, blubber, and ingested milk were collected from five nursing fur seal pups on November 10, 1969. The tissues were kept frozen from the time of collection until analysis.

Blubber, brain, liver, and muscle tissues were analyzed for BHC, heptachlor, aldrin, heptachlor epoxide, toxaphene, methoxychlor, dieldrin, endrin, and the *o,p'*- and *p,p'*-isomers of DDE, DDD, and DDT. Samples of the thawed tissues weighing approximately 10 g each were mixed with anhydrous sodium sulfate in a blender. Each mixture was extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and partitioned with acetonitrile. The acetonitrile was evaporated just to dryness at room temperature and the residue eluted from a Florisil column (*8*). Milk samples were analyzed by the method described by Giuffrida (*4*).

[1] National Marine Fisheries Service, Marine Mammal Biological Laboratory, Seattle, Wash. 98115.

[2] Gulf Breeze Laboratory, Department of the Interior, Gulf Breeze, Fla. 32561.

Sample extracts were then identified and quantified by gas chromatographs equipped with electron capture detectors and 5′ x ⅛″ o.d. glass columns. Operating conditions are outlined in Table 1:

Laboratory tests indicated recovery rates greater than 85% for pesticides found in tissues. Data in this report do not include a correction factor for percentage recovery. The lower limit of sensitivity is 0.01 ppm (mg/kg, wet weight). All residues reported are on a wet-weight basis except milk, which was calculated on a fat basis.

Sample extracts had to be concentrated to small volumes (volumes less than that required to obtain 0.010 ppm sensitivity for pesticides) in order to evaluate the level of polychlorinated biphenyl compounds (PCB's). These compounds were present at levels that would not significantly interfere with the quantitation of DDT and its metabolites. Thin-layer chromatography was used to separate and confirm the presence of these organochlorine compounds.

Results

Pesticides were found in every sample of tissue and ingested milk collected from the nursing fur seal pups. All 20 of the tissue samples contained DDE; 14 contained DDD; 17, DDT; and 5 contained dieldrin; all except those from the brain had trace amounts of polychlorinated biphenyls (Table 2). The highest concentration of a single pesticide was 45 ppm of DDE in the blubber of one pup. Brain tissue contained the lowest concentrations of DDT and its metabolites. On the average, the levels of DDE, DDD, and DDT tended to be lower in brain and liver tissues of fur seal fetuses (*2*) than in those of nursing pups in the present study (Table 3). Samples of blubber and muscle were not collected from the fetuses. All of the samples of milk contained DDE, DDD, and DDT; four contained dieldrin. Trace amounts of PCB's were also found in all samples of ingested milk.

Pesticides in the milk, especially residues of DDE, varied widely in amount, but nursing pups with the largest amounts of pesticides in the milk generally had the largest amounts of pesticides in body tissues.

See Appendix for chemical names of compounds mentioned in this paper.

Acknowledgments

We thank Jerrold Forester and Johnnie Knight, Bureau of Commercial Fisheries, Pesticide Field Station, Gulf Breeze, Fla., for their assistance in the chemical analysis, and Lavrenty Stepetin, Bureau of Commercial Fisheries, St. Paul Island, Alaska, for collecting the samples.

LITERATURE CITED

(*1*) *Abegglen, C. E., A. Y. Roppel, A. M. Johnson, and F. Wilke. 1961.* Fur seal investigations, Pribilof Islands, Alaska. Report of field activities, June-November 1961. 148 p. (U.S. Fish Wildlife Serv., Marine Mammal Biological Laboratory, Seattle, Wash.)

(*2*) *Anas, R. E. and A. J. Wilson, Jr. 1970.* Organochlorine pesticides in fur seals. Pesticides Monit. J. 3(4):198-200.

(*3*) *Ashworth, U. S., G. D. Ramaiah, and M. C. Keyes. 1966.* Species difference in the composition of milk with special reference to the northern fur seal. J. Dairy Sci. 49(10): 1206-1211.

(*4*) *Giuffrida, L., D. C. Bostwick, and N. F. Ives. 1966.* Rapid cleanup techniques for chlorinated pesticide residues in milk, fats, and oils. J. Ass. Offic. Agr. Chem. 49(3):634-638.

(*5*) *George, J. L. and D. E. H. Frear. 1966.* Pesticides in the Antarctic. J. Appl. Ecol. 3(Suppl.):155-167.

(*6*) *Holden, A. V. and K. Marsden. 1967.* Organochlorine pesticides in seals and porpoises. Nature 216(5122): 1274-1276.

(*7*) *Bureau of Commercial Fisheries, Marine Mammal Biological Laboratory.* In press. Fur seal investigations, 1967. U.S. Fish Wildlife Serv. Spec. Sci. Rep. Fish. 597.

(*8*) *Mills, P. A., J. H. Onley, and R. A. Gaither. 1963.* Rapid method for chlorinated pesticide residues in nonfatty foods. J. Ass. Offic. Agr. Chem. 46(2):186-191.

(*9*) *Sladen, W. J. L., C. M. Menzie, and W. L. Reichel. 1966.* DDT residues in Adelie penguins and a crabeater seal from Antarctica. Nature 210(5037):670-673.

TABLE 1.—*Operating conditions for analysis by gas chromatography*

Liquid phase	3% DC 200	5% QF-1	1:1 3% DC 200/ 5% QF-1	2% DEGS
Solid support	60/80 Gas Chrom Q	60/80 Gas Chrom Q	80/100 Gas Chrom Q	80/90 Anakrom ABS
Oven temperature	190 C	185 C	185 C	190 C
Injector and detector temperature	210 C	210 C	210 C	210 C
N_2 flow rate	30 ml/minute	25 ml/minute	25 ml/minute	25 ml/minute

TABLE 2.—*Pesticides in tissues and ingested milk of nursing northern fur seal pups, Pribilof Islands, Alaska, November 10, 1969*

[nd = not detectable; T = trace]

Tissue and Pup Number	Residues in ppm (Mg/kg, wet weight) [1]				
	DDE	DDD	DDT	Dieldrin	PCB
Muscle					
1	8.1	0.33	0.34	0.038	T
2	0.58	0.060	0.068	nd	T
3	0.19	0.015	0.022	nd	T
4	1.0	0.051	0.084	nd	T
5	0.069	nd	0.019	nd	T
Brain					
1	0.34	nd	0.030	nd	—
2	0.12	nd	nd	nd	—
3	0.18	nd	0.013	nd	—
4	0.058	nd	nd	nd	—
5	0.012	nd	nd	nd	—
Liver					
1	6.4	0.13	0.22	nd	T
2	1.3	0.085	0.11	nd	T
3	1.9	0.14	0.30	nd	T
4	0.22	0.012	0.024	nd	T
5	0.12	0.023	0.057	nd	T
Blubber					
1	45	1.5	1.4	0.089	T
2	11	0.77	0.76	0.042	T
3	14	0.70	0.83	0.046	T
4	2.3	0.29	0.35	0.049	T
5	0.35	0.071	0.22	nd	T
Ingested milk					
1	5.1	0.13	0.20	nd	T
2	4.9	0.12	0.19	0.033	T
3	2.4	0.17	0.20	0.020	T
4	0.32	0.024	0.059	0.020	T
5	0.039	0.017	0.032	0.013	T

[1] Tissues only—residues in milk were calculated on a fat basis.

TABLE 3.—*Pesticides in liver and brain tissues of fetal and nursing northern fur seal pups*

[nd = not detectable]

Tissue and Type of Pup	Sample Size	Residues in PPM (Mg/kg, wet weight)							
		DDE		DDD		DDT		Dieldrin	
		Median	Range	Median	Range	Median	Range	Median	Range
Liver									
Fetus [1]	7	nd	nd-0.10	—	nd	—	nd	—	nd
Nursing	5	1.3	0.12-6.5	0.085	0.01-0.13	nd	0.02-0.22	—	nd
Brain									
Fetus [1]	7	nd	nd-0.04	—	nd	—	nd	—	nd
Nursing	5	0.12	0.01-0.34	—	nd	nd	nd-0.01	—	nd

[1] Collected off Washington, February-March 1969 (*2*).

International Cooperative Study of Organochlorine Pesticide Residues in Terrestrial and Aquatic Wildlife, 1967/1968

A. V. Holden[1]

ABSTRACT

A two-part collaborative study of organochlorine residues in wildlife was carried out by 17 laboratories in 11 countries. The first part involved the analysis of four test samples containing organochlorine residues; one sample was a solution of standard chemicals, while the other three were cod-liver oil, chicken egg, and sprat (Clupea Sprattus) *homogenate. This part provided a basis for comparison of the relative efficiency and accuracy of the laboratories in residue analysis.*

The second part of the program consisted of the sampling and analysis of four species of wildlife from areas believed to be free of pesticide usage in each country. The results indicate the degree of background contamination of the environment, as exemplified by the species selected, which were the starling, pike, marine mussel, and dogfish.

The range of variation of residues among individuals of a natural population is much larger than that due to analytical errors or to differences between laboratories. Distributions within populations are non-Gaussian, necessitating large samples for the detection of relatively small interpopulation differences.

Introduction

At a meeting sponsored by the Organization for Economic Cooperation and Development (O.E.C.D.) in Paris in June 1966, attended by representatives of 17 member countries, concern was expressed regarding the possible effects on the environment caused by the occurrence of pesticide residues in areas where there is no local usage of pesticides. To establish the presence of these residues in wildlife, a preliminary cooperative study was made voluntarily by laboratories in 11 member countries. The first O.E.C.D. Technical Conference on Pesticides in the Environment [sponsored jointly with The Natural Environment Research Council (London)] was held in Scotland in September 1967 to discuss the results of this preliminary study. At this conference a decision was made to proceed with a further, more extensive study during the period 1967/1968. Reported here are the results of the extended study presented at the second O.E.C.D. Technical Conference on Pesticides in the Environment [sponsored jointly with T.N.O. (The Hague)], held in the Netherlands in September 1969.

The main objective of the study was to establish the levels of pesticide contamination existing in selected species sampled at prearranged times in a prescribed manner in areas believed to be free of pesticide contamination arising from any local usage of organochlorine pesticides. The earlier study in 1966/1967 had demonstrated that certain types of pesticide residues were detectable in several species of wildlife in areas where no known usage of pesticides existed, but no attempt was made to compare the analytical abilities of the laboratories involved. The second study therefore included a program of analysis of different types of samples exchanged among the participating laboratories of several member countries of O.E.C.D., to establish a common basis for the comparison of their efficiency and accuracy with regard to both the qualitative and quantitative aspects of residue analysis. Each laboratory employed the techniques and apparatus which it normally used for pesticide residue analyses.

A total of 17 laboratories took part in the analytical program, 16 in 10 member countries of O.E.C.D. and also the analytical laboratory of Euratom at the European Community Commission Joint Research Center, Ispra, Italy. All had participated in the preliminary study in 1966/1967. The laboratories are identified in Table 1.

[1] Freshwater Fisheries Laboratory, Pitlochry, Scotland (acting as coordinator).

TABLE 1.—*Laboratories participating in international cooperative study of organochlorine pesticide residues in terrestrial and aquatic wildlife, 1967/1968*

Country	Participating Laboratories
Canada	Ontario Research Foundation, Sheridan Park, Ontario, Canada.
Euratom	Analytical Laboratory, Euratom, European Community Commission Joint Research Center, Ispra, Italy.
Finland	State Veterinary Medical Institute, Box 10 368, Helsinki 10, Finland.
Ireland	The Agricultural Institute, Oak Park, Carlow, Ireland.
Netherlands	Central Institute for Nutrition and Food Research (CIVO), Utrechtseweg 48, Zeist, Netherlands.
	National Institute of Public Health (RIV), Sterrenbos 1, Utrecht, Netherlands.
	Institute for Veterinary Pharmacology and Toxicology (Vet. Tox.), University of Utrecht, Biltstraat 172, Utrecht, Netherlands.
Norway	Veterinary College of Norway, Department of Pharmacology, Oslo 4, Norway.
Portugal	Laboratory for Phytopharmacology, Quinta do Marques, Oeiras, Portugal.
Spain	Institute of General Organic Chemistry, Calle Juan de la Cierua 3, Madrid-6, Spain.
Sweden	Institute of Analytical Chemistry, University of Stockholm, Roslagsvägen 90, Stockholm 50, Sweden.
United Kingdom	Laboratory of the Government Chemist (LGC), Cornwall House, Stamford Street, London S.E. 1.
	Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory (MAAF), Remembrance Avenue, Burnham-on-Crouch, Essex.
	The Nature Conservancy (NC), Monks Wood Experimental Station, Abbots Ripton, Huntingdonshire.
	Department of Agriculture and Fisheries for Scotland, Freshwater Fisheries Laboratory (FFL), Faskally, Pitlochry, Perthshire.
United States	U.S. Department of the Interior, Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Md. 20810, U.S.A.
	U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Md. 20705 U.S.A.

Interlaboratory Quality Evaluation Program for Pesticide Residue Analysis

DESCRIPTION OF TEST SAMPLES

Four types of test samples were circulated among the participating laboratories. These were analyzed by the methods in current use at the respective laboratories; details of the methods of analysis and confirmation are given in Table 2. As can be seen, the variations in technique were considerable. In addition, two samples containing polychlorinated biphenyls (PCB's) were provided for confirmation of the identity of unknown residues detected in some of the test samples.

Sample No. 1—a mixture of six organochlorine insecticides or derivatives, a 5-ml volume of hexane solution of the mixture being supplied in a glass ampoule.

This sample was distributed by the Institute of Veterinary Pharmacology and Toxicology, University of Utrecht, Netherlands. On receipt of a report on the analysis of the sample from each laboratory, the Institute provided the correct analysis, as calculated from the amounts originally dissolved. This enabled analysts to correct any errors in standards or technique originating in their laboratories before proceeding with the other tests and wildlife samples.

Sample No. 2—two ampoules, one containing a solution of the PCB formulation Clophen A.50 in hexane at a stated concentration and the other a solution in hexane of five PCB isomers at stated concentrations, these having been isolated by preparative gas chromatography and estimated by mass spectrometry.

The solutions could be used for approximate quantitative estimation of some PCB residues in wildlife and were distributed by the Institute of Analytical Chemistry, University of Stockholm, Sweden. Clophen A.50 (Bayer, Germany) is a commercial formulation comprising a series of PCB isomers and containing 50% chlorine by weight. It is similar to the Aroclors 1254, manufactured by Monsanto, which contains 54% chlorine by weight.

Sample No. 3—an ampoule containing cod-liver oil, distributed by the Laboratory of the Government Chemist, London.

Sample No. 4—a sample containing 15 g of fresh chicken egg, uniformly ground with anhydrous sodium sulphate, supplied in an aluminum tube by the laboratory in Utrecht as for Sample No. 1.

The eggs were produced in 1 week from 5 chickens which had been treated previously with a mixture of organochlorine insecticides for 1 month. The contents were homogenized and thoroughly mixed with anhydrous sodium sulphate for shipping.

Sample No. 5—a sample containing 15 g of a dried homogenate of 71 specimens of sprat (Clupea sprattus) *caught in the Dutch Wadden Sea in June 1968.*

The homogenate was mixed thoroughly with anhydrous sodium sulphate for shipping in aluminum tubes and was supplied from Utrecht as for Sample No. 1.

ANALYTICAL PROCEDURES

The methods used varied widely, particularly in extraction and cleanup. Some laboratories made a preliminary group separation of residues by column chromatography (CC) before analyzing by gas-liquid chromatography (GLC). Confirmation techniques included the preliminary (CC) separation, two or more GLC columns, thin-layer chromatography (TLC), acidification to destroy dieldrin and endrin, and alkaline hydrolysis of TDE, DDT, and γ-BHC. The techniques are summarized in Table 2.

Extraction involved cold or hot solvents of various types, for short or long periods, in vertical columns using an adsorbent material, in Soxhlet extractors or

sometimes in open dishes. Cleanup usually employed some form of liquid-liquid partition, commonly between hexane and acetonitrile or dimethylformamide, followed by passage through an adsorbent column. In a few cases the partition stage was omitted and fat removed on the column. The most common adsorbent was Florisil, and in many cases successive elutions with solvents of different polarity provided a pre-GLC separation. Other adsorbents were alumina and silica.

Some analysts obtained pre-GLC separations by thin-layer plates or by treatment with acid or alcoholic KOH. The GLC analysis generally employed two different types of column, providing further confirmation.

DISCUSSION OF RESULTS

Although distribution of the samples began in April 1968, many laboratories found difficulty in completing the analyses of the test samples by the end of 1968. This was partly due to their inability to give priority to the samples and partly to difficulties presented by sample No. 5. Some laboratories experienced difficulty with certain samples to which they were not accustomed and which required additional cleanup or separation techniques. Sample No. 1 was the only one analyzed by all participants.

The results of the analyses of test samples No. 1, 3, 4, and 5 are given in Tables 3-6. Where analyses by two methods were reported, the mean value is listed. For the mixture of pure pesticides (No. 1) the analyses are given to one decimal place, but for the other test samples, which contained lower concentrations, the data are usually given to two decimal places.

The manner of reporting inevitably differed widely, with some laboratories stating only the concentrations of residues found in appreciable amounts and others mentioning levels of residues not fully confirmed, while some analysts reported pesticides to be absent at the level of detection obtainable by their current methods of analysis. The values reported were generally uncorrected for losses incurred in extraction.

As might be expected, agreement between the laboratories on the analysis of the mixture of pure pesticides was reasonably good. In a few instances, analysts were encountering residues not normally found in their routine work, e.g., heptachlor epoxide and endrin, and this necessitated preliminary investigation of the appropriate GLC separation and the possibility of breakdown of endrin. The other exchange samples showed a wider variation between laboratories, both qualitatively and quantitatively. These samples required full processing (extraction, cleanup, and confirmation) which introduced varying degrees of error.

Sample No. 1

Some analysts reported that they were doubtful of the purity of their own standards, in particular heptachlor epoxide and DDT. Assuming that the distributing laboratory employed only high-purity chemicals, others using standards of lower purity for calibration would be likely to report excessively high concentrations; but errors in dilution and errors due to GLC injection variations would be random.

The results (Table 3) show that distributions of the values for all six pesticides were approximately normal, suggesting that any errors due to impurity of standards must be small. Only the values obtained by direct determination have been used in the statistical evaluation of the data, presented in Table 7. This table gives the true value for each pesticide, the mean calculated from the analyses, standard deviation, coefficient of variation, and the number of laboratories which reported values within $\pm$ 5% of the mean.

With the exception of endrin, the means of the concentrations reported were very close to those stated to have been present by the distributing laboratory. No explanation has been found for the anomalous value of the mean for endrin, but the distributing laboratory, using the same standard for analysis, obtained a result similar to those reported by many other laboratories. Two laboratories, Ireland and Netherlands RIV, reported exceptionally high values for endrin. Omission of these values produces a distribution which is closer to normal, with a coefficient of variation of $\pm$ 11.9%. The mean value of the coefficient of variation for the six residues is then approximately $\pm$ 9%. By comparison, the coefficient of variation for replicate GLC injections by one analyst is, at best, usually of the order of $\pm$ 2% and often as high as $\pm$ 5%. (A 2% error is equivalent to a one-division error for a GLC peak giving 50% of full-scale deflection on a 100-division recorder scale.)

Examining the data in more detail, none of the 17 laboratories reported values consistently within $\pm$ 5% of the means, although 5 reported only one value outside these limits. The deviations from the means for individual laboratories were not random; however, Euratom reported five low values, Norway four low values, and Spain five high values (all in excess of 5% error). It is possible that the particular methods of diluting the original sample for analysis which were in use in the individual laboratories may have produced the biased results, and the methods of calculation may also have differed. For example, some analysts use a calibration graph derived from a series of concentrations of a standard, but others calculate from only one such concentration. The latter method is liable to give a significant error unless the standard and sample concentrations are similar.

Sample No. 2 (PCB)

The two samples provided were examined by most laboratories, but only three gave details of the results obtained. In the first sample 5 major peaks and a number of small peaks were detected, and in the second at least 14 peaks could be found. Most analysts used these peaks as references (by relative retention times) for the unknown peaks found in test sample Nos. 3 and 5.

Sample No. 3 (cod-liver oil)

This sample presented some difficulty in cleanup, but the proportion of PCB residues was less than the pesticide residues. Interference between these two groups was recognized by most analysts, and a variety of methods was used to overcome this difficulty.

The 14 laboratories analyzing this sample reported 12 pesticide residues apart from PCB's. The frequency of reporting was as follows:

Dieldrin	12	*o,p'* - DDT	6	Endrin	2
p,p' - DDE	14	α - BHC	6	Aldrin	1
p,p' - TDE	13	γ - BHC	4	Heptachlor epoxide	2
p,p' - DDT	13	β - BHC	1	*o,p'* - TDE	1
		PCB's	9 positive or suspected		

The laboratories were in general agreement on the presence of four of these compounds, dieldrin and the three members of the *p,p'* - DDT group. The distributions of the data for these residues were approximately normal, and statistical analysis gives the following information:

	Mean	Range	SD	CV
Dieldrin	0.139	< 0.05 - 0.37	± 0.114	± 82%
p,p' - DDE	0.357	0.16 - 0.58	± 0.120	± 34%
p,p' - TDE	0.541	0.13 - 0.80	± 0.205	± 38%
p,p' - DDT	0.628	0.17 - 1.44	± 0.338	± 54%

Mean, range, and standard deviation in ppm

The standard deviation for a single determination is thus ± 0.1 to ± 0.3 ppm, the percentage error being higher for dieldrin and DDT than for DDE and TDE. The range of values reported is considered to be excessively large, but this seems to be only partly due to the problems caused by the presence of PCB's.

Sample No. 4 (chicken egg)

Analysts found this the easiest of the three "wildlife" samples to analyze, with no PCB interference and relatively high pesticide concentrations. Little cleanup was found to be necessary—some analysts using an unprocessed extract in solvent for direct injection on a GLC column.

The 16 laboratories analyzing this sample reported 10 residues. The number of laboratories reporting each residue was as follows:

Dieldrin	16	Endrin	14	Aldrin	1
p,p' - DDE	16	γ - BHC	5	*o,p'* - DDT	2
p,p' - TDE	12	HCB	5		
p,p' - DDT	16	β - BHC	1		

The analysts were in general agreement on the presence of dieldrin, *p,p'*-DDE, *p,p'*-DDT, and endrin, but a few did not report *p,p'*-TDE, which was in a smaller concentration. One laboratory probably incorrectly identified endrin as *o,p'*-DDT since endrin interferes with *o,p'*-DDT on some types of GLC columns. The γ-BHC and hexachlorobenzene (HCB) peaks are probably identical and were confirmed by some analysts as HCB, e.g., by separation on silica. Peaks at this early stage of a chromatogram are not always attempted by analysts.

The data for the five major residues were approximately normally distributed, with statistical analysis giving the following results (values in terms of homogenate were not included):

	Mean	Range	SD	CV
Dieldrin	0.162	0.08 - 0.24	± 0.046	± 28%
Endrin	0.136	0.07 - 0.36	± 0.073	± 54%
p,p' - DDE	0.127	0.08 - 0.23	± 0.040	± 32%
p,p' - TDE	0.046	0.02 - 0.10	± 0.023	± 51%
p,p' - DDT	0.445	0.14 - 0.62	± 0.140	± 31%

Mean, range, and standard deviation in ppm

One laboratory reported an exceptionally high value for endrin which, if omitted reduces the coefficient of variation to ± 25%, and the standard deviation to ± 0.029 ppm. The agreement between analysts is thus appreciably better than that for the cod-liver oil sample, the coefficient of variation being in the range of 25-30% with the exception of *p,p'*-TDE, which was in a significantly lower concentration. This measure of agreement, irrespective of the type of extraction or other processing, is probably due to the absence of PCB residues and problems of cleanup.

Sample No. 5 (sprat)

This sample proved to be the most difficult to analyze, due partly to the low levels of pesticide residues present

in relation to PCB's and partly to difficulties experienced in cleanup.

Fourteen laboratories analyzed this sample and reported 12 residues, in addition to PCB's, as follows:

Dieldrin	14	*p,p'* - DDT	12	Heptachlor epoxide	4
Endrin	13	γ - BHC	8	Aldrin	2
p,p' - DDE	12	α - BHC	3	HCB	2
p,p' - TDE	12	β - BHC	2	*o,p'* - DDT	1

PCB's 13 positive

Once again the laboratories were in general agreement on the presence of dieldrin, endrin, and the three members of the *p,p'*-DDT group, although interference by PCB peaks with *p,p'*-TDE and *p,p'*-DDT made their quantitative estimation difficult. As with test sample No. 4, γ-BHC and HCB are probably the same residue, but few analysts are familiar with HCB.

The data for the five major residues showed less agreement between analysts than for test samples No. 3 and 4; but, assuming normal distribution of the values, statistical analysis gives the following results (values in terms of homogenate were not included):

	Mean	Range	SD	CV
Dieldrin	0.122	0.02 - 0.22	± 0.060	± 50%
Endrin	0.132	0.09 - 0.21	± 0.039	± 29%
p,p' - DDE	0.068	0.01 - 0.17	± 0.052	± 76%
p,p' - TDE	0.118	0.04 - 0.28	± 0.064	± 54%
p,p' - DDT	0.113	0.02 - 0.25	± 0.083	± 73%

Mean, range, and standard deviation in ppm

Two exceptionally high values were reported for DDE; the omission of these reduces the coefficient of variation to ± 56%. One very high TDE value was reported, and omitting this reduces the coefficient of variation to ± 37%. The interference of a PCB peak with DDT is the most likely reason for the high variation among the values for DDT, and with the omission of this residue the coefficient of variation is of the order of ± 25% to ± 50%, slightly greater than the values for test sample No. 4.

A study of the data for samples No. 3, 4, and 5 shows no evidence that any particular technique for extraction, cleanup, or pre-GLC separation consistently gave values higher or lower than average for a particular pesticide. The greatest degree of agreement was reached with test sample No. 4 (chicken egg) which required little or no cleanup and was free from PCB interference. This group of compounds, commonly found in marine samples, interfered in test samples No. 3 (cod-liver oil) and 5 (sprat), and most separation techniques in common use do not separate PCB's from the members of the *p,p'*-DDT group. Where such a separation is effective, as with a silica column, TDE and DDT values can be estimated more accurately.

CONCLUSIONS

The results of the analyses of the standard mixture of pesticides, which required only appropriate dilution before GLC determination, show that at this stage of the procedure laboratories agreed on the identity of the common pesticide residues and on the amounts present with a coefficient of variation of 7-10%. In the analysis of wildlife samples, which required extraction, cleanup, and sometimes pre-GLC separation before GLC determination, the coefficient of variation was considerably greater, ± 30% to ± 60% for the more difficult samples, and no better than ± 25% to ± 30% for the least difficult.

In the wildlife samples, agreement on the identity of residues was good only for the major residues found, which were dieldrin, *p,p'*-DDE, *p,p'*-TDE, and *p,p'*-DDT. These are normally the compounds which are of the most concern to ecologists, and the accuracy of the analyses is generally sufficient for most ecological studies. However, the detection of year-by-year trends in contamination levels or of small differences between populations in different areas would require a greater accuracy. This could probably be achieved by greater efficiency of recovery in cleanup techniques, together with a method of separation of the interfering PCB's from the true pesticide residues where necessary.

———*Organochlorine Residues in Wildlife Samples*———

SAMPLING PROCEDURES

This study involved the sampling and analysis of one or more of the following species—starling (*Sturnus vulgaris*), pike (*Esox lucius*), mussel (*Mytilus edulis*), and dogfish (*Squalus acanthias*)—or appropriate ecological equivalents. The species were to be sampled as far as possible at stated periods, from areas where there was no history of local pesticide usage. The preferred specimen sizes were indicated in some instances as well as the methods of preparing samples for analysis. In some cases analyses of individuals or groups of individuals were planned to permit statistical evaluation of the results.

The detailed procedures for sample collection and preparation were outlined as follows:

Starling, Sturnus vulgaris—adults of the species to be sampled on arrival at breeding areas. Where possible, 30 or 35 specimens to be taken, numbered individually, and the weight of each bird recorded. If such a large number of specimens is not available, or if analytical time is limited, 10 specimens should be numbered, and weighed individually. Remove 10 g of breast muscle from each bird for analysis.

For 10 specimens, analyze each bird individually, 2 in duplicate but not consecutively.

For 30 or 35 specimens, analyze 10 individually as above, and analyze 20 (or 25) in 5 groups of 4 (or 5), mixing the 10-g aliquots in each group uniformly before analysis.

Mussel, Mytilus edulis—Specimens to be sampled in the estuaries of, or at the mouths of, large rivers during November or December 1967, or as soon as possible afterwards, but before the spawning period (spring 1968).

A total of 10 large or 25 small specimens to be collected, numbered individually, and the overall shell length of each recorded. The soft tissue to be used for analysis.

Where possible, 10 specimens should be analyzed individually, 2 analyses in duplicate but not consecutively.

If specimens are too small for individual analyses, tissues should be mixed in five groups of five specimens and analyzed, two groups in duplicate but not consecutively.

Pike, Esox lucius—Ten adults, 4 years of age or older, sampled in April or before spawning; length, weight, sex, and age recorded.

A 100-g portion of lateral muscle to be taken at midsection for analysis, each fish being analyzed separately. Samples from two fish to be analyzed in duplicate but not consecutively.

Dogfish, Squalus acanthias—Large specimens to be taken as available; length, weight, sex, and age recorded if possible. Remove lateral muscle and liver from each specimen for analysis. (This species was chosen because of its wide distribution in the marine environment. Its examination was in the nature of a reconnaissance, but it was hoped that those countries able to obtain and analyze specimens easily would do so.)

Data from 10 individual specimens represent the minimum requirement for statistical analysis of the populations sampled. Although not required, it was hoped that some of the participating laboratories would also analyze composited samples for comparison of data with results obtained from analysis of individual specimens.

ANALYTICAL PROCEDURES

The methods of analysis employed for the wildlife samples were usually the same as those for the exchange samples, but the following major differences were noted by participating laboratories.

Canada	Method (a) used for pike, method (b) for starlings and mussels.
Netherlands	For starlings, Vet. Tox. Laboratory used method of Onley and Bertuzzi(*1*), acetone/methylcellosolve extraction, DMF partition, transfer to 40-60 C petroleum ether, Florisil column, GLC analysis on 5% DC-11 and 5% QF-1 columns on Chromosorb W at 185 C.
Spain	For dogfish, Onley and Bertuzzi(*1*) cleanup method used.
United Kingdom	Starlings analyzed by NC laboratory. Mussels analyzed by MAFF, DAFS laboratories. Pike and dogfish analyzed by DAFS laboratory.
United States	Analyses at Laurel laboratory only. GLC analysis employed 10% QF-1 column at 160 C in place of 12% DEGS column.

RESULTS

Ten countries sampled and analyzed the starling and pike, and all sampled mussels. Euratom, Ireland, Norway, Spain, Sweden, and the United Kingdom examined dogfish. There was difficulty, in some countries, in obtaining the full number of specimens required for analysis, particularly of the starling, and the specified program for analysis of starlings (single specimens and groups of 4 or 5) was not always completed.

Summaries of results of analyses for the four wildlife species are given in Tables 8 to 11. These indicate the sizes of the specimens analyzed; the mean values and ranges of values obtained for the four major pesticide residues—dieldrin, DDE, TDE, and DDT; the presence or otherwise of PCB residues; and any necessary qualifications. Where residues were not detected, the limit of detection was stated. Unfortunately, analytical procedures at some laboratories did not permit determination of all four residues.

All sampling sites were considered to be free of local pesticide usage. However, the movement of starling flocks may result in some ingestion of pesticides from other areas. Further, it is impossible to ensure freedom from pollution in estuaries where mussels are obtainable; and the catchment areas of lakes from which pike were sampled may also contain unknown sources of pesticides.

Although the ranges of values for each pesticide found in individuals of a population vary considerably, the mean values can give some indication of the degree of contamination of the population. Certain conclusions may be drawn from the data in the tables, and these are discussed below. For the purpose of comparison, levels between 0.001 and 0.01 ppm could be considered as the minimum degree of contamination now found in the environment, and levels above 0.1 ppm could be regarded as evidence of local pollution.

DIELDRIN

Mean levels less than 0.01 ppm in starlings were recorded only by Euratom, Finland, Portugal, Spain, and Sweden, and some U.S. and Portuguese samples had mean levels over 0.1 ppm. In the pike, all mean values were below 0.01 ppm except in Ireland, but in the dogfish the values reported were above 0.01 ppm. For mussels almost half of the samples had less than 0.01 ppm, but in the Netherlands, Ireland, United Kingdom, Portugal, and the United States sample values up to 0.06 ppm were obtained. This insecticide is nevertheless a minor contaminant in most countries.

DDE

In starlings this residue was always in excess of 0.01 ppm, but much higher values were found in Canada, the United States, Italy (Euratom), the Netherlands, Portugal, and Sweden.

In pike several countries reported levels below 0.01 ppm; Euratom and Spain both found a high level of contamination. In mussels, Scandinavia reported values below 0.01 ppm, and only in the United States did levels exceed 0.1 ppm. Of the six countries examining dogfish, Euratom and Spain reported values (in muscle) above 0.1 ppm and Sweden, Norway, Ireland, and the United Kingdom above 0.01 ppm. Also, the laboratories (Euratom, Sweden, and the United Kingdom) that examined liver reported values above 0.1 ppm.

TDE

This residue was not always reported, and no high values were found in the mussel or starling. Most countries reported values above 0.01 ppm in the mussel, and in the dogfish all values reported exceeded 0.01 ppm with values exceeding 0.1 ppm in Italy (Euratom) and Spain. For pike, Spain and Euratom reported some values above 0.1 ppm, but most found levels below 0.01 ppm.

DDT

In starlings, levels were above 0.1 ppm in the Netherlands and Spain and above 0.01 ppm in most other countries. In pike, only Euratom reported values above 0.1 ppm. In dogfish, all six laboratories found levels exceeding 0.1 ppm. In mussels, residues in the range of 0.01-0.1 ppm were found in all countries, with the exception of a low level (0.0036 ppm) in Norway and a high level (0.184 ppm) in Portugal.

GENERAL

The impression is gained that the Mediterranean area may contain a higher level of DDT group contamination than elsewhere in marine species, but the North Sea also has a significant level of pollution. Both Sweden and the Netherlands reported high DDT group levels in starlings, but pike generally showed only slight contamination from this chemical group.

PCB

These residues were not always reported. From the limited data reported, however, residues in starlings appear to be very small or below detection, and residues in pike were also at low levels. PCB's were present generally in mussels and in dogfish, with levels in mussels being somewhat higher than in other samples. It is not possible with current analytical techniques to identify individual PCB's, but those occurring in wildlife mainly elute on GLC columns after dieldrin. The PCB residues in this study were quantified, where mentioned, by comparison with Clophen A.50 (test sample No. 2).

STATISTICAL ANALYSIS OF DATA

A satisfactory statistical analysis of the data obtained from the wildlife samples was only possible where (a) the values measured were large enough to be useful, and (b) there were sufficient individuals in a sample to provide an indication of the appropriate type of distribution. These requirements limited the statistical analyses to the data for DDE concentrations in starlings and pike from most countries, although in some instances the data for other residues were also examined.

The majority of the population distributions were found to be of a non-Gaussian type, but a lognormal distribution was considered to be appropriate. Tables 12 and 13 give the results obtained from the DDE analyses of the various starling and pike samples. The variance in each case is in the logarithmic form, but the standard deviation is the antilog of the square root of the variance. The 95% confidence limits are calculated from the variance (log form) by deriving the antilog of twice the square root of the variance (log form). The geometric mean is then divided and multiplied by this value, giving the lower and upper limits, respectively. The precision of the mean is calculated in a similar manner, from the variance (log form) divided by the number of observations, i.e., the variance of the mean. The antilog of twice the square root of this value (log form) is used to obtain the 95% limits of the mean, by division and multiplication.

The variance for the starling populations appears to be dependent on the level of contamination existing, and the standard deviations ranged from 1.4 to 3.2. These indicate that a wide range of values can be expected for the DDE contents of individuals in a population of starlings, the upper limit being as much as one hundred times the lower limit for 95% of the population. For the purpose of assessing trends in contamination levels or for comparing the degrees of contamination in populations from two areas, the sample size will depend upon the percentage difference which it is required to detect, if it exists. The tables give the approximate number of specimens required to determine differences of 25%-200% between two means, based on the analyses for both those countries with the higher variance and those with the lower variance.

The geometric mean values for DDE in the starlings fall into two groups, those from Canada, the United States, and the Netherlands giving an overall mean of 0.230 ppm with a variance (log form) of 0.22, and all the other values giving an overall mean of 0.072 ppm with a variance (log form) of 0.060. The range of values to be found among 95% of the population would in the first case be from 0.026 to 2.0 ppm and in the second case from 0.023 to 0.23 ppm. The DDE values in the pike were all low, with the sole exception of those from Euratom. Excluding the latter, the overall geometric mean is 0.0096 ppm with a variance (log form) of 0.052. The 95% limits in this case are from 0.0033 to 0.028 ppm.

The natural range of values of a pesticide residue among individuals of a population is clearly large, although in theory this might be reduced if the individuals could be selected for age, sex, size, etc. Such a selection is normally impractical, and the large error inherent in any estimate of the overall degree of contamination must be accepted. A comparison with the coefficients of variation determined from the various exchange samples analyzed in the first part of this study, however, suggests that the errors due to analysis, to variations between techniques or laboratories, would be relatively unimportant in any comparison of population means, either within or between different countries.

The estimates of the numbers of individuals to be sampled in any population in order to detect reasonably large differences between populations are high. For example, the analysis of 50 individuals from each of 2 populations to enable detection of a 25% difference in the geometric means would entail a considerable analytical effort. It may be possible to reduce this effort to some extent by analyzing several homogenates of small numbers of individuals, such as 10 groups each of 5 individuals, but this requires a more detailed examination of the population structure than is possible from the present data. The major obstacle to any further increase in the number of individuals sampled would, however, in most cases, be that such sampling would be unacceptable in the case of many wildlife species, especially those considered to be at risk. Any international program of monitoring the environment for pesticide or other contamination would require consideration of the following:

(a) the sample size required to detect a percentage change in the contamination level which may be biologically significant

(b) whether a species can be sampled to this degree without suffering damage in the process

(c) whether the analytical facilities are available to handle the large numbers of individuals involved.

CONCLUSIONS

The second O.E.C.D. study program has shown that the ability of analysts in different countries to detect and estimate the major pesticide contaminants of the environment is reasonably good and sufficiently adequate for the purpose of monitoring wildlife populations. A further improvement can be expected as techniques become more uniform and as methods of separating interfering PCB compounds from organochlorine pesticide residues are more widely used.

The estimates of the coefficient of variation to be expected in the analysis of different types of samples for dieldrin and the DDT group of residues are:

One analyst, replicate analyses, standard solution	± 2-3%
One analyst, replicate analyses, wildlife sample	± 15-30%
Between analysts, standard solution	± 7-10%
Between analysts, wildlife samples requiring minimum processing	± 25-30%
Between analysts, wildlife samples requiring maximum processing	± 30-60%

The distribution between residues in individuals of a wildlife population is of a lognormal rather than a normal form, and the number of individual analyses required is consequently large if a reasonably accurate estimate of the mean level of contamination is to be obtained. For the detection of small changes in the contamination level, as in an international monitoring program, the analytical effort required would be considerable, and the size of the population sample could be unacceptably high or impracticable for many species. The detection of annual changes of the order of 50% would, however, be feasible.

Current levels of the residues of dieldrin, DDE, TDE, and DDT in the wildlife species examined in areas free from the direct use of organochlorine pesticides, are generally in the range of 0.01-0.1 ppm, although in a few countries levels above 0.1 ppm were found. Pike were the least contaminated, and starlings usually the most, presumably a reflection of the relative freedom of movement of the species in the environment.

See Appendix for chemical names of compounds mentioned in this paper.

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The statistical analyses were made at the Marine Laboratory, Aberdeen, Scotland, by Mr. W. B. Hall, to whom the author is indebted for the information in that section of the paper.

LITERATURE CITED

(1) *Onley, J. H. and P. F. Bertuzzi. 1966.* Rapid extraction procedure for chlorinated pesticide residues in raw animal tissues and fat and meat products. J. Ass. Offic. Anal. Chem. 49(2):370-374.

TABLE 2.—*Analytical techniques*

Laboratory	Methods of Extraction			Cleanup			Pre-GLC Separation	G L C			Confirmation		
	Cold	Hot	Time	Partition	Column	Other		Packing	Length	Temp. (C)	GLC	TLC	Chem.
Canada	b) A/N-hexane	a) ether-hexane	2 hrs.		Florisil	coldbath	6% and 20% ether in PE	6% QF-1 4% SE-30 on Chromosorb W	6 feet	200		+	+
Euratom	a) A/N in column			A/N-PE	Florisil		6% and 15% ether in PE	1) 5% DOW-11 7.5% QF-1 on Gas Chrom Q	10 feet	175	+		+
	b) A/N			A/N-PE	Florisil		6% and 15% ether in PE	2) 5% DOW-11 on Gas Chrom Q	5 feet	175	+		+
	c) PE				Florisil		6% and 15% ether in PE						
Finland	ether in column					a) H_2SO_4	TLC	1) 4% SF-96 on Chromosorb W	5 feet	176			
						b) TLC		2) 8% QF-1 on Chromosorb W		180			
Ireland		H-acetone 2:1		H/DMF	Alumina			1) 5% DC-200 on Aeropak 30	5 feet	185	+		
								2) 5% QF-1 on Chromosorb W		185			
Netherlands CIVO		hexane		H/DMF	Florisil		6% and 15% ether in PE	1) 5% DC-200 on Gas Chrom Q	6 feet	200	+		
								2) 2% DC-200 3% QF-1 on Gas Chrom Q	6 feet	200	+		
RIV	A/N on Florisil			A/N to PE			6% and 15% ether in PE	5% DC-200 on Aeropak 30	5 feet	200			
Vet. Tox.		PE	6 hrs.	H/DMF	Florisil		a) hexane b) ether in hexane	5% DC-200 7% QF-1 on Gas Chrom Q		175		+	
Norway	a) PE on column b) ether on column			PE/DMF		H_2SO_4 alcoholic KOH		1) 10% QF-1 on Chromosorb W	6 feet	175-180			
								2) 4% SF-96 on Gas Chrom P	5 feet	170-175			
Portugal		hexane	6 hrs.	H/DMF	Alumina		a) first 50 ml b) next 40 ml	1) 10% DC-200 on Gas Chrom Q	3 feet	200		+	
								2) 5% QF-1 on Gas Chrom Q	3 feet	185			
Spain		hexane		A/N	Florisil on alumina			5% DC-200 7.5% QF-1 on Chromosorb G	5 feet	190			+
Sweden		PE	3½ hrs.		a) Silica	b) 25% KOH c) $AgClO_4$ in fuming H_2SO_4		1) 1.75% SF-96 3.75% QF-1 on Gas Chrom P	5 feet	180-190			+
								2) SF-96	5 feet	180-190			+

TABLE 2.—*Analytical techniques*—Continued

Laboratory	Methods of Extraction			Cleanup			Pre-GLC Separation	GLC			Confirmation		
	Cold	Hot	Time	Partition	Column	Other		Packing	Length	Temp. (C)	GLC	TLC	Chem.
United Kingdom													
LGC	b) DMSO on column	a) 2:1 hexane-acetone	3 hrs.	DMF	Alumina silica			1) SE-52 2) Apiezon L 3) XE-60				+	
NC	hexane and acetone alternately			hexane DMF	Alumina			1) Apiezon L on Gas Chrom Z 2) SE-30 on Gas Chrom Z	2½ feet	188			+ +
MAFF		hexane		hexane DMF	Alumina			1) 2% Oronite 128 0.2% Epikote 1001 on Gas Chrom Q 2) 2.5% SE-30 0.25% Epikote 1001 on Chromosorb G	4 feet 4 feet	168 168			
FFL		hexane	30 min.		Alumina		Silica	1) 10% DC-200 on Chromosorb W 2) 5% DC-200 7.5% QF-1 on Chromosorb W	5 feet 5 feet	200 200			
U.S.A.													
Laurel		PE	8 hrs.	A/N hexane	Florisil		TLC	1) 3% OV-17 on Gas Chrom Q 2) 3% XE-60 on Gas Chrom Q 3) 12% DEGS on Gas Chrom Q	6 feet 6 feet 6 feet	90 170 200		+	
Beltsville	a) A/N			hexane			Florisil	1) 5% DC-200 on Aeropak 30	6 feet	190		+	
		b) chloroform methanol	16 hrs.	hexane and A/N			TLC	2) XE-60 on Gas Chrom Q	5 feet	175		+	

Note: A/N = acetonitrile
PE = petroleum ether
H = hexane
Ether = diethyl ether
DMSO = dimethylsulfoxide
DMF = dimethylformamide

TABLE 3.—*Analyses of test sample no. 1—standards*

Laboratory	Concentrations in mg/liter					
	Heptachlor Epoxide	Dieldrin	Endrin	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT
Canada	5.0	5.2	5.0	4.7	9.9	10.2
Euratom	4.3	5.2	4.9	4.0	9.1	9.3
Finland	[1] 3.2	5.4	6.8	5.1	10.5	10.8
Ireland	4.8	5.9	7.9	[1] 4.0	10.2	10.4
Netherlands						
CIVO	4.7	5.2	5.6	5.1	11.5	9.8
RIV	6.0	5.0	8.2	5.5	9.1	9.7
Vet. Tox.	4.4	5.3	5.2	5.0	10.0	9.3
Norway	[2] NC	5.1	5.0	4.1	8.7	9.0
Portugal	[3] 5.3	[3] 5.1	[3] 5.7	[3] 5.2	[3] 10.5	[3] 9.1
Spain	5.8	5.5	6.7	6.0	10.7	11.8

TABLE 3.—*Analyses of test sample no. 1—standards*—Continued

Laboratory	Concentrations in mg/liter					
	Heptachlor Epoxide	Dieldrin	Endrin	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT
Sweden	3.8	5.5	6.6	5.1	10.4	10.2
United Kingdom						
LGC	4.7	5.2	4.9	5.1	10.1	9.9
MAFF	5.0	4.2	5.0	4.7	10.6	10.0
NC	4.9	5.5	5.5	4.7	9.7	9.3
FFL	4.9	4.8	5.9	4.5	10.2	10.0
United States						
Laurel	5.1	5.3	6.0	5.1	9.0	9.5
Beltsville	5.0	5.9	5.9	5.0	9.2	10.0
TRUE VALUES	4.95	5.24	7.05	4.87	10.04	9.95

[1] Calculated indirectly.
[2] NC = present, but not calculated.
[3] Means of 2 methods.

TABLE 4.—*Analyses of test sample No. 3—cod-liver oil*

Laboratory	Concentrations in PPM														
	α-BHC	γ-BHC	β-BHC	Heptachlor	Aldrin	H-epoxide	Dieldrin	Endrin	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT	*o,p'*-DDT	*o,p'*-TDE	Hexachloro-benzene	PCB
Canada	0.075						0.10	0.087	0.32	0.41	0.50	0.055			(?)
Euratom									0.58	0.72	0.79				(?)
Finland							0.12		[1] 0.41	0.62	0.48	0.11			1.39
Ireland	0.14	0.05				0.02	0.37		0.51	0.68	1.44	(?)			NC
Netherlands															
CIVO															
RIV															
Vet. Tox.							≤0.05		0.31						T
Norway							[2] 0.17		[2] 0.33	[2] 0.58	[2] 0.50				
Portugal	0.07		0.11				0.016		0.50	0.66	0.63				
Spain	T	0.10			0.30				0.20	0.80	0.70				
Sweden							0.09		0.40	0.49	0.55	0.085	[1] 0.24		NC
United Kingdom															
LGC	0.03	0.01				0.02	0.08		0.35	0.50	0.65	0.20			NC
MAFF	0.056	0.16					0.33	? 0.20	0.37	0.80	1.10				
NC															
FFL							0.16		0.35	0.39	0.41				NC
U.S.A.															
Laurel		<0.005					<0.05		0.16	0.13	0.17	0.04			NC
Beltsville							0.18		0.21	0.25	0.25	0.22			

[1] + PCB interference.
[2] Means of two methods.
NOTE: ? = Suspected
T = Trace
NC = Present, but not calculated

TABLE 5.—*Analyses of test sample no. 4—chicken egg*

Laboratory	Concentrations in PPM														
	α-BHC	γ-BHC	β-BHC	Heptachlor	Aldrin	H-epoxide	Dieldrin	Endrin	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT	*o,p'*-DDT	*o,p'*-TDE	Hexachlorobenzene	PCB
Canada							0.18	0.11	0.12	0.035	0.51	0.11			
Euratom							0.20	0.16	0.11	0.02	0.51				
Finland							0.21	0.15	0.14	0.028	0.59				
Ireland		0.025					0.11	(?)	0.09	0.04	0.17				
Netherlands															
CIVO							0.16	0.14	0.13		0.47			0.039	
RIV		[1] 0.08					0.23	0.36	0.16	0.07	0.62			T	
Vet. Tox.							0.17	0.11	0.09	0.04	0.39			0.14	
Norway							0.135	0.15	0.088	0.028	0.42				
Portugal		0.03	0.05				0.18	0.11	0.17	0.03	0.39				
Spain		0.04			0.14		0.13		0.10	0.05	0.50	0.21			
Sweden							[2] 0.044	[2] 0.039	[2] 0.034	[2] 0.004	[2] 0.10				
United Kingdom															
LGC							[3] 0.145	[3] 0.09	[3] 0.105		[3] 0.36			[3] 0.035	
MAFF		0.009					0.15	0.11	0.23	0.10	0.53				
NC															
FFL							0.24	0.13	0.16	0.06	0.58			0.09	
U.S.A.															
Laurel							0.08	0.07	0.08		0.49				
Beltsville							0.11	0.08	0.12		0.14				

[1] + HCB.
[2] Homogenate only.
[3] Means of two methods.
NOTE: ? = Suspected
T = Trace

TABLE 6.—*Analyses of test sample no. 5—sprat*

Laboratory	Concentrations in PPM														
	α-BHC	γ-BHC	β-BHC	Heptachlor	Aldrin	H-epoxide	Dieldrin	Endrin	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT	*o,p'*-DDT	*o,p'*-TDE	Hexachlorobenzene	PCB
Canada		0.016					0.19	0.13	0.083	0.10	0.087				NC
Euratom							0.18	0.17		0.09	0.05				0.30 approx.
Finland					0.005		0.12	0.13	NC	0.067	0.061				1.70
Ireland	0.02	0.02	0.04			0.02	0.04	0.21	0.15	0.14	0.20				NC
Netherlands															
CIVO							0.10	0.09	0.07						0.80
RIV															
Vet. Tox.							0.11	0.10	0.025					0.04	0.57
Norway							[1] 0.075	[1] 0.12	[1] 0.068	[1] 0.093	[1] 0.050				NC
Portugal															

TABLE 6.—*Analyses of test sample no. 5—sprat*—Continued

Laboratory	Concentrations in PPM														
	α-BHC	γ-BHC	β-BHC	Heptachlor	Aldrin	H-epoxide	Dieldrin	Endrin	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT	*o,p'*-DDT	*o,p'*-TDE	Hexachloro-benzene	PCB
Spain	0.098	0.022			0.046		0.024	T	0.025	0.043	0.11	T			0.65
Sweden		0.03					0.10		0.06	0.15	0.15				2.0
United Kingdom															
LGC	[2] 0.02	[2] 0.015	[2] 0.005			[2] 0.005	[2] 0.09	[2] 0.09	[2] 0.03	[2] 0.04	[2] 0.02				NC
MAFF		0.014					0.13	0.12	0.17	0.28	0.25				
NC															
FFL							0.22	0.18	0.072	[3] 0.16	[3] 0.23			0.10	NC
U.S.A.															
Laurel		0.01				0.005	0.20	0.10	<0.04	0.08	<0.07				>0.50
Beltsville		0.01				0.01	0.10	0.10	0.01	0.10	0.02				0.80 approx.

[1] Means of two values.
[2] Homogenate only.
[3] Identity uncertain.
NOTE: T = Trace; NC = Present, but not calculated.

TABLE 7.—*Summary of analyses of test sample no. 1*

Residue	Concentrations in mg/liter					
	No. of Determinations	True Concentration	Means of Analyses	Standard Deviation	Coefficient of Variation	Proportion within ±5% of Mean
Heptachlor epoxide	15	4.95	4.913	±0.54	±11.0%	9 out of 15
Dieldrin	17	5.24	5.253	±0.39	± 7.4%	13 out of 17
Endrin	17	7.05	[1] 5.929	±1.01	±17.1%	5 out of 17
p,p'-DDE	16	4.87	4.931	±0.49	± 9.9%	11 out of 16
p,p'-TDE	17	10.04	9.965	±0.75	± 7.5%	9 out of 17
p,p'-DDT	17	9.95	9.900	±0.69	± 7.0%	10 out of 17

[1] See discussion of results for Sample No. 1 in text.

TABLE 8.—*Analyses of starlings*

Location		Weight (grams)	Mean Values and Ranges in PPM				
			Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
Canada							
New Brunswick	singly (10)	71-96	0.045 (0.014-0.151)	0.349 (0.058-1.28)	0.021 (0.004-0.092)	0.030 (0.004-0.166)	
	5 groups of 5	71-97	0.095 (0.023-0.262)	0.481 (0.126-0.803)	0.020 (0.014-0.025)	0.016 (0.008-0.023)	
British Columbia	Singly (10)	71-97	0.036 (0.004-0.125)	0.520 (0.039-3.30)	0.024 (0.006-0.124)	0.038 (0.005-0.174)	
	5 groups of 5	74-100	0.035 (0.007-0.057)	0.306 (0.111-0.636)	0.011 (0.005-0.019)	0.008 (0.004-0.012)	

TABLE 8.—*Analyses of starlings*—Continued

Location		Weight (grams)	Mean Values and Ranges in PPM				
			Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
Canada—Continued							
Ontario	singly (10)	73-88	0.014 (0.003-0.040)	0.567 (0.075-1.51)	0.006 (0.002-0.012)	0.011 (0.007-0.015)	
	5 groups of 5	79-105	0.009 (0.003-0.015)	0.423 (0.146-0.861)	0.017 (0.007-0.050)	0.022 (0.004-0.056)	
Euratom							
Ispra	singly (10)	72-93	≤0.001	0.172 (0.05-0.35)	0.043 (0.03-0.06)	0.065 (0.04-0.12)	
	5 groups of 4	72-91	≤0.001	0.164 (0.12-0.24)	0.048 (0.03-0.06)	0.066 (0.05-0.08)	
Finland							
Evo	singly 5	80-96	0.013 (<0.01-0.03)	0.09 (0.07-0.11)	<0.01	0.034 (<0.01-0.11)	0.15-0.19
Ryttylä	singly (18)	61-77	<0.01	0.085 (0.04-0.35)	<0.01	0.01 (<0.01-0.03)	0.04-0.35
	5 groups of 5		0.02 (<0.01-0.08)	0.10 (0.08-0.12)	<0.01	0.018 (<0.01-0.04)	0.11-0.19
Netherlands							
Oude Molen	singly (10)	72-80	0.029 (0.01-0.07)	0.29 (0.05-1.50)	NC	0.10 (0.06-0.23)	
	6 groups of 4	62-90	0.027 (0.02-0.04)	0.19 (0.11-0.31)	NC	0.15 (0.10-0.18)	
Eext	singly (10)	74-90	0.04 (0.01-0.09)	0.24 (0.03-1.29)	NC	0.16 (0.07-0.41)	
	2 groups of 5	62-85	0.01 (<0.01-0.02)	0.23 (0.08-0.38)	NC	0.12 (0.10-0.13)	
Beers Mill	singly (10)	70-84	0.05 (<0.01-0.13)	0.30 (0.09-1.26)	NC	0.23 (0.12-0.41)	
	4 groups of 4	62-83	0.03 (0.02-0.04)	0.18 (0.08-0.26)	NC	0.12 (0.08-0.16)	
Norway	singly (24)	72-100					
Method (a)			0.042 (0.01-0.14)	0.073 (0.02-0.17)	<0.01	<0.001	
Method (b)			0.032 (<0.01-0.17)	0.091 (0.03-0.18)	<0.01	<0.001	<0.01-0.03
Portugal							
Ourique	singly (3)	84-89	0.003 (0.001-0.007)	0.044 (0.035-0.150)	0.003 (0.003)	0.007 (0.006-0.008)	
Campo Maior	singly (4)	78-90	0.012 (0.004-0.024)	1.03 (0.61-1.20)	0.0045 (<0.001-0.011)	0.004 (<0.001-0.015)	
S. Mansor	singly (2)	—	0.245 (0.137-0.353)	<0.001	<0.001	<0.001	
Spain							
Maderuelo (Segovia)	singly (5)	84-99	0.013 (0.010-0.020)	0.042 (0.022-0.063)	0.057 (0.035-0.080)	0.099 (0.055-0.150)	T
	4 groups of 5	88-97	T	0.071 (0.030-0.140)	0.076 (0.062-0.096)	? 0.241 (? 0.047-0.860)	
Sweden							
Krankesjön	singly ♂ (17)	25-97	0.013 (T-0.062)	0.124 (0.039-0.300)	<0.003	<0.003	0.066 (0.027-0.180)
	♀ (13)	50-97	0.012 (T-0.065)	0.069 (0.037-0.110)	<0.003	<0.003	0.033 (0.017-0.056)
Villingsberg	singly (10)	72-91	0.005 (<0.001-0.017)	0.072 (0.015-0.130)	<0.003	<0.003	0.132 (0.049-0.300)
Kvismaren	singly (10)	80-93	0.013 (T-0.037)	0.074 0.026-0.110)	<0.003	<0.003	0.078 (0.030-0.200)
Boda Bruk	singly (10)	76-94	0.037 (<0.001-0.200)	0.081 (0.024-0.230)	<0.003	<0.001	0.114 (0.020-0.360)

TABLE 8.—*Analyses of starlings*—Continued

Location		Weight (grams)	Mean Values and Ranges in PPM				
			Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
United Kingdom							
Nature conservancy	singly (7)	81-98	0.027 (0.01-0.09)	0.049 (0.02-0.08)	—	(0.055) (<0.01-0.10)	
	4 groups of 5	78-94	0.045 (0.01-0.09)	0.053 (0.03-0.08)	—	0.030 (0.01-0.07)	
United States							
Patuxent	singly (10)	83-93	0.11 (<0.02-0.42)	0.32 (0.07-0.92)	<0.007	0.05 (<0.02-0.26)	T
	5 groups of 5	82-86	<0.01 (<0.01-0.09)	0.17 (0.11-0.29)	<0.01	<0.01 (<0.01-0.03)	

NOTE: T = Trace
NC = Present, but not calculated.
? = Suspected

TABLE 9.—*Analyses of mussels*

Location	Weight (grams)	Length of Shell (centimeters)	Mean Values and Ranges in PPM				
			Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
Canada							
Portage Island (10)		6.3-6.8	0.001 (<0.0001-0.001)	0.016 (0.006-0.041)	0.012 (0.004-0.016)	0.010 (0.002-0.053)	
St. John (10 groups of 5)		2.6-3.2	0.001 (<0.0001-0.002)	0.027 (0.009-0.091)	0.016 (0.008-0.030)	0.057 (0.020-0.104)	
British Columbia (10)	Fat 0.5%	4.0-5.0	0.002 (<0.0001-0.006)	0.021 (0.007-0.106)	0.010 (0.002-0.062)	0.038 (0.015-0.126)	
Euratom							
Magra-Tillaro, 7 groups of 5	6.5-16.5	4.2-6.5	T ≤0.005	0.015 (0.012-0.021)	0.026 (0.020-0.030)	0.078 (0.046-0.099)	Absent
Ireland							
Clonakilty (10) singly		6.0-8.0	0.03 (0.01-0.05)	0.02 (<0.01-0.07)	<0.01	<0.01	
(10) group		6.2-7.3	0.03	0.02			
Kinsale (10) singly		6.9-8.3	0.016 (<0.01-0.03)	0.01 (<0.01-0.02)	<0.01	<0.01	
(10) group			0.01	0.02			
East Ferry: (10) singly		7.1-7.5	0.04 (0.02-0.06)	0.02 (0.01-0.07)	<0.01	<0.01	
(10) group			0.04	0.05			
Finland							
6 groups of 10	5.02-6.71	2.8-3.1	<0.001	<0.01 (<0.01-0.01)	<0.01 (<0.01-0.01)	0.017 incl. *o,p'*-DDT (<0.01-0.03)	0.06
Netherlands							
1967 IJmuiden	5.5-14.5		0.016 (0.010-0.020)	0.020 (0.013-0.025)	NC	NC	0.44
Scheveningen	12.7-15.1		0.042 (0.031-0.057)	0.032 (0.022-0.041)	NC	NC	0.47
Grevelingendam W	10.0-24.3		0.009 (0.006-0.011)	≤0.009	NC	NC	0.26
Grevelingendam E	13.5-18.8		0.006 (0.004-0.007)	0.022 (0.018-0.031)	NC	NC	0.46

TABLE 9.—*Analyses of mussels*—Continued

Location	Weight (grams)	Length of Shell (centimeters)	Mean Values and Ranges in PPM				
			Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
Netherlands—Continued							
1968 Wadden Sea	24.6		<0.007	NC	NC	NC	
Schiermonnikoog	18.1		0.013	NC	NC	NC	
Den Helder	26.5		0.018	NC	NC	NC	
IJmuiden	25.2		0.018	NC	NC	NC	
Scheveningen	17.9-18.4		0.046 (0.039-0.053)	NC	NC	NC	Mean 0.46
Hoek van Holland	21.7		0.034	NC	NC	NC	
Grevelingendam N	29.4		0.015	NC	NC	NC	
Grevelingendam E	34.2		0.010	NC	NC	NC	
Westkapelle	29.4		0.012	NC	NC	NC	
Norway							
singly	52-113	7.3-8.9	0.0021 (0.001-0.003)	0.0015 (0.001-0.002)	0.0033 (0.002-0.006)	0.0036 (<0.001-0.020)	
3 groups of 3, 5, 6		2.2-3.2	T	0.005 (0.004-0.006)	<0.001	0.009 (<0.001-0.027)	0.004
Portugal							
Aveiro		5.8-8.3	0.004 (0.001-0.005)	0.00º (0.004-0.013)	0.004 (0.002-0.006)	0.012 (0.005-0.017)	
Setubal		6.6-9.2	0.006 (0.003-0.010)	0.024 (0.020-0.034)	0.020 (0.009-0.035)	0.059 (0.037-0.084)	
Cascais		3.0-6.1	0.034 (0.022-0.047)	0.036 (0.020-0.062)	0.059 (0.039-0.083)	0.184 (0.128-0.278)	
Spain							
Vigo	12.03-40.30	7.52-9.20	0.001	0.014	0.046	0.053	
Barcelona	2.74-5.11	4.16-5.30	<0.001	0.033	0.304	0.140	T
Sweden							
Fiskebäckskil	10.51-20.87	6.5-7.9	<0.001	0.004 (0.001-0.013)	<0.01	0.010 (0.004-0.026)	0.048
Graddö			<0.001	0.003	<0.01	0.010	0.032
Askö	4.5-6.6	3.14-3.36	<0.001	0.008 (0.002-0.017)	<0.01	0.015 (0.006-0.023)	0.036
Lundakrabukten	4.53-6.19	3.63-3.76	<0.001	0.007 (0.005-0.009)	<0.01	0.007 (0.003-0.011)	0.029
United Kingdom							
R. Roach			0.0230 (0.012-0.047)	0.0254 (0.018-0.033	0.0304 (0.019-0.055)	0.011 (0.008-0.018)	
R. Crouch			0.0204 (0.012-0.036)	0.0230 (0.005-0.072)	0.0328 (0.010-0.073	0.005 (0.002-0.012)	
Loch Linnhe		5.0-6.0	0.00335 (0.002-0.006)	0.0214 (0.013-0.037)	0.0157 (0.009-0.032)	<0.016 (<0.01-<0.02)	
Lochgoilhead		5.8-7.3	0.0168 (0.006-0.035)	0.0240 (0.007-0.087)	0.0279 (0.007-0.042)	<0.018 (<0.01-0.3)	T
United States							
Modiolus demissus, groups	8.20-14.20	4.1-4.9	0.02 (<0.02-0.04)	0.13 (0.08-0.18)	0.10 (0.07-0.13)	0.08 (0.05-0.12)	T

NOTE: T = Trace; NC = Present, but not calculated (due to PCB interference).

TABLE 10.—*Analyses of pike*

Location	Weight (kilograms)	Length (centimeters)	Age (year)	Means and Ranges in PPM				
				Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
Canada								
Saskatchewan	1.02-2.61	53.4-67.3	4-5	0.000 (<0.0001-0.001)	0.024 (0.007-0.049)	0.010 (0.003-0.023)	0.035 (0.013-0.079)	
Quebec	1.14-3.79	58.5-76.2	4-6	0.000 (<0.0001-0.001)	0.009 (0.005-0.016)	0.003 (0.001-0.006)	0.013 (0.005-0.030)	

TABLE 10.—*Analyses of pike*—Continued

Location	Weight (kilograms)	Length (centimeters)	Age (year)	Means and Ranges in PPM				
				Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
Euratom								
Ispra	0.49-6.00	39-100	2-6	<0.005	1.36 (0.26-4.13)	0.18 (0.06-0.53)	0.41 (0.08-1.57)	
Finland								
Lintuselkä	1.06-1.73	55-65	4	<0.005	<0.01	<0.01	0.018 (0.01-0.03)	
Hoikanlahti	0.61-1.45	48-60	4-6	<0.005	<0.01	<0.01	<0.01	
Ireland								
L. Carra	1.56-8.16	53-94	5-9	0.044 (0.01-0.22)	0.020 (<0.01-0.05)	T	T	
Netherlands	0.262-0.364	30.9-34.5		0.0022 (0.0010-0.0034)	0.0057 (0.004-0.007)	T	T	Mean 0.046
Norway								
Method (a)	0.31-3.05	36-86	2-11	<0.001	0.0094 (0.004-0.019)	0.0021 (0.001-0.006)	0.0026 (0.001-0.006)	
Method (b)				<0.001	0.0138 (0.005-0.045)	<0.001	0.0041 (T-0.010)	0.002-0.011
Spain								
Santillana (2)	0.73-1.20	42.5-54.0	2	<0.001	0.026 (0.018-0.034)	0.049 (0.023-0.076)	0.010 (<0.001-0.020)	T
Buendia (1)	2.10	66.5	3	<0.001	0.090	0.028	0.036	T
Garcia Sola (2)	4.65-1.76	78.5-60.5	2-4	<0.001	0.815 (0.790-0.840)	0.310 (0.130-0.490)	0.018 (<0.001-0.037)	T
Sweden								
Lake Bolmen	0.600-0.975	39-44		T	0.011 (0.005-0.016)	<0.01	0.031 (0.015-0.045) (included *o,p'*-DDT)	0.024
United Kingdom								
Loch Tulla	0.67-1.43	46-55		0.0011 (<0.001-0.003)	0.0146 (0.006-0.021)	0.0046 (0.002-0.008)	0.0043 (0.002-0.008)	
Loch Choin	0.45-1.67	40-62		<0.001 (<0.001-0.001)	0.0057 (0.003-0.009)	0.0034 (0.002-0.006)	0.0037 (0.002-0.008)	
Loch Skiach	1.85-6.13	64-93		<0.001 (<0.001-0.002)	0.0166 (0.008-0.040)	0.0024 (0.001-0.005)	0.0055 (0.003-0.010)	
Loch Glassie	0.45-1.48	42-64		<0.0018 (<0.001-0.002)	0.0036 (0.002-0.007)	0.0032 (0.001-0.011)	0.0044 (0.002-0.008)	
United States								
Garrison Reservoir (2)	2.72-3.49	73.7-79.2	4	<0.002-0.003	0.006-0.01	0.003-0.009	0.006-0.02	T

NOTE: T = Trace.

TABLE 11.—*Analyses of dogfish*

Location		Weight (grams)	Length (centimeters)	Means and Ranges in PPM				
				Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
Euratom								
La Spezia	lat. muscle (4)	588-9500	54-104	<0.005	0.15 (<0.01-0.38)	0.09 (0.004-0.21)	0.26 (0.019-0.57)	T
	liver (4)			<0.005	3.72 (1.5-9.3)	1.50 (0.62-3.94)	6.76 (2.05-18.6)	
Ireland								
Dingle	muscle (2)	917-950	60	0.03 (0.02-0.04)	0.025 (0.02-0.03)	T	0.20 (0.06-0.35)	
Howth	muscle (5)	843-966	62-65	0.04 (0.01-0.07)	0.03 (0.02-0.04)	T	0.31 (0.08-0.66)	

TABLE 11.—*Analyses of dogfish*—Continued

Location			Weight (grams)	Length (centimeters)	Means and Ranges in PPM				
					Dieldrin	DDE	TDE	*p,p'*-DDT	PCB
Norway	muscle	(10)	2700-5400	86-107					
Method (a)					0.022 (<0.01-0.04)	0.030 (0.01-0.06)	0.032 (0.01-0.04)	0.092 (0.020-0.20)	
Method (b)					<0.016 (<0.01-0.03)	0.063 (0.03-0.12)	0.023 (0.01-0.06)	0.185 (0.04-0.38)	0.01-0.04
Spain									
Barcelona	muscle	(4)	588-735	53-60	<0.001	0.318 (0.242-0.468)	0.344 (0.298-0.386)	0.221 (T-0.404)	T
Vigo (*S. Blainvillei*)	muscle	(4)	770-1370	55-65	<0.001	T	0.016 (0.009-0.044)	0.013 (T-0.053)	T
Sweden									
Lysekil	muscle	(7)	1690-5950	81-111	<0.001	0.051 (0.012-0.110)	T	0.091 (0.015-0.210)	0.145
	liver	(7)			<0.001	0.187 (0.044-0.720)	T	0.310 (0.039-1.100)	0.350
United Kingdom									
E. of Shetland	muscle	(5)			0.014 (<0.01-0.02)	0.058 (0.02-0.11)	0.036 (0.01-0.07)	0.102 (0.02-0.23)	T
	liver	(5)			0.01 (<0.01-0.04)	0.104 (0.03-0.19)	0.108 (0.03-0.24)	0.192 (0.05-0.51)	

NOTE: T = Trace.

TABLE 12.—*Statistical analysis of DDE residue data from starlings*

Country	Area	No. of Specimens	Geometric Mean	Variance	Standard Deviation
Canada	N. Brunswick	10	0.217	0.19	2.75
	British Columbia	10	0.193	0.33	3.76
	Ontario	10	0.340	0.24	3.12
U.S.A.		10	0.157	0.21	2.88
Netherlands	Oudemolen	10	0.140	0.25	3.19
	Eext	10	0.120	0.24	3.12
	Beers Mill	10	0.215	0.10	2.07
Euratom		11	0.157	0.060	1.76
Finland	Ryttla 1	7	0.072	0.052	1.69
	Ryttla 2	18	0.070	0.063	1.78
Norway	Method 1	25	0.064	0.055	1.72
	Method 2	23	0.078	0.046	1.64
Sweden	Krankesjon M	19	0.104	0.059	1.75
	Villingsberg	12	0.053	0.080	1.92
	Kvismaren	12	0.058	0.073	1.86
	Boda bruk	12	0.055	0.114	2.18
	Krankesjon F	13	0.066	0.020	1.38
Spain		6	0.040	0.025	1.44
U Kingdom		7	0.044	0.043	1.62

Sample sizes required to detect a given percent difference between two means.

	25%	50%	75%	100%	150%	200%
Canada, U.S.A., Netherlands		60		25	15	10
All other countries	50	20	10	8		

TABLE 13.—*Statistical analysis of DDE residue data from pike*

Country	Area	No. of Specimens	Geometric Mean	Variance	Standard Deviation
Canada	Saskatchewan	12	0.018	0.11	2.15
	Quebec	10	0.0083	0.05	1.64
Euratom		11	0.941	0.13	2.28
Netherlands		10	0.0056	0.009	1.24
Norway	Method 1	11	0.0078	0.06	1.74
	Method 2	12	0.011	0.10	2.04
Sweden		10	0.0098	0.04	1.54
U Kingdom	Loch Tulla	12	0.013	0.03	1.52
	Loch Choin	12	0.0054	0.02	1.42
	Loch Skiach	12	0.014	0.05	1.69
	Loch Glassie	12	0 0032	0.04	1.59

Sample sizes required to detect a given percent difference between two means

	25%	50%	75%	100%
All countries	50	20	10	8

Organochlorine and Heavy Metal Residues in Bald Eagle Eggs

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ABSTRACT

Bald eagle eggs collected in 1968 from nests in Wisconsin, Maine, and Florida all contained residues of DDE, DDD, dieldrin, heptachlor epoxide, and polychlorinated biphenyls. Many also contained traces of DDT. Eggs from five nonproductive nests sampled in Maine contained much higher residues than did eggs collected from either productive or nonproductive nests in Wisconsin and Florida.

Introduction

During the past 2 decades, bald eagles (*Haliaeetus leucocephalus*) have declined throughout the United States, exclusive of Alaska, and have had reduced reproductive success in many areas (*1,3,13*). A number of other raptorial and fish-eating birds at the top of ecological food chains have declined similarly (*6*). Various authors have related these declines to widespread distribution of organochlorine pesticides in the environment (*6,7,10*). Accumulation of pesticide residues by eagles has been shown (*11,14*), and mortality due to pesticide poisoning has been recorded (*12*).

This study was undertaken to explore the relationship between reproductive success of eagles and the residue content of their eggs. The approach was to compare residues in eagle eggs from Maine, where nesting success of bald eagles has been poor for many years, with residues in eggs from Wisconsin and from the Everglades Park area of Florida where eagles were known to nest more successfully.

This paper reports the results from egg collections made in 1968 from 20 nests in Wisconsin, Maine, and Florida, and from 1 nest in Maine in 1967. It includes results of chemical analyses for organochlorine pesticides and certain heavy metals, shell thickness measurements, and histories of nesting success.

Sampling Procedures

Reproductive histories of bald eagle nests in Wisconsin and Maine came from the records of the National Audubon Society and were obtained during their nationwide surveys. Histories of the Florida nests, all within the Everglades National Park in southern Florida, came from National Park Service records. These reproductive histories are shown in Table 1.

One randomly selected egg was collected from each of 21 different nests—10 in Wisconsin, 5 in Maine, and 6 in Florida. Both producing and nonproducing nests were included from Wisconsin and Florida, but we were not successful in obtaining eggs from any successful nests in Maine, so only nonproducing nests were sampled there. After the normal incubation period, five additional unhatched eggs were collected from three of the Wisconsin nests.

Nine of the Wisconsin eggs were collected in the early stages of incubation, between March 21 and April 1. Five had no visible signs of development, and four contained embryos 3-12 days old. Four late, unhatched eggs collected on June 1 and 2 from three of these same nests showed no signs of development. The egg from the 10th Wisconsin nest (Osgood Spring Lake), collected June 2, showed no signs of development.

Maine eggs were collected between April 28 and May 22 in 1968 and on July 13, 1967; all were addled or dry.

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TABLE 1.—*Reproductive histories of bald eagle nests from which eggs were taken in 1967 and 1968*

[blanks = no data available]

Nest Location	Number of Young Produced Per Year							
	1960	1961	1962	1963	1964	1965	1966	1967
WISCONSIN								
Pickerel Lake				2	2			2
Cranberry Lake			2	1	0	0	1	2
Mud Lake			0	0			0	0
Windigo Lake	2	2	1	0	1	0	2	2
Deer Lake	2	2		2	1	0	3	1
Pacwawong Lake				2	2	0	2	1
Barnes Lake				0		0	0	0
Sanborn Lake			0	0	0	1	0	0
Rainbow Flowage	2		0	0	2	1	2	2
Osgood Spring Lake		2		1	0	1	1	2
Average young/nest	2.0	2.0	0.6	0.8	1.0	0.2	1.2	1.2
MAINE								
Little Swan Island					0	0	0	0
Birch Island					0	0	0	0
Kennebec River				0	0	0	0	0
Brandy Pond						0	0	0
Swan Island			0	0	0	0	0	0
Average young/nest					0	0	0	0
FLORIDA								
Buoy Key	1	1	1	2	0	0	1	1
Cormorant Key	0	1	1	0	1	0	0	1
Palm Key		0	0	0	0	0	0	1
Deer Key	2	2	2	1	2	0	1	2
Crab Key	2	1	1	1	1	2	0	2
Manatee Key	1	1	0	2	1	2	2	0
Average young/nest	1.2	1.0	0.8	1.0	0.8	0.7	0.7	1.2

Eggs from the Florida nests were collected January 11 and 12. One showed no signs of development, and five contained embryos 6-31 days old.

After collection in the field, each egg was wrapped in several layers of aluminum foil, individually packed in a can filled with vermiculite, and shipped to Patuxent, where subsequent examinations were made. Most eggs were not frozen prior to shipment and examination. Shells were washed with acetone before opening. The age of developing embryos was estimated on the basis of the 34-35 day incubation period suggested by Herrick (*5*). Shells were dried in an herbarium dryer at 80 F for 16 hours; shell thickness was measured at the waist, using a Starrett 1010M dial gauge micrometer graduated in units of 0.01 mm.

Egg volume was measured by water displacement, and these values were used to compute the parts per million (ppm) concentration of pesticides, taking 1.0 as an assumed specific gravity, as described by Stickel *et al.* (*14*). This procedure provides more nearly comparable readings for eggs that have lost different amounts of moisture in the field than do uncorrected weights. Greatest length and breadth were measured for each egg, and these measurements were used to estimate the volume of the five eggs whose volume was not measured. The equation for this estimate was volume (ml) = 3.73 x length (cm) x breadth (cm) —35.3 (*L. F. Stickel and S. N. Wiemeyer, manuscript in preparation*).

The difference between measured volume and computed volume in a test series of 24 eagle eggs averaged 1.9%.

Analytical Procedures

Eggs were analyzed individually for residues of organochlorine pesticides and for heavy metals, including lead, copper, zinc, cadmium, iron, and nickel. Each egg was mixed in an Omnimixer; three 20-g aliquots were taken, one for pesticide analysis, one for heavy metal analysis, and one for storage for possible future needs.

The efficiency of the mixing procedure and the effect of sample size on the recovery of organochlorine pesticides were evaluated experimentally with ringneck pheasant (*Phasianus colchicus*) eggs before the analysis of eagle eggs was begun. Pheasant eggs were from birds fed *p,p'*-DDD or *p,p'*-DDE. Six separate pools of eggs were prepared, each containing an equal number of eggs from DDD-fed birds and from DDE-fed birds. The pools weighed 91-111 g each (similar to the weight of the contents of an eagle egg) and contained four fresh eggs or six embryonated eggs. Each pool was mixed in an Omnimixer, and two aliquots were taken—one of 70 g and one of 20 g. Each aliquot was fortified with dieldrin to a concentration of 4 ppm. Three of the pools were from fresh eggs and three from eggs that contained embryos in various stages of development.

Samples were ground with anhydrous sodium sulfate and extracted for 7 hours with petroleum ether in Soxhlet apparatus. Extracts were concentrated, taken up in 50 ml of hexane and partitioned four times with 50-ml portions of acetonitrile. The acetonitrile was evaporated to dryness at room temperature, and the pesticides were eluted on a 2 x 15 cm column of partially inactivated Florisil with 200 ml of 3:1 hexane-benzene mixture. The pesticides in the clean extract were separated and removed in four fractions from a thin layer plate as described by Mulhern (*8*). The four fractions were then analyzed separately by electron capture gas chromatography.

All four fractions were analyzed on an OV-17 column, and the residues were confirmed on either a 3% XE-60 column or a 12% DEGS column. Details of the analytical procedure and column specifications for the OV-17 and XE-60 columns are given by Reichel *et al.* (*12*). The DEGS column support was Anakrom SD, 100/110 mesh size; nitrogen flow rate was 85 ml/minute; column temperature was 190 C; retention time of dieldrin on this column is 9.5 minutes. Average recovery by this method is 85-96%. The lower limit of sensitivity was approximately 0.05 ppm wet weight. Residues were not

corrected for percent recovery. In addition to zonal separation by thin layer chromatography (TLC) and analysis on two gas chromatographic columns, residues in 20% of the samples were confirmed by TLC (silica gel plate—2% ethyl ether in hexane solvent).

Recovery from the 20-g and 70-g aliquots was compared by t-tests on the paired observations from each pool (Table 2). Recovery of *p,p'*-DDE from the 70-g, fresh-egg aliquots was significantly ($P<0.05$) lower than from the 20-g aliquots. This may have been related to the combination of the higher lipid content and the greater microgram quantities. No such differences occurred between recoveries of DDE from different sized aliquots of embryonated eggs. There were no significant differences in recovery of DDD or dieldrin from the aliquots of the two sizes in either fresh or embryonated eggs.

TABLE 2.—*Recovery of organochlorine pesticides from 20-g and 70-g aliquots of pools of pheasant eggs*

Sample Pool	Condition of Eggs	Concentrations in ppm (Wet Weight)					
		p,p'-DDE		*p,p'*-DDD		Dieldrin	
		Aliquot Size		Aliquot Size		Aliquot Size	
		20 g	70 g	20 g	70 g	20 g	70 g
1	Fresh	78.16	[1]58.53	0.40	0.59	3.46	3.70
2	Fresh	75.22	[1]50.48	0.56	0.39	3.99	3.39
3	Fresh	80.43	[1]52.20	0.57	0.48	4.03	4.04
4	Embryonated	86.40	73.36	0.83	0.83	4.09	3.84
5	Embryonated	93.01	94.48	0.61	0.63	3.78	3.78
6	Embryonated	72.46	75.17	0.63	0.38	4.01	3.84

[1] Recovery significantly lower ($P<0.05$) than from the 20-g aliquot.

It was concluded that the mixing procedure was adequate and that a 20-g sample was sufficient. Eagle egg aliquots of 20-g each, therefore, were analyzed by the procedures described above.

For the metal analyses, a 20-g aliquot of the homogenized egg was weighed and dried at 110 C to constant weight. Removal of organic matter and dissolution of ash for subsequent analysis were as described by Bagley *et al.* (*2*). The Perkin-Elmer atomic absorption spectrophotometer, Model 303, was used for all determinations. Instrument settings were essentially those recommended in the Analytical Methods for Atomic Absorption Spectrophotometry (*9*).

Results

The results of analysis for organochlorine pesticides are shown in Table 3. In addition, all eggs contained residues of polychlorinated biphenyl (PCB) compounds as evidenced by comparing the numerous peaks on the gas chromatograms with peaks produced by commercial preparations of PCB's. The presence of PCB's was confirmed in one egg each from Wisconsin, Maine, and Florida by combined gas chromatograph and mass spectrographic analysis. The various PCB compounds contained three to eight atoms of chlorine per molecule.

Eggs taken in 1968 from the four nonproductive nests in Maine contained DDE concentrations averaging 21.76 ppm (range 13.20-27.55 ppm) and dieldrin averaging 1.41 ppm (range 0.31-312 ppm). The 1967 egg contained 18.00 ppm of DDE and 2.54 ppm of dieldrin.

Residue concentrations in eggs from Florida and Wisconsin were similar to each other except for the one egg from Buoy Key, Fla. which contained 27.93 ppm of DDE. DDE concentration in Florida eggs averaged 10.72 ppm including the Buoy Key egg and 7.27 ppm (range 4.29-11.66 ppm) excluding it. DDE concentration in Wisconsin eggs averaged 4.76 ppm (range 1.81-15.27 ppm). Dieldrin concentrations in Florida eggs averaged 0.21 ppm (range 0.11-0.28 ppm) and in Wisconsin eggs averaged 0.37 ppm (range 0.07-1.20 ppm); they were not significantly different from each other.

Reproductive histories of the nests are shown in Table 1. Although eagles tend to return to the same nest year after year, some may shift nest sites (*4*). There also may be replacement of birds in a mated pair. Therefore, the relationship between successful nesting in past years and residues found in eggs during 1968 is not a precise one on a nest by nest basis. Also, residue levels in the food supply in a local area change from year to year and further complicate interpretation.

The residues in eggs from Florida nests with various histories showed no obvious relationship to these histories, and the same was true for eggs from Wisconsin (Tables 1 and 3). All eggs from Maine came from unsuccessful nests, so this comparison could not be made for that area.

Shell thickness of Wisconsin eggs averaged 0.55 mm and that of Maine eggs, 0.53 mm. These measurements were very similar despite the higher concentrations of both DDE and dieldrin in the Maine eggs. Shell thickness of Florida eggs, which averaged 0.50 mm, cannot be compared directly with shell thickness of eggs from Wisconsin and Maine because of the likelihood of latitudinal differences and because of the smaller egg size.

The question of whether the 1968 eagle eggs had thinner shells than eggs from the same areas in the years before 1947 will be dealt with in a publication by D. W. Anderson on the measurements of an historical series. Preliminary results suggest that such thinning has occurred in Florida. (*7*).

The results of analysis for copper, iron, and zinc are shown in Table 4. No detectable quantities of lead, cadmium, or nickel were found. These results are presented for the record only, since data are inadequate for interpretation.

Conclusion

Eagle eggs collected from five nonproductive nests in Maine contained much higher residues of DDE and of dieldrin than did eggs collected from either productive or nonproductive nests in Wisconsin and Florida.

See Appendix for chemical names of compounds mentioned in this paper.

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TABLE 3.—*Residues of organochlorine pesticides in bald eagle eggs collected in Wisconsin, Maine, and Florida in 1968*

[T = <0.05 ppm]

Location	Shell Thickness (mm)	Egg Volume (ml)	Residues in ppm [1]				
			p,p'-DDE	*p,p'*-DDD	*p,p'*-DDT	Dieldrin	Heptachlor Epoxide
WISCONSIN							
Pickerel Lake	0.60	123	1.81	0.10	T	0.10	0.01
Cranberry Lake (1)	0.54	111	15.27	0.49	0.13	0.65	0.06
(2)	0.50	[2][111]	3.36	0.44	—	0.48	0.04
Mud Lake (1)	0.60	129	7.63	0.38	0.22	0.12	0.02
(2)	0.59	[2][120]	3.06	0.30	—	0.11	0.01
(3)	0.62	134	3.58	0.24	—	0.07	0.006
Windigo Lake (1)	0.48	123	6.91	0.22	0.16	0.19	0.03
(2)	0.55	125	7.32	0.23	T	0.12	0.02
(3)	0.53	108	8.56	0.40	—	0.18	0.01
Deer Lake	0.56	116	3.08	0.25	T	0.15	0.01
Pacwawong Lake	0.52	130	3.61	0.24	—	0.33	0.01
Barnes Lake	0.52	110	5.22	0.31	0.23	0.19	0.01
Sanborn Lake	0.60	122	2.85	0.12	T	0.07	0.01
Rainbow Flowage	0.54	126	4.33	0.48	T	0.84	0.04
Osgood Spring Lake	0.56	[2][86]	5.10	0.69	—	1.20	0.02
Average [3]	0.55		4.76	0.32	0.06	0.37	0.02
MAINE							
Little Swan Island	0.54	138	13.20	0.49	—	1.18	—
Birch Island	0.55	[2][123]	21.04	0.44	0.49	0.31	0.02
Kennebec River	0.50	[2][112]	25.25	1.58	1.38	3.12	0.04
Brandy Pond	0.52	135	27.55	0.77	0.35	1.02	0.03
Swan Island [4]	—	[2][122]	18.00	1.61	0.30	2.54	0.09
Average	0.53		21.76	0.82	0.56	1.41	0.02
FLORIDA							
Buoy Key	0.50	125	27.93	0.31	—	0.16	0.01
Cormorant Key	0.44	97	7.84	0.80	0.67	0.28	0.02
Palm Key	0.52	71	6.49	0.58	T	0.22	0.02
Deer Key	0.53	108	6.09	0.80	T	0.24	0.02
Crab Key	0.50	107	4.29	0.20	—	0.11	0.005
Manatee Key	0.54	107	11.66	0.72	0.38	0.27	0.02
Average	0.50		10.72	0.57	0.18	0.21	0.02

[1] Wet weight, adjusted as described in text to compensate for differential drying of contents. Total micrograms per egg can be computed by multiplying egg volume by ppm for each chemical.

[2] Estimated as described in text.

[3] Averages computed on a nest basis. Parentheses designate different eggs from the same nest.

[4] The data for the Swan Island egg are not included in the averages; it was collected July 13, 1967. Shell thickness not measured.

TABLE 4.—*Heavy metal residues in bald eagle eggs collected in 1968 from Wisconsin, Maine, and Florida*

Nest Location		Residues in ppm					
		Copper		Iron		Zinc	
		Dry Wt.	Wet Wt.[1]	Dry Wt.	Wet Wt.[1]	Dry Wt.	Wet Wt.[1]
WISCONSIN							
Pickerel Lake		8.0	1.1	73	10.8	56	8.2
Cranberry Lake	(1)	5.0	0.7	114	15.0	51	6.7
	(2)	6.0	0.7	86	9.3	50	5.5
Mud Lake	(1)	8.6	1.1	89	11.6	51	6.6
	(2)	6.0	0.8	49	6.4	50	6.6
	(3)	5.0	0.5	76	8.6	43	4.9
Windigo Lake	(1)	6.7	1.0	78	11.7	42	6.3
	(2)	4.0	0.6	43	5.9	30	4.1
	(3)	4.0	0.6	70	9.8	35	4.9
Deer Lake		6.0	0.9	84	13.0	48	7.4
Pacwawong Lake		9.0	1.2	73	10.0	36	4.9
Barnes Lake		3.7	0.6	89	14.1	43	6.8
Sanborn Lake		4.0	0.7	59	9.2	46	7.2
Rainbow Flowage		8.0	1.1	64	9.3	44	6.4
Osgood Spring Lake		3.0	0.5	83	14.5	47	8.2
MAINE							
Little Swan Island		2.0	0.3	59	7.8	38	5.1
Birch Island		3.0	0.4	42	4.7	32	3.7
Kennebec River		4.0	0.5	118	16.2	52	7.1
Brandy Pond		5.0	0.6	79	10.7	37	5.0
FLORIDA							
Buoy Key		4.9	0.7	74	10.2	36	4.6
Cormorant Key		7.0	1.0	117	16.7	36	5.1
Palm Key		7.0	1.0	86	13.3	42	6.5
Deer Key		4.8	0.7	103	14.9	49	7.1
Crab Key		7.7	0.9	147	18.3	65	8.1
Manatee Key		8.0	1.2	100	14.4	38	5.5

[1] The wet weights were adjusted as described in text.

LITERATURE CITED

(1) *Abbott, J. M. 1967.* The Chesapeake bald eagles. Atlantic Nat. 22(1):20-25.

(2) *Bagley, G. E. and L. N. Locke. 1967.* The occurrence of lead in tissues of wild birds. Bull. Environ. Contamination Toxicol. 2(5):297-305.

(3) *Broley, C. L. 1958.* The plight of the bald eagle. Audubon Mag. 60(4):162-171.

(4) *Hensel, Richard J., and Willard A. Troyer. 1964.* Nesting studies of the bald eagle in Alaska. Condor 66: 282-286.

(5) *Herrick, F. H. 1933.* Daily life of the American eagle: early phase (concluded). Auk 50(1):35-53.

(6) *Hickey, J. J., Editor. 1969.* Peregrine falcon populations: their biology and decline. Univ. Wis. Press, Madison. 596 p.

(7) *Hickey, J. J. and D. W. Anderson. 1968.* Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 162(3850):271-273.

(8) *Mulhern, B. M. 1968.* An improved method for the separation and removal of organochlorine insecticides from thin-layer plates. J. Chromatogr. 34(1968): 556-558.

(9) *Perkin-Elmer Corp. 1966 (Revised).* Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer Corp., Norwalk, Conn.

(10) *Ratcliffe, D. A. 1967.* Decrease in eggshell weight in certain birds of prey. Nature 215(5097):208-210.

(11) *Reichel, W. L., E. Cromartie, T. G. Lamont, B. M. Mulhern, and R. M. Prouty. 1969.* Pesticide residues in eagles. Pesticides Monit. J. 3(3):142-144.

(12) *Reichel, W. L., T. G. Lamont, E. Cromartie, and L. N. Locke. 1969.* Residues in two bald eagles suspected of pesticide poisoning. Bull. Environ. Contamination Toxicol. 4(1):24-30.

(13) *Sprunt, A., IV and F. J. Ligas. 1966.* Audubon bald eagle studies—1960-1966. Proc. 22nd Annu. Conv. Nat. Audubon Soc., Sacramento, Calif.

(14) *Stickel, L. F., N. J. Chura, P. A. Stewart, C. M. Menzie, R. M. Prouty, and W. L. Reichel. 1966.* Bald eagle Pesticide relations. Trans. 21st N. Amer. Wildlife Natur. Resources Conf., p. 190-200.

Organochlorine Residues and Autopsy Data From Bald Eagles 1966-68 [1]

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ABSTRACT

Sixty-nine bald eagles found moribund or dead in 25 States during 1966-68 were analyzed for pesticide residues. Residues of polychlorinated biphenyls and DDE were detected in all samples of eagle carcasses; residues of dieldrin were detected in 68 and residues of DDD in 64; DDT, heptachlor epoxide, and DCBP were detected less frequently. Eight specimens had levels of dieldrin in the brain within the lethal range, and another probably died of DDT poisoning. Autopsy revealed that illegal shooting was the most frequent cause of mortality of these eagles; electrocution, impact injuries, probable lead poisoning, and infectious avian diseases were other causes of mortality.

Introduction

An earlier report (*6*) presented the pesticide residue data obtained by this laboratory for bald eagles (*Haliaeetus leucocephalus*) found dead in 1964 and 1965. The purpose of this paper is to report and evaluate residues and other data on bald eagles for 1966 through 1968.

Sampling and Autopsy Procedure

A systematic sampling scheme for bald eagles cannot be undertaken because of the relatively low populations of these birds and their protected status. However, bald eagles found dead or moribund are collected by Federal, State, and private cooperators, packed in dry ice, shipped air express to this laboratory, and stored, intact in plastic bags, in a freezer at —25 C. Specimens that are decomposed or have been held in captivity are discarded. The collection areas for the 69 samples included in this report are shown in Table 1. A total of 21 birds were collected in 1966, 22 birds in 1967, and 26 birds in 1968.

TABLE 1.—*Distribution of eagles collected, by State and year of death, 1966-68*

State	Number of Eagles Collected		
	1966	1967	1968
Alaska	1	2	
Arkansas	1		
Connecticut		1	
Florida	1		2
Idaho			1
Illinois			2
Iowa	4	2	1
Maine		1	
Maryland			2
Massachusetts	1		1
Michigan	4	3	1
Minnesota	2	5	3
Missouri	1		2
Montana	1		
New Jersey			1
New York		1	
Ohio	1		
Oregon		1	
South Carolina		1	1
South Dakota			2
Tennessee		1	
Utah			2
West Virginia		1	
Wisconsin	4	3	4
Wyoming			1
Total	21	22	26

Autopsy examinations were performed on all specimens to determine possible cause of death. Tissues for histological study were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with either hematoxylin and eosin, Ziehl-Neelsen acid-fast, periodic-acid Schiff (PAS), Giemsa, Perl's Prussian blue, or the Van Kossa stain. The entire brain was removed and placed in an acetone-rinsed glass jar, and the remaining carcass (except for skin, feet, wings, liver, and gastro-intestinal

[1] Bureau of Sport Fisheries and Wildlife, Patuxent Wildlife Research Center, Laurel, Md. 20810.

tract) was wrapped in aluminum foil. The samples were usually processed for residue analysis within 1 week after dissection.

Analytical Procedure

The remaining carcass was ground and homogenized in a Hobart food cutter. A 20-g aliquot of the carcass and the entire brain were mixed separately with anhydrous sodium sulfate in a blender and extracted for 7 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated, taken up in 50 ml of acetonitrile-saturated hexane and partitioned four times with 50 ml of hexane-saturated acetonitrile in a 500-ml separatory funnel with a teflon stopcock. The combined acetonitrile was evaporated to dryness in a 500-ml Phillips beaker at room temperature. The remaining residue was dissolved in hexane, and the pesticides were eluted on a Florisil column (2 x 18 cm, 200-ml reservoir) with 200 ml of 3:1 hexane-benzene mixture. Florisil was partially deactivated with 2-5% water as determined by recovery of pesticide standards. The clean eluate was concentrated, divided in half, streaked on a thin layer (TL) plate, and the pesticides were separated and removed in four fractions as described by Mulhern (*4*). The four fractions would contain, if present, the following: I—dieldrin, lindane, heptachlor epoxide, endrin, 4,4′-dichlorobenzophenone (DCBP), methoxychlor, and dicofol; II—*p,p′*- and *o,p′*-DDD; III—*o,p′*-DDE, *p,p′*- and *o,p*-DDT; IV—*p,p′*-DDE, heptachlor, aldrin, and mirex.

The TL fractions were analyzed separately by gas chromatography (GC) using three columns of different polarity; the operating parameters are shown in Table 2. The four fractions were analyzed on the OV-17 column, and the pesticides detected in fractions II through IV were confirmed on the XE-60 column. The residues detected in fraction I were confirmed on the DEGS column.

TABLE 2.—*Chromatographic operating conditions using electron capture detection*

	Columns, Glass 6″x¼″ O.D.		
	A	B	C
Liquid Phase	3% OV-17	3% XE-60	12% DEGS
Support	Gas Chrom Q	Gas Chrom Q	Anachrom SD
Mesh Size	100/120	60/80	100/110
N_2 Flow Rate (ml/min)	100	100	85
Temperature (C)	190	170	190
Retention Time of dieldrin (minutes)	14.0	16.3	11.0

In addition to the TL zonal separations and dual-column gas chromatographic confirmation, the residues in 10% of the samples were confirmed by TLC. This TLC confirmation also showed that the DCBP detected by GC was not the GC breakdown product of dicofol. Some samples were also treated with alkali to confirm DDT.

The polychlorinated biphenyl (PCB) compounds were present in fraction IV and in considerably smaller amounts in fraction III. Inspection of the GC profiles for fraction IV from these specimens indicated that the maximum ratio of PCB's (Aroclor 1254 as reference) to DDE encountered was 1:1. Therefore, a study was made to determine if PCB's could interfere in the analysis of DDE. Standards were prepared to contain the following ratios of PCB to DDE: 1:1, 2:1, 3:1, and 5:1. The standards were zoned on the TL plate, as described above, and DDE was analyzed on the OV-17 column. PCB compounds did not interfere in the quantitative determination of DDE unless the ratio exceeded 2:1.

To determine the recovery efficiency of the analytical procedure, 20-g aliquots of an eagle carcass homogenate containing trace quantities of pesticides were spiked to contain the following: 15 ppm DDE, 7.5 ppm DDD, 4.5 ppm DDT, 5.0 ppm dieldrin, 4.0 ppm heptachlor epoxide, 2.0 ppm DCBP, and 3.5 ppm *o,p′*-DDT. The average recoveries were: 95% DDE, 102% DDD, 110% DDT, 106% dieldrin, 112% heptachlor epoxide, 75% DCBP, and 107% *o,p′*-DDT. In addition, experimental quail tissues containing biologically incorporated carbon-14-labeled *p,p′*-DDT and dieldrin were analyzed by liquid scintillation techniques. The average recovery from all tissues was 80% for DDT and 79% for dieldrin (*5*). The residue levels reported for the eagle samples were not corrected for recovery; the lower limit of sensitivity was approximately 0.05 ppm wet weight.

Results and Discussion

RESIDUES

The chlorinated pesticide residues found in 69 bald eagle carcasses and brains are summarized in Table 3. Median values are presented instead of means because of the skewness of the data. All eagle carcass samples contained DDE; 68 contained dieldrin; and 64 contained DDD. In addition, 39 samples contained DDT; 34 contained heptachlor epoxide; and 24 contained DCBP. All samples contained PCB compounds; their presence was confirmed in six samples by gas chromatography-mass spectrographic (GC-MS) analysis. The characterization of the individual PCB compounds for two of these samples has been reported by Bagley *et al.* (*1*).

The median value of DDE in the carcass samples was lower in 1968 than in the preceding years. However, it cannot be concluded that a general average decrease did, in fact, occur due to (1) the wide range in DDE levels, (2) the wide distribution of collection sites, and (3) the relatively small number of samples collected in any one area over the 3-year period. For example, 16 States were represented in only 1 of the 3 years, and

TABLE 3.—*Pesticide residues in bald eagles, 1966-68*

[T = <0.05 ppm]

Compound	Year	Residues in ppm [1]					
		Carcass			Brain		
		Median	Range	N [2]	Median	Range	N [2]
p,p'-DDE	1966	11.80	0.5-136.0	21	1.50	T- 35.9	21
	1967	16.55	0.6-263.0	22	1.81	T-149.0	21
	1968	4.92	0.4-104.0	26	0.92	T-113.9	26
p,p'-DDD	1966	1.10	T- 13.8	21	0.17	T- 1.5	14
	1967	1.09	<0.1- 79.2	20	0.70	T- 14.4	14
	1968	0.85	T- 24.8	23	1.00	T- 3.8	12
p,p'-DDT	1966	0.20	T- 1.3	14	T	T	4
	1967	0.20	T- 14.1	15	T	T- 20.3	3
	1968	0.15	T- 0.5	10	0.42	0.1- 0.7	2
Dieldrin	1966	0.59	T- 2.1	21	0.10	T- 0.6	17
	1967	0.60	<0.1- 8.2	21	0.27	T- 9.5	16
	1968	0.47	T- 22.3	26	0.77	T- 7.0	17
Heptachlor epoxide	1966	0.07	T- 0.25	13	0.02	T- 0.04	5
	1967	0.08	T- 0.34	12	0.19	T- 0.2	5
	1968	0.08	T- 0.21	9	0.07	T- 0.21	5
Dichlorobenzophenone	1966	1.20	0.3- 5.0	5	0.20	T- 2.0	4
	1967	0.71	0.1- 3.5	9	0.60	T- 1.9	5
	1968	0.53	T- 6.4	10	0.45	0.2- 1.2	6

[1] Calculated on a wet-weight basis.
[2] Number of specimens that contained residues; the median is based on this number.
NOTE: A total of 21 birds were collected in 1966, 22 birds in 1967, and 26 birds in 1968.

5 States were represented in only 2 of the 3 years. Four contiguous States—Iowa, Michigan, Minnesota, and Wisconsin—were represented in each year of the reporting period. The concentrations of DDE in individual carcass samples from these States are shown in Table 4. Analysis of variance of the log-transformed data showed no significant difference ($P=0.05$) between years, either for the four States combined or when Minnesota or Wisconsin were tested separately. A trend may become more evident upon the analysis of samples collected in 1969 and 1970.

One of the principal findings of these analyses is that eight specimens had concentrations of dieldrin in the brain that were in the lethal range. Experimental studies have shown that residues of about 4 ppm of dieldrin in the brain is the lower lethal level *(9)*. These specimens contained 3.6 to 9.5 ppm of dieldrin in the brain (Table 5); seven were collected in 1968, one in 1967, and none in 1966.

Autopsy revealed no injuries or infectious diseases in six of these specimens, and death was probably due to dieldrin poisoning. Two showed lesions of infectious disease. The Missouri sample had typical lesions of the mesenteric form of avian tuberculosis, and the Maryland sample had a generalized bacterial infection subsequent to an infection of an old gunshot injury. However, the levels of dieldrin in the brains of these two eagles suggests that dieldrin poisoning was at least an important contributory cause of death. The identity of dieldrin for all specimens listed in Table 5 was confirmed by GC-MS analysis.

TABLE 4.—*DDE residues in carcasses of individual bald eagles collected in States from which samples were available in each year, 1966-68*

State	DDE Residues in ppm (Wet Weight)		
	1966	1967	1968
Iowa	0.5	1.4	0.64
	2.3	8.7	
	4.7		
	32.1		
Michigan	1.9	15.8	1.2
	18.4	44.8	
	27.4	68.2	
	136.0		
Minnesota	12.8	1.2	0.7
	27.5	1.7	7.9
		22.0	11.0
		25.8	
		40.0	
Wisconsin	1.3	1.1	0.8
	19.0	1.6	5.0
	19.7	17.3	13.4
	24.0		44.1
Median	18.7	15.8	5.0

TABLE 5.—*Data on suspected cases of dieldrin poisoning*

State	Year	Age [1]	Sex [2]	Dieldrin in Brain (PPM)	Autopsy Diagnosis
Minnesota	1967	Ad	F	9.5	Open [3]
South Carolina	1968	Im	U	3.6	Open
Missouri	1968	Ad	F	4.1	Avian T.B.
Maryland	1968	Ad	F	4.3	Old gunshot wound, secondary bacterial infection
Wisconsin	1968	Ad	F	4.4	Open
	1968	Ad	F	4.4	Open
Florida	1968	Ad	F	5.5	Open
	1968	Ad	F	7.0	Open

[1] Ad = adult, Im = immature.
[2] F = female, U = unknown.
[3] Open = no diagnosis could be made on the basis of autopsy findings.

One eagle, an immature female from Connecticut, contained 20.3 ppm of DDT plus 14.4 ppm of DDD in the brain and is suspected of having died from DDT poisoning. Experimental and field studies have shown that 30 ppm of DDT plus DDD in the brain will cause lethal poisoning *(8)*. The pesticide contents of the specimen from Connecticut mentioned above and the specimen from Florida that contained 7 ppm of dieldrin have also been previously reported elsewhere *(7)*.

AUTOPSY DATA

The results of the autopsy examinations of the 69 specimens are summarized in Table 6. Illegal shooting still remains the most frequent single cause of mortality among the bald eagles examined in this laboratory *(3)* and during the period 1966-68 was responsible for the death of 40% of the specimens. Impact injuries, resulting from the eagles striking some object, frequently a power line, accounted for 10 deaths. Two eagles were known to have been electrocuted by a power line in Alaska. Two were accidentally captured by traps and subsequently killed. One adult female from Missouri died of fowl cholera *(Pasteurella multocida)*. An emaciated immature female collected in Maryland had ingested two lead shot; one was found in the esophagus and the other in the stomach. Analysis by atomic absorption revealed that the liver contained 21 ppm, kidney 5 ppm, and pancreas 22 ppm of lead (wet weight).

TABLE 6.—*Causes of bald eagle mortality as determined by autopsy*

Cause of Death	Age		Sex [1]			Total
	Adult	Immature	F	M	U	
Shot	10	18	13	11	4	28
Open [2]	11	9	15	4	1	20
Impact	2	8	4	4	2	10
Infectious Disease	2		2			2
Trapped	1	1		2		2
Lead Poisoning		1	1			1
Electrocution		2	2			2
Nephrosis		1		1		1
Choked		1	1			1
Visceral Gout	1		1			1
Fecal Impaction	1		1			1
						69

[1] F = female, M = male, U = sex could not be determined.
[2] Open = no diagnosis could be made on the basis of autopsy findings.

An immature male eagle from Minnesota had a marked nephrosis with microcalculi formation and necrosis of the kidney tubules, pathological changes suggestive of heavy metal poisoning. The kidney sample contained a low level of lead; therefore, aliquots of the carcass and kidney were analyzed for mercury residues by neutron activation at Gulf General Atomic, Inc. The carcass contained 59 ppm and the kidney 130 ppm of mercury (wet weight). These levels are considered dangerously high when compared with the levels of mercury reported in wildlife and experimental samples by Swedish workers *(2)*.

Of the 20 eagles for which there was no diagnosis upon completion of the autopsy, 6 were later found to have high levels of dieldrin in the brain, and another eagle had a high level of DDT, as reported above.

Conclusion

The presence of PCB's and DDE in all samples from 25 States and the accumulation of high concentrations of dieldrin in the brains of 8 eagles and DDT in another demonstrates widespread environmental contamination. The bald eagle, located as it is at the top of food chains, is the final recipient of accumulations of environmental pollutants.

See Appendix for chemical names of the compounds mentioned in this paper.

Acknowledgments

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LITERATURE CITED

(1) Bagley, G. E., W. L. Reichel, and E. Cromartie. 1970. Identification of polychlorinated biphenyls in two bald eagles by combined gas chromatography-mass spectrometry. J. Ass. Offic. Agr. Chem. 53(2):251-261.

(2) Borg, K., H. Wanntorp, K. Erne, and E. Hanko. 1969. Alkyl mercury poisoning in terrestrial Swedish wildlife. Viltrevy 6(4):301-379.

(3) Coon, N. C., L. N. Locke, E. Cromartie, and W. L. Reichel. 1970. Causes of bald eagle mortality, 1960-1965. J. Wildlife Dis. 6(1):72-76.

(4) Mulhern, B. M. 1968. An improved method for the separation and removal of organochlorine insecticides from thin layer plates. J. Chromatogr. 34:556-558.

(5) Prouty, R. M. and E. Cromartie. 1970. Recovery of DDT and dieldrin from tissues of Coturnix japonica stepwise during residue analysis. Environ. Sci. Tech. 4(9):768-769.

(6) Reichel, W. L., E. Cromartie, T. G. Lamont, B. M. Mulhern, and R. M. Prouty. 1969. Pesticide residues in eagles. Pesticides Monit. J. 3(3):142-144.

(7) Reichel, W. L., T. G. Lamont, E. Cromartie, and L. N. Locke. 1969. Residues in two bald eagles suspected of pesticide poisoning. Bull. Environ. Contamination Toxicol. 4(1):24-30.

(8) Stickel, L. F. and W. H. Stickel. 1969. Distribution of DDT residues in tissues of birds in relation to mortality, body condition and time. Ind. Med. Surg. 38(3):91-100.

(9) Stickel, W. H., L. F. Stickel, and J. W. Spann. 1969. Chemical fallout; current research on persistent pesticides. Proc. First Rochester Conf. on Toxicity p. 174-204.

PESTICIDES IN SOIL

Monitoring Pesticides in Soils From Areas of Regular, Limited, and No Pesticide Use

Lynn J. Stevens,[1] Carroll W. Collier,[2] and Donald W. Woodham[2]

ABSTRACT

Pilot studies were conducted nationwide at 51 locations in 1965, 1966, and 1967 to determine existing pesticide residue levels in soils. Samples were collected from 17 areas in which pesticides are used regularly, 16 areas with a record of at least one pesticide application, and in 18 areas with no history of pesticide use. The samples were analyzed by gas chromatography.

A wide variety of pesticides were found in soils from areas of regular use. Residues of DDT and dieldrin were found in soils where pesticide use has been limited. Except for a small amount of DDT found in soil from one No Use Area, all other samples from the No Use Areas were negative.

Introduction

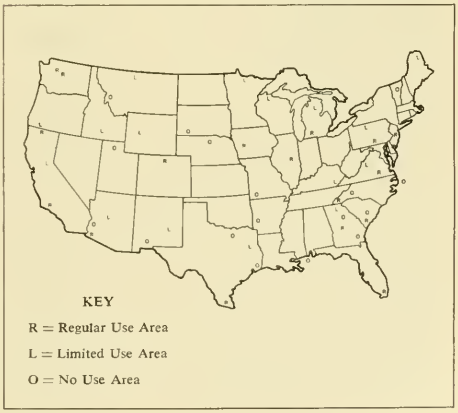
The Plant Protection Division of the Agricultural Research Service expanded its pesticides monitoring activities in 1965 to include the sampling of soil at 51 locations distributed over the conterminous United States (Fig. 1). The objective of this expanded study was to determine existing pesticide residue levels in soils from a variety of locations and to detect any significant changes in those levels.

Wherever possible, the sampling areas were established to coincide with sites being monitored by other Federal agencies so that residue data for soil could become a part of the mosaic picture of pesticides in the environment.

Included in the 51 locations were 17 areas in which pesticides are used on a regular basis as a part of normal cropping practices. These areas, hereafter referred to as Regular Use Areas, were selected on the basis of the availability of pesticide use records. The Regular Use Areas were established, whenever possible, at locations where the U.S. Public Health Service was monitoring pesticides in people.

The Regular Use Areas fit into three broad categories of crops grown: (1) vegetable- and/or cotton-producing areas; (2) tree fruit-producing areas; and (3) areas where various crops are produced (included were sugar beets, peanuts, potatoes, corn, and soybeans).

FIGURE 1.—*Sampling locations: areas of regular, limited, and no pesticide use*



[1] Plant Protection Division, U.S. Department of Agriculture, Hyattsville, Md.

[2] Plant Protection Division, U.S. Department of Agriculture, Gulfport, Miss.

The Regular Use Areas and their principal crops include:

Vegetable and/or Cotton

Lower Rio Grande Valley of Texas — cotton and vegetables
Dade County, Fla. — vegetables
Eastern South Carolina — vegetables
Monmouth County, N. J. — vegetables
Kern County, Calif. — cotton and vegetables

Tree Fruit

Western North Carolina — apples
Central Georgia — peaches
Adams County, Pa. — apples
Berrien County, Mich. — mixed
Yuma County, Ariz. — citrus
Wenatchee, Wash. — apples

Other

Eastern Virginia — peanuts
Urbana, Ill. — corn
Western Iowa — corn and soybeans
Weld County, Colo. — sugar beets and potatoes
Quincy, Wash. — root crops
Tulelake, Calif. — small grains and root crops

For purposes of comparison, 16 sampling areas were established at locations where pest infestations have required only periodic control. Each of the 16 areas, hereafter referred to as Limited Use Areas, had been treated at least once, and none had been treated more than four times.

The following are the Limited Use Areas listed by principal treatments and pests:

Klamath County, Oreg.
(aldrin, dieldrin) — grasshoppers
Lincoln County, Idaho
(aldrin, dieldrin) — grasshoppers
Phillips County, Mont.
(aldrin, dieldrin) — grasshoppers
Fremont County, Wyo.
(aldrin, dieldrin) — grasshoppers
Pike County, Ky.
(dieldrin) — Japanese Beetle
Davy Crockett National Forest, Tex.
(2,4-D) — undesirable trees
Manistee-Huron National Forest, Mich.
(DDT) — forest pests
Thomas Jefferson National Forest, Va.
(DDT) — forest pests
Chippewa National Forest, Minn.
(DDT) — forest pests
Coconino National Forest, Ariz.
(DDT) — forest pests
Lincoln National Forest, N. Mex.
(DDT) — forest pests
Stanislaus National Forest, Calif.
(DDT) — forest pests
Chequamegon National Forest, Wis.
(DDT) — forest pests
Allegheny National Forest, Pa.
(DDT) — forest pests
Eagle Lake State Forest, Maine
(DDT) — forest pests
Chattahoochee National Forest, Ga.
(DDT) — forest pests

To determine whether or not pesticides might be distributed into areas not directly exposed to pesticide treatment, 18 areas were established for soil sampling in locations with no known previous use of pesticides. Criteria for selection of these areas, hereafter called No Use Areas, were as follows:

(1) uncultivated land that had not been cultivated within the past 10 years
(2) sampling areas at least 1 mile from any known treated areas
(3) land remote from currently cultivated land.

Included in the No Use Areas were 10 National Wildlife Refuges, 6 National Forests, and 2 National Grasslands; these are as follows:

National Wildlife Refugees

Gulf Islands National Wildlife Refuge, Miss.
Kofa National Wildlife Refuge, Ariz.
Okefenokee National Wildlife Refuge, Ga.
Seney National Wildlife Refuge, Mich.
Ravalli National Wildlife Refuge, Mont.
Ft. Niobrara National Wildlife Refuge, Nebr.
San Andres National Wildlife Refuge, N. Mex.
Anahuac National Wildlife Refuge, Tex.
Mississquoi National Wildlife Refuge, Vt.
Pea Island National Wildlife Refuge, N. C.

National Forests

Pisgah National Forest, N. C.
Oconee National Forest, Ga.
Francis Marion National Forest, S. C.
Ozark National Forest, Ark.
Mark Twain National Forest, Mo.
Cache National Forest, Utah

National Grasslands

Cross Timbers National Grasslands, Tex.
Buffalo Gap National Grasslands, S. Dak.

Sampling Procedures

REGULAR USE AREAS

In each Regular Use Area, one field of 20 acres or more from each of five farms was selected for study. Within each field, five 1-acre plots were laid out and sampled. Plot sampling was done on a stratified random basis

with a sample consisting of 50 soil cores 2 inches in diameter and 3 inches deep taken in a grid pattern from each plot.

Regular Use Areas were sampled annually after the pest control season, and in a few of the areas samples were taken before the control season as well. The schedule of sampling is shown in Table 1.

LIMITED AND NO USE AREAS

In each of the Limited and No Use Areas, 1 square mile was selected for sampling, and ten 1-acre plots were laid out in each square mile. Plot samples in these areas were taken in the same manner as those in the Regular Use Areas. Limited and No Use Areas were sampled once each year at approximately the same time of year in the late summer or fall.

The 50-core composites were each passed through a ¼-inch mesh screen three times to facilitate mixing and to remove stones, twigs, roots, and other debris from the soil. A representative portion of the mixed, screened soil was retained in a new 1-gallon paint can with an airtight lid. Each sample can was labeled with a field sample number and collection date. Soil samples were then stored at room temperature until processed for pesticide analyses.

Careful sanitation practices were followed in the field and in the laboratory. Equipment was thoroughly cleaned before collection or handling of each sample to avoid cross-contamination.

Analytical Methods

EXTRACTION

Soil subsamples of 300 g wet weight were placed in 2-quart fruit jars with 600 ml of 3:1 hexane-isopropanol solvent and concentrically rotated 4 hours at 30 rpm. After the soil settled, about 200 ml of the extract solution was filtered into a separatory funnel. The isopropanol was removed by washing twice with distilled water. The hexane was then filtered into a clean glass bottle through a funnel containing glass wool and anhydrous sodium sulfate (Na_2SO_4). The bottle was capped until analysis. For the most part, no further cleanup was needed prior to analysis.

ANALYSIS

Gas Chromatography: Chlorinated Pesticides. Where it was practical, instrument amplification was set to provide half-scale deflection of aldrin at the 0.5 ng level.

Sample extracts showing high levels of pesticides were diluted to bring them within the linear range of the electron capture detectors. Low levels of pesticides were reported when peak heights greater than twice the "signal to noise ratio" were obtained.

Dual-column confirmations (nonpolar vs mixed polar-nonpolar or polar columns) were made in all cases when a pesticide without a clear-cut history of use was recovered.

A system of controls was designed to detect inadvertent contamination during processing and to provide recovery

TABLE 1.—*Soil sampling schedule for the Regular Use Areas, 1965-67*

Sampling Site	1965		1966		1967	
	Spring	Fall	Spring	Fall	Spring	Fall
VEGETABLE AND/OR COTTON						
Kern County, Calif.			x	x		
Lower Rio Grande Valley, Tex.	x	x		x		
Monmouth County, N. J.			x	x		x
Dade County, Fla.		x		x	x	
Eastern South Carolina		x	x	x		
TREE FRUIT						
Wenatchee, Wash.	x	x		x		x
Adams County, Pa.		x	x	x		
Berrien County, Mich.		x	x	x		x
Western North Carolina		x	x	x		
Yuma County, Ariz.	x		x	x		
Central Georgia		x	x	x		
OTHER						
Quincy-Moses Lake, Wash.	x	x		x		x
Tulelake, Calif.		x		x		x
Weld County, Colo.	x	x		x		x
Urbana, Ill.	x	x	x	x		
Western Iowa	x	x	x	x		
Eastern Virginia		x	x	x		

values for suspected or known pesticides. These controls included: (1) fortified solvent, unprocessed; (2) fortified solvent, processed; (3) unfortified solvent, processed; (4) composite sample, fortified and processed; (5) composite sample, unfortified but processed. Recovery corrections were usually determined by subtraction of residues in control 5 from residues in control 4, and comparing the difference with residues from control 1. The sensitivity level was 0.01 ppm.

Typical columns used and their operating parameters were:

DC-200: 3% on 100/120 mesh Gas Chrom Q; 180 C; flow rate 80 ml/min

UCW-98: 3.8% on 100/120 mesh Gas Chrom Q; 180 C; flow rate 80 ml/min

QF-1: 9% on 100/120 mesh Gas Chrom Q; 180 C; flow rate 30 ml/min

Mixed (OV-17/QF-1): 11% on 100/120 mesh Gas Chrom Q; 210 C; flow rate 80 ml/min

These parameters were based on a 6-foot, U-shaped column (glass), ¼ inch o.d. x 5/32 inch i.d.

The initial toxaphene analyses were done with a colorimetric method *(1)*. That method, however, was subject to manifold interferences. The gas chromatographic method finally adopted was based on taking average values from the four predominant toxaphene peaks on a nonpolar column and comparing them to the corresponding peaks from the chromatogram of a known standard. If one peak was obscured, the remaining three were used. This method appears to be accurate down to the 0.05 ppm level.

Gas Chromatography: Organophosphate Pesticides. Analyses for phosphate pesticides were made by flame thermionic, flame photometric, or electron capture detection. In most cases, a sample splitter system with dual detection was used. This method allowed parathion detection in the presence of sulfur, which would normally mask that phosphate in electron capture.

In general, only those organophosphates that were amenable to chlorinated pesticide cleanup were detected. No serious attempts were made to quantitate metabolites or oxygen analogs of the organophosphates.

The columns used for phosphate analyses were identical to those described for the chlorinated pesticide complex.

Arsenic Analysis. Arsenic was detected by atomic absorption following (a) hydrochloric acid extraction, (b) reduction to the plus three valence form, (c) partitioning into benzene, and (d) back partitioning into water. The initial analyses, however, were done by a modified colorimetric method *(2)*.

2,4-D Analysis. The analysis of 2,4-D was done using the method described by Woodham, *et al.* in 1967 *(3)*.

Confirmation Techniques. In addition to dual-column gas chromatographic confirmations, the following techniques were also used to verify questionable results: thin layer chromatography, combined TLC-GLC, p-value comparisons, sulfonations, nitrations, oxidations, saponifications, and special column cleanup procedures.

Results and Discussion

Analytical results are reported herein as parts per million (ppm) on a dry-weight basis. Because of the soil sampling method employed, ppm are directly convertible to pounds per 3-inch acre. Data summaries are used for purposes of discussion; however, the data are presented in more detail in Supplement I.

The records of pesticide use contained in this report are as accurate as could be obtained. Securing exact historical treatment records, however, is seldom possible. The analytical findings indicate that the records obtained are accurate but certainly not complete. Pesticides other than those listed in Supplement I were used on many of the study areas. These are listed in Supplement II.

REGULAR USE AREAS (Table 2)

Vegetable and/or Cotton-Growing Areas. DDT and dieldrin were the most consistently detected pesticides in soils from vegetable and/or cotton-growing areas. DDT was found in all 25 fields sampled in amounts ranging from 0.29 ppm to 15.63 ppm. Dieldrin was found in 80% of the fields sampled in amounts ranging from 0.02 ppm to 3.08 ppm. Aldrin, on the other hand, was detected in only one field at 0.01 ppm. Residues of endrin ranging from 0.01 ppm to 1.73 ppm were found in soils from 44% of the fields sampled. Chlordane (0.05-2.52 ppm) was found in 36% of the fields; toxaphene/Strobane (0.66-9.38 ppm) was found in 60% of the fields; and heptachlor epoxide (0.01-0.58 ppm) was found in 24% of the fields. Endosulfan was found in soils from about one-third of the fields sampled. Trifluralin was found in 12% of the fields sampled at levels ranging from 0.08 ppm to 0.24 ppm. Widely ranging amounts of arsenic were found in soils from all of the fields analyzed for arsenic (20 fields).

Tree Fruit Areas. Soils from every orchard sampled contained residues of DDT. The amounts detected ranged from 0.07 ppm to 245.4 ppm. The largest amounts found were in apple orchards located in Washington, Michigan, and Pennsylvania. The smallest amounts were found in North Carolina apple orchards, Arizona citrus groves, and Georgia peach orchards.

Dieldrin was found in soils from 70% of the orchards sampled. Residue levels ranged from 0.02 ppm to 2.84

ppm. Residues of endrin, ranging from 0.02 ppm to 12.61 ppm, were detected in soils from nearly half of the orchards sampled. Endrin was used for rodent control in those orchards. Endosulfan residues were found in soils from 30% of the orchards. Chlordane and toxaphene were each found in one orchard.

Residues of arsenic, ranging from 1.27 ppm to 219.2 ppm, were detected in soils from all of the orchards sampled.

Small Grain and Root Crop-Growing Areas. DDT in amounts ranging from 0.01 ppm to 9.23 ppm was found in soils from nearly two-thirds of the fields sampled in the six small grain and root crop-growing areas. Dieldrin was found in 85% of the fields sampled from 0.002 ppm to 0.60 ppm. Aldrin (0.002 ppm to 0.47 ppm) was detected in soils from about half as many fields as dieldrin. Residues of endrin were found in soils from about one-third of the fields sampled in amounts ranging from 0.002 ppm to 1.68 ppm. Other pesticides detected were: endosulfan in 9% of the fields; chlordane in 27% of the fields; heptachlor epoxide in 15%; toxaphene/Strobane in 12%; and trifluralin in 12%.

Soils from 48.5% of the fields were analyzed for arsenic, and all were positive. The amounts detected ranged from 1.38 ppm to 26.64 ppm.

LIMITED USE AREAS (Table 3)

Residues of DDT were detected in soils from all of the Limited Use Areas on which it was used. In addition, DDT was found in one area reportedly treated with dieldrin. The average amount of DDT found at all locations in 1965 was 0.22 ppm and in 1966, 0.168 ppm. The average amounts found ranged from 0.001 ppm to 0.99 ppm.

Dieldrin was detected in soil from one of the areas where it was used and in soil from an area reportedly treated with DDT. In both areas, the average amount detected was 0.001 ppm.

TABLE 2.—*Ranges of residues detected in soil in regular use areas and percent of fields containing residues, 1965-67*

Pesticide	Vegetable and/or Cotton		Tree Fruits		Small grains and Root Crops	
	ppm	Percent	ppm	Percent	ppm	Percent
DDT	.29-15.63	100	.07-245.41	100	.01 - 9.23	65
Dieldrin	.02- 3.08	80	.02- 2.84	70	.002- .60	85
Aldrin	.01	5			.002- .47	42
Endrin	.01- 1.73	44	.02- 12.61	47	.002- 1.68	33
Endosulfan	.01- 1.22	32	.02- 4.63	30	.07 - .92	9
Chlordane	.05- 2.52	36	.10	3	.01 - .15	27
Heptachlor Epoxide	.01- .58	24			.002- .09	15
Toxaphene/Strobane	.66- 9.38	60	7.72	3	.11 - 2.01	12
Trifluralin	.08- .24	12			.002- .48	12
Arsenic	1.50-54.10	[1] 100	1.27-219.20	[1] 100	1.38 -26.64	[1] 100

[1] Number of fields analyzed for arsenic: Vegetable and/or Cotton, 20 fields; Tree Fruits, 30 orchards; Small Grain and Root Crops, 16 fields.
NOTE: Empty spaces indicate no residues detected.

TABLE 3.—*Average concentration of DDT and dieldrin in soils from Limited Use Areas, 1965-1966*

Limited Use Areas	Residues in ppm			
	Combined DDT		Dieldrin	
	1965	1966	1965	1966
Klamath County, Oreg.		.001		.001
Manistee-Huron Nat'l Forest, Mich.	.04			
Thomas Jefferson Nat'l Forest, Va.	.50	.99		
Chippewa Nat'l Forest, Minn.	.34			
Coconino Nat'l Forest, Ariz.	.04	.05		.001
Lincoln Nat'l Forest, N. Mex.	.16	.03		
Stanislaus Nat'l Forest, Calif.	.06	.12		
Allegheny Nat'l Forest, Pa.	.62	.30		
Eagle Lake Nat'l Forest, Maine	.61	.43		
Chattachoochee Nat'l Forest, Ga.		.22		

NOTE: Empty spaces indicate no residues detected.

NO USE AREAS

DDT (0.001 ppm) was found in soil from one No Use Area (Cache National Forest). No pesticide residues were detected in soils from any of the other No Use Areas.

The pesticides detected in soils were primarily restricted to the chlorinated hydrocarbon series. Arsenic was found in every sample analyzed for it. This is not uncommon because there is a natural level of arsenic in most soils.

The most commonly detected pesticides in the Regular Use Areas were combined DDT and dieldrin. The amounts detected generally reflected the amounts that had been used. The only evidence of buildup was in some of the orchards that have been repeatedly treated with DDT over a number of years. Residues of the magnitude found in some of the orchards sampled would result in serious problems if those orchards were taken out of fruit production and put into hay, oilseed, or root crops.

Comparatively small amounts of DDT and dieldrin were found in soils from 10 of the 16 Limited Use Areas. These residues corresponded to history of pesticide use in 8 of the 10. In the other two, there is a transposition of what was used and what was found. This may be due to an error in the use records.

A relatively small amount of DDT was found in soil from one No Use Area; all other data from the No Use Areas were negative.

LITERATURE CITED

(1) *Yates, W. E. and W. F. Barthel. 1968.* Analysis for toxaphene and Strobane. USDA-ARS 81-28.

(2) *Kingsley, G. R. and R. R. Schaffert. 1951.* Microdetermination of arsenic and its application to biological material. Anal. Chem. 23(6):914-919.

(3) *Woodham, D. W., G. F. Gardner, and W. F. Barthel. 1967.* Gas chromatographic analysis of herbicides I. Residue analysis for 2,4-D in soil, water, and sediment. USDA-ARS 81-17, 6 p.

SUPPLEMENT I

TABLE 1.—*Record of pesticide applications on four fields in Kern County, Calif.*

Pesticide	Pounds Actual/Acre											
	Field 2			Field 3			Field 4			Field 5		
	1962-1964	1965	1966	1960-1964	1965	1966	1961-1964	1965	1966	1955-1964	1965	1966
DDT				X			2.50			X		
Aldrin							.001					
Endrin	17.50			6.00								
Endosulfan		7.50										
Toxaphene							2.00					
Azinphosmethyl		3.00										
Demeton										0.80		
Diazinon		1.00	1.00									
Malathion			3.60									
Methyl Demeton			1.50									
Methyl Parathion	17.50	11.25	3.00									
Parathion			6.00									
Phorate				10.00+	20.00	10.00			10.00			
Trifluralin				.75	.75	.75						

NOTE: Records from Field 1 were not available; X denotes unknown amount.

TABLE 2.—*Pesticide residues in soils from five fields in Kern County, Calif.*

Pesticide	Residues in ppm									
	Field 1		Field 2		Field 3		Field 4		Field 5	
	May 1966	Nov. 1966	May 1966	Nov. 1966	May 1966	Nov. 1966	June 1966	Nov. 1966	May 1966	Nov. 1966
DDT	1.26	1.75	1.17	1.44	1.90	3.08	0.59	0.88	0.29	0.41
TDE	.59		.13		.43	.08	.04	.004		
Dieldrin					.05				.02	
Endrin	1.70	1.73	.10	.11	.03				.05	.06
Endosulfan	.44	.49								
Trifluralin			.24	.10					.09	.28

NOTE: Empty spaces indicate no residues detected.

TABLE 3.—*Record of pesticide applications on five fields in the lower Rio Grand Valley*

Pesticide	Pounds Actual/Acre																			
	Field 1				Field 2				Field 3				Field 4				Field 5			
	1956-1964	1965	1966	1967	1958-1964	1965	1966	1967	1958-1964	1965	1966	1967	1955-1964	1965	1966	1967	1956-1964	1965	1966	1967
DDT	33.45	1.50	1.00		37.79	3.50	2.50		51.40	4.50	0.50		61.50				80.12			
TDE									10.00											
Dieldrin					.38				.75				26.25				15.86			
Endrin	.54				.25	.15	.25		2.98				1.70				9.83			
BHC	3.90				2.50				.60				1.35				1.32			
Endosulfan																	1.34			
Heptachlor	1.00				16.00				1.25				3.50				1.07			
Chlordane							1.00													
Perthane																	.35			
Strobane									1.70											
Toxaphene	16.20	3.00	2.00		47.00	7.00	1.25		34.00	9.00	1.00		29.25				39.16			
Azinphosmethyl	.80				3.25				6.00				5.00	0.28			5.62	0.28		
Demeton	.13												.48				.36			
Malathion													1.00				.15			
Methyl parathion	11.53	5.12	8.55		15.25	10.50	12.37	10.40	24.65	3.75	16.25		9.25	7.50	45.00	13.00	30.80	7.50	9.40	
Parathion	1.00		1.12			1.00			1.30	3.00			4.25	9.60			20.80	9.60	19.00	
2,4-D														1.00						
Trifluralin						.62		.75								.75				
As Pentoxide					.50											9.00				
Ca Arsenate	65.00	10.00															9.10			
Na Arsenite									3.00											
DEF					1.50		.50		1.50					3.00	1.20		14.37	3.00	2.00	
PCNB							1.00													
Sulfur					55.00															

TABLE 4.—*Pesticide residues in soils from five fields in the lower Rio Grande Valley*

Pesticide	Residues in ppm														
	Field 1			Field 2			Field 3			Field 4			Field 5		
	Apr. 1965	Oct. 1965	Oct. 1966	Apr. 1965	Oct. 1965	Oct. 1966	Apr. 1965	Oct. 1965	Oct. 1966	May 1965	Oct. 1965	Oct. 1966	May 1965	Fall 1965	Oct. 1966
DDT	3.19	4.75	3.67	3.06	3.93	3.52	2.74	3.22	2.49	2.70	3.45	2.67	4.55	5.97	3.82
TDE			.26			.11	.38	.33	.25			.11			.22
Dieldrin	.03		.02	.04						.10			.05		
Endrin			.27												
Toxaphene/Strobane	—	—	2.90	—	—	1.98	—	—	1.77	—	—	2.01	—	—	2.43
Methyl parathion															
Parathion															
Arsenic	11.70	—	10.22	2.24	—	2.78	2.48	—	2.20	2.00	—	2.28	5.20	—	5.30

NOTE: Empty spaces indicate no residues detected; — = no samples analyzed.

TABLE 5.—*Record of pesticide applications on five fields in Monmouth County, N.J.*

Pesticide	Pounds Actual/Acre														
	Field 1			Field 2			Field 3			Field 4			Field 5		
	1958-1964	1965	1967	1960-1964	1965	1967	1960-1964	1965	1967	1959-1964	1965	1967	1961-1964	1965	1967
DDT	18.00						5.25			8.00			1.50		
Aldrin	3.00														
Dieldrin				0.88						1.50					
Endosulfan	3.00	1.00	3.38	12.00		2.25	4.00	0.50	1.50	4.00	2.50	2.00	4.00	1.50	1.50
Azinphosmethyl	3.38	1.13	.50	3.50	0.25	1.00	6.00	.50	.50	5.25	.75		4.50	.38	
Parathion	.57		2.25		.50	.75	2.72	.38	.75	4.62		1.00	.80	.40	.75
Dimethoate		.40			1.00			.67			.07				

NOTE: 1966 records not available.

TABLE 6.—*Pesticide residues in soils from five fields in Monmouth County, N.J.*

Pesticide	Residues in ppm														
	Field 1			Field 2			Field 3			Field 4			Field 5		
	Mar. 1966	Aug. 1966	Oct. 1967	Mar. 1966	Aug. 1966	Oct. 1967	Mar. 1966	Aug. 1966	Oct. 1967	Mar. 1966	Aug. 1966	Oct. 1967	Mar. 1966	Sept. 1966	Oct. 1967
DDT	1.27	1.63	2.40	0.85	1.21	1.84	2.47	2.61	3.95	4.00	2.24	6.92	0.46	0.62	1.17
Aldrin															.01
Dieldrin	.04	.08	.15	.11	.12	.11	.14	.08	.11	.01		.04	.24	.24	.24
Chlordane			.06				.05		.07	.05		.06			
Endosulfan	.09	.42	.31	.10	.19	.15	.28	.79	.45	.15	.55	.43	.17	.57	.39
Heptachlor			.006						.004						
Heptachlor epoxide	.09	.07	.06				.05	.03	.04	.07	.05	.06		.02	
Arsenic	13.24	18.50	27.56	24.82	29.20	32.82	17.58	23.30	33.56	21.98	30.50	35.78	18.36	21.10	27.20

NOTE: Empty spaces indicate no residues detected.

TABLE 7.—*Record of pesticide applications on five fields in Dade County, Fla.*

Pesticide	Pounds Actual/Acre														
	Field 1			Field 2			Field 3			Field 4			Field 5		
	1955-1964	1965	1966	1958-1964	1965	1966	1965	1966	1962-1964	1965	1966	1962-1964	1965	1966
DDT	18.75	2.00		124.60	12.00		1.00			13.00		15.54	8.18	
TDE												5.14	2.13	
Aldrin				10.00										
Chlordane									0.40			10.90	14.17	
Endosulfan	67.50	7.50		3.00	1.50				.38			3.10	1.30	
Endrin							1.60							
Lindane												3.50	1.20	
Toxaphene		4.00		8.00	4.00	2.00	4.00		2.20	9.00		31.59	19.90	
Azinphosmethyl	16.88	1.88							4.85			1.90	.54	
Diazinon				3.50	2.00									
Parathion				4.00		.50			1.80	.50	4.00	36.66	18.16	
Phorate					2.00		3.00							
2,4-D	1.50													
Lead Arsenate												.91	.42	
Sodium Arsenite	62.00	10.00	1.00			1.70		3.00						
Dimethoate	2.66	1.33	1.00			1.30	1.60	2.67	.25	.25	.25	.34	1.10	
PCNB										2.00				
Sulfur									30.00	18.50	13.20			

TABLE 8.—*Pesticide residues in soils from five fields in Dade County, Fla.*

Pesticide	Residues in ppm														
	Field 1			Field 2			Field 3			Field 4			Field 5		
	Oct. 1965	Nov. 1966	Mar. 1968	Oct. 1965	Nov. 1966	Mar. 1968	Oct. 1965	Nov. 1966	Mar. 1968	Oct. 1965	Nov. 1966	Mar. 1968	Oct. 1965	Nov. 1966	Mar. 1968
DDT	0.80	2.45	1.64	15.63	10.92	4.37	2.70	3.59	1.56	5.19	7.05	3.13	1.27	0.87	1.65
Chlordane		T	2.52		T	.48		T	.93			.15		.36	1.60
Dieldrin				.38	.75	.29	.48	.45	.31			.05			
Endrin						.05			.05			.01			
Endosulfan	1.22												.11		
Heptachlor epoxide		.58			.04			.08							
Toxaphene	—		1.21	—		2.64	—		.66	—	4.42	4.14	—	9.38	7.00
Trifluralin												.09			
Arsenic	—	—	50.92	—	—	28.55	—	—	54.10	—	—	7.89	—	—	4.04

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.
T = <.01 ppm.

TABLE 9.—*Record of pesticide applications on five fields in eastern South Carolina*

Pesticide	Pounds Actual/Acre														
	Field 1			Field 2			Field 3			Field 4			Field 5		
	1952-1964	1965	1966	1953-1964	1965	1966	1956-1964	1965	1966	1957-1964	1965	1966	1956-1964	1965	1966
DDT	65.00			15.00	2.50	2.50	20.00			8.00			72.00		
TDE										40.00	8.00				
Endrin	.50	0.50			1.00	1.00							3.00	1.00	
Chlordane	6.00			12.00						3.00					
Lindane							4.30	1.50							
Toxaphene	3.00						15.00		3.00	47.00	9.00	18.00	38.00		
Azinphos-methyl										5.00	1.00				
Diazinon					2.00	2.00				15.00	3.00				
Parathion	5.70			9.00			7.50			23.50	4.50	1.50	5.00		
2,4-D			0.25												0.50

TABLE 10.—*Pesticide residues in soils from five fields in eastern South Carolina*

Pesticide	Residues in PPM														
	Field 1			Field 2			Field 3			Field 4			Field 5		
	Aug. 1965	Mar. 1966	Aug. 1966	Aug. 1965	Mar. 1966	Aug. 1966	Aug. 1965	Mar. 1966	Aug. 1966	Aug. 1965	Mar. 1966	Aug. 1966	Aug. 1965	Mar. 1966	Aug. 1966
DDE	0.98			0.91			0.31			0.30					
DDT	7.69	6.96	10.84	7.13	8.61	12.29	2.59	3.92	4.15	2.22	2.52	2.71	3.15	2.83	3.11
TDE		.11	.14		.19	.30	.81	.75	1.49	.21	.11	.18		.31	.12
Dieldrin					.08					.14	.08	.09	.13	.06	.06
Endrin		.39						.30					.28	.33	.13
Chlordane															.47
Toxaphene	—	—	2.99	—	—	1.05	—	—	5.64	—	—	.99	—	—	2.04
Arsenic	4.00	2.24	9.34	6.22	6.66	24.24	3.75	3.90	7.88	1.85	1.50	4.82	2.84	4.00	9.34

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 11.—*Record of pesticide applications on five apple orchards near Wenatchee, Wash.*

Pesticide	Pounds Actual/Acre											
	Orchard 1				Orchard 2				Orchard 3			
	1961-1964	1965	1966	1967	1956-1964	1965	1966	1967	1958-1964	1965	1966	1967
DDT				5.00	52.00+	14.00		1.00	18.00	6.00		12.00
Dieldrin					2.00+				4.50			
Endrin	1.50	1.13	0.38									
Endosulfan					2.00	.75			2.00			
Dilan									1.25			
Perthane									20.00	8.00	8.00	X
Tetradifon	6.00				4.00+				11.00			
Azinphosmethyl	18.00	6.00	2.00	5.00	2.50	2.50	2.00		27.25	2.50	2.00	X
Diazinon		5.00	2.00	5.00				8.00	5.50			8.00
Ethion	8.00				4.00				4.00	4.00	8.00	16.00
Parathion				X	26.00+	2.00		1.50	23.25	2.00		20.00
2,4-D										.05		
2,4,5-T								X				
Binapacryl	6.00	10.00	2.00		8.00	3.00	4.00		2.00	4.00	2.00	
Dinocap		3.50	3.50	X	3.00	.75			1.50	1.50		4.50
Ovex					2.66							
Oxythioquinox				1.25		4.00		5.20		2.00		10.20
Sulfur					6.00				4.66			

Pesticide	Pounds Actual/Acre							
	Orchard 4				Orchard 5			
	1959-1964	1965	1966	1967	1963-1964	1965	1966	1967
DDT	29.40			8.00	6.00	3.00		
Dieldrin	1.60							
Endrin							1.60	
Endosulfan	6.00	0.75			3.00	1.50		
Dilan	1.00				1.00			
Perthane	7.00		4.00	X				
Tetradifon	9.00				1.00			
Azinphosmethyl	4.50	1.25	1.50	12.00	1.25	1.50	1.25	2.50
Diazinon				1.00			3.00	9.00
Ethion	14.00			1.00	2.00	4.00		
Parathion	10.00	2.50		12.00				
2,4-D								
2,4,5-T								
Binapacryl	6.00	1.00	.50		3.00	3.00		
Dinocap	1.00							
Ovex								
Oxythioquinox		3.00		10.70		1.50	1.50	5.00
Sulfur	18.00						5.00	

NOTE: X denotes unknown amount.

TABLE 12.—*Pesticide residues in soils from five apple orchards near Wenatchee, Wash.*

Pesticide	Residues in ppm											
	Orchard 1				Orchard 2				Orchard 3			
	June 1965	Oct. 1965	Sept. 1966	Oct. 1967	June 1965	Oct. 1965	Sept. 1966	Oct. 1967	June 1965	Oct. 1965	Sept. 1966	Oct. 1967
DDT	75.88	71.84	108.74	127.01	76.29	68.08	113.74	107.14	82.16	76.83	88.70	92.91
Dieldrin							.26	.16	2.55	2.84	1.84	1.09
Endrin	3.43	5.37	1.97	1.15			.49	.40			4.36	2.40
Endosulfan					1.99	1.66	.94	.21	4.63	2.95	.71	.19
Toxaphene	—	—			—	—			—	—		
Ethion												.15
Ovex								.61				.45
Arsenic	205.96	—	—	—	71.04	—	—	—	95.52	—	—	—

Pesticide	Residues in ppm							
	Orchard 4				Orchard 5			
	June 1965	Oct. 1965	Sept. 1966	Oct. 1967	June 1965	Oct. 1965	Sept. 1966	Oct. 1967
DDT	46.64	64.76	62.39	62.21	59.24	87.62	106.99	92.98
Dieldrin	.83	1.61	.75	.47			.13	
Endrin			.24	.13			1.14	.51
Endosulfan	2.09	1.70	.83	.40	3.32		.08	.13
Toxaphene	—	—			—	—		7.72
Ethion	3.16							
Ovex				.74				
Arsenic	80.88	—	—	—	31.68	—	—	—

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 13.—*Record of pesticide applications on five orchards in Adams County, Pa.*

Pesticide	Pounds Actual/Acre														
	Orchard 1			Orchard 2			Orchard 3			Orchard 4			Orchard 5		
	1955-1964	1965	1966	1955-1964	1965	1966	1955-1964	1965	1966	1955-1964	1965	1966	1955-1964	1965	1966
DDT	12.00			56.00			56.00			44.80			56.00		
TDE				62.00	2.00		62.00	2.00		55.20			62.00	2.00	
Dieldrin				4.00			4.00			3.20			4.00		
Endrin				21.00	.72		21.00			20.40	1.79		21.00	1.79	
Tetradifon				3.00	1.00		3.00	1.00		3.75			3.75	1.00	
Azinphosmethyl	2.48			17.38	2.25		17.38	2.25		17.20	2.12		17.38	2.25	
Demeton											.50				
Ethion				1.00			1.00			1.00			1.00		
Malathion				2.00			2.00			2.00			2.00		
Parathion	9.52	0.78		15.06	1.25		15.06	1.25		12.82	.60		15.06	1.25	
Lead Arsenate	36.23	3.50		88.36	22.56		88.36	22.56		88.36	8.40		88.36	22.56	
Dinocap				.17			.68								
Ovex				7.50			7.50			6.70			7.50		
Oxythioquinox					.25			.25			.30			.25	
Sulfur		7.00		1.90	.12		1.90	4.68		1.90			1.90		

TABLE 14.—*Pesticide residues in soils from five orchards in Adams County, Pa.*

Pesticide	Residues in ppm														
	Orchard 1			Orchard 2			Orchard 3			Orchard 4			Orchard 5		
	Oct. 1965	Mar. 1966	Sept. 1966	Oct. 1965	Mar. 1966	Oct. 1966	Oct. 1965	Mar. 1966	Oct. 1966	Oct. 1965	Mar. 1966	Oct. 1966	Oct. 1965	Mar. 1966	Oct. 1966
DDT	12.40	11.78	14.78	76.84	139.35	141.95	121.36	136.45	220.76	26.75	21.18	35.14	62.20	67.10	81.81
TDE	.64	1.02	1.02	17.91	26.63	24.25	20.32	19.07	24.65	10.82	8.99	15.51	17.71	18.61	23.96
Dieldrin	.52	.34	.37			.84	.50	.73	.56	.26			.75	T	.52
Endrin	2.41	2.55	1.63	10.84	12.61	9.17	9.29	7.14	5.64	6.39	4.07	5.14	8.43	9.14	5.89
Arsenic	47.10	98.90	129.70	93.90	162.58	219.20	70.90	56.26	113.40	18.60	19.38	41.70	54.60	82.40	118.00
Ovex				2.93	2.55	1.93	1.83	1.32	1.41	3.00	1.53	1.82	2.60	1.60	2.06

NOTE: Empty spaces indicate no residues detected.
T = <.01 ppm.

TABLE 15.—*Record of pesticide applications on five orchards in Berrien County, Mich.*

Pesticide	Pounds Actual/Acre											
	Orchard 1				Orchard 2				Orchard 3			
	1954-1964	1965	1966	1967	1955-1964	1965	1966	1967	1956-1964	1965	1966	1967
DDT	54.50			10.50					2.00		3.20	12.00
TDE	28.50											
BHC									X			
Dieldrin	5.00				12.50	1.00	2.16	1.50	.50		3.20	
Endrin									.24			
Methoxychlor	1.00								34.65	10.00		
Tetradifon	3.00	0.50	0.80	1.76					3.75	1.25	.25	
Azinphosmethyl	17.64	21.60	8.40	5.24				2.25	8.86	10.75	8.00	1.52
Carbophenothion									X		.40	
Demeton	.70			3.50					X			
Diazinon	2.00								X			
Ethion	.50								4.50	3.20		
Malathion	12.75								X			
Parathion	13.64		3.20						2.75			.75
Phorate		.80										
2,4-D		.25										
Lead Arsenate	4.00				100.00	10.00	8.40	11.76	20.00	10.00	25.60	8.73
Dimethoate									X			
Ovex	4.25											
Oxythioquinox		1.80	.20								.40	.38
Sulfur	56.00								57.40	11.80	36.50	11.72

TABLE 15.—*Record of pesticide applications on five orchards in Berrien County, Mich.*—Continued

Pesticide	Pounds Actual/Acre							
	Orchard 4				Orchard 5			
	1955-1964	1965	1966	1967	1957-1964	1965	1966	1967
DDT	69.75	2.25	3.00	8.36				
TDE								
BHC								
Dieldrin					17.55			
Endrin								
Methoxychlor					7.80			
Tetradifon								
Azinphosmethyl	23.67	3.02	3.75		21.00		3.00	1.50
Carbophenothion								
Demeton								
Diazinon					12.20			
Ethion								
Malathion								
Parathion	1.28	1.10	.45	2.89	2.90			
Phorate								
2,4-D								
Lead Arsenate					97.60			
Dimethoate								
Ovex								
Oxythioquinox								
Sulfur	452.00	12.50	99.75	76.41	184.75	20.50	26.60	11.40

NOTE: X denotes unknown amount.

TABLE 16.—*Pesticide residues in soils from five orchards in Berrien County, Mich.*

Pesticide	Residues in ppm											
	Orchard 1				Orchard 2				Orchard 3			
	Nov. 1965	Mar. 1966	Nov. 1966	Oct. 1967	Nov. 1965	Mar. 1966	Nov. 1966	Sept. 1967	Nov. 1965	Mar. 1966	Nov. 1966	Oct. 1967
DDT	12.32	4.26	14.04	8.07	1.12	0.67	1.26	0.76	63.56	61.66	45.29	25.29
TDE	6.11	9.51	3.37	1.04	.29	.54	.41	.18	14.61	23.83	8.28	3.04
Dieldrin	.44	.33	.37	.20	.55	.51	.64	.53	.12	.15	.39	.31
Endrin				.36								.92
Arsenic	—	—	—	3.40	43.00	40.46	43.60	38.50	59.73	57.30	32.84	22.50

Pesticide	Residues in ppm						
	Orchard 4			Orchard 5			
	Nov. 1965	Mar. 1966	Sept. 1967	Nov. 1965	Mar. 1966	Nov. 1966	Oct. 1967
DDT	6.28	2.44	7.88	2.21	1.73	3.05	1.39
TDE	1.16	4.23	1.93	3.37	3.19	2.11	2.02
Dieldrin	.31	.42	.36	2.27	2.60	2.33	.88
Endrin							.59
Arsenic	—	—	10.70	51.58	49.32	52.40	35.20
Ethion	.20						

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 17.—*Record of pesticide applications on five apple orchards in western North Carolina*

Pesticide	Pounds Actual/Acre														
	Orchard 1			Orchard 2			Orchard 3			Orchard 4			Orchard 5		
	1955-1964	1965	1966	1955-1964	1965	1966	1955-1964	1965	1966	1955-1964	1965	1966	1955-1964	1965	1966
DDT	36.00		—	36.00		—	36.00		—	36.00		—	42.00		—
Endrin	25.60	3.20	—	19.20		—	16.00	3.20	—	9.60	3.20	—			—
Azinphosmethyl	60.00	12.00	—			—	36.00	12.00	—			—			—
Demeton	18.00	6.00	—			—			—			—			—
Malathion	56.00	8.00	—	40.00		—	48.00	8.00	—	56.00	8.00	—	24.00	8.00	—
Parathion	84.00	12.00	—	60.00		—	60.00	12.00	—	33.00	11.00	—	33.00	11.00	—

NOTE: Records for 1966 not available.

TABLE 18.—*Pesticide residues in soils from five apple orchards in western North Carolina*

Pesticide	Residues in ppm														
	Orchard 1			Orchard 2			Orchard 3			Orchard 4			Orchard 5		
	Oct. 1965	Apr. 1966	Oct. 1966	Sept. 1965	Apr. 1966	Oct. 1966	Sept. 1965	Apr. 1966	Nov. 1966	Oct. 1965	Apr. 1966	Oct. 1966	Oct. 1965	Apr. 1966	Oct. 1966
DDT	13.47	19.89	12.61	0.12	0.19	0.13	7.59	8.57	3.72	1.87	4.38	6.59	1.69	0.90	1.48
Endrin	3.13	3.36	1.34				.67	3.29	1.50	1.53	.55	.93	.19		
Chlordane						.10									
Arsenic	—	85.74	104.24	—	47.30	39.44	—	60.06	24.62	—	33.70	62.90	—	69.48	76.88

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 19.—*Record of pesticide applications on five citrus groves near Yuma, Ariz.*

Pesticide	Pounds Actual/Acre														
	Grove 1			Grove 2			Grove 3			Grove 4			Grove 5		
	Pre 1965	1965	1966	Pre 1965	1965	1966	Pre 1965	1965	1966	Pre 1965	1965	1966	Pre 1965	1965	1966
DDT	—			—			—			—			—		7.50
Diazinon	—			—	0.25		—			—			—		
Sulfur	—	50.00	85.00	—	50.00		—	37.50		—			—		33.30

NOTE: Records for "Pre 1965" not available.

TABLE 20.—*Pesticide residues in soils from five citrus groves near Yuma, Ariz.*

Pesticide	Residues in ppm														
	Grove 1			Grove 2			Grove 3			Grove 4			Grove 5		
	Apr. 1965	Jan. 1966	Nov. 1966	Apr. 1965	Jan. 1966	Nov. 1966	Apr. 1965	Feb. 1966	Nov. 1966	Apr. 1965	Feb. 1966	Nov. 1966	Apr. 1965	Jan. 1966	Nov. 1966
DDE	0.09	0.10	0.06	0.06	0.17	0.11	0.31	0.40	0.47	0.09	0.13	0.07	0.55	0.55	1.59
DDT	.04	.09	.02	.02	.07	.07	.29	.29	.30				2.39	3.05	7.02
TDE	—	—	.01	—	—	—	—	—	.04	—	—	—	—	—	.24
Dieldrin	.15	.19	.12	.08	.05	.02									
Arsenic	1.59	—	—	1.59	—	—	3.65	—	—	1.27	—	—	1.75	—	—

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 21.—*Record of pesticide applications on five peach orchards in central Georgia*

Pesticide	Pounds Actual/Acre														
	Orchard 1			Orchard 2			Orchard 3			Orchard 4			Orchard 5		
	1955-1964	1965	1966	1957-1964	1965	1966	1955-1964	1965	1966	1955-1964	1965	1966	1964	1965	1966
DDT	8.75												5.00		
Dieldrin	1.50	0.75	0.75	1.48	0.74	0.38	3.75	0.75	0.75	1.50	0.75	0.38	1.50	0.75	0.75
BHC	1.40												.80		
Endosulfan	3.00	1.00		3.00	1.00	1.00	.75		.75	3.00		1.00	3.00	1.00	1.00
Mirex										.01					
Malathion									.75						
Parathion	16.65	1.35	1.35	15.30	1.35	1.35	18.00	2.70	2.70	17.30	1.35	1.35	12.15	1.35	1.35
Lead Arsenate							42.00	6.00	3.00			6.00			
Sulfur	545.60	77.99	78.00	439.20	66.00	54.80	410.40	79.20	81.00	288.00	45.80	54.80	409.20	77.90	78.00

TABLE 22.—*Pesticide residues in soils from five peach orchards in central Georgia*

Pesticide	Residues in ppm														
	Orchard 1			Orchard 2			Orchard 3			Orchard 4			Orchard 5		
	Sept. 1965	Feb. 1966	Sept. 1966	Sept. 1965	Feb. 1966	Sept. 1966	Sept. 1965	Feb. 1966	Sept. 1966	Sept. 1965	Feb. 1966	Sept. 1966	Sept. 1965	Feb. 1966	Sept. 1966
DDT	0.51	0.52	0.38	0.15	0.15	0.30	0.17		0.21	0.48	0.34	0.51	0.51	0.49	0.39
TDE						.01						.08			
Dieldrin	.49	.59	.43	.40	.50	.39	.56	0.44	.54	.31	.37	.37	.61	.92	.95
Endosulfan	.62			1.45	.83	1.63	.50		.30	2.80	.40	2.38	1.08	.10	.80
Arsenic	—	6.52	5.32	—	6.26	4.54	—	7.32	7.04	—	6.98	7.20	—	6.50	8.98

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 23.—*Record of pesticide applications on five fields near Quincy and Moses Lake, Wash.*

Pesticide	Pounds Actual/Acre															
	Field 1				Field 2				Field 3				Field 4				Field 5
	1963-1964	1965	1966	1967	1963-1964	1965	1966	1967	1961-1964	1965	1966	1967	1956-1964	1965	1966	1967	1964
DDT					5.00				X				5.00				5.00
Aldrin	0.25								0.25								.25
Dieldrin		0.25															
Endosulfan									2.00	2.00							
Heptachlor					X												
Toxaphene					3.00					1.50			27.00				
Azinphosmethyl							2.00										
Demeton													4.50				
Ethion					1.00												
Parathion	6.00				2.00		.50		2.00								
2,4-D																	1.50
Sulfur									X	17.50							
MCPA			0.25						.25								.50

NOTE: X denotes unknown amount.

TABLE 24.—*Pesticide residues in soils from five fields near Quincy and Moses Lake, Wash.*

Pesticide	Residues in ppm																			
	Field 1				Field 2				Field 3				Field 4				Field 5			
	June 1965	Oct. 1965	Sept. 1966	Oct. 1967	June 1965	Oct. 1965	Sept. 1966	Oct. 1967	Sept. 1965	Oct. 1965	Oct. 1966	Oct. 1967	June 1965	Oct. 1965	Sept. 1966	Oct. 1967	June 1965	Oct. 1965	Sept. 1966	Oct. 1967
DDT	0.04	0.07	0.06		0.08	0.09	0.06	0.03	0.02	0.04	0.06	0.14	2.02	1.40	2.84	2.08	0.23	2.67	2.18	1.69
Aldrin	.07			0.14				.004												
Dieldrin	.14	.18	.19	.21	.08	.05	.14	.06	.17	.10	.18	.18			.01		.07	.10	.07	.06
Endrin																			.01	
Endosulfan										.08		.16				.36			.07	
Toxaphene	—	—			—	—			—	—			—			.95	—	—		
Trifluralin				.003				.48												
Arsenic	3.12	—	—	3.05	3.36	—	—	2.78	26.64	—	—	12.60	3.60	—	—	1.54	2.76	—	—	—

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 25.—*Record of pesticide applications on seven fields near Tulelake, Calif.*

Pesticide	Pounds Actual/Acre																			
	Field 1 [1]		Field 2				Field 3 [1]		Field 4				Field 5 [1]		Field 6		Field 7			
	1955-1964	1965	1955-1964	1965	1966	1967	1955-1964	1965	1955-1964	1965	1966	1967	1955-1964	1965	1966	1967	1966	1967		
DDT	4.50																			
Endrin									0.20				0.20							X
Endosulfan								0.25												
Demeton		0.25	0.50	0.25				.25		0.25				0.25						
Malathion			2.50	2.50	4.00	4.00					4.00									
2,4-D	1.25		.50				1.00		1.00				1.50							

[1] No pesticides applied in 1966 or 1967.
NOTE: X denotes unknown amount.

TABLE 26.—*Pesticide residues in soils from seven fields near Tulelake, Calif.*

Pesticide	Residues in ppm																			
	Field 1			Field 2			Field 3			Field 4			Field 5			Field 6		Field 7	
	Nov. 1965	Oct. 1966	Sept. 1967	Nov. 1965	Oct. 1966	Sept. 1967	Nov. 1965	Oct. 1966	Sept. 1967	Nov. 1965	Oct. 1966	Oct. 1967	Nov. 1965	Oct. 1966	Sept. 1967	Fall 1966	Oct. 1967	Fall 1966	Sept. 1967
DDT	0.50	0.36	0.47			0.07	0.01	0.01		0.01		0.01				0.02	0.01		
Aldrin																			
Dieldrin		.01	.002													.04	.05		
Endrin		.04	.08		0.07	.002	.31	.42	0.50	.16	0.10	.10	0.18	0.06	0.12	.04	.09	0.60	1.39
Toxaphene			.58																
Chlordane								T	.02										
Parathion									.06										
Arsenic	4.20	—	—	2.40	—	—	3.62	—	—	5.32	—	—	3.42	—	—	—	—	—	—

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.
T = <.01 ppm.

TABLE 27.—*Record of pesticide applications on five fields in Weld County, Colo.*

Pesticide	Pounds Actual/Acre																			
	Field 1				Field 2				Field 3				Field 4				Field 5			
	1949-1964	1965	1966	1967	1962-1964	1965	1966	1967	1960-1964	1965	1966	1967	1958-1964	1965	1966	1967	1955-1964	1965	1966	1967
DDT	22.50				10.00+															
Aldrin	8.00				3.00	1.00			0.25											
Dieldrin									1.50				0.50				0.25			
Endrin	X	0.50											.75							
Toxaphene											X									
Azinphos-methyl												X	X							
Demeton							8.00													
Diazinon		8.00			1.00															
M. Parathion		.50																		
Parathion													1.00+							
Phorate				1.00													10.00			
Sulfur												X	X		10.00					

NOTE: X denotes unknown amount.

TABLE 28.—*Pesticide residues in soils from five fields in Weld County, Colo.*

Pesticide	Residues in ppm																			
	Field 1				Field 2				Field 3				Field 4				Field 5			
	May 1965	Oct. 1965	Oct. 1966	Sept. 1967	May 1965	Oct. 1965	Sept. 1966	Sept. 1967	May 1965	Oct. 1965	Oct. 1966	Sept. 1967	May 1965	Oct. 1965	Sept. 1966	Sept. 1967	May 1965	Oct. 1965	Sept. 1966	Sept. 1967
DDT	3.43	3.74	4.30	3.16	0.55	0.53	1.20	0.72	1.49	1.28	1.83	1.52	0.11	0.12	0.17	0.28	0.68	0.31	0.89	0.84
Aldrin	.19	.02		.01	.04			.01				.01								
Dieldrin	.41	.44	.36	.29	.25	.22	.31	.19		.03	.06	.06			.04	.02	.02	.02	.03	.03
Endrin		.02		.06			.03		.08						.01	.01			.01	.02
Endosulfan											.11	.37								
Chlordane				.01				.01			.07	.07				.01				
Toxaphene	—	—			—	—			—	—	.78	2.01	—	—			—	—		.11
Malathion				.03																

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 29.—*Record of pesticide applications on five fields near Urbana, Ill.*

Pesticide	Pounds Actual/Acre																	
	Field 1			Field 2			Field 3			Field 4			Field 5					
	1962-1964	1965	1966	1958-1964	1965	1966	1962-1964	1965	1966	1961-1964	1965	1966	1960-1964	1965	1966			
Aldrin	1.00	1.00		0.15	0.63		0.25	1.75	1.40	3.00	1.25			1.75	1.60			
Dieldrin										.50								
Heptachlor				1.00			1.30					0.75						
2,4-D									.31	3.20			1.50		.37			
Trifluralin			1.00															

TABLE 30.—*Pesticide residues in soils from five fields near Urbana, Ill.*

Pesticide	Residues in ppm											
	Field 1				Field 2				Field 3			
	May 1965	Oct. 1965	May 1966	Winter 1966	May 1965	Nov. 1965	May 1966	Apr. 1967	Apr. 1965	Oct. 1965	May 1966	Apr. 1967
DDT												
Aldrin	0.11	0.01	0.16	0.47	0.01	0.02	0.02	0.02	—	0.01	0.01	0.07
Dieldrin	.12	.11	.13	.17	.02	.08	.07	.12	—	.01	.02	.09
Chlordane					.02	.03			0.06	0.15		
Heptachlor							.002	.03			.01	.02
Heptachlor epoxide					.01	.02	.03	.07	.01	.02	.02	.04
2,4-D												.004
Trifluralin				.02			.002					

Pesticide	Residues in ppm							
	Field 4				Field 5			
	May 1965	Nov. 1965	May 1966	Winter 1966	May 1965	Oct. 1965	May 1966	Nov. 1966
DDT	0.07	0.07						
Aldrin	.09	.09	0.19	0.35			0.01	0.26
Dieldrin	.28	.35	.35	.36		0.02	.04	.06
Chlordane								
Heptachlor				.002				
Heptachlor epoxide				.002				
2,4-D								
Trifluralin								

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 31.—*Record of pesticide applications on five fields in western Iowa*

Pesticide	Pounds Actual/Acre														
	Field 1			Field 2			Field 3			Field 4			Field 5		
	1960-1964	1965	1966	1961-1964	1965	1966	1960-1964	1965	1966	1961-1964	1965	1966	1957-1964	1965	1966
Aldrin	9.00			2.00			4.00			4.00		1.60	0.20		
Diazinon														1.00	
Phorate	1.00	1.00	0.80	1.00	1.00	1.00	1.00	1.00	1.00		1.00				
2,4-D	2.50	.25	.25		.33	.20		.33	.20	.50			2.50	.50	
Trifluralin															0.50

TABLE 32.—*Pesticide residues in soils from five fields in western Iowa*

Pesticide	Residues in ppm																	
	Field 1				Field 2				Field 3				Field 4				Field 5	
	May 1965	Nov. 1965	Apr. 1966	Nov. 1966	May 1965	Nov. 1965	Apr. 1966	Oct. 1966	May 1965	Nov. 1965	Apr. 1966	Nov. 1966	May 1965	Nov. 1965	Apr. 1966	Oct. 1966	Apr. 1966	Oct. 1966
Aldrin	0.03	0.11	0.01	0.01		0.07	0.01		0.07	0.03	0.13	0.002	0.06	0.02	0.002	0.04	0.04	0.01
Dieldrin	.02	.06	.04	.05	0.01	.01	.01	0.01	.06	.05	.03	.04	.10	.04	.01	.08	.22	.27
Chlordane														.11				
Heptachlor															.05	.08		
Heptachlor epoxide			.002												.05	.02		
Trifluralin																	.03	.09

NOTE: Empty spaces indicate no residues detected.

TABLE 33.—*Record of pesticide applications on six fields in eastern Virginia*

Pesticide	Pounds Actual/Acre																
	Field 1			Field 2			Field 3			Field 4			Field 5			Field 6	
	1962-1964	1965	1966	1960-1964	1965	1966	1962-1964	1965	1966	1960-1964	1965	1966	1963-1964	1965	1966	1960	
DDT				24.75						6.80							
Aldrin				1.50													
Dieldrin																	4.00
Diazinon	3.50			2.50	3.80					6.00							
Parathion		2.20									2.00			1.90			
Phorate		.80															
CDEC				X													
2,4-D									0.75	9.00							
Sulfur	72.00	72.00		796.25	97.00		800.00	45.00		170.00	101.25		11.25	91.25			

TABLE 34.—*Pesticide residues in soils from six fields in eastern Virginia*

Pesticide	Residues in ppm																
	Field 1			Field 2			Field 3			Field 4			Field 5			Field 6	
	Oct. 1965	Mar. 1966	Nov. 1966	Oct. 1965	Mar. 1966	Oct. 1966	Oct. 1965	Mar. 1966	Oct. 1966	Oct. 1965	Mar. 1966	Nov. 1966	Oct. 1965	Mar. 1966	Oct. 1966	Oct. 1966	
DDT	0.17	0.06		0.67	1.11	0.74	0.10	0.13		0.30	0.38	0.24	0.11	0.20	0.17	0.91	
Aldrin										.02							
Dieldrin	.12	.21	0.13	.08	.13	.07	.08	.14	0.06	.16	.24	.14	.12	.19	.11	.60	
Chlordane						.10											
Arsenic	1.64	—	1.68	3.48	—	4.06	1.85	—	1.38	2.06	—	1.52	3.20	—	3.88	—	

NOTE: Empty spaces indicate no residues detected; — = No samples analyzed.

TABLE 1.—*Additional pesticides used at the vegetable and/or cotton-growing areas*

KERN COUNTY, CALIF.

Aramite	Magnesium Chlorate
Carbaryl	Mevinphos
Dichloropropane	Panogen
Dichloropropene	Sodium Chlorate
Dicofol	Trichlorofon

LOWER RIO GRANDE VALLEY, TEX.

Azodrin	Endothall
Bidrin	Magnesium Chlorate
Calcium Cyanamide	Pentachlorophenol
Carbaryl	Sodium Chlorate
Disulfoton	Trichlorofon

DADE COUNTY, FLA.

Atrazine	Dithane M-45
Captan	Dyrene
Carbaryl	Maneb
Copper	Zineb

MONMOUTH COUNTY, N. J.

Carbaryl	Maneb
Dinitro compounds	Mevinphos
Di-Syston	Phosphamidon
Dithane	Polyram

EASTERN SOUTH CAROLINA

Atrazine	Linuron
Carbaryl	Maneb
Dichloropropene	Mevinphos
Eptam	Pyrethrum
Folpet	Rotenone

NOTE: Only Aramite and captan were analyzed for.

TABLE 2.—*Additional pesticides used at the fruit-growing areas*

WENATCHEE, WASH.

Amitrole	Fenson	Simazine
Carbaryl	Genite 923	Tepp
Dalapon	Glyodin	Vernolate
Dicofol	Lime Sulfur	Zinc
Dinitro compound	Mevinphos	Zinc Phosphate
Dinitrocresol	Paraquat	Zinc Sulfate
Diuron	Polysulfide	Ziram
Dodine		

ADAMS COUNTY, PA.

Amitrole	Dicofol	Glyodin
Aramite	Dinitro compound	Lime Sulfur
Captan	Dodine	Simazine
Copper Sulfate	Ferbam	Tepp
Dichlone	Folpet	Zineb

BERRIEN COUNTY, MICH.

Aramite	Kepone
Benzene Sulfamate	Kolofog
Benzene Sulfonic Acid	Kolospray

TABLE 2.—*Additional pesticides used at the fruit-growing areas*—Continued

Captan	Lime Sulfur
Carbaryl	Maneb
Chlorbenside	Mercury
Copper	Metacide
Cu Oxychloride Sulfate	Mevinphos
$CuSO_4$	NAA
Cu Sulfide	Naled
Dichlone	Niacide
Dicofol	Phenylmercuric Triethanol-ammonium Lactate
Dinitrocresol	Phosphamidon
Dodine	Prolate
Dormant Oil	70 Sec. Viscous Oil
Ferbam	Silvex
Fixed Copper	Tepp
Genite 923	
Glyodin	

WESTERN NORTH CAROLINA

Carbaryl

YUMA COUNTY, ARIZ.

Dicofol
Sabadilla

CENTRAL GEORGIA

Carbaryl
Dichlone
Dichloran
Zinc

NOTE: Only Aramite, captan, Kepone, and Genite 923 were analyzezd for.

TABLE 3.—*Additional pesticides used at small grain and root crop-growing areas*

QUINCY-MOSES LAKE, WASH.

Aramite	Eptam
Captan	Naled
Dicofol	Tepp
Di-Syston	

TULELAKE, CALIF.

Captan	Di-Syston
Dichloropropene	Panogen
1080	

WELD COUNTY, COLO.

Carbaryl	Maneb
Copper Sulfate	Pebulate
Diallate	Polyram
Dichloropropene	Trichlorofon
Di-Syston	Zinc

URBANA, ILL.

Amiben	CDAA
Atrazine	NPA

WESTERN IOWA

NPA

EASTERN VIRGINIA

Atrazine	Di-Syston
Carbaryl	Ethylene Dibromide
Copper	Pebulate
Dichloropropane	Vernolate

NOTE: Only Aramite, captan, and naled were analyzed for.

Pesticides referred to in this report:

Aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Amiben	3-amino-2,5-dichlorobenzoic acid
Amitrole	3-amino-1,2,4-triazole
Aramite	2-(*p-tert* butylphenoxy)-1-methylethyl-2-chloroethyl sulfite
Arsenic pentoxide	As_2O_5
Atrazine	2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine
Azinphosmethyl	*0,0*-dimethyl *S*-(4-oxo-1,2,3-benzotriazine-3,(4*H*)-ylmethyl) phosphorodithioate
Azodrin	3-hydroxy-*n*-methyl-*cis*-crotonamide, dimethyl phosphate
Benzine sulfonic acid, *p*-chloro-3,4-dichlorobenzyl ester	*p*-chlorobenzenesulfonic acid, 3-4-dichlorobenzyl ester
BHC (benzene hexachloride)	1,2,3,4,5,6-hexachlorocyclohexane consisting of several isomers and containing a specified percent of gamma BHC
Bidrin	3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide, dimethyl phosphate
Binapacryl	2-*sec*-butyl-4,6-dinitrophenyl 3-methyl-2-butenoate
Calcium arsenate	$Ca_3(AsO_4)_2$
Calcium cyanamide	$CaCN_2$
Captan	*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
Carbaryl	1-naphthyl methylcarbamate
Carbophenothion	3-[(*p*-chlorophenylthio)methyl] *0,0*-diethyl phosphorothioate
CDAA	2-chloro-*N,N*-diallylacetamide
CDEC	2-chlorallyl diethyldithiocarbamate
Chlorobenside	*p*-chlorobenzyl *p*-chlorophenyl sulfide
Chlordane	1,2,3,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
Copper oxychloride sulfate	mixture of copper chlorides and sulfates
Copper sulfate	$CuSO_4 \cdot 5H_2O$
2,4-D	2,4-dichlorophenoxyacetic acid
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane
DEF	*S,S,S*-tributyl phosphorotrithioate
Demeton	mixture of *0,0*-diethyl *S* and *0* [2-(ethylthio)] ethyl phosphorothioates
Diallate	*S*-(2,3-dichloroallyl) diisopropylthiocarbamate
Diazinon	*0,0*-diethyl *0*-(2-isopropyl-6-methyl-4-pyramidyl) phosphorothioate
Dichlone	2,3-dichloro-1,4-naphthoquinone
Dichloran	2,6-dichloro-4-nitroaniline
Dichloropropane	1,2-dibromo-3-chloropropane
Dichloropropene	1,3-dichloropropene
Dicofol	4,4′-dichloro-*alpha*-trichloromethyl benzhydrol
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethano=naphthalene
Dilan	mixture of 1,1-bis(*p*-chlorophenyl)-2-nitrobutane (i.e. Bulan) and 2-nitro-1,1-bis (*p*-chlorophenyl) propane (i.e. Prolan)
Dimethoate	*0,0*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate
Dinitrocresol	4,6-dinitro-*o*-cresol
Dinocap	2-(1-methyl-*n*-heptyl)-4,6-dinitrophenyl crotonate
Di-Syston	*0,0*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate
Dithane M-45	coordination product of Zn ion and manganese ethylene Bis dithiocarbamate 80%
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethyl urea
Dodine	*n*-dodecylguanidine acetate
Dyrene	2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine
Endosulfan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepan 3-oxide
Entothall	7-oxabicyclo-(2,2,1)-heptane-2,3-dicarboxylic acid
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
EPTC	*S*-ethyl di-*N,N*-propylthiocarbamate
Ethion	*0,0,0′,0′*-tetraethyl *S,S′*-methylene bis-phosphorodithioate
Ethylene dibromide	1,2-dibromoethane
Ethyl parathion (parathion)	*0,0*-diethyl-*0*-*p*-nitrophenyl phosphorothioate

Fenson	*p*-chlorophenyl ester of benzene sulfonic acid
Ferbam	ferric dimethyldithiocarbamate
Folpet	*N*-[(trichloromethyl)thio] phthalimide
Genite 923	2,4-dichlorophenyl ester of benzene sulfonic acid
Glyodin	2-heptadecyl-2-imidazolene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Kepone	decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta (*cd*) pentalen-2-one
Lead arsenate	$PbHAsO_4$
Lime sulfur	30% Ca polysulfide and various small amounts of Ca thiosulfate, water, and free sulfur
Lindane	gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane of 99+% purity
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
Magnesium chlorate	$Mg(ClO_3)_2 \cdot 6H_2O$
Malathion	*S*-[1,2-bis (ethoxycarbonyl) ethyl] *0,0*-dimethyl phosphorodithioate
Maneb	manganese ethylenebisdithiocarbamate
MCPA	4-chloro-2-methylphenoxyacetic acid
Metacide	mixture of 20% *0,0*-diethyl *0*-*p*-nitrophenyl phosphorothioate (i.e. parathion) and 80% of *0,0*-dimethyl *0,p*-nitrophenyl phosphorothioate (i.e. methyl parathion)
Methoxychlor	1,1,1-trichloro-2,2-bis (*p*-methoxyphenyl) ethane
Methyl demeton	*S* (and *0*) (2-ethylthio) ethyl *0,0*-dimethyl thiophosphate
Methyl parathion	*0,0*-dimethyl *0*-*p*-nitrophenyl phosphorothioate
Mevinphos	2-carbomethoxy-1-propen-2-yl dimethyl phosphate
Mirex	dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta (*cd*) pentalene
NAA	*alpha*-naphthaleneacetic acid
Naled	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Niacide	mixtures of manganous dimethyl dithiocarbamate and mercaptobenzothiazole
NPA	*N*-1-naphthylphthalamic acid
Ovex	*p*-chlorophenyl *p*-chlorobenzenesulfonate
Oxythioquinox	6-methyl-2,3-quinoxalinedithiol cyclic *S,S*-dithiocarbonate
Panogen	methylmercuric dicyandiamide ($C_4H_8N_4Hg$)
Paraquat	1,1′-dimethyl-4,4′-bipyridinium
PCNB	pentachloronitrobenzene
PCP	pentachlorophenol
Pebutate	*S*-propyl butylethylthiocarbamate
Perthane	1,1-dichloro-2,2-bis (*p*-ethylphenyl)ethane
Phorate	*0,0*-diethyl *S*-(ethylthio) methyl phosphorodithioate
Phosphamidon	1-chloro-diethylcarbamoyl-1-propen-2-yl dimethyl phosphate
Polyram	mixture of ethylene bis dithiocarbamic acid and zinc salts and sulfides
Prolate	*0,0*-dimethyl *S*-phthalimidomethyl phosphorodithioate
Pyrethrum	
Rotenone	$C_{23}H_{22}O_6$
Sabadilla	
Silvex	2-(2,4,5-trichlorophenoxy) propionic acid
Simazine	2-chloro-4,6-bis (ethylamino)-*s*-triazine
Sodium arsenite	$NaAsO_2$
Sodium chlorate	$NaClO_3$
Strobane	terpene polychlorinates (65-66% chlorine)
Sulfur	S
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
TDE	2,2-bis (*p*-chlorophenyl)-1,1-dichloroethane
Tepp	tetraethyl pyrophosphate
Tetradifon	*p*-chlorophenyl 2,4,5-trichlorophenyl sulfone
Toxaphene	technical chlorinated camphene (67-69% chlorine) octachlorocamphene
Trichlorofon	dimethyl (1-hydroxy-2,2,2-trichloroethyl) phosphonate
Trifluralin	*alpha, alpha, alpha*-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine
Vernolate	*S*-propyl dipropylthiocarbamate
Zinc	Zn
Zinc phosphide	Zn_3P_2
Zineb	zinc ethylenebis[dithiocarbamate]
Ziram	zinc dimethyldithiocarbamate

APPENDIX

*Chemical Names of Compounds Mentioned in This Issue**

ALDRIN	Not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
AMITROLE	3-amino-1,2,4-triazole
DCBP	4,4′-dichlorobenzophenone
DICOFOL	4,4′-dichloro-*a*-(trichloromethyl)benzhydrol
DDD (TDE)	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; technical DDD contains some *o,p′*-isomer also.
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
DDT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; technical DDT consists of a mixture of the *p,p′*-isomer and the *o,p′*-isomer (in a ratio of about 3 or 4 to 1)
DIELDRIN	Not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethano-naphthalene
ENDRIN	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
HEPTACHLOR	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
HEPTACHLOR EPOXIDE	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
LINDANE	1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer
METHOXYCHLOR	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane
MIREX	dodecachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalene
ZINEB	zinc ethylenebis[dithiocarbamate]
2,4-D	2,4-dichlorophenoxyacetic acid
2,4-DB	4-(2,4-dichlorophenoxy)butyric acid
2,4,5-TP	2-(2,4,5-trichlorophenoxy)propionic acid

*Does not include chemical names of compounds mentioned in the papers by (1) P. E. Cornelissen and (2) Lynn J. Stevens *et al.* Since the lists of compounds mentioned in these papers were extensive, they have been included in tables in the individual papers.

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ANNOUNCEMENT

Please note that the *Information for Contributors* has been revised to include an invitation for the submission of "brief" reports for publication in the Journal. It is the consensus of the Editorial Advisory Board and the Monitoring Panel that a useful purpose will be served in providing a place for the publication of data of a preliminary nature or studies of limited scope. Frequently, although such studies do not warrant publication as full reports, they do provide timely and informative data that should be brought to the attention of the readers. These papers will be identified as BRIEFS at the end of each issue.

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Residues of Polychlorobiphenyls in Biological Samples[1]

A. Richardson, J. Robinson, A. N. Crabtree, and M. K. Baldwin

ABSTRACT

The presence of polychlorobiphenyls (polychlorinated biphenyls) in samples of aquatic origin has been demonstrated and confirmed by combined gas-liquid chromatography—mass spectroscopy. The relevance of this finding to the determination of organochlorine insecticides is discussed.

Introduction

During the past 15 years the detection and quantitation of organochlorine insecticides in biological material has received considerable attention. With the introduction of the electron capture detector, used in conjunction with gas-liquid chromatography, it has become possible to detect residues of less than one part per hundred million. However, the electron capture detector is not specific for organochlorine compounds *(1)*, and even if it were, the occurrence of polyhalogenated hydrocarbons other than organochlorine insecticides in the environment would make the detection and quantitation of chlorinated pesticides difficult.

The presence of chlorinated compounds, other than insecticides, as residues in environmental samples has been suspected for some years. Roburn *(2)* showed that the total organochlorine content of some wildlife samples was greater than the organochlorine insecticide content determined by gas-liquid chromatography and inferred the presence of other organochlorine compounds. Eidelman *(3)* examined Norwegian cod liver oil and reported the presence of compounds "in the region of DDE, TDE and DDT" on gas chromatograms. Paper chromatography also indicated the presence of halogenated compounds which were not known insecticides. Harrison *(4)* reported the presence of compounds in biological samples which interfered with the determination of *p,p'*-DDT and *p,p'*-TDE. Robinson *et al. (5)* detected compounds of unknown identity in many marine organisms; these compounds, which also gave a response with the microcoulometric detector, were of low polarity and had retention times similar to or longer than that of *p,p'*-DDE.

The identification of a group of polychlorinated compounds by Jensen *et al. (6,7)* and Widmark *(8)* in the Swedish environment using a combination of gas chromatography and mass spectroscopy first drew attention to polychlorobiphenyls as environmental pollutants. The presence of these materials has also been reported in Britain *(9,10)* and North America *(11)*; the conclusions drawn by these reports were based upon the chromatographic behavior of the compounds in the samples analyzed and should, therefore, only be regarded as tentative. Koeman *et al. (12)*, however, examined samples collected in Holland by mass spectrometer and produced clear evidence of the presence of polychlorobiphenyls, together with other unidentified organochlorine compounds, and subsequently Bagley *et al. (13)* have used a similar technique to identify polychlorobiphenyls in bald eagle samples in the United States.

More recently, Reynolds *(14)* described a procedure for the separation of the more polar chlorinated insecticides from the polychlorobiphenyls, but his report stated that no positive identification had been obtained from polychlorobiphenyls in wildlife. Holden *et al. (15)* also described a similar procedure, but *p,p'*-DDE is not separated from the polychlorobiphenyls by the procedure of Reynolds *(14)* or Holden *et al. (15)*.

The composition of commercial blends of polychlorobiphenyls is not known, but mass spectral examination of a particular commercial product, Aroclor 1254, indicated the presence of chlorinated biphenyls containing three to seven chlorine atoms per molecule *(16)*. A synthetic mixture of mono- and di-chlorobiphenyls has

[1] From the Tunstall Laboratory, Shell Research Limited, Sittingbourne, Kent, England.

been separated on capillary columns *(17)*, and there is also a report on the separation of the components of a commercial sample of chlorinated biphenyls in the distillation range 325-360 C by programmed temperature, capillary GLC *(18)*.

Recently we investigated the presence of organochlorine insecticides in the environment. Since the largest residues in the United Kingdom occur in birds associated with the aquatic and marine environment, tissues of the shag and the heron have been analyzed for content of organochlorine insecticides. Herring oil has also been examined because of the obvious importance of fish in the diet of these birds. The results of these monitoring surveys will be published later.

Experimental Design

The method used routinely for the determination of chlorinated pesticides in environmental samples is given below. The extraction and cleanup procedure used is a modification of that described by de Faubert Maunder *et al. (19)*. It is suitable for the determination of γ-BHC, aldrin, heptachlor, *p,p'*-DDE, *p,p'*-TDE, *p,p'*-DDT, heptachlor epoxide, endrin, and dieldrin at concentrations of one part per hundred million in animal tissues, fats, oils, and eggs. The procedure consists of extraction with hexane or hexane/acetone followed by cleanup with dimethylformamide, hexane partition, liquid-solid chromatography on Florisil, and determination of the chlorinated insecticides by gas-liquid chromatography with electron capture detection *(20)*.

APPARATUS

Separating funnels:	100-ml, 250-ml, and 1-liter capacity.
Volumetric flasks:	10-ml, 20-ml, 25-ml, 50-ml, and 100-ml capacity.
Flat bottom flasks:	150 ml.
Graduated stoppered cylinders:	25-ml capacity.
Chromatographic column:	The column consists of thick wall glass tubing 55 cm long x 0.5 cm bore. A female S29 ball joint is fused at the bottom. The outlet of the column is made from a female S13 joint. The top of the column is connected to a S29 male joint which fits to an air line.
Soxhlet extraction apparatus	
High speed homogenizer	
Centrifuge	
Beakers	

REAGENTS

Sodium sulphate, anhydrous granular. General purpose reagent. Wash with hexane prior to use.

Hexane petroleum fraction SBP 60-70. Redistill and collect fraction boiling between 64 and 66 C.

Acetone. General purpose reagent, redistilled.

Sand. Horticultural sharp sand, hexane washed.

Diethyl ether. Analytical reagent, redistilled.

Florisil 60/100 mesh. Activate by heating overnight at 120 C. Deactivate by addition of 3% v/w distilled water into conical flask, stopper, and shake for 30 minutes.

Distilled water.

Potassium oxalate. General purpose reagent.

Methanol. Analytical reagent grade.

All reagents and apparatus should be examined by gas-liquid chromatography of a hexane extract to ensure freedom from contamination.

EXTRACTION

Animal fats, muscle tissue, kidney, liver, and brain. Freeze sample by storing in polystyrene box with Cardice. With sharp knife chop up frozen sample, mix well, and weigh 4 g into a 250-ml beaker. Add 10 g of sand and grind with a heavy glass rod with flattened end; add sufficient sodium sulphate and grind again to give a dry uniform granular mass. Warm the ground material successively with 50, 30, 30, and 30 ml volumes of 2:1 v/v hexane:acetone on a steam bath stirring carefully until the solvent boils gently. Decant the extract into a 150-ml flat-bottom flask and evaporate the bulked extracts to near dryness on a steam bath. Add 25 ml of hexane and re-evaporate to remove all traces of acetone. Transfer to a 100-ml volumetric flask by filtering through a filter funnel containing a glass wool plug covered with sodium sulphate and when cool make up to the mark with hexane. Treat a 25-ml solution of this by DMF partition.

Eggs. Weigh the egg contents, or the whole egg if contents are dehydrated. If the eggs are fluid, incorporate sufficient sodium sulphate to give a granular mass. If they are dehydrated and solid, grind them with sand and sodium sulphate. Transfer the granular mass to a suitable extraction thimble and extract for 2½ hours in a Soxhlet extractor with acetone:hexane (1:2). Evaporate the contents of the extraction flask to near dryness on a steam bath. Add 25 ml of hexane and evaporate to remove all traces of acetone. Transfer to a 100-ml volumetric flask by filtering through a filter funnel containing a glass wool plug covered with sodium sulphate and when cool make up to volume with hexane.

Fish Oil. Dissolve 4 g of fish oil in hexane in a graduated 100-ml flask and make up to volume with hexane. Take 25 ml for DMF partition.

LIQUID-LIQUID PARTITION PROCESS

Place 10 ml of DMF in a 100-ml separatory funnel. Allow a little of the DMF to run through into a lower 100-ml separatory funnel to lubricate both taps with clean DMF. This ensures that any leaking taps can be discarded without loss of sample. Place 25 ml of the hexane extract of the biological sample into the upper 100-ml separatory funnel, shake vigorously for 1 minute, allow the layers to separate, and run off the DMF phase into the lower 100-ml separatory funnel. Shake the hexane extract with two further 10-ml portions of DMF and add the DMF extracts to the first DMF extract. Discard the hexane phase. Wash the combined DMF extracts with 10 ml of hexane saturated with DMF. The hexane used for this washing is then extracted with an additional 10 ml of DMF. This extract and the original 30 ml of DMF extract are added to a 250-ml separatory funnel containing 180 ml of 2% aqueous sodium sulphate solution and 10 ml of hexane. Shake the aqueous sodium sulphate/DMF/hexane mixture and allow to settle for 40 minutes. Run off the aqueous phase and wash the hexane with two further 20-ml portions of distilled water. Dry the hexane extract by using a small quantity of sodium sulphate before the next cleanup procedure.

LIQUID-SOLID CHROMATOGRAPHY

Using a plug of hexane-washed cotton wool as a support, add 3 g of Florisil to the column and top with 1 cm of sodium sulphate.

Run on to the column the hexane solution obtained from the DMF partition and wash in with a little hexane. Using a 25-ml graduated flask as a receiver, elute with hexane to a volume of 25 ml. Replace the receiver with another 25-ml flask and elute with 25 ml of 10% diethyl ether in hexane. The first fraction will contain any γ-BHC, heptachlor, aldrin, *p,p'*-DDE and PCB's, *p,p'*-DDD, and *p,p'*-DDT which may be present in the original extract. The second fraction contains any heptachlor epoxide, dieldrin, and endrin which may be present.

GAS-LIQUID CHROMATOGRAPHY

Instrument conditions for first fraction:

Column:	1 m x 3 mm i.d., all glass
Column packing:	3.8% SE-30 on Diatoport S 80/100 mesh
Carrier gas:	N_2, oxygen free
Inlet pressure:	1.75 kg/cm^2
Detector:	Electron capture
Temperature:	184 C

Instrument conditions for second fraction:

Column:	1.5 m x 3 mm i.d., all glass
Column packing:	2% Oronite polybutene + 0.2% Epikote 1001 on Celite 85/100 mesh
Carrier gas:	N_2, oxygen free
Inlet pressure:	1.75 kg/cm^2
Detector:	Electron capture
Temperature:	184 C

Standard solutions:

Standard 1 (a)—A hexane solution containing 0.004 μg/ml of γ-BHC, 0.004 μg/ml aldrin, and 0.04 μg/ml *p,p'*-DDT.

Standard 1 (b)—A hexane solution containing 0.004 μg/ml of heptachlor, 0.02 μg/ml *p,p'*-DDE, and 0.02 μg/ml *p,p'*-DDD.

Standard 2—A hexane solution containing 0.01 μg/ml of heptachlor epoxide, 0.02 μg/ml dieldrin, and 0.02 μg/ml endrin.

METHOD

Inject 20 μl of sample into the appropriate column and record the chromatogram. The sample may then be diluted or concentrated to give a peak of the same height as in the appropriate standard.

Because of the decrease in sensitivity of the detector during prolonged running, it is impracticable to construct a calibration curve for the estimation of insecticide present. To overcome this difficulty it is essential to inject a standard after every two samples and to average the two standard peak heights for calculating the concentration of the insecticide in the biological samples between the standard samples.

Calculations:

Let mass of sample $= W$ g

Initial volume of hexane containing W g $= 100$ ml

Volume taken for cleanup $= 5$ ml

Final volume after cleanup (20 or 25 ml) $= Z$ ml

Volume of standard = volume of sample injected $= 20$ μl

Concentration of standard $= C$ μg

Dilution or concentration of final volume $= \frac{D}{Z}$

Mean peak height of standard at $R_T = P_S$ cm

Peak height of sample at $R_T = P_E$ cm

Then ppm of each component $=$

$$\frac{P_E \times 100 \times Z \times D \times C}{P_S \times W \times 5 \times Z} \text{ ppm}$$

EXTRACTION AND CLEANUP OF SAMPLES FOR GAS-LIQUID CHROMATOGRAPHY—MASS SPECTRAL ANALYSIS

Fish Oil. The crude herring oil (220 g) was dissolved in 500 ml of hexane and dried over anhydrous sodium sulphate.

Eggs. The total egg weight was 67 g, and the final hexane extract volume prior to the DMF partition was 100 ml.

CHROMATOGRAPHIC CLEANUP

Fish Oil. The solution in hexane was concentrated to 50 ml and percolated through a column 1 cm wide containing 20 g of activated Florisil. The column was eluted with hexane until there was no further elution of peaks corresponding to those occurring in the first fraction of routinely analyzed samples. The resulting eluate was concentrated to 10 ml and passed down an activated Florisil column (6 g), eluted with hexane, and fractions collected until no further peaks were eluted, as shown by GLC electron capture. This process was repeated three times.

The hexane eluate obtained as above contains *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, polychlorobiphenyls, and non-oxygenated "drin" insecticides. The more polar compounds such as dieldrin, endrin, and heptachlor epoxide are not eluted under these conditions. The solvent was evaporated from the eluate, and the residue was nitrated with a mixture of nitric and sulphuric acid 4:1 v/v. The acid mixture was poured into water and extracted with hexane. The resulting hexane solution was again chromatographed through activated Florisil, and the eluate was evaporated to 1 ml.

Heron Egg. Purification of the heron egg extract, following DMF partition, was achieved by chromatography on a deactivated Florisil column (25 g) using hexane (150 ml) as eluent. The concentrated eluate (10 ml) was further purified by chromatography on activated Florisil (6 g) using hexane as eluent.

Portions of the above samples were examined by gas-liquid chromatography with electron capture detection, microcoulometric detection, and mass spectrometric and flame ionization detection.

Standards of Aroclor 1254 were also examined by GLC with the same column conditions and detection procedures.

Gas chromatographic operating conditions for electron capture detection and microcoulometric detection were as follows:

Instrument:	Pye 104
Column 1:	152 cm × 0.3 cm glass tube packed with 3.8% SE-30 on Diatoport S
Inlet pressure:	1.25 kg/cm^2
Temperature:	185 C
Carrier gas:	N_2, oxygen free
Electron capture detector:	63 Ni source with pulsed DC supply and 150 microsecond pulse interval
Microcoulometric detector:	Inlet temperature 200 C Furnace temperature 900 C
Electrolyte:	70% v glacial acetic acid: 30% v water
Oxygen flow rate:	60 ml/minute

The other columns mentioned were operated under the following conditions:

Column 2:	152 cm × 0.3 cm glass tube packed with 2% GEXE60 on 80/100 mesh Gas Chrom Q
Temperature:	185 C
Inlet pressure:	1.4 kg/cm^2
Column 3:	152 cm × 0.3 cm glass tube packed with 2% WF1 on 100/120 mesh Gas Chrom Q
Temperature:	185 C
Inlet pressure:	1 kg/cm^2

The GLC/mass spectrometer operating conditions were:

Instrument:	Pye 104 chromatograph with flame ionization detector and 1:1 split between detector and mass spectrometer, an AEI MS 12
Chromatographic column:	152 cm × 0.3 cm glass column packed with 2% Silicone SE-30 and 0.2% Epikote 1001 on 85/100 mesh Diatoport S
Carrier gas:	Helium
Flow rate:	40 ml/minute
Temperature:	184 C
Ionization voltage:	70 volts

Results and Discussion

Gas chromatography on SE-30 columns followed by electron capture detection gave complex chromatograms for the hexane-eluted fractions of herring oil and heron

egg samples (Fig. 1 and 2). Comparison of relative retention times of these compounds with those obtained by the gas chromatography of Aroclor 1254 (Fig. 3) further suggested the possibility that chlorinated biphenyls may be present, and this was supported by the detection of peaks with similar retention values by the microcoulometric detector (Fig. 4).

Positive identification of the components responsible for interference in the determination of the organochlorine insecticides was made by mass spectroscopy coupled with gas chromatography. An example of part of the mass spectra obtained for two peaks from heron eggs is shown in Fig. 5 in comparison with two peaks of the same relative retention values on SE-30 derived from Aroclor 1254. The assignment of molecular weights and number of chlorine atoms per molecule for both samples is given in Table 1. This indicates the molecular weight (Cl = 35) and number of chlorine atoms per molecule of chlorinated compounds eluted at the quoted relative retention volumes (RRV *p,p'*-DDE = 1.00). Where more than one component was present in a peak this is indicated by a second or third row of values. For comparison, the molecular weights and number of chlorine atoms per molecule of polychlorobiphenyls is given in Table 2. Apart from confirming the occurrence of polychlorobiphenyls in environmental samples it also shows that on SE-30 columns *p,p'*-DDE has a similar retention time to that of a pentachlorobiphenyl. It is of interest that an extract of Coho salmon from Lake Michigan extracted and cleaned up in the same way as the two samples of European origin also showed interference by a pentachlorobiphenyl on the *p,p'*-DDE when examined by mass spectroscopy following gas chromatography on SE-30.

FIGURE 1.—*GLC/EC herring oil extract*

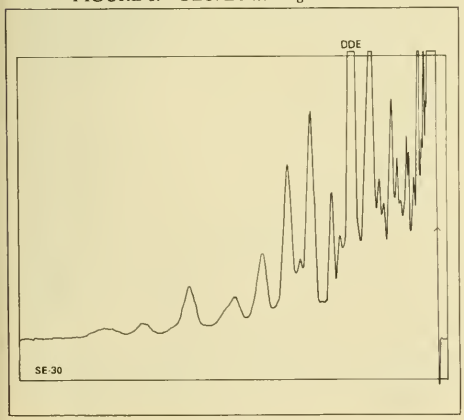


FIGURE 2.—*GLC/EC heron egg extract*

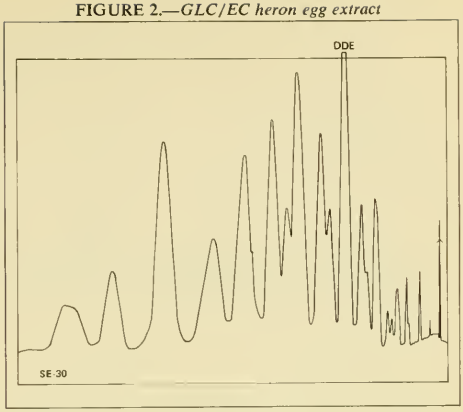


FIGURE 3.—*Comparison of relative retention times, Aroclor 1254, heron egg, and herring oil*

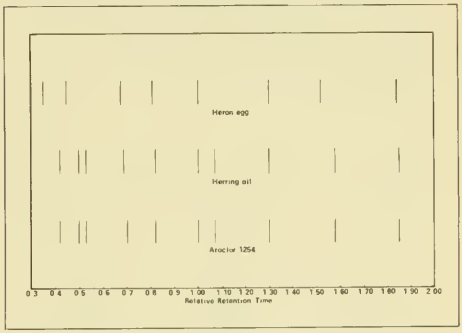


FIGURE 4.—*GLC/MC herring oil and heron egg extract*

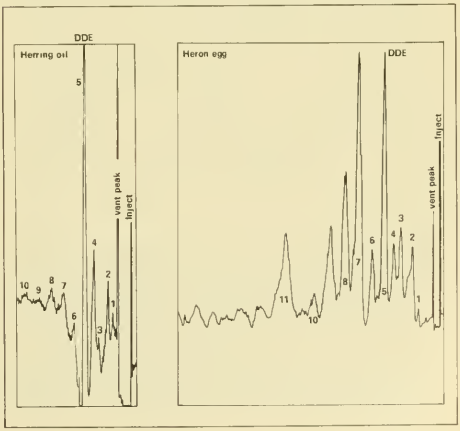


FIGURE 5.—*Comparison of mass spectra obtained from heron egg extract and Aroclor*

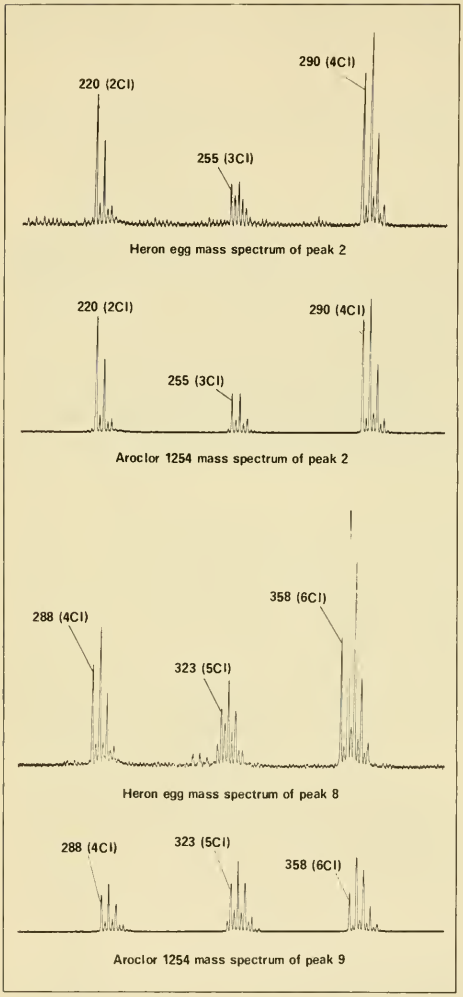
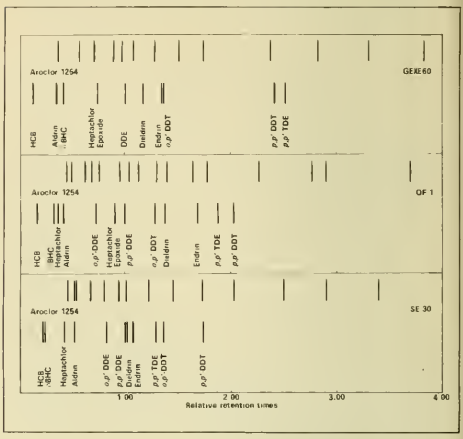


FIGURE 6.—*Comparison of relative retention times, $R_{DDE} = 1.00$ of Aroclor 1254 and chlorinated insecticides on GEXE60, QF-1, and SE-30*



Retention time data for some organochlorine insecticides and metabolites, and Aroclor 1254 were obtained on three liquid phases, silicone gum SE-30, GEXE60 and QF-1. These are compared in Fig. 6. The main interest is in the compounds of the DDT group since in general they occur, together with dieldrin, most widely in wildlife samples. However, since dieldrin can be easily separated from the polychlorobiphenyls by liquid-solid chromatography, the quantitation of dieldrin in the presence of these compounds presents no difficulty. There are considerable difficulties, on the other hand, in the case of DDT-type compounds and the PCB's. Eluate 1 of our procedure contains all the less polar compounds, i.e., lindane, aldrin, heptachlor, *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, and the polychlorobiphenyls. On all three of the stationary phases studied in this investigation there are some similarities in the retention times of some of the polychlorobiphenyls and one or more of the DDT-type compounds. Preliminary experiments have indicated the feasibility of separating PCB's from *p,p'*-DDT and *p,p'*-DDD by liquid-solid chromatography in a manner similar to Reynolds *(14)* and Holden *(15)*, but we have not, to date, been able to separate *p,p'*-DDE in this manner. The use of thin layer chromatography as described by Reichel *et al. (21)* has been unsuccessful in achieving the separation also.

The presence of halogenated compounds other than organochlorine insecticides in environmental samples presents a complex analytical problem which may not be solvable by gas chromatography. It would seem desirable to carry out a separation based on the chemical differences between the two groups of compounds such as resistance of PCB's to oxidation and/or alkaline hydrolysis and treat the DDT compound or compounds produced in this manner as a measure of DDT and its congeners.

TABLE 1.—*Molecular weight and number of chlorine atoms per molecule of components separated by GLC*

Relative retention volumes (p,p'-DDE = 1.00)— Molecular weights and corresponding number of Cl atoms per molecule										
AROCLOR 1254										
0.42 256(3) 290(4)	0.50 290(4)	0.53 324(5)	0.70 324(5)	0.82 324(5)	1.00 358(6) 324(5)	1.07 358(6)	1.30 358(6)	1.58 358(6)	1.85 358(6)	2.25 392(7) 358(6)
HERRING OIL										
0.42 *	0.50 290(4)	0.53 *	0.70 324(5)	0.82 324(5)	1.00 324(5) 316(4)	1.07 358(6)	1.30 358(6)	1.58 358(6)	1.85 392(7)	2.22 392(7)
HERON EGG										
0.35 256(3)	0.45 290(4)	0.69 324(5) 290(4)	0.81 324(5)	1.00 316(4) 324(5)	1.30 358(6) 324(5) 316(4)	1.52 358(6) 324(5)	1.84 358(6)	2.05 392(7) 358(6)	2.42 392(7)	2.76 392(7)	3.07 392(7)	3.75 426(8)

* Mass spectra not obtained.

TABLE 2.—*Molecular weight of polychlorinated biphenyls*

$C_{12}H_{(10-n)}Cl_n$	
No. of chloro-substituents	Corresponding molecular weight
1	188
2	222
3	256
4	290
5	324
6	358
7	392
8	426
9	460
10	494

At the present time there are no results to indicate the relative metabolic biodegradation rates of polychlorobiphenyls, and neither are pure components of the polychlorobiphenyls available. It is therefore impossible using current analytical procedures to quantitatively determine these materials in the environment with any degree of confidence.

Generally, in the North American continent, p,p'-DDT and its metabolites predominate over the PCB's, and therefore the determination of DDT compounds presents less difficulty than in the case of some European samples where the PCB concentration is much higher than the total DDT content. In the latter cases any results for the least polar chlorinated insecticides, particularly p,p'-DDE, must be considered as tentative until a method of separating the two groups of compounds is available.

See Appendix for chemical names of compounds mentioned in this paper.

Acknowledgment

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LITERATURE CITED

(1) *Lovelock, J. E. 1960.* An ionisation detector for permanent gases. Nature 187:49-50.

(2) *Roburn, J. 1965.* A simple concentration cell technique for determining small amounts of halide ions and its use in the determination of residues of organochlorine pesticides. Analyst 90:467-475.

(3) *Eidelman, M. 1963.* Determination of micro quantities of some chlorinated organic pesticide residues in edible fats and oils. J.A.O.A.C. 46:182-186.

(4) *Harrison, R. B. 1966.* The detection and determination of organochlorine pesticide residues in wildlife with special reference to endrin. J. Sci. Food Agr. 17:10-13.

(5) *Robinson, J., A. Richardson, A. N. Crabtree, J. C. Coulson, and G. R. Potts. 1967.* Organochlorine residues in marine organisms. Nature 214:1307-1311.

(6) *Jensen, S. 1966.* A new chemical hazard. New Sci. 32: 612.

(7) *Jensen, S., A. G. Johnels, M. Olssem, and G. Otterlind. 1969.* DDT and PCB in marine animals from Swedish Waters. Nature 224:247-250.

(8) *Widmark, G. 1967.* Possible interference by chlorinated biphenyls. J.A.O.A.C. 50.1069.

(9) *Holmes, D. C., J. H. Simmons, and J. O'G. Tatton. 1967.* Chlorinated hydrocarbons in British wildlife. Nature 216:227-229.

(10) *Holden, A. V. and K. Marsden. 1967.* Organochlorine pesticides in seals and porpoises. Nature 216:1274-1276.

(11) *Riseborough, R. W., P. Rieche, D. B. Peakall, S. G. Herman, and M. N. Kirven. 1968.* Polychlorinated biphenyls in the global ecosystem. Nature 220:1098-1102.

(12) *Koeman, J. H., M. C. Ten Noever de Braun, and R. H. de Vos. 1969.* Chlorinated biphenyls in fish, mussel and birds from the River Rhine and the Netherlands Coastal Area. Nature 221:1126-1128.

(13) *Bagley, G. E., W. L. Reichel, and E. Cromartie. 1970.* Identification of polychlorinated biphenyls in two bald eagles by combined gas-liquid chromatography—mass spectrometry. J.A.O.A.C. 53:251-261.

(14) *Reynolds, L. M. 1969.* Polychlorinated biphenyls (PCB's) and their interference with pesticide residue analysis. Bull. Environ. Contamination Toxicol. 4:128-143.

(15) *Holden, A. V. and K. Marsden. 1969.* Single stage clean-up of animal tissue extracts for organochlorine residue analysis. J. Chromatogr. 44:481-492.

(16) *Shell Research Ltd. 1956.* The use of chlorine isotopes in the interpretation of mass spectra—The analysis of polychlorinated aromatic hydrocarbons. Thornton Research Centre, Res. Rep. R.722.

(17) *Weingarten, H., D. W. Ross, J. M. Schlater, and G. Wheeler, Jr. 1962.* Gas chromatographic analysis of chlorinated biphenyls. Anal. Chim. Acta 26:391-394.

(18) *Perkin-Elmer Ltd. 1964.* Analysis of chlorinated biphenyls with open tubular (Golay) columns. Gas Chromatogr. Appl. Sheet No. GC-DS-011.
(19) *de Faubert Maunder, M. J., A. Egan, E. W. Godly, E. W. Hammond, J. Roburn, and J. Thompson. 1964.* Clean-up of animal fats and dairy products for the analysis of chlorinated pesticide residues. Analyst 89: 168-174.
(20) *Richardson, A. 1967.* The determination of organochlorine insecticides in animal tissues and fluids. Paper presented at VIth International Plant Protection Congress, Vienna.
(21) *Reichel, W. L., T. G. Lamont, E. Cromartie, and L. N. Locke. 1969.* Residues in two bald eagles suspected of pesticide poisoning. Bull. Environ. Contamination Toxicol. 4:24-30.

Dieldrin and Endrin Concentrations in a Louisiana Estuary

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ABSTRACT

The general objective of this study was to determine the endrin and dieldrin concentrations in water, bottom sediment, and oysters in an estuarine area of Louisiana.

Sampling was conducted on approximately a semimonthly basis from October 1968 through May 1969 in Grand Bayou, Hackberry Bay, and Creole Bay. Creole Bay is about 14 miles above the Gulf of Mexico.

Samples of oysters, sediment, and water were analyzed for residues of dieldrin and endrin using electron capture gas chromatography. Identification was made first on a nonpolar column and then on a polar column.

The median concentration of dieldrin and endrin present in the oysters collected from Grand Bayou, Hackberry Bay, and Creole Bay was 1.3 ppb and less than 1 ppb, respectively, while the maximum concentration was 3.4 ppb and 2.4 ppb. Water samples from all stations on every sampling date contained less than 1 ppb of both dieldrin and endrin; the highest level of dieldrin detected in the bottom sediment was 4 ppb, and the maximum concentration of endrin was less than 5 ppb.

Levels in oyster samples collected in this study were compared to those for samples collected in the same general area between 1964-66.

Introduction

The use of pesticides has been a principal factor in increased agricultural production as well as control of insect vectors of disease. However, some pesticide residues often persist in the environment and can eventually appear in water resources far from the area of initial application. In addition, aquatic organisms have the ability to concentrate trace substances at levels many times greater than those present in the aqueous environment. Because of their stationary habits and sensitivity to the environment, oysters are of particular concern as concentrators of pesticides. Oysters and other mollusks are, in fact, so uniquely efficient in concentrating chlorinated hydrocarbon pesticides that methods for using mollusks to monitor pesticide pollution have been investigated *(1)*. Fortunately, the oyster is capable of purging itself of absorbed pesticides and other toxins when the pollutant is removed from the environment. Physiological recovery of the oyster can occur if the damage has not been too extensive *(2)*.

The general objective of this study was the determination of endrin and dieldrin concentrations in the water, bottom sediment, and oysters of an estuarine area of Louisiana. Of major concern were the concentrations in oysters, specifically *Crassostrea virginica,* the American oyster which is sometimes referred to as the eastern oyster. As shown in the inset to Fig. 1, the study area was near Barataria Bay located some 40 miles south of New Orleans.

In 1963 a major fish kill occurred in the lower Mississippi River with endrin identified as the causative agent. The possibility of pesticides reaching the estuarine waters was considered quite possible, especially in view of the westerly tides which may carry Mississippi River water into Barataria Bay. Because of the public health implications of pesticides in water and shellfish, an investigation was conducted in 1964-1965 by the Gulf Coast Shellfish Sanitation Research Center in cooperation with the Louisiana State Board of Health, Louisiana Wildlife and Fisheries Commission, Louisiana Stream Control Commission, and the Federal Water Pollution Control

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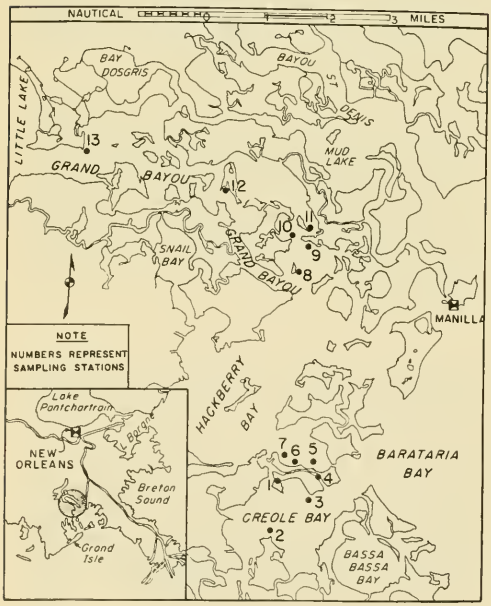
Administration *(3)*. Oysters, water, and bottom sediment from oyster-growing areas west of the lower Mississippi River were analyzed for dieldrin, endrin, and other chlorinated hydrocarbon pesticides. In most instances, chlorinated pesticides were not detected or were present in very low concentrations. The median concentrations of endrin and dieldrin in the oyster samples were less than 10 ppb.

Hammerstrom *et al. (3)* reported the concentrations of dieldrin and endrin detected in oysters during a 1965-1966 study of southern Louisiana estuaries. The highest concentration of endrin reported was 70 ppb, and the median value was less than 10 ppb. The highest concentration of dieldrin was 90 ppb, and the median value was less than 10 ppb.

Bugg *et al. (4)* reported a 1964-66 study of pesticides in the South Atlantic and Gulf of Mexico. Oysters were collected from estuarine areas of South Carolina, Georgia, Florida, Mississippi, Texas, and Louisiana. Pesticide concentrations were determined by electron capture gas chromatography, and chlorinated pesticides were either not detected or were present in relatively low concentrations. The Louisiana samples had a median concentration of dieldrin of 10 ppb.

FIGURE 1.—*Study area*



Study Area

As shown in Fig. 1, the estuarine area selected for the study is approximately 40 miles south of New Orleans; sampling sites are located in Grand Bayou, Hackberry Bay, and Creole Bay. Creole Bay is about 14 miles above the Gulf of Mexico.

The entire watershed above the study area eventually drains through Grand Bayou, Hackberry Bay, and Creole Bay. This watershed encompases the region between Bayou Lafourche on the west and the Mississippi River on the north and east. The calculation of the amount of fresh water reaching the study area from the watershed is very complicated and, in fact, is not attempted by the U. S. Geological Survey. Their hydrographical studies are not applicable to areas affected by tidal backwater and interconnected drainage *(Sauer, V. B., U.S. Geological Survey, Baton Rouge, La. personal communication, 1969)*.

The study area is typically estuarine in nature, with average depths of 4 to 5 feet, and stratification of salinity levels. The 1963 total marketable oyster harvest for Barataria Bay and vicinity was estimated to be 111,000 barrels (3.73 ft.^{3}/barrel) *(5)*.

Agricultural use of pesticides is common above Barataria Bay, especially along Bayou Lafourche and the Mississippi River where the ground is high and fertile. The principal crops are sugar cane, soybeans, and rice. Dieldrin, endrin, and DDT have been used in the past to control insect pests such as the sugar cane borer. Dieldrin, never extensively used, is not presently applied in this area. The use of endrin has been curtailed since 1968 upon recommendation of the Louisiana State Department of Agriculture, although small amounts of endrin may have been applied in some areas during 1969. The major economic poisons in current use in the drainage area are carbaryl for sugar cane; toxaphene, DDT, and silvex for soybeans; and propanil, 2,4-D, and 2,4,5-T for rice.

Sampling Procedures and Environmental Analyses

As shown in Fig. 1, 13 sampling stations were selected in Grand Bayou, Hackberry Bay, and Creole Bay. The stations were divided into four groups, with Group A (Stations 1-7) being closest to the Gulf, Group B (Stations 8-11) further up in the estuarine area, and Group C (Station 12) and D (Station 13) being still further upstream.

Sampling was conducted 12 times on an approximately semimonthly basis from October 1968 through May 1969. At about noon on each sampling date approximately 10 oysters (the average edible portion per oyster

was 10 g), 50 g of bottom sediment, and 32 oz of water were collected at each station. The oysters and bottom sediment were collected using a dredge; the sediment samples were obtained at a depth of 3 to 4 inches; and surface water samples were collected by standard techniques.

Field measurements, using instrumental analyses, were made on each sampling date at each station for the following environmental parameters: temperature, salinity, dissolved oxygen, and pH. Temperature and salinity measurements were made with a commercial salinometer, dissolved oxygen with a dissolved oxygen probe, and pH with a pH meter. The turbidities of the water samples were measured at the Riverside Research Laboratories with a turbidimeter.

Analytical Procedures

The oyster, sediment, and water samples were analyzed for residues of dieldrin and endrin using electron capture gas chromatography. A Micro-Tek Gas Chromatograph Model MT-220 was used, and identification was made first on a nonpolar column *(9)* (DC 200 on Anakrom ABS) and then on a polar column (4% SE-30 and 6% QF-1 on Anakrom ABS).

The sediment samples were ground to a fine powder, and at least a 15% moisture content was maintained in order to enhance the release of the volatile pesticides.

The analytical procedures used for oysters, including the extraction of dieldrin and endrin and their detection by gas chromatography, were minor modifications of methods published in the *Guide to the Analysis of Pesticide Residues (6)*. The analyses of water and sediment samples were carried out in accordance with established procedures *(7,8)*.

The oyster and soil extracts were cleaned up by filtering through columns made up of successive layers of anhydrous sodium sulfate and Florisil. The Florisil was specially prepared for use with the micro-modification of the Mills cleanup method. The columns were washed with hexane which would elute the PCB's but not the pesticide residues *(9)*. The pesticides were then eluted from the column with diethyl ether in petroleum ether. In any event the concentrations obtained for dieldrin and endrin represent maximums since PCB's, if present, would exert a positive interference. The eluate from the columns was then concentrated by evaporation to a small volume by a stream of air at room temperature. The volume was then made up to 10 ml, and an aliquot of 5 μl injected into the gas chromatograph.

Periodically, samples were spiked with either dieldrin or endrin; the spiked samples were then carried through the appropriate analytical procedure and percent recovery of the pesticides calculated. Recovery was 82% or better for the oysters, 95% or better for the water, and 50% or better for the sediment. The concentrations reported herein have been corrected for recovery.

The chemical standards used in these analyses were obtained from the Pesticides Research Laboratory, USPHS, Perrine, Fla. The sample size used for GLC injection was selected to provide detection and confirmation of residues at or above 1 ppb for the oysters and water and 2 ppb for the sediment.

A statistical evaluation of the number of sampling stations and number of oysters per station indicated that approximately 15 stations with 10-15 oysters per station would be adequate to secure meaningful data.

Results of sample analyses for pesticides were expressed in one of four ways: (1) as an identifiable concentration, (2) as a concentration less than a given value, (3) as a trace amount, or (4) as having less than the minimum detectable limit for the method. The first three ways are considered as positive responses for the presence of pesticides; however, only the first one is considered as a positive, identifiable response. The levels of pesticides found were reported in parts per billion (μg/kg) of drained weight for the oysters and on the air-dried soil weight. Residues in the water samples were reported in parts per billion (μg/liter).

Environmental Data

A summary of the environmental data collected during the study period is contained in Table 1. The temperature, pH, and dissolved oxygen values were very similar at each of the four groups of stations. Salinity values decreased as the distance from the Gulf of Mexico increased; whereas, the turbidity increased as salinity decreased. The overall environmental conditions during the study period were conducive to satisfactory oyster growth and reproduction.

TABLE 1.—*Environmental data summary*

Parameter	Mean Value			
	Group A	Group B	Group C	Group D
Temperature (C)	17.5	17.6	17.0	17.2
pH (units)	8.0	8.0	8.1	8.1
Salinity (‰)	15.4	12.5	10.1	8.6
Turbidity (JTU)	58	67	73	126
Dissolved Oxygen (mg/liter)	8.8	8.8	8.9	8.8
(% saturation)	99	98	97	96

TABLE 2.—*Pesticide detection in environmental samples*

Pesticide	Medium	No. of Samples with Positive Concentration	No. of Samples with Concentration Less than a Given Value	No. of Samples with Trace Amounts	Total No. of Positive Samples	Total No. of Samples	Percent of Positive Samples
Dieldrin	Water	0	2	2	4	148	3
	Sediment	0	8	1	9	45	20
	Oysters	93	20	0	113	113	100
Endrin	Water	2	2	4	8	148	5
	Sediment	0	7	1	8	44	18
	Oysters	3	62	12	77	111	69

Dieldrin Concentrations

A summary of the data for water, sediment, and oyster samples positive for dieldrin is contained in Table 2.

A total of 148 water samples were analyzed for the presence of dieldrin during the survey. Responses of less than the minimum detectable limit for the method (1 ppb) were obtained in 144 samples (97% of the total water samples). Two samples yielded positive results of less than 0.2 ppb, and trace quantities were detected in two other samples.

A total of 45 sediment samples were analyzed for the presence of dieldrin. Responses of less than the minimum detectable limit were obtained for 36 samples (80% of the total sediment samples). Positive results were obtained as follows: one sample contained less than 4 ppb, one sample less than 2 ppb, and six samples less than 1 ppb. A trace amount of dieldrin was detected in one sample.

A total of 113 oyster samples were analyzed for the presence of dieldrin, and all yielded positive results. Twenty samples gave positive results with levels less than a given concentration, and 93 samples exhibited positive, identifiable concentrations. Of the 20 samples, 4 contained levels of less than 2 ppb, 3 had less than 1.1 ppb, 7 had less than 1 ppb, 1 had less than 0.9 ppb, and five had less than 0.5 ppb. The mean concentration in the 93 samples with identifiable concentrations was 1.4 ppb, and the median value was 1.3 ppb. The maximum measured concentration was 3.4 ppb.

The variation of dieldrin concentrations in oysters collected at each sampling date is shown in Table 3. In general, the concentrations were greater in the groups of stations which were further removed from the Gulf of Mexico (Groups C and D).

TABLE 3.—*Dieldrin concentrations in oysters*

Sampling Date	Average Dieldrin Concentration in PPB			
	Group A	Group B	Group C	Group D
10-21-68	0.8	1.0	1.0	—
11-17-68	0.8	0.7	1.7	2.2
12-1-68	*	*	—	—
12-15-68	0.8	0.4	1.5	1.1
12-29-68	1.1	1.4	—	—
2-2-69	1.3	1.6	—	*
2-16-69	1.9	1.7	2.2	1.7
3-2-69	1.5	1.8	—	1.2
3-24-69	1.9	1.8	1.1	1.5
4-14-69	2.0	2.0	2.2	2.7
5-11-69	1.1	1.1	—	1.1

NOTE: * = less than the detectable limit of 1 ppb; — = no sample.

Endrin Concentrations

A summary of the data for water, sediment, and oyster samples positive for endrin is contained in Table 2. A total of 148 water samples were analyzed for the presence of endrin during the survey. Responses of less than the minimum detectable limit for the method (1 ppb) were obtained in 140 samples (95% of the total water samples). Two samples yielded identifiable levels of 0.09 and 0.2 ppb; two samples were positive with levels of less than 0.2 ppb; and trace quantities were detected in four other samples.

A total of 44 sediment samples were analyzed for the presence of endrin. Responses of less than the minimum detectable limit were obtained in 36 samples (82% of the total sediment samples). Five samples were positive with levels of less than 5 ppb; and two samples had less than 4 ppb. A trace amount of endrin was detected in one sample. The sediment contained 31% sand (over 50 microns in diameter), 25% silt (2 to 50 microns in diameter), and 16% clay (less than 2 microns in diameter) with the balance being organic material and soluble compounds.

A total of 111 oyster samples were analyzed for the presence of endrin. Responses of less than the minimum detectable limit for the method (1 ppb) were obtained in 34 samples (31% of the total oyster samples). A total of 62 samples were positive with levels of less than a given concentration: 3 samples exhibited positive, identifiable concentrations; and 12 samples contained trace quantities. Of the 62 samples, 1 sample yielded a positive response of less than 6 ppb, 10 samples less than 5 ppb, 5 samples less than 4 ppb, 1 sample less than 2 ppb, 32 samples less than 1 ppb, 1 sample less than 0.8 ppb, and 12 samples less than 0.5 ppb. The mean concentration in the three samples with identifiable concen-

trations was 1.8 ppb, with the lowest measured value being 0.9 ppb and the highest 2.3 ppb.

Discussion and Conclusions

The dieldrin and endrin concentrations in *C. virginica* oysters, water, and bottom sediment samples collected during 1968-1969 from a southeastern Louisiana oyster-growing estuarine area were low; however, they must be evaluated in terms of the tolerance levels in food for these pesticides. The overall mean concentrations of dieldrin and endrin in over 100 oyster samples was 1.4 ppb and less than 1 ppb, respectively; and the median values were 1.3 ppb and less than 1 ppb, respectively. Dieldrin was detected in a positive, identifiable concentration in 93 of 113 samples; whereas, endrin was found in like manner in only 3 of 111 samples. The maximum concentration of dieldrin and endrin present in the oysters collected from Grand Bayou, Hackberry Bay, and Creole Bay was 3.4 ppb and 2.3 ppb, respectively.

Water samples from all stations on every sampling date contained less than 1 ppb of both dieldrin and endrin. The highest level of dieldrin detected in the bottom sediment was less than 4 ppb. The maximum concentration of endrin in this medium was less than 5 ppb; however, in the case of endrin the only definite maximum value detected was 1.9 ppb.

Upon comparison of pesticide concentrations found in this study with those from similar surveys conducted in 1964-1966 and 1965-1966, it seems evident that the pesticide influx into the study area has decreased since the earlier work *(3,4)*. A comparison of the results is contained in Table 4. The 1968-1969 median dieldrin concentration is less that the 1964-1966 level by a factor of 7, and the maximum is less by a factor of 26 than the 1965-1966 level. The 1968-1969 maximum endrin concentration is 29 times less than the 1965-1966 level.

TABLE 4.—*Pesticides in oysters*

Survey	Residues in PPB			
	Dieldrin		Endrin	
	Median	High	Median	High
1964-1966 (*4*)	10	—	—	—
1965-1966 (*3*)	<10	90	<10	70
1968-1969	1.3	3.4	<1	2.4

The decrease in pesticide concentrations found in oysters is due to a decrease in the amounts of chlorinated hydrocarbons applied in the selected drainage area as well as to decreasing pesticide concentrations in the nearby Mississippi River. Recent pesticide concentrations detected in the Mississippi River at New Orleans were generally near zero and considerably less than amounts detected previously.

In order to determine the major source of pesticides in the study area, an attempt was made to evaluate the effect of the measured environmental conditions on the level of pesticides in the oysters at any given date. Due to the lack of sufficient definite results for endrin concentrations in the oysters, only the time variations of dieldrin concentrations were examined.

Considering the recent nondetectable levels of dieldrin and endrin measured in the Mississippi River at New Orleans, it was considered that the major influx of pesticides to the study area was in runoff from the drainage basin. Pesticides in the runoff could come from soil residues or from rainout or washout. Rainfall data from two rain gauge stations located in the drainage basin were obtained for the period from October 1968 through May 1969. One station (Diamond 4N W) was located approximately 15 miles northeast of the center point of the oyster-sampling area; and the other station (Galliano) was located about 15 miles northwest of the center point. The measurement of rainfall over 7-day periods prior to the collection of samples is summarized in Table 5.

TABLE 5.—*Rainfall in drainage area*

Sampling Date	7-day rainfall prior to sampling (inches)		Average 7-day Rainfall (inches)
	Diamond 4 NW	Galliano	
10-21-68	0.42	—	0.21
11-17-68	0.50	1.30	0.90
12-1-68	1.52	2.00	1.76
12-15-68	1.15	0.49	0.82
12-29-68	0.36	0.65	0.50
1-19-69	3.18	2.50	2.84
2-2-69	0.20	0.10	0.15
2-16-69	1.75	1.33	1.54
3-2-69	0.12	0.28	0.20
3-24-69	0.63	1.42	1.02
4-14-69	3.34	1.68	2.51
5-11-69	4.77	4.48	4.62

The effect of the 7-day rainfall on the measured environmental characteristics on a given sampling day was examined. No apparent effects on water temperature, pH, or dissolved oxygen were found. However, as would be expected, the levels of salinity and turbidity at each group of stations varied depending on the rainfall just preceding sampling. These variations are shown in Fig. 2.

In general, turbidity increased and salinity decreased as the distance separating the stations and the Gulf of Mexico increased. Considering turbidity only, there was a greater variation between Group A stations (closest to Gulf) and Group D stations (farthest from the Gulf) for 7-day rainfalls between 1 and 5 inches than for rains between 0 and 1 inch.

If the major pesticide influx into the sampling area was from land drainage, then the dieldrin concentrations in the oysters should be greater following heavier rains. This was found to be true as is indicated in Table 6 and shown in Fig. 3. Pesticide concentrations in the oysters increased with increasing distance from the Gulf of Mexico, and the average levels at all groups of stations were higher after heavier rains during the 7-day period preceding sampling. If westerly tides forcing Mississippi River water into the area caused the greatest influx of dieldrin, the trends indicated in Fig. 3 should be reversed.

TABLE 6.—*Influence of rainfall on dieldrin in oysters*

7-day Rainfall (inches)	Residues of Dieldrin in PPB			
	Group A	Group B	Group C	Group D
0-1	1.0	1.2	1.4	1.6
1-5	1.4	1.3	1.8	1.8

FIGURE 2.—*Variation of turbidity and salinity with rainfall*

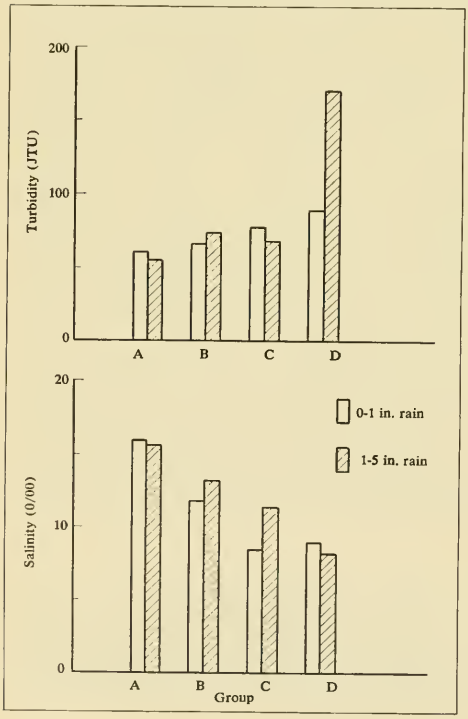
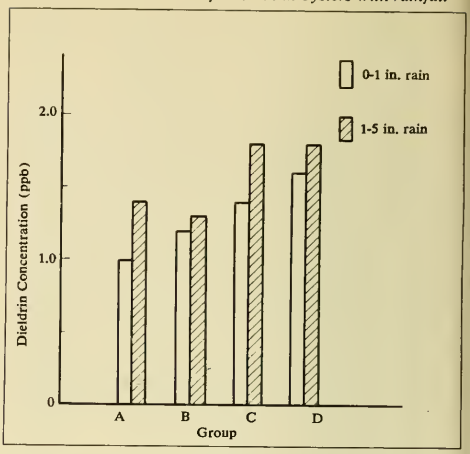


FIGURE 3.—*Variation of dieldrin in oysters with rainfall*



The influence of water temperature on dieldrin uptake by oysters was examined, and the results are shown in Table 7. On December 15, 1969, the average water temperature at all stations was 8.5 C; the average dieldrin concentrations were less at all groups of stations than they were at any other temperatures. The 7-day rainfall prior to December 15 was in the 0- to 1-inch range. At about 8 C the American oyster ceases to feed and is essentially dormant *(10);* therefore, the low levels of dieldrin in the oysters observed on December 15, 1969, can be attributed to oyster inactivity as well as to the low level of rainfall in the 7-day period preceding sampling.

TABLE 7.—*Influence of temperature on dieldrin in oysters*

Water Temp. (C)	Number of Days	Dieldrin Concentration in PPB			
		Group A	Group B	Group C	Group D
8.5	1	0.8	0.4	1.5	1.1
10-15	4	1.5	1.6	2.2	1.4
15-25	4	1.6	1.6	1.6	1.8

Although levels of dieldrin and endrin in the media investigated by this study are of great concern, the use of these pesticides has declined since 1965. However, at the time of this study, DDT was being utilized in this drainage area; and data for DDT could be gathered from the extractions and chromatographs from this investigation if funds were available. Peaks did appear on the chromatographs; however, efforts at that time could not be directed to analyzing this information.

See Appendix for chemical names of compounds mentioned in this paper.

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LITERATURE CITED

(1) *U.S. Department of the Interior. 1965.* Effects of pesticides on fish and wildlife—1964 research findings of Fish and Wildlife Service. Circ. 226. 77 p.

(2) *Butler, P. A., A. J. Wilson, Jr., and A. J. Rick. 1960.* Effect of pesticides on oysters, Proc. Nat. Shellfish. Ass., 51:23-32.

(3) *Hammerstrom, R. J. et al. 1967.* Study of pesticides in shellfish and estuarine areas of Louisiana, Public Health Service Publ. 999-UIH-2. 26 p.

(4) *Bugg, J. C., E. Higgins, and E. A. Robertson. 1967.* Chlorinated pesticide levels in the eastern oyster *(Crassostrea virginica)* from selected areas of the South Atlantic and Gulf of Mexico. Pesticides Monit. J. 1(3):9-12.

(5) *Louisiana State Board of Health, 1964.* Louisiana oyster water survey area III-B, Barataria Bay and vicinity.

(6) *Burchfield, H. P., D. E. Johnson, and E. E. Storrs. 1965.* Guide to the analysis of pesticide residues, Vol. 1, (Sec. II: A. 3 a) U. S. Dep. of Health, Educ. and Welfare, Washington, D.C.

(7) *Warnick, S. L. and A. R. Gaufin. 1965.* Determination of pesticides by electron capture gas chromatography. J. Amer. Water Works Ass.57(8): 1023.

(8) *Warnick, S. L., R. F. Gaufin, and A. R. Gaufin. 1966.* Concentration and effects of pesticides on aquatic environments. J. Amer. Water Works Ass. 158 (5): 601.

(9) *Reynolds, L. M. 1969.* Polychlorobiphenyls (PCB's) and their interference with pesticide residue analysis. Bull. Environ. Contamination Toxicol., 4(3):128-143, 1969.

(10) *Galtsoff, Paul. 1964.* The American Oyster, Crassostrea virginica Gmelin. Fish. Bull. Fish Wildlife Serv. Vol. 64: 70, 152-180, 404-405, 407.

Monitoring Ecological Conditions Associated With Wide-Scale Applications of DMA 2,4-D to Aquatic Environments[1]

T. A. Wojtalik, T. F. Hall, and Larry O. Hill

ABSTRACT

Over 18,000 surface acres of Nickajack and Guntersville Reservoirs were treated with about 170,000 gallons of dimethylamine salt of 2,4-D during April—June 1969 to control invading Eurasian watermilfoil (Myriophyllum spicatum L.). *The DMA 2,4-D was applied at the rates of 20 and 40 lb of 2,4-D acid equivalent (a.e.) per acre. Representative habitat types were selected and monitored for 2,4-D content in water, plankton, and sediment and for plankton species composition, distribution, abundance, change, and response.*

The applications of liquid DMA 2,4-D applied at a rate of either 20 or 40 lb a.e. per acre to milfoil colonies achieved excellent control within 3 to 4 weeks but did not seriously affect other submersed aquatics, and the treated water had no apparent effects on most marginal plants. No harmful or distinguishable response to the herbicide was observed in zooplankton, phytoplankton, benthic macroinvertebrates, or fish. Water from treated areas continued to be used for domestic purposes during this period without user complaints. Since the lower rate of application achieved good results in large block applications, it was concluded that reduced amounts of liquid 2,4-D may be used efficiently in such areas, provided that hydrolic flows and other characteristics are carefully considered. Dimethylamine salt of 2,4-D appears to be a noncumulative herbicide in that only small amounts are translocated along and through food chains or food webs. Plankton sorbed large amounts and retained it for extended periods. Finished drinking water from municipal treatment plants on occasion contained 2,4-D.

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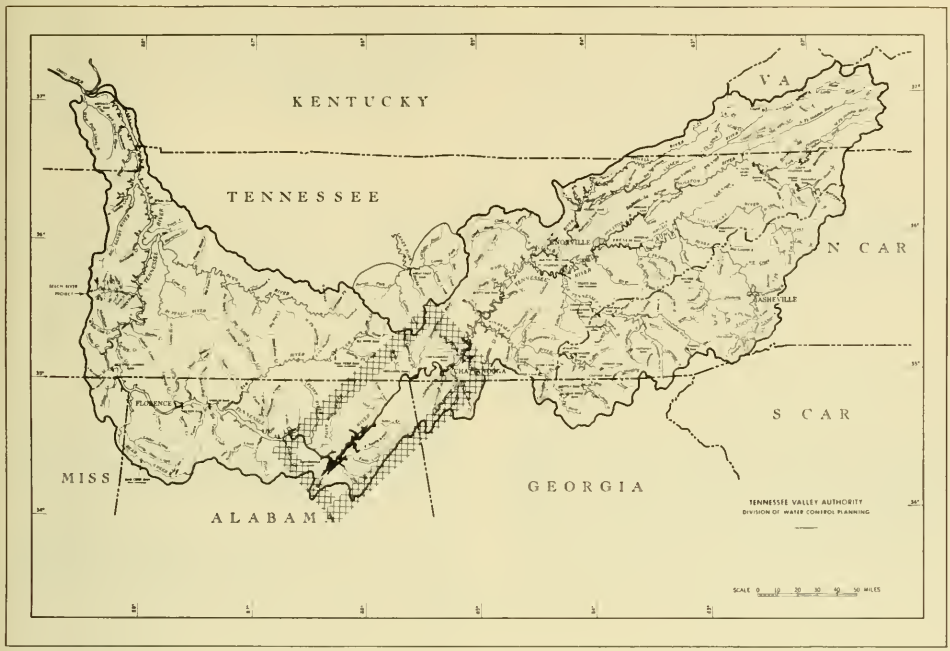
Introduction

Photogrammetric study and field inspections in the fall of 1968 showed that more than 18,000 acres of Nickajack and Guntersville Reservoirs on the Tennessee River were densely colonized by Eurasian watermilfoil *(Myriophyllum spicatum* L.) (Fig. 1). The colonies had formed in shallow water zones extending to depths as great as 16 feet below top summer pool level. Herbicidal treatment of all known surviving colonies was scheduled for the spring of 1969 after special water level manipulation produced an extra 2-foot winter drawdown. The objectives of the herbicidal application were: (1) to restore watermilfoil-choked areas to more desirable open water-use areas, and (2) to help prevent the vegetative spread of this nuisance macrophyte through the Tennessee River system.

The herbicide used was a liquid concentrate of a dimethylamine salt (DMA 2-4-D) which contained 4 lb of 2,4-D acid equivalent (a.e.) per gallon. Helicopters applied the herbicide at rates of 20 to 40 lb a.e. per acre. The applications of the liquid herbicide which is less costly and possibly more direct acting than the granular herbicide achieved excellent control within 3 to 4 weeks but did not control other submersed aquatics, and the treated water had no apparent effects on marginal plants. Spraying began on Guntersville Reservoir on April 1, 1969, and was terminated on May 29, 1969. Nickajack was treated June 2-5, 1969. Because of the magnitude of the watermilfoil control program, a broad monitoring project was developed on Guntersville Reservoir, starting in March before treatment and continuing with post-treatment sampling and evaluation through April 1970.

FIGURE 1.—*Main river reservoirs with heavy infestations of Eurasian watermilfoil in March 1969*



The possible effects of the herbicidal applications on certain aquatic macrophytes, other aquatic organisms, and potentially on man were studied and evaluated.

Study Areas

Five study areas having extensive colonies of watermilfoil were established in Guntersville Reservoir. Four of the study areas were treated with herbicide: an embayment which was landlocked except for a limited flow through a road culvert; an island and shallow water channel complex on an overbank exposed to main river flow; a large embayment with limited flow-through from a tributary stream; and a slough off the channel on the overbank enclosed between channel edge islands and the shoreline. These areas were located at Jagger Branch, Ossa-win-tha, North Sauty Creek, and Comer Bridge, respectively (Fig. 2). The fifth area, Sublett Ferry Slough, which was similar to Jagger Branch, was left untreated and used mainly for observing watermilfoil colonization progression and phenological aspects.

Sampling Techniques

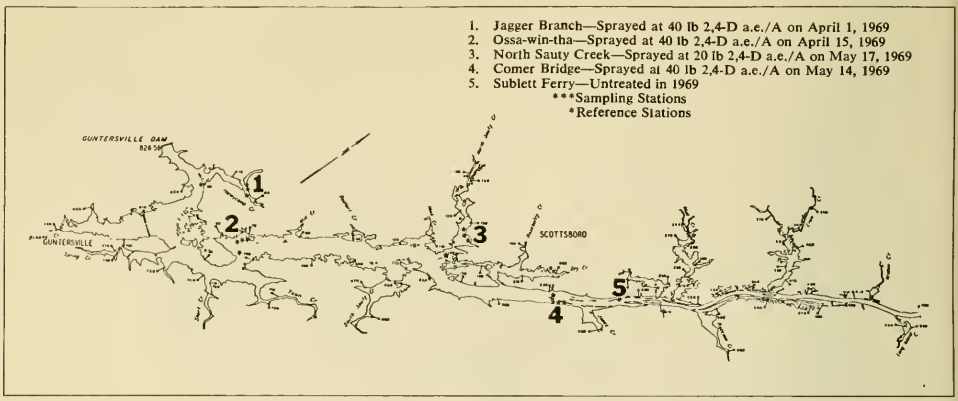
Three sampling sites designated as A, B, and C were selected in each of the four treatment areas, fieldmarked with styrofoam floats, and plotted on vertical aerial photographs which showed the watermilfoil infestations. In each study area at each collection time, composite water samples were made up by stratum source using equal volumes from sites A, B, and C for each layer. Samples were placed in 1-liter acid-washed glass bottles and then kept on ice until extracted within 24 hours (usually 8-10 hours).

Water samples were collected before treatment and during the posttreatment period at approximately 1, 8, and 24 hours; at 2 and 4 weeks; and at approximately 2, 3, 6, and 12 months. These samples were analyzed for the herbicide with a Varian Aerograph chromatograph equipped with a concentric tritium foil detector after processing by the stepwise procedure given in Supplement A. The acid concentrations can be converted to DMA 2,4-D concentrations by multiplying the acid values by 1.2.

Plankton, chlorophyll, and carbon-14 samples were collected by standard techniques. Processing and data computations were by procedures commonly used by limnologists.

Plankton tows below the surface were made with a ½-meter Wisconsin-type net of No. 20 bolting cloth

FIGURE 2.—*Location of main study areas in evaluation of DMA 2,4-D applications for control of Eurasian watermilfoil at Guntersville Reservoir, March-October 1969*



and a 150-micron mesh bucket. The large volumes of plankton collected were placed in 1-liter acid-washed bottles and stored on crushed ice. Upon arrival at the analytical laboratory 8 to 10 hours later, all except the Jagger Branch 1-, 8-, and 24-hour samples were immediately filtered through 0.3-micron glass fiber filters and washed with 20-30 ml of distilled water. The filtrates and filters were subsequently analyzed separately.

A set of three samples was collected with an Ekman dredge from each of the sampling sites A, B, and C within each study area. Each set of samples was later composited, providing a single composite sample for each of the three sampling sites. Immediately after collection, a surface 1-inch skim was removed with a plexiglass skimmer and placed in labeled double plastic bags. Plastic bags are recognized as less than ideal containers, but it was felt that the bulk volume of the sample could saturate the plastic retention surfaces and still provide a "normal" residual content without interference from compounds potentially added by the plastic. The samples were iced until arrival at the laboratory where they were placed in freezers until analyzed by procedures given in Supplement B. The material analyzed for 2,4-D was subsampled, and an aliquot was dried at 105 C, weighed, and incinerated at 600 C in a muffle furnace to obtain an estimate of volatile solids and ash-free dry weight. The estimates of 2-4-D content and volatile solids or ash-free dry weight from the same samples provided data on the correlation of 2,4-D present before treatment, after treatment but before plant breakup, and after breakup and remnant accumulation. Before plant control a given amount of organic material and volatile solids existed in the milfoil beds. After 2,4-D treatment, as the plants responded and died, volatile solids, organics, and 2,4-D increased in the sediments. Higher accumulations could be expected after the plants fragmented or lost their leaves.

The efficacy of an herbicide is influenced by water temperature, hydrogen ion concentration, light penetration, dissolved oxygen, and alkalinity. Data were obtained on these parameters for top, middle, and lower water strata at each of the three sites of the four study areas prior to treatment and up to nine times during the 1-year evaluation following treatment. The samples were collected in a manner and time sequence to provide matching data for herbicidal concentrations and milfoil response. Temperatures were determined with a Whitney thermistor equipped with a graduated suspension line. Hydrogen ion concentrations were measured with an Orion pH meter. Light penetration was determined with a submarine photometer. Dissolved oxygen was measured by the azide modification of the Winkler method. Alkalinity was measured as parts per million of calcium carbonate by titration of the samples with N/50 sulfuric acid to an end point of 4.8.

Samples of Eurasian watermilfoil were taken from three sites of each of the four study areas during the pretreatment and posttreatment sampling periods. Surface and bottom strata samples were taken at each site with an Ekman dredge (0.05 m^2) at selected times during the 1-year sampling cycle. At least five bottom and five surface samples were collected and inspected for watermilfoil during each sampling period. Supplementary observations were also made on watermilfoil colonies at an intermediate level.

Submersed aquatics in all study areas were also sampled with a dragchain sampling device. Thirty to sixty

samples were taken at each treatment area per inspection. Observations were made of the persistence of watermilfoil in the untreated Sublett Ferry Slough and in the adjacent main river. Samples of watermilfoil for biomass determinations were collected in September 1969 at the untreated Sublett Ferry area for comparison with posttreatment watermilfoil populations in the four treated study areas.

Supplemental Observations

In conjunction with the sampling activities, general observations were also made for possible response to exposure by filamentous green algae, other macrophytes, macroinvertebrates, and zooplankters in the different reservoir treatment areas. Observations made on fish were of a more limited nature because of their behavioral change and mobility as the watermilfoil decomposed.

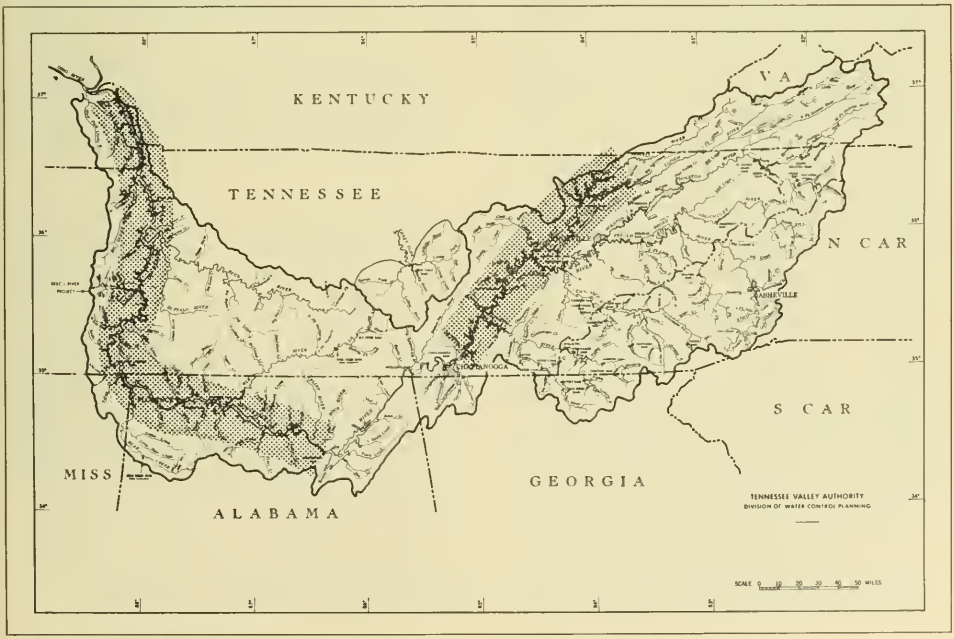
Possible herbicidal toxicity to fish was investigated by confining bluegill, *Lepomis macrochirus* Rafinesque; redear sunfish, *Lepomis microlophus* Gunther; and fathead minnows, *Pimephales promelas Rafinesque* in nylon and wooden cages placed in treated and untreated areas 24 hours before treatment and observed for 96 hours after treatment. Ten live specimens of bluegill and redear sunfish were taken from the cages at the end of the test and frozen for later analysis of residual 2,4-D in the whole fish (Supplement C).

In addition to the cage test, native fish were collected before and after treatment at three locations. Four gill nets (two each, 1½- and 2-inch bar mesh) were set for one night at each station. Selected species were removed, frozen, and shipped to the laboratory for 2,4-D analysis.

Each month, gill netting and electro-fishing were used to collect gizzard shad, channel catfish, largemouth bass, and redear sunfish to determine if 2,4-D was increasing in the flesh of the fish in treated portions of the lower reservoir. Herbicidal treatment began on the lower end of the reservoir and proceeded upstream.

Divers hand-picked mussels from their places of residence in the riverbed (Fig. 3). Several individuals of a species were collected at each station in March before treatment and in June and December after all treatment ended in upstream reservoirs. It was impossible to select mussels by species and size and, thus, the mussels obtained for analysis did not represent the same species and the same sizes at all stations during each sampling period, nor did they represent the same species and sizes

FIGURE 3.—*Downstream and upstream collection areas of commercial mussels analyzed for 2,4-D content*



on successive sampling dates. The meats were removed from the shells, labeled, frozen, and shipped to the laboratory for 2,4-D analysis (Supplement D).

Samples of the epiphytic and benthic macroinvertebrates inhabiting watermilfoil beds and the sediments under beds were obtained with the same Ekman dredge used for soil sampling. The Ekman dredge was used to clip the milfoil at the surface to a depth of 20 cm (8 inches), again between 60 and 80 cm (24 to 32 inches), and finally from the bottom upward to 20 cm (8 inches). Of the study areas, only Jagger Branch contained surface-breaking watermilfoil or watermilfoil within 15 to 30 cm (6 to 12 in) of the bottom when treated. A minimum of five samples of benthic materials was obtained from each site, A, B, and C, producing a minimum total of 15 for the area.

In Jagger Branch during pretreatment and the first month of posttreatment sampling. 15 epiphytic samples at both the surface and at mid-depth were also taken. The samples were washed in a 30 mesh/inch screen in the field, preserved in alcohol, then sorted in the laboratory after a second washing in a 30-mesh screen. The macroinvertebrates were identified and enumerated, and their distribution in horizontal and vertical locations was plotted.

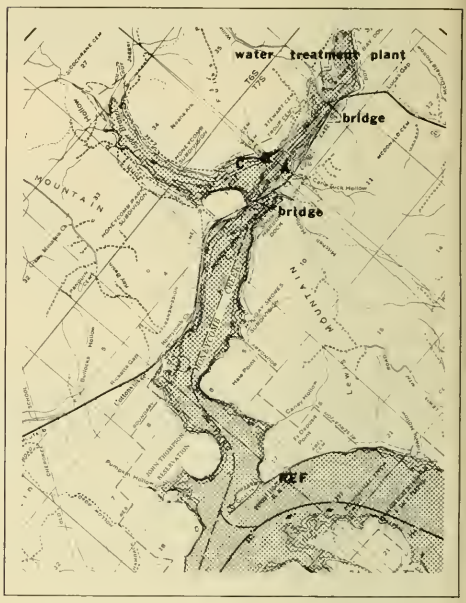
Discussion

Pretreatment monitoring was conducted during March 1969. Jagger Branch was the most enclosed of the four study areas and appeared to offer the most stringent test conditions since relatively little herbicide dilution was expected (Fig. 4). Waterborne soluble dimethylamine salt was monitored as it penetrated macrophyte beds following surface application and during the posttreatment period as its residual concentration decreased and disappeared. After 8 hours, residual material in the water filtrate was assumed to be that neither actively adsorbed nor absorbed by the milfoil, other aquatic life, or silt. No attempt was made to measure the passage of water masses at the sampling points.

A vertical stratification of 2,4-D occurred within Jagger Branch between the time of application and the 8-hour sample. Nearly 5 mg/liter was present at the surface while only 1.5 mg/liter occurred at the level of the root-crowns following treatment at a rate of 40 lb 2,4-D a.e. per acre. Within two weeks the 2,4-D content in water was uniformly 0.65 mg/liter and at 1 month it was 0.001 mg/liter. These concentrations eliminated surface-breaking colonies within a month after treatment and prevented regrowth for at least 12 months.

A site adjacent to Jagger Branch was intensively monitored for 4 weeks. No acute toxicity was evident to any nontarget form of aquatic life while levels of 2.4-D exceeded 5 mg/liter for 5 days and 1 mg/liter for an additional 3 days. No apparent gross injury was found on unsprayed marginal plants even though their root systems were in, at, or near the waterline of herbicidal treatment areas.

FIGURE 4.—*Enclosed Jagger Branch study area showing sampling station A, B, and C, the reference station on the main reservoir, and the confined hydraulic situation for the intake of the test water treatment plant, Guntersville Reservoir*



A strong decrease in pH (2.1 units) occurred between 1 and 14 days after treatment in watermilfoil beds which were in the early stages of breakup at Jagger Branch (Table 2); however, plankton counts showed no significant population change at any time during the study. The hydrogen ion concentration returned to its normal level approximately 1 month after treatment, when breakup was almost complete. Oxygen levels also decreased during the period of breakup with a minimum of 5.9 mg/liter. Following watermilfoil breakup and settling of suspended matter, light penetration at 1 meter below the water surface increased from 16 to 66%. The release of nutrients from milfoil upon breakup was not followed by explosive growth of algae or other plants.

The Ossa-win-tha area is associated with offshore islands adjacent to the main channel where conditions are favorable for rapid herbicide dilution. Less than 0.87 mg/liter was present within 24 hours after treatment at a

rate of 40 lb DMA 2,4-D and there was less than 0.005 mg/liter within 14 days. Even with lower 2,4-D concentration and shorter exposure time, only scattered rootcrowns survived at 6 months, and only a few floating fragments remained 12 months after treatment.

The Comer Bridge study area is on the flooded overbank of the old channel where it receives lateral inflow from the main river and upstream inflow from a large tributary. These inflows produced high dilutions of the 2,4-D application at 40 lb per acre; content in water was 0.44 mg/liter or less within 24 hours. Again, even with limited persistence and low concentrations, watermilfoil control was achieved for 6 months to 1 year.

The North Sauty study area was in a large lateral arm of the reservoir with downstream flow through narrow bridges. Application at a rate of 20 lb 2,4-D per acre achieved persistent water concentrations of 0.72 mg/liter or more for 2 weeks. Living watermilfoil plants were eliminated within 1 month, and control persisted for more than 1 year into the posttreatment period even though abundant viable milfoil seed was present in the area.

Chemical parameter values other than 2,4-D concentrations at Ossa-win-tha, Comer Bridge, and North Sauty Creek were similar to those at Jagger Branch. Oxygen levels were adequate at all four locations before, during, and after the period of breakup. General observations gave no evidence of fish kills or acute toxicity of the herbicide to other aquatic forms at any of the study areas. Changes in macroinvertebrate populations shown in preliminary analyses appeared to result from observed emergence of maturing insects and/or loss of the watermilfoil substrate, epiphytic food, and cover for snails and some insects as the watermilfoil macrophytes died.

As well as could be determined under field conditions, herbicidal concentrations in the lake water had no apparent control effect on most other aquatic plants. Direct observations revealed that plants survived lakewater 2,4-D concentrations at the 40 lb a.e. per acre rate, exclusive of direct spraying on their aerial portions. Plants surviving and flourishing included all common marginal woody species; common grasses, rushes, and sedges; submersed species such as coontail, crispy-leaf pondweed, and *Chara*; filamentous green algae such as *Cladophora* and *Spirogyra*; and common phytoplankton. One macrophyte, *Justicia americana* (L.) Vahl, showed a marked decline in areal extent following treatment, but even some colonies of this species persisted in treatment areas.

The data suggest that concentration and residence time are closely related to water flow rates. Jagger Branch was the site with the most restricted water exchange, while North Sauty Creek had restricted water exchange but a larger watershed. Both of these embayments had higher concentrations and longer 2,4-D residence times than were found at Ossa-win-tha and Comer Bridge where high flow-through rates resulted in greater water exchange, greater dilution, and a more rapid loss from sites of application. The loss at these sites was such that less than 1.0 mg/liter remained at 24 hours.

It appears that reduced amounts of liquid 2,4-D may be used effectively in applications over large areas, provided that careful consideration is given to the hydraulic flows and other characteristics of watermilfoil habitats. The authors believe that milfoil control may be achieved through underwater injections near the rootcrowns if the absorbed herbicide is transported upward in watermilfoil from the application zone. The heavy application was used to assure that the herbicide applied to the water surface would reach all parts of the milfoil for a duration sufficient to assure control. It is evident that relatively uniform concentrations in the water reached all levels in the watermilfoil beds especially in the enclosed embayments. In the center of the enclosed embayment away from sprayed weed beds the 24-hour after-treatment water concentrations of 2,4-D were uniformly 0.32 mg/liter throughout the 4-meter depth.

It is also evident that treatment rates under "low-flow" conditions in the enclosed embayments were excessive even at the 20-lb per acre rate. Control in these sites was so effective that no living watermilfoil could be found for at least 1 year after treatment. In contrast, the 40-lb rate was less effective at Comer Bridge where flow-through conditions associated with the main river were accompanied by some survival of watermilfoil. As a general rule, watermilfoil control was attained more readily with the herbicide in minimal dilution embayments than in main stream high dilution areas. Other plants present in the treated areas continued to grow without the stress of competition with milfoil. None reached significant population levels. These plants included *Potamogeton crispus, P. nodosus, Ceratophyllum demersum, Najas minor, N. guadalupensis,* and *Chara.*

Removal of liquid DMA 2,4-D from water by plankters that adsorb or absorb the herbicide is an important form of loss of the applied material. The plankters removed approximately 24% of the herbicide within 1 hour and a proportional amount during the next 7 hours, while the 2,4-D content of raw water was increasing 19 times. Data 24 hours after application indicated that nearly 100% of the 2,4-D extracted from the raw water samples was apparently bound within plankters. Filtrates from the plankton samples contained only limited amounts of herbicide the first day and relatively insig-

nificant amounts at 30 and 60 days. Filtrates sampled at 1 hour after treatment contained 73% of the waterborne 2,4-D but less than 25% at 8 hours and less than 10% at 24 hours. The apparent differences, as shown in Table 8, among the analyses of water, plankton, and plankton filtrates result from the fact that the plankters were collected by towing nets which concentrated and possibly damaged the dispersed plankton in the surface meter of water. When the plankton tow samples were filtered by vacuum filtration, some leakage of 2,4-D from the plankters into the filtrate may have occurred. From the viewpoint of nontarget organism accumulation of 2,4-D, the plankters readily accumulated the herbicide and retained it for more than 6 months, while the water with its small content of widely dispersed plankters lost its DMA 2,4-D load in approximately 2 weeks. Maximal herbicidal content of raw water occurred after only 8 hours.

In fish taken from the treatment areas, only the grazing and filter-feeding gizzard shad and one of its predators (largemouth bass) showed a slight 2,4-D content. At no time were unusual tastes or odors reported in fish. Fishermen, boat dock operators, and Alabama conservation officials reported that 1969 was one of the best fishing years ever for crappie, redear sunfish, bluegill, and largemouth bass in Guntersville Reservoir. The peak of the fishing season coincided with the treatment period, and no complaints were received from fishermen that the treatment was affecting fishing. In addition to sport fishing, thousands of pounds of commercial fish, (channel catfish, smallmouth buffalo, carp, and drum) were harvested by commercial fishermen; herbicidal treatment did not affect either their catch or market acceptance during the operation.

Because of the observed uptake of 2,4-D in plankton, it was expected that filter-feeding and possibly grazing fish, macroinvertebrates, and crustaceans would concentrate the herbicide. No large beds of commercial mussels occur in the major portions of Guntersville and Nickajack Reservoirs that were treated, nor does the Asiatic clam, *Corbicula*, yet occur in abundance in the embayments. However, since small *Corbicula* are the main food of redear sunfish, spawning redear sunfish were sampled in one study area. They were analyzed for 2,4-D content after stomach analyses confirmed *Corbicula* were consumed. As shown in Table 11, the redear did not accumulate DMA 2,4-D.

Caged fish tests for acute toxicity were conducted in two areas but gave ambiguous results, because the fish were damaged by mechanical handling.

Frequent observations revealed no significant effects upon either individual or populations of juvenile and mature fish in the study areas. Gill net catches at Stations 2 and 3 showed more fish caught per net-night after treatment (Tables 9 and 10).

Commercial mussels taken from colonies located on overbank edges or channel slopes downstream from Guntersville Reservoir (Table 14) apparently accumulated 2,4-D with high efficiency. A trend of progressive downstream dilution of the waterborne 2,4-D was apparent. Mussels in beds in the path of the waterborne herbicide filtered and accumulated either the soluble 2,4-D or particles containing or having the 2,4-D adhering to them. During the interval of actual application, the amounts of 2,4-D accumulated were less than 1 mg/kg wet weight, and the amounts progressively decreased downstream for about 214 miles to TRM 135, at which point a significant increase in 2,4-D was found. Subsequent investigation revealed that these colonies are downstream of the confluence of the Beech and Tennessee Rivers which drain an area where many people used 2,4-D compounds to treat stumps, brush, and weeds as part of tributary area development and new use of the Beech watershed. It appears that runoff contamination from such areas reached the Beech River, and through it entered the Tennessee River. Again a progressive dilution and decreasing accumulation trend was found from TRM 135 to Kentucky Dam, a distance of 114 river miles. A small local contribution of the herbicide to the area below Kentucky Dam may be occurring, but efforts to document its source have given negative results.

One anomalous situation was noted in the 2,4-D values obtained from the mussels. A pretreatment mussel sample collected below Guntersville Dam contained the highest 2,4-D concentration found at any time during the monitoring. Several aliquots run from the same sample indicated the same results, and simultaneous determinations of extraction coefficients for spiked mussel samples confirmed the high value. No significant amount of granular 2,4-D was applied by TVA to Guntersville Reservoir to control Eurasian watermilfoil or to test various formulations of 2,4-D in 1968. At present, the source of the 2,4-D in the mussel sample of March 1969 is unknown.

The 2,4-D added to the waters of Nickajack and Guntersville Reservoirs was apparently rapidly diluted in downstream receiving waters since the mussels in colonies at all points below Guntersville Dam (TRM 349) showed rapidly decreasing flesh concentrations of 2,4-D. It is assumed that mussels do not selectively remove 2,4-D over and above the amounts received in the filtering-feeding system directly from the water. Mussels from each successive colony downstream appear to concentrate 2,4-D in the same proportion as received. Either increases in distance from the treatment area or increases in posttreatment time, or both, are accompanied by lower 2,4-D levels in the mussels.

The maximum concentration of 2,4-D in mussels after treatment approached 1 mg/kg wet weight. Most concentrations were generally much lower during the treatment interval and thereafter than those found in pretreatment samples. Higher pretreatment concentrations in downstream areas are attributed to local non-TVA additions of herbicide prior to TVA's large-scale upstream treatments.

Water treatment plants, regardless of intake location, processing, or special holding methods, apparently removed little DMA 2,4-D from the raw water.

The 2,4-D concentrations at water intakes can be reduced by applying DMA 2,4-D outside the water treatment plant buffer zone and then changing to granular applications of butoxyethanol ester (BEE) of 2,4-D within the buffer zone. The reductions can be accomplished without change in application rate and are largely related to the ester being relatively insoluble in water, whereas salt is highly water-soluble.

Preliminary analysis of macroinvertebrate samples from Jagger Branch indicated neither acute toxicity nor definable chronic toxicity; however, population changes of genera and species were apparent. These changes were apparently due to the rapid collapse of the milfoil which provided a substrate, epiphytic food in the form of diatoms (as much as 75% of the ash-free dry weight of milfoil samples), as well as cover from fish predators. Extremely large hatches of damselflies, dragonflies, mayflies, midges, and other dipterans were observed during the time of actual spraying and for 4 months after spraying.

Large cladocerans, snails, some diptera, and some lepidoptera larvae were absent 2 to 4 weeks after treatment mainly because there were no tangled stems and leaf mats to afford a suitable substrate. The same forms could be collected by plankton towing or benthic sampling, but only scattered individuals occurred after milfoil leaves fell from their stems.

See Appendix for chemical names of compounds mentioned in this paper.

TABLE 1.—*Content of 2,4-D acid equivalent in Guntersville Reservoir water samples during 1969*

Area	Concentration in Mg/Liter							
	Pre-treatment	Posttreatment						
		Hours			Weeks		Months		
		1	8	24	2	4	2	3	6
Jagger Branch [1] (Treated 4/1/69)									
Top—A+B+C	0.023	0.25	4.8	1.8	0.63	0.001	0.028	0.042	<0.001
Mid—A+B+C	0.008	1.2	3.1	1.5	0.67	—	0.023	0.036	<0.001
Low—A+B+C	0.005	0.22	1.4	1.1	0.66	0.001	0.020	0.023	—
Ref 1+2	0.006	0.014	—	0.003	0.077	<0.001	—	0.009	0.003
Plankton filtrate		0.22	0.20	0.23		<0.001	0.013		<0.001
Comer Bridge [1] (Treated 5/14/69)									
Top—A+B+C	<0.001	0.96		0.043	<0.001	0.024	0.002	—	<0.001
Mid—A+B+C	0.002	0.46		0.044	<0.001	0.021	0.004	—	<0.001
Low—A+B+C	0.001	0.52		0.039	—	0.015	0.009	—	0.011
Ref 1+2	<0.001	0.005		0.021	0.002	<0.001	<0.001	—	<0.001
Plankton filtrate		0.58		0.053	0.002	0.002			0.002
Ossa-win-tha [1] (Treated 4/15/69)									
Top—A+B+C	—	—	1.4	0.63	<0.001	0.001	0.002		0.002
Mid—A+B+C	—	—	1.2	0.63	0.005	0.001	0.004		<0.001
Low—A+B+C	—	1.5	1.5	0.87	0.001	<0.001	0.002		<0.001
Ref 1+2	—	0.002	0.025	0.55	<0.001	<0.001	0.18		<0.001
Plankton filtrate	0.064	0.039	0.41	0.33	0.17	0.002	0.001		0.005
North Sauty [2] (Treated 4/17/69)									
Top—A+B+C	<0.001	2.0		1.6	0.72	<0.001	0.003		<0.001
Mid—A+B+C	—	2.1		1.6	0.66	<0.001	0.003		<0.001
Low—A+B+C	<0.001	1.9		0.91	0.67	<0.001	0.007		<0.001
Ref 1+2	—	<0.001		0.002	0.20	<0.001	0.004		<0.001
Plankton filtrate	0.068	0.041		0.33	<0.001	<0.002	0.004		0.010

[1] Application rate 40 lb/A 2,4-D a.e. DMA 2,4-D.
[2] Application rate 20 lb/A 2,4-D a.e. DMA 2,4-D.
NOTE: Ref 1+2 is equi-volume samples composited from 1- and 2-meter depths at main channel untreated reference point for each study area.
A+B+C is equi-volume samples composited from three sub-areas of a treatment area—composited within each stratum.
Blanks = Not sampled.
— = Sample lost.

TABLE 2.—*Magnitude of physical and chemical parameters of Jagger Branch which may have influenced efficacy of 2,4-D*

Parameters	Pre-treatment	Posttreatment							
		Hours			Weeks		Months		
		1	8	24	2	4	2	3	6
Light—%									
Top	66.0	75.6	75.6	85.0	72.5	72.8			84.0
Mid	14.0	37.8	37.8	18.8	16.2	12.1			66.0
Low	2.0	5.3	5.3	5.6	4.7	7.0			24.0
Reference [1]					80.0	82.1			
Temperature—F									
Top	53.0	59.2	66.3	60.3	61.0	68.3	79.5	93.0	
Mid	53.0	56.8	59.0	57.8	61.0	67.8	77.8	89.2	
Low	53.0	55.5	55.7	55.3	60.7	67.2	75.7	86.3	
Reference [1]		55.5	60.0	56.0	62.0	65.5	77.0	86.0	
Oxygen—mg/liter									
Top	9.4	13.6	13.8	12.7	6.5	6.3	8.1	8.6	9.0
Mid	9.6	12.3	12.7	11.6	6.6	6.2	7.9	8.2	9.0
Low	9.5	11.0	12.4	11.7	5.9	6.1	7.8	7.5	9.1
Reference [1]		10.0	10.5	10.9	8.9	6.4	8.6	8.6	8.3
Alkalinity—mg/liter									
Top	54.0	62.7	60.0	63.7	61.3	64.7	56.7	57.3	56.0
Mid	54.0	60.0	57.7	57.7	59.7	65.3	57.7	55.7	56.0
Low	54.0	60.7	58.0	62.0	61.7	64.0	57.3	56.0	55.0
Reference [1]		47.0	47.0	49.0	50.0	49.0	47.0	44.0	55.0
pH									
Top	6.9	8.4	9.3	8.5	6.4	8.1	7.8	8.7	7.5
Mid	6.9	7.9	9.1	7.7	6.4	8.0	7.7	8.6	7.4
Low	6.9	7.9	8.7	7.4	6.3	8.0	7.4	8.3	7.5
Reference [1]		6.8	6.7	7.2	7.2	8.0	7.6	7.9	7.9

[1] All reference samples were collected from nearby untreated areas (such as shown in Fig. 4) at a depth of 1 meter or similar depth to mid-samples taken from treated areas.
NOTE: Blanks = Not sampled.

TABLE 3.—*Abundance of watermilfoil during 1969-1970 in four study areas and in an untreated area of Guntersville Reservoir*

Area	Pre-treatment	Posttreatment							
		Weeks		Months					
		2	4	2	3	4	5	6	12
Jagger Branch (Treated April 1, 1969, full rate)									
Station I, A+B+C—Top	Ab	M	0	0	0	—	—	0	0
Station I, A+B+C—Mid	Ab	M	0	0	0	—	—	0	0
Station I, A+B+C—Low	Ab	M	0	0	0	—	—	0	0
Comer Bridge (Treated May 14, 1969, full rate)									
Station III, A+B+C—Top	0	0	T	T	T	—	—	0	0
Station III, A+B+C—Mid	0	0	0	0	0	—	—	0	0
Station III, A+B+C—Low	Ab	M	T	T	T	—	—	T	T
Ossa-Win-Tha (Treated April 15, 1969, full rate)									
Station II, A+B+C—Top	T	0	T	T	—	T	0	—	T
Station II, A+B+C—Mid	T	0	0	0	—	0	0	—	0
Station II, A+B+C—Low	Ab	M	T	T	—	T	T	—	0
North Sauty (Treated May 17, 1969, half rate)									
Station V, A+B+C—Top	T	0	—	0	—	0	0	—	0
Station V, A+B+C—Mid	T	0	—	0	—	0	0	—	0
Station V, A+B+C—Low	Ab	M	—	0	—	0	0	—	0
Control Area—Sublett Ferry									
Station IV, A+B+C—Top	← Abundant at all times →							M	Ab
Station IV, A+B+C—Mid	← Abundant at all times →							M	Ab
Station IV, A+B+C—Low	← Abundant at all times →								Ab

NOTE: Ab = Abundant—Dense colonies of healthy milfoil.
M = Moderate—Thinning colonies of milfoil in early stages of breakup.
T = Trace—Only an occasional living healthy-appearing milfoil root crown or floating fragment found following breakup (breakup practically complete at 4 weeks in treated plots).
0 = Living milfoil not found—All inspections negative for floating fragments and/or negative for root crowns.
A+B+C represents three sub-areas within a study area.

Application rates DMA 2,4-D at 40 lb/A a.e. for Jagger Branch, Comer Bridge, and Ossa-win-tha areas, 20 lb/A a.e. for North Sauty.

TABLE 4.—*Concentration of total 2,4-D acid equivalent in posttreatment plankton tow samples collected from Jagger Branch, Guntersville Reservoir, 1969*

Sampling Location	Plankton Sample (Grams)	Posttreatment Sampling Time	Total 2,4-D a.e. (mg/kg, wet weight)
I, A+B+C	3.3	1 Hour	0.06
I, A+B+C	1.7	8 Hours	0.88
I, Mid Channel-Ref [1]	0.87	24 Hours	0.35
I, A+B+C	0.38	24 Hours	1.8
I, A+B+C	0.97	14 Days	2.6
I, A+B+C (center of sampling site) [2]	0.11	30 Days	3.6
I, A+B+C	0.09	60 Days	2.2
I, A+B+C	0.16	4 Months	1.1
I, Plankton-Ref [3]	0.02	6 Months	<0.10
I, A+B+C	0.71	6 Months	0.37

[1] The plankton tow was conducted in open channel at mid-embayment of Jagger Branch.
[2] "Chemical mowing" had sufficiently progressed so that plankton tow could be conducted through the center of sampling sites without obstructive effects from viable milfoil.
[3] Reference sample collected in untreated area near the confluence of Honeycomb Creek with Guntersville Lake.

TABLE 5.—*Algal identity, abundance, chlorophyll content, and productivity in Jagger Branch, Guntersville Reservoir, during 1969 sampling*

Dominant Genera at 1-m Depth	Identity and Abundance (No. Cells/ml)						
	Hours			Weeks		Months	
	1	8	24	2	4	2	3
Cocconeis	41	198	113	85	34	0	0
Melosira	7	7	0	160	10	352	126
Navicula	14	61	0	58	7	17	61
Chlorella	7	99	48	412	72	525	610
Cosmarium	7	7	0	55	113	239	72
Scenedesmus	3	0	0	34	7	68	41
Tetraspora	177	273	0	829	0	0	0
Raphidiopsis	38	78	116	160	280	130	222
Synedra	0	34	14	7	0	709	304
Biflagellate	0	3	68	720	0	477	597
Merismopedia	0	3	0	0	0	58	392
Bluegreen filament	0	3	17	0	0	3	351

Chlorophyll A Content as Standing Stock (mg/m³) for Jagger Branch and Main Stream Reference Samples

	Pre-treatment	Hours			Weeks		Months		
		1	8	24	2	4	2	3	6
Jagger Branch									
Surface	2.13	2.17	3.17	2.82	8.91	7.61	6.70	6.97	—
1 meter	1.04	2	5.13	3.78	10.05	7.61	8.06	14.45	8.83
2 meters	1.04	1.09	4.74	3.17	5.87	5.92	8.70	17.67	9.27
Reference [1]									
Surface	5.17	[2]	3.83	13.31	10.66	[2]	4.48	[2]	2.86
1 meter	6.31	[2]	[2]	18.10	8.27	5.08	4.69	10.98	3.18
2 meters	8.26	[2]	[2]	18.10	9.31	[2]	5.26	[2]	3.43

Productivity as Carbon 14 Fixation Rate (mg C/m³/hr) for Posttreatment Samples

	Treatment	24 Hours	Weeks		Months	
			2	4	2	3
Jagger Branch	16.65	14.66	15.35	37.42	3.78	—
Main stream reference	201.02	—	127.72	29.94	4.53	—

[1] Samples at untreated main channel.
[2] Broken sample.
NOTE: — = Sample lost during processing.

TABLE 6.—*Zooplankters in surface samples for pretreatment and posttreatment periods, Jagger Branch, Guntersville Reservoir*

	No. per milliliter of water						
	Pre-treatment	Posttreatment					
		Hours		Weeks		Months	
		1	8	2	4	2	3
Rotifera							
Monogononta							
Ploima							
Asplanchna					1		
Branchionidae					62	5	7
Keratella	2		2	2	238	359	76
Polyanthra						2	7
Hexanthra							5
Arthropoda							
Crustacea							
Cladocera							
Bosmina	4	5	4	61	71	5	3
Daphnia				2			
Diaphanosoma	2	1		3	1		
Leptodora			2				1
Copepoda							
Cyclopoida	2	1	2	6	5		
Nauplii			1	4	44	73	

NOTE: Main channel zooplankton populations were high during pretreatment and early posttreatment, then declined through the summer.

TABLE 7.—*Levels of 2,4-D and volatile solids in pretreatment and posttreatment sediment samples, Jagger Branch, Guntersville Reservoir, 1969*

Location Number	Water depth for sample (feet)	Total 2,4-D a.e. (mg/kg, dry weight)	Percent Volatile Solids
I-A-Pre	Unknown	<0.10	6.5
I-A-Pre	6	<0.10	6.0
I-A-Pre	5	<0.10	9.3
I-A-Pre	6	<0.10	2.3
I-A-24 hours	5	<0.10	4.4
I-B-24 hours	5	0.13	5.6
I-C-24 hours	5	0.10	6.7
I-A-2 weeks	6	0.33	6.0
I-B-2 weeks	6.5	0.25	4.6
I-C-2 weeks	5	0.18	7.6
I-A-4 weeks	5	<0.10	3.3
I-B-4 weeks	6	<0.10	4.6
I-C-4 weeks	5	0.13	6.8
I-A-2 months	7	0.30	5.6
I-B-2 months	7	0.16	6.0
I-C-2 months	7	0.45	4.2
I-A-3 months	8	<0.10	5.8
I-B-3 months	7	0.37	5.0
I-C-3 months	7	0.28	5.0
I-A-6 months	6	<0.10	4.5
I-B-6 months	6	<0.10	6.0
I-C-6 months	6	<0.10	5.0

NOTE: It was anticipated that residual 2,4-D would accumulate in the sediments by sorption and by plant fragment accumulation bringing sorbed 2,4-D to the bottom as the plants broke up. Although leaf and plant fragments accumulated their organic and volatile solids, composition was not sufficient to register an increase in sediment samples. 2,4-D did, however, accumulate for a short period.

TABLE 8.—*Summary of 2,4-D concentrations in surface water samples, plankton tow samples at and near surface, plankton filtrate, and sediment, Jagger Branch, Guntersville Reservoir, 1969*

	Pre-treatment	Posttreatment Time							
		Hours			Weeks		Months		
		1	8	24	2	4	2	3	6
H_2O (mg/liter)	0.023	0.25	4.8	1.8	0.63	0.001	0.028	0.042	<0.001
Plankton (mg/kg)	—	0.06	0.88	1.8	2.6	3.6	2.2	1.1	0.37
Filtrate (mg/liter)		.22	.20	.23		<0.001	0.013		<0.001
% in plankton		24.0	18.3	100	(1)	(1)	(1)	(1)	(1)
% in H_2O		(1)	(1)	(1)	24.2	0.003	0.012	0.038	0.002
Soil (mg/kg)	<0.10	(1)	(1)	0.11	.25	0.11	0.30	0.25	<0.10

[1] Insufficient amount.
NOTE: Blanks = Not sampled.
— = Sample lost.

TABLE 9.—*Pretreatment gill net catches, Guntersville Reservoir, March 26, 1969*

Species	TRM 352 Honeycomb Creek			TRM 364 Ossa-win-tha			TRM 386 Comer Bridge		
	Number	Length/Range (inches)	Wt. (lb)	Number	Length/Range (inches)	Wt. (lb)	Number	Length/Range (inches)	Wt. (lb)
Sauger	1	15.6	1.5	—	—	—	3	14.4-16.0	3.2
Largemouth bass	1	14.5	1.9	—	—	—	—	—	—
White bass	—	—	—	—	—	—	1	9.2	0.5
Yellow bass	1	9.4	0.5	5	8.7-9.7	2.2	4	8.5-9.1	1.6
Redear sunfish	—	—	—	2	7.4-9.7	1.4	2	7.8-8.1	0.8
Warmouth	—	—	—	—	—	—	1	9.0	0.7
Green sunfish	1	7.4	0.5	—	—	—	—	—	—
Channel catfish	36	11.2-18.6	30.4	14	12.5-20.0	17.4	3	11.3-16.7	2.6
Blue catfish	2	12.4-15.6	2.0	1	15.8	1.3	—	—	—
Yellow bullhead	—	—	—	2	10.6-10.7	1.0	—	—	—
Drum	—	—	—	3	10.3-13.8	2.2	—	—	—
White sucker	—	—	—	—	—	—	1	15.2	1.3
Spotted sucker	—	—	—	—	—	—	3	12.2-14.9	3.6
Mooneye	—	—	—	—	—	—	1	10.9	0.6
Golden shiner	1	10.3	0.6	—	—	—	—	—	—
Skipjack herring	10	12.4-17.8	13.1	2	14.5-14.7	2.1	4	13.7-16.3	4.3
Gizzard shad	31	9.6-13.8	14.5	41	9.8-12.2	15.3	11	9.5-13.7	5.5
Total	84	—	65.0	70	—	42.9	34	—	24.7
Catch per net-night	21		18.3	17.5		10.7	8.5		6.2
Game fish	4		4.4	7		3.6	11		9.4
Percent game fish	4.8		6.8	10.0		8.4	32.4		38.1
Dominant species by percent	(Gizzard shad, 36.9%)			(Gizzard shad, 58.6%)			(Gizzard shad, 32.4%)		

TABLE 10.—*48-hour posttreatment gill net catches, Stations 2 and 3, Guntersville Reservoir, 1969*

Species	Station 2, TRM 364, 4/18/69			Station 3, TRM 386, 5/17/69		
	Number	Length/Range (inches)	Wt. (lb)	Number	Length/Range (inches)	Wt. (lb)
Sauger	1	14.0	0.8	—	—	—
Largemouth bass	1	12.0	0.9	1	19.6	4.0
White bass	—	—	—	1	12.7	0.9
Yellow bass	—	—	—	2	9.5-10.6	1.1
White crappie	2	9.2-9.7	0.7	1	10.2	0.6
Redear sunfish	1	9.2	0.5	4	7.5-9.1	1.3
Bluegill	4	7.0-7.7	1.1	12	6.8-7.5	3.1
Warmouth	—	—	—	1	8.8	0.4
Channel catfish	3	12.0-16.7	2.5	3	12.3-18.6	3.6
Blue catfish	1	19.8	3.0	—	—	—
Yellow bullhead	1	11.1	0.7	2	9.6-10.5	0.8
Smallmouth buffalo	1	20.4	4.8	5	12.6-21.0	13.4
Drum	—	—	—	1	10.0	0.3
Carp	1	22.8	8.3	2	12.2-12.7	3.4
Spotted sucker	—	—	—	3	12.6-18.6	5.2
Golden shiner	—	—	—	5	9.3-10.2	1.9
Mooneye	—	—	—	1	11.6	0.6
Spotted gar	1	20.0	1.2	7	21.4-32.9	21.9
Skipjack	—	—	—	1	19.4	1.7
Gizzard shad	197	9.6-12.6	79.9	70	9.9-11.1	26.6
Total	214	—	104.4	122	—	90.8
Catch per net-night	53.5	—	26.1	30.5	—	22.7
Game fish	9	—	4.0	22	—	11.4
Percent game fish	4.2	—	3.8	18.0	—	12.6
Dominant species by percent		(Gizzard shad, 92.1%)			(Gizzard shad, 57.4%)	

TABLE 11.—*Number of samples and 2,4-D acid equivalent content in fish collected from Honeycomb Creek below Jagger Branch treatment area during 1969*

Species	Pretreatment		Posttreatment									
			4 Weeks		2 Months		3 Months		4 Months		6 Months	
	No.	mg/kg wet wt.	No.	mg/kg wet wt.	No.	mg/kg wet wt.	No.	mg/kg wet wt.	No.	mg/kg wet wt.	No.	mg/kg wet wt.
Gizzard shad	5	<0.10	20	0.34	6	<0.10	6	0.22	—	—	6	<0.10
Bluegill	40	<0.10	12	<0.10	10	<0.10	9	<0.10	4	<0.10	6	<0.10
White crappie	13	<0.10	—	—	—	—	—	—	—	—	—	—
Largemouth bass	5	0.15	4	<0.10	8	<0.10	6	<0.10	7	<0.10	6	<0.10
Channel catfish	—	—	11	<0.10	5	<0.10	3	<0.10	—	—	3	<0.10
White bass	—	—	1	<0.10	—	—	—	—	—	—	—	—
Sauger	—	—	1	<0.10	—	—	—	—	—	—	—	—
Redear sunfish	—	—	—	—	—	—	7	<0.10	8	<0.10	6	<0.10

TABLE 12.—*Concentrations of 2,4-D acid equivalent in raw and treated water from water treatment plants on Guntersville Reservoir and at Huntsville, Ala. April 1-June 26, 1969*

Location	Date	mg/liter 2,4-D a.e.			Percent Operating Efficiency
	1969	Background	Raw	Finished	
North Marshall	4-2	0.002 (3-31)	1.9	1.3	32
(First plant used as	4-3		5.4	3.4	37
test facility in relation	4-4		5.9	3.7	37
to spray operations)	4-5		5.9		
	4-5 (short intake)		5.7		
	4-6		5.2	5.8	
	4-7		3.0 (8 a.m.)	1.8 (11 a.m.)	40
	4-7		1.6 (11 a.m.)	1.6 (4:30 p.m.)	0
	4-8		1.5	1.4	7
	4-9		1.1	0.92	16
	4-10		0.42	0.54	
	4-11		0.23	0.14	39
	4-14		0.048	(1)	
	4-15		0.053	(1)	
	4-16		0.036	(1)	
	4-17		0.014		
	4-21		0.009		
	4-24		0.016		
	4-28		0.020		
	5-1		0.003		
Guntersville No. 1	4-1		0.006	0.006	0
(Spring Creek)	4-2		0.003	0.002	33
	4-3		0.005	0.003	40
	4-6		0.015	0.017	
	4-7		0.005	0.002	60
	4-8		(1)	0.084	
	4-9		0.008	(1)	
	4-10		0.008	(1)	
	4-13		0.089	(1)	
	4-14		0.043	0.026	40
	4-15		—	(1)	
	4-16		0.020		
	4-17		0.014		
	4-21		0.021		
	4-23		0.002		
	4-27		0.015		
	5-1		<0.001		
	5-8		0.081		
	5-22		0.001		
	5-29		0.008		
	6-5		0.006		
	6-12		0.039		
	6-19		0.003		
	6-26		0.012		

TABLE 12.—*Concentrations of 2,4-D acid equivalent in raw and treated water from water treatment plants on Guntersville Reservoir and at Huntsville, Ala. April 1-June 26, 1969*—Continued

Location	Date	mg/liter 2,4-D a.e.			Percent Operating Efficiency
	1969	Background	Raw	Finished	
Guntersville No. 2 (Browns Creek)	4-1	0.003 (3-31)	0.006	0.002	67
	4-2		0.008	0.010	
	4-3		0.022	0.013	41
	4-6		0.32	0.20	37
	4-7		0.12	0.074	38
	4-8		[1]	[1]	
	4-9		0.097	[1]	
	4-10		0.100	[1]	
	4-12		0.094	[1]	
	4-13		0.12	0.089	
	4-14		0.15	0.12	
	4-15		0.11	0.076	
	4-16		0.010		
	4-16 (short intake)		0.012		
	4-17		0.018		
	4-21		0.007		
	4-24		0.001		
	4-27		0.017		
	4-30		<0.001		
	5-8		0.004		
Arab	4-1	0.002	0.002	0.001	50
	4-2		0.005	0.002	60
	4-3		0.007	0.007	0
	4-6		0.100	0.019	81
	4-7		0.11	0.041	63
	4-8		[1]	0.057	
	4-9		0.082	[1]	
	4-10		0.097	[1]	
	4-13		0.11	0.064	42
	4-14		0.097	0.062	
	4-15		0.092	[1]	
	4-16		0.030	[1]	
	4-21		0.018		
	4-23		<0.001		
	4-28		0.005		
	5-1		<0.001		
	5-21		0.001		
	5-29		<0.001		
	6-5		<0.001		
Albertsville	4-16	<0.001			
	4-28		0.002		
	5-1		<0.001		
	5-8		<0.001		
Scottsboro	5-5	0.003			
	5-8	0.002			
	5-15		0.046		
	5-22		0.002		
	5-29		<0.001		
	6-5		0.23		
	6-9		0.005	[2] 0.039 & 0.012	
	6-10		0.002		
	6-11		0.009	0.090	
	6-12		0.002	0.013	
	6-13		<0.001	[1]	
	6-16		<0..001	0.002	
	6-17		0.002	0.002	
	6-18		0.002		
	6-26		0.003		
Section	5-1	0.006			
	5-15		<0.001		
	5-22		0.002		
	5-29		<0.001		
	6-5		0.18		

TABLE 12.—*Concentrations of 2,4-D acid equivalent in raw and treated water from water treatment plants on Guntersville Reservoir and at Huntsville, Ala. April 1-June 26, 1969*—Continued

Location	Date	mg/liter 2,4-D a.e.			Percent Operating Efficiency
	1969	Background	Raw	Finished	
Section—Continued	6-9		[2] 0.014 & 0.050	0.005	
	6-11		0.002	0.015	
	6-12		0.002	[1]	
	6-13		<0.001	[1]	
	6-16		0.001	0.002	
	6-17		0.001	<0.001	
	6-18		<0.001	<0.001	
	6-26		0.002		
Widows Creek	6-11		0.002		
Bridgeport	6-11		0.001	0.008	
	6-12		0.039	0.005	
	6-13		[2] 0.054 & 0.001	[1]	
	6-16		0.006	[2] 0.011 & 0.002	
	6-17				
	6-19		0.005	0.003	
	6-20		[1]		
South Pittsburg	5-29	<0.001			
	6-5		0.032		
	6-11		<0.001	0.099	
	6-12		0.13	0.002	
	6-13		<0.001	<0.001	
	6-16		0.009	0.001	
	6-17		<0.001	0.002	
	6-19		<0.001	<0.001	
	6-26		0.001		
Huntsville	4-1	0.003	0.003	0.003	0
	4-2		0.004	0.002	50
	4-3		0.004	0.003	25
	4-6		0.034	0.022	35
	4-7		0.017	0.016	6
	4-8		[1]	[1]	
	4-10		0.018	[1]	
	4-13		0.042	[1]	
	4-14		0.025	[1]	
	4-16		0.016		
	4-21		0.001		
	4-23		0.011		
	4-27		0.005		
	5-1		<0.001		
	5-7		0.006		
	5-14		<0.001		
	5-21		<0.001		
	5-29		0.002		
	6-4		0.003		
	6-11		0.003		
	6-18		<0.001		
	6-24		0.003		

[1] Sample not analyzed.
[2] First sample collected in the morning; second in the afternoon.
NOTE: Blanks = Not sampled.

TABLE 13.—*Normal treatment facilities and procedures in municipal water treatment plants on Guntersville Reservoir and at Huntsville, Ala. 1969*

Water treatment plant	Removal of iron	Coagulation	Sedimentation	Filtering	Aeration	Carbon
Albertsville		x	x	x		
Arab	x	x	x	x		
Guntersville		x	x	x	x	
Huntsville		x	x	x	x	
Scottsboro		x	x	x		
Widows Creek		x	x	x		
North Marshall		x	x	x		x

TABLE 14.—*Content of 2,4-D acid equivalent in mussel samples from below and above Guntersville and Nickajack Reservoirs before and after April 1969 applications (Guntersville Dam—TRM 349)*

Station (TRM)	Species	March		June		December	
		No. of Mussels	2,4-D a.e. (mg/kg Wet Wt.)	No. of Mussels	2,4-D a.e. (mg/kg Wet Wt.)	No. of Mussels	2,4-D a.e. (mg/kg Wet Wt.)
348.0	*Amblema plicata*	—	—	14	0.050	—	—
348.0	*Plagiola lineolata*	7	0.15	3	<0.050	—	—
348.0	*Pleurobema cordatum*	—	—	—	—	12	0.26
348.0	*Fusconaia flava* f. *undata*	12	<0.050	11	0.050	—	—
256.0	*Plagiola lineolata*	8	0.070	6	0.055	15	<0.050
256.0	*Fusconaia ebenus*	18	2.7	18	0.83	17	<0.050
205.5	*Fusconaia ebenus*	14	0.51	19	0.57	11	0.075
205.5	*Amblema plicata*	6	0.14	12	0.47	—	—
193.4	*Fusconaia ebenus*	18	0.11	18	0.10	17	<0.050
193.4	*Elliptio crassidens*	—	—	6	0.33	15	<0.050
135.2	*Fusconaia ebenus*	12	0.54	—	—	13	<0.050
135.2	*Quadrula quadrula*	9	1.4	—	—	10	<0.050
135.2	*Amblema plicata*	—	—	2	0.10	5	<0.050
101.7	*Obliquaria reflexa*	14	0.095	—	—	—	—
101.7	*Fusconaia ebenus*	12	0.16	11	0.41	15	0.055
101.7	*Amblema plicata*	—	—	2	0.10	—	—
96.7	*Quadrula quadrula*	13	0.45	18	<0.050	12	0.13
96.7	*Pleurobema cordatum*	—	—	8	0.97	—	—
21.5	*Amblema plicata*	12	0.29	12	0.150	10	0.26
21.5	*Fusconaia ebenus*	12	0.20	4	0.10	—	—
529.0	Mixed species	—	—	—	<0.050	—	—
120.0	*Plagiola lineolata*	—	—	—	—	10	0.11

SUPPLEMENT A

Method for Analysis of Raw Lake Water for Determining the Concentrations of 2,4-D Acid Equivalent of the Dimethylamine Salt of 2,4-D

The water samples were processed according to the following procedure and finally analyzed with a Varian Aerograph gas chromatograph, using a concentric tritium foil detector.

1. Add an appropriately sized sample aliquot to a separatory funnel—a 1-liter sample is adequate for the low ppb range.
2. *Alkalize the water sample to pH 12.0 to 12.5 with NaOH (about 10 ml of 4N NaOH).
3. *Employ intermittent and vigorous shaking for about 10 minutes to effect hydrolysis of any BEE ester.
4. Acidify the water sample to a pH of approximately 1.5 with 20 ml of phosphoric acid.
5. Employ intermittent and vigorous shaking for 2 minutes.
6. Partition with 100 ml of nanograde chloroform and employ continuous and moderate shaking for 2 minutes.
7. Filter chloroform layer through an "acidified Na_2SO_4" funnel into a 250-ml round-bottom flask. Pre-rinse the "acidified Na_2SO_4" funnel with about 20 ml of nanograde chloroform. This filtrate is discarded.
8. Add 10 ml of saturated Na_2SO_4 to the aqueous layer.
9. Partition with 50 ml of nanograde chloroform. Employ continuous and moderate shaking for 2 minutes.
10. Filter chloroform layer and any emulsion layer (less about 0.5 ml) through the "acidified Na_2SO_4" funnel into the 250-ml flask.
11. Break up the acidified Na_2SO_4 crystals in the funnel by gently punching with the tip of a stainless steel spatula.
12. Rince "acidified Na_2SO_4" funnel with about 20 ml of nanograde chloroform. Filtrate is also added to the 250-ml round-bottom flask.
13. Carefully evaporate the chloroform extracts just to dryness with a rotary vacuum evaporator. Water bath temperature should not exceed 45 C. Use a temperature range of 40 to 45 C.
14. After allowing the round-bottom flask to *cool*, take up the residue with three 5-ml aliquots of diethyl ether, and *quantitatively* transfer to a 15-ml conical centrifuge tube using a small funnel.
15. Evaporate ether solution to dryness with zero nitrogen. If a high concentration of 2,4-D is expected, evaporate an aliquot of the ether solution.
16. With a pipette, add an additional 2 ml of nanograde ether to the centrifuge tube ensuring that the walls of the tube are rinsed thoroughly.
17. Evaporate ether solution to dryness with zero nitrogen.
18. Esterify residue with 1.0 ml of BF_3-methanol mixture for 30 minutes at 70 C. Centrifuge tube must be *tightly* stoppered and intermittent swirling of the tube contents must be employed during esterification.
19. *Cool* tube contents, unstopper, and add 2 ml of 2% Na_2SO_4 solution.
20. Add 10 ml of nanograde hexane and shake tube contents vigorously.
21. Centrifuge the tube for 5 minutes at approximately 4,000 rpm to remove emulsions and any turbidity from the hexane layer.
22. Construct a calibration curve using esterified standards or inject esterified standards in appropriate quantities so that a given peak height closely approaches the peak height of the unknown. In this fashion the ratio-and-proportion principle may be employed. The latter method should be used only in the linear portion of the standing current.

* Omit if BEE 2,4-D is known to be absent.

23. Average individual results if multiple injections are employed and calculate concentration of 2,4-D in mg/liter ensuring that esterification aliquot, sample aliquot, and extraction coefficient factors are appropriately incorporated.
24. When utilizing electron capture detection with *good* sensitivity, this method has a limit of detectability of 1 ppb. Report data as follows:

Concentration Range	*Report to Nearest Value*
0.100 mg or less	0.001 mg
0.10 mg to 1.00 mg	0.01 mg
1.0 mg or greater	0.1 mg

EXTRACTION COEFFICIENT

Determine extraction efficiency by extracting and analyzing 2,4-D standards (duplicates) identical to the sample procedure. Compare with an unextracted, esterified standard. Select an amount which is most representative of the concentration range of samples which are being analyzed.

REAGENTS

Esterifying reagent—Add 45 ml of nanograde methanol to 5 ml of 14% BF_3-CH_3OH solution. Carefully add 0.5 ml of concentrated hydrochloric acid to this mixture.

Acidified sodium sulfate—Add ether to 1 lb of anhydrous Na_2SO_4 until crystals are just covered. Add a few drops (approximately three at a time) of concentrated sulfuric acid to the ether solution. To determine the pH value after each acid addition, a small quantity of the slurry is removed, the ether evaporated, water is added to cover the crystals, and the pH is measured on the aqueous mixture. A pH value of 4.0 is desirable. Use Whatman 2V filter paper to prepare the "Na_2SO_4 funnel."

SUPPLEMENT B

Extraction and Analysis of DMA 2,4-D and/or BEE 2,4-D to Determine the Total 2,4-D Acid Equivalent in Sediment Samples

1. Weigh approximately 35 g of sediment into an aluminum weighing dish.
2. *Quantitatively* transfer the sample to a 500-ml Erlenmyer flask utilizing small aliquots of distilled water. For spiked samples, add 2,4-D standard to flask and allow to dry. Then add the sediment aliquot.
3. To the flask contents, add 1 ml of 20% H_2SO_4, 15 ml of acetone, 25 ml of petroleum ether, and 100 mg of diethyl ether. Insert a 1½ inch magnetic stir bar and "cap" the flask with aluminum foil.
4. Place flask on a magnetic stirring device and stir at medium speed for 1 hour. Occasionally swirl flask contents manually to inhibit a "dryness ring." Also use small amounts of distilled water and diethyl ether to "rinse" flask wall.
5. When 5 minutes remain on the 1-hour hydrolysis period, add 10 g of Celite 545 to flask contents. Rinse the flask well with small aliquots of distilled water.
6. Vacuum filter the flask contents through a Whatmann #1 pad. Wash remaining residue from flask with small aliquots of distilled water. Rinse filter cake with two 25-ml aliquots of 1:1 diethyl and petroleum ether solution.
7. *Quantitatively* transfer filtrate to a 1-liter separatory funnel utilizing 10-ml aliquots of the 1:1 ether solution.
8. Partition the filtrate with one 50-ml aliquot and two 25-ml aliquots of phosphate buffer (pH 6.8). Collect the buffer extracts in a beaker. Discard the organic phase.
9. Acidify the buffer solution with 20% H_2SO_4 (dropwise) to a pH value of approximately 2.0 units. Execute this manipulation using magnetic stirring.
10. *Quantitatively* transfer the buffer solution to a 1-liter separatory funnel.
11. Partition the acidified buffer solution with three 50-ml aliquots of chloroform. Add the $CHCl_3$ extracts to a 250-ml round-bottom flask via an "acidified Na_2SO_4" funnel. Wash funnel with an additional 10 ml of chloroform.
12. With a rotary vacuum evaporate the chloroform extracts "just" to dryness. Utilize a water bath with a temperature range of 40 to 45 C.
13. Cool the round-bottom flask. Take up the residue with three 5-ml aliquots of diethyl ether, and *quantitatively* transfer to a 15-ml conical centrifuge tube using a small funnel.
14. Evaporate ether solution to dryness with zero nitrogen. If a high concentration of 2,4-D is expected, evaporate an aliquot of the ether solution.
15. With a pipette, add an additional 2 ml of nanograde diethyl ether to the centrifuge tube insuring that the walls of the tube are rinsed thoroughly.
16. Evaporate ether solution to dryness with zero nitrogen.
17. Esterify residue with 1 ml of BF_3-methanol mixture for 30 minutes at 70 C. Centrifuge tube must be *tightly* stoppered and intermittent swirling of tube contents must be employed during esterification.
18. *Cool* tube contents, unstopper, and add 2 ml of 2% Na_2SO_4.
19. Add 10 ml of nanograde hexane and shake tube contents vigorously.
20. Centrifuge the tube for 5 minutes at approximately 4,000 rpm to remove any emulsions or particulate matter from the hexane layer.
21. Construct a calibration curve using esterified standards or inject esterified standards in appropriate quantities so that a given peak height closely approximates the peak height of the unknown. In the latter fashion the ratio-and-proportion principle may be employed. This method should be used only in the linear portion of the standing current.
22. Average individual results if multiple injections are employed and calculate concentration of 2,4-D in mg/kg by dry weight insuring that esterification aliquot, sample aliquot, percent moisture, and extraction coefficient factors are appropriately incorporated.
23. When utilizing electron capture detection with good sensitivity, this analytical method should provide a limit of detectibility of 100 ppb or 0.10 mg/kg. Data should be reported as follows:

Concentration Range	*Report to Nearest Value*
0.10 mg to 1.0 mg	0.01 mg
1.0 mg or greater	0.1 mg

Extraction Coefficient

Determine extraction efficiency by extracting and analyzing a sample which has been spiked with a known quantity of 2,4-D. Compare with the amount recovered from an unspiked portion of the sample. Select a "spike" amount which is most representative of the concentration range of the sample being analyzed.

Percentage Moisture

Weigh approximately 35 g of the subject sample into an aluminum weighing dish. Dry this aliquot in an oven at 110 C for 20 hours. Cool, desiccate to a constant weight, and then determine the dry weight.

Reagents

Esterifying reagent—Add 45 ml of nanograde methanol to 5 ml of 14% BF_3-CH_3OH solution. Carefully add 0.5 ml of concentrated HCl to this mixture.

Acidified sodium sulfate—Add nanograde ether to 1 lb of anhydrous Na_2SO_4 until crystals are just covered. Add a few drops (approximately three at a time) of concentrated sulfuric acid to the ether solution. To determine the pH value after each acid addition, a small quantity of the slurry is removed, the ether is evaporated, water is added to cover the crystals, and the pH is measured on the aqueous mixture. A pH value of 4.0 is desirable. Use Whatman 2V filter paper (fluted) to prepare the "acidified Na_2SO_4" funnel.

Phosphate Buffer—pH of 6.8 units.

Sodium sulfate—20 g of anhydrous Na_2SO_4 dissolved in distilled water and diluted to 1 liter.

SUPPLEMENT C

Extraction and Analysis of DMA 2,4-D and/or BEE 2,4-D to Determine the Total 2,4-D Acid Equivalent in Fish Tissue

The fish samples were processed according to the following procedure and finally analyzed with a Varian Aerograph gas chromatograph, using a concentric tritium foil detector.

1. Frozen fish specimens were diced into ¼- to ½-inch segments utilizing a heavy-duty meat cleaver. After compositing manipulations, an aliquot of the frozen pieces of flesh was homogenized in a Waring blendor. Homogenization was effected by mixing frozen segments of the whole fish body with dry ice chips. The dry ice-to-fish tissue ratio for blending was approximately 3:1 by weight. Allow dry ice in the thoroughly disintergrated sample to completely sublime.
2. *Quantitatively* transfer a 25-g aliquot of fish tissue into a 400-ml beaker which contains 50 ml of ethanol. Use approximately 15 ml of distilled water to effect the transfer.
3. With magnetic stirring, heat the mixture for 5 minutes at 65 C in a water bath. After 4 minutes of stirring, add 15 g of Celite which has been mixed with approximately 60 ml of *hot* distilled water into the extraction mixture. Vacuum filtrate, while hot, through a Whatman #1 pad. Filter to cake dryness.
4. *Carefully* return the filter cake to the 400-ml beaker, add 10 ml of ethanol and 40 ml of distilled water, reslurry with magnetic stirring for 5 minutes at 65 C. Immediately vacuum filter to cake dryness through a Whatman #1 pad.
5. *Quantitatively* transfer the filtrate to a freshly rinsed 400-ml beaker. Add 10 ml of 10% KOH to the mixture and hydrolyze for 15 minutes. Use magnetic stirring at room temperature.
6. After alkaline hydrolysis, add 20% H_2SO_4 (dropwise) stirring until a pH of 2.0 units is reached. Stir for an additional minute. Precipitated protein can be removed from the pH electrode by utilizing "jet-like" spurts of distilled water from a plastic bottle dispenser.
7. With stirring, add 10 ml of 20% PTA and 2.0 g of Celite. Place mixture in 65 C water bath and stir for 10 minutes to effect acid hydrolysis.
8. While hydrolyzing mixture is hot, vacuum filter through a Whatman #1 pad. Rinse the filter cake with two 30-ml aliquots of hot distilled water.
9. *Quantitatively* transfer the filtrate to a 1-liter separatory funnel and add 25 ml of saturated Na_2SO_4.
10. Partition the filtrate with one 50-ml aliquot and two 25-ml aliquot of nanograde chloroform.
11. Collect the $CHCl_3$ extracts in a 250-ml beaker. *Quantitatively* transfer the $CHCl_3$ extracts to a freshly rinsed 1-liter separatory funnel.
12. Partition the $CHCl_3$ extracts with two 25-ml aliquots of buffer (pH 6.8). Discard the $CHCl_3$ layer and retain the buffer extracts. Be sure no chloroform remains in the buffer extracts.
13. Add 20% H_2SO_4 (dropwise) to the buffer extracts until a pH of 2.0 units is obtained.
14. *Quantitatively* transfer the acidified aqueous phase to a 1-liter separatory funnel and partition with two 20-ml aliquots of a 1:1 mixture of diethyl ether and petroleum ether solution. Carefully transfer each ether extract into a 25-ml graduated cylinder via a small "acidified Na_2SO_4" funnel. Add 15 ml of ether solution to the separatory funnel as a rinse. Also add ether rinse to the graduated cylinder via the "acidified Na_2SO_4" funnel.
15. Residue cleanup is effected by pouring the 1:1 ether extract from the graduated cylinder into a "Florisil-acidified Na_2SO_4" column. The column is prepared by plugging a 25-ml burette with glass wool. It is charged with 6 cm of activated Florisil and approximately 1 cm of acidified Na_2SO_4. The column is initially rinsed with diethyl and methanol elutions of approximately 50 ml each. After cylinder contents are introduced into the column, contaminants are eluted with 20 ml of diethyl ether.
16. Elute the acid equivalent with 20 ml of methanol into a 25-ml graduated cylinder.
17. Evaporate the methanol eluate utilizing a stream of zero nitrogen. The cylinder should be placed in a beaker of hot water to expedite evaporation.
18. With a pipette, add 2 ml of nanograde diethyl ether to the cylinder insuring that the walls are thoroughly rinsed.
19. Evaporate to dryness with a stream of zero nitrogen.
20. Esterify the residue with 2 ml of BF_3-methanol solution for 30 minutes at 70 C.
21. Cool cylinder contents, unstopper, and add 2% Na_2SO_4 up to the 15-ml increment marking.

22. Add 10 ml of nanograde hexane and shake cylinder contents vigorously.
23. Construct a calibration curve using esterified standards or inject esterified standards in appropriate quantities so that a given peak height approaches the peak height of the unknown. The latter method should be used only in the linear portion of the standing current.
24. Average individual results if multiple injections are employed and calculate the concentration of the 2,4-D in mg/kg insuring that esterification aliquot, sample aliquot, and extraction coefficient factors are appropriately incorporated.
25. When utilizing electron capture detection with good sensitivity, this method should provide a limit of detectability of 100 ppb or 0.10 mg/kg. Data should be reported as follows:

Concentration Range	*Report to Nearest Value*
0.10 mg to 1.0 mg	0.01 mg
1.0 mg or greater	0.1 mg

Extraction Coefficient

Determine extraction efficiency by extracting and analyzing a sample which has been spiked with a known quantity of 2,4-D. Compare with the amount recovered from an unspiked portion of the sample. Select a "spike" amount which is most representative of the concentration range of the sample being analyzed.

Reagents

Esterifying reagent—Add 45 ml of nanograde methanol to 5 ml of 14% BF_3-CH_3OH solution. Carefully add 0.5 ml of concentrated HCl to this mixture.

Acidified sodium sulfate—Add nanograde ether to 1 lb of anhydrous Na_2SO_4 until crystals are just covered. Add a few drops (approximately three at a time) of concentrated sulfuric acid to the ether solution. To determine the pH value after each acid addition, a small quantity of the slurry is removed, the ether is evaporated, water is added to cover the crystals, and the pH is measured on the aqueous mixture. A pH value of 4.0 is desirable. Use Whatman 2V filter paper to prepare the "Na_2SO_4 funnel."

Phosphotungstic Acid (20% by weight)—Add 100 g of PTA to distilled water and dilute to 500 ml.

10% KOH—100 g of KOH dissolved in distilled water and diluted to 1 liter.

SUPPLEMENT D

Extraction and Analysis of DMA 2,4-D and/or BEE 2,4-D to Determine the Total 2,4-D Acid Equivalent in Mussel Flesh

1. *Quantitatively* transfer 25 g of thoroughly homogenized flesh into a 400-ml beaker using approximately 30 ml of distilled water.
2. Add 40 ml of 5% ethanolic KOH. Stir on a hot water bath at 65 C for 5 minutes to effect alkaline hydrolysis.
3. With *rapid* stirring, add 20% H_2SO_4 (dropwise) slowly until a pH of 2.0 is reached. Remove precipitated protein from pH electrode with "jet-like" spurts of distilled water from a plastic bottle.
4. With *rapid* stirring, add 10 ml of PTA (dropwise) to the mixture. Continue stirring action for an additional 1 minute.
5. Add 15 g of Celite with stirring. Rinse beaker walls with distilled water. Stir until complete dissolution is reached.
6. Acid hydrolyze with stirring for 15 minutes in a hot water bath at 65 C.
7. Cool the resultant mixture with stirring in an ice bath (aluminum pan) for 5 minutes.
8. *Quantitatively* vacuum filter the vessel contents through a Whatman #5 filter. When dryness is reached, return the "filter cake" carefully to the 400-ml beaker utilizing a small spatula. Reslurry the filter cake in 100 ml of distilled water. Heating is not necessary. Quantitatively vacuum filter the beaker contents through another Whatman #5 filter pad. Before "cake" dryness is reached, add beaker rinsings and filter the cake to complete dryness.
9. *Quantitatively,* transfer the filtrate to a 1-liter separatory funnel by using small rinses of 1:1 ethyl ether and petroleum ether solution.
10. Add 25 ml of saturated Na_2SO_4 to the separatory funnel contents.
11. Partition the filtrate *twice* with 50-ml aliquots of the 1:1 ether solution. Discard the aqueous phase.
12. Combine the ether extracts and partition the organic phase with 25 ml of phosphate buffer (pH 6.8). Add the buffer phase to a 250-ml beaker.
13. Partition the ether extracts a second time with 25 ml of phosphate buffer. Add the buffer phase to the 250-ml beaker.
14. Rigorously rinse the separatory funnel with hot, cold, and distilled water rinses. Return the buffer extracts to the separatory funnel and partition the aqueous phase with 25 ml of chloroform.
15. Discard the chloroform wash layer. Dispense the chloroform-washed buffer layer into a 250-ml beaker.
16. With stirring, add dropwise 20% H_2SO_4 to the buffer solution until a pH of 2.0 is reached.
17. *Quantitatively* transfer the acidified aqueous phase to the freshly rinsed 1-liter separatory funnel and partition with two 20-ml aliquots of 1:1 ether solution. Transfer each ether extract into a 25-ml graduated cylinder via a small "acidified Na_2SO_4" funnel. Add 15 ml of the ether solution to the separatory funnel as a rinse. Also add the rinse to the graduated cylinder via the "acidified Na_2SO_4" funnel.
18. Evaporate the ether solution in the graduated cylinder to dryness with zero nitrogen.
19. With a pipette, add 2 ml of nanograde ethyl ether to the graduated cylinder insuring that the walls of the vessel are rinsed thoroughly.
20. Evaporate the ether solution to dryness with zero nitrogen.
21. Esterify the residue with 2 ml of BF_3-methanol mixture for 30 minutes at 70 C.
22. Cool cylinder contents, unstopper, and add 2% Na_2SO_4 up to the 15-ml increment marking.
23. Add 10 ml of nanograde hexane and shake cylinder contents vigorously.

24. Construct a calibration curve using esterified standards or inject esterified standards in appropriate quantities so that a given peak height approaches the peak height of the unknown. In this fashion, the ratio-and-proportion principle may be employed. The latter method should be used only in the linear portion of the standing current.
25. Average individual results if multiple injections are employed and calculate the concentration of 2,4-D in mg/kg insuring that esterification aliquot, sample aliquot, and extraction coefficient factors are appropriately incorporated.
26. When utilizing electron capture detection with good sensitivity, this method should provide a limit of detectability of 50 ppb or 0.050 mg/kg. Data should be reported as follows:

Concentration Range	*Report to Nearest Value*
0.100 mg to 0.050 mg	0.005 mg
0.10 mg to 1.00 mg	0.001 mg
1.0 mg or greater	0.1 mg

Extraction Coefficient

Determine extraction efficiency by extracting and analyzing a sample which has been spiked with a known quantity of 2,4-D. Compare with the amount recovered from an unspiked portion of the sample. Select a "spike" amount which is most representative of the concentration range of the sample being analyzed.

Reagents

Esterifying reagent—Add 45 ml of nanograde methanol to 5 ml of 14% BF_3-CH_3OH solution. Carefully add 0.5 ml of concentrated HCl to this mixture.

Acidified sodium sulfate—Add nanograde ether to 1 lb of anhydrous Na_2SO_4 until crystals are just covered. Add a few drops (approximately three at a time) of concentrated sulfuric acid to the ether solution. To determine the pH value after each acid addition, a small quantity of the slurry is removed, the ether evaporated, water is added to cover the crystals, and the pH is measured on the aqueous mixture. A pH value of 4.0 is desirable. Use Whatman 2V filter paper to prepare the "Na_2SO_4 funnel."

Ethanolic KOH—Add 50 g of KOH and 300 ml of absolute ethanol to 500 ml of distilled water, dissolve, and dilute volume to 1 liter with distilled water.

Phosphotungstic Acid (20% by weight)—Add 100 g of PTA to distilled water and dilute to 500 ml.

SELECTED REFERENCES

Federal Water Pollution Control Administration. 1968. Report of the Committee on Water Quality Criteria, p. 159.

Hiltibran, R. C. 1967. Effects of some herbicides on fertilized fish eggs and fry. Trans. Amer. Fish. Soc. 96(4): 414-416.

House, W. B. et al. 1967. Assessment of ecological effects of extensive or repeated use of herbicides. Midwest Research Institute, Kansas City, Mo. Distributed by Clearinghouse for Federal Scientific and Technical Information, Springfield, Va. 22151.

Hughes, Janice S. 1964. Effects of selected herbicides on bluegill sunfish. Proc. 18th Ann. Conf. S.E. Ass. Game and Fish Comm., p. 480-482.

Hughes, Janice S. and J. T. Davis. 1963. Variations in toxicity to bluegill sunfish of phenoxy herbicides. Weeds 11(1): 50-52.

Kearney, P. C. and D. D. Kaufman (edited by). 1969. Degradation of Herbicides. Marcel Dekker, Inc., New York, NY.

Klingman, G. C. 1965. Weed Control: As a Science. John Wiley and Sons, Inc., New York, NY.

Menzie, C. M. 1969. Metabolism of Pesticides. Bureau of Sport Fisheries and Wildlife, Special Scientific Report, Wildlife No. 127, U. S. Dep. of the Interior, Washington, D. C., July.

Muzik, T. J. 1970. Weed Biology and Control. McGraw Hill Book Co., New York, N. Y.

Robson, T. O. 1968. The control of adquatic weeds. Ministry of Agriculture, Fisheries and Food Bulletin No. 194, Her Majesty's Stationery Office, London.

Smith, G. E. and B. Isom. 1967. Investigation of effects of large-scale applications of 2,4-D on aquatic fauna and water quality. Pesticides Monit. J. 1(3):16-21.

Walker, Charles R. 1970. Problems in clearance and registration of herbicides for aquatic areas (mimeo). Presented 1970 Ann Meeting of Weed Science Society of America, Montreal, Quebec, Canada.

PESTICIDES IN AIR

Volatilization of Soil-Applied DDT and DDD From Flooded and Nonflooded Plots[1]

G. H. Willis, J. F. Parr, and S. Smith

ABSTRACT

A mixture of 42% DDT and 16% DDD was incorporated into Commerce silt loam to a 6-inch depth and also surface-applied to achieve an average concentration of 42.3 ppm of DDT and 16.2 ppm of DDD. Atmospheric concentration gradients and cumulative recovery for each pesticide were then monitored continuously for 6 months at 10 and 30 cm above the water or soil surface of uncropped, flooded and nonflooded plots.

Within the first 2 days the atmospheric concentration of DDT at 10 cm dropped from a maximum value of 1977 to 58 ng/m³ above the flooded plot and from 2041 to 100 ng/m³ above the nonflooded plot, while corresponding levels of DDD decreased from 405 to 30 ng/m³ and from 575 to 92 ng/m³, respectively. Concentrations at 30 cm were comparatively lower throughout the study, denoting the existence of concentration gradients which were easily characterized. Except for the first few days following application, pesticide concentrations at either height seldom exceeded 100 ng/m³. Subsequent changes in the atmospheric concentration of the pesticides appeared to be related to climatological factors.

The higher atmospheric concentration and cumulative recovery of DDT compared with DDD apparently reflected the relative amounts of each pesticide in the original mixture, although other possibilities are discussed. It is evident that the flooding treatment effectively retarded the volatilization of both pesticides.

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Introduction

A number of chlorinated hydrocarbon pesticides such as DDT tend to persist and accumulate in agricultural soils because of their resistance to biological degradation. These pesticide residues are currently regarded as a potential health hazard to humans, because they can be absorbed by roots and leaves of crop plants. Recent reports (*1*,*2*,*4*,*5*) indicate that some chlorinated hydrocarbon pesticides, including DDT, degrade more rapidly under biologically active anaerobic conditions than in well-aerated environments. Thus, flooding or submergence has been suggested as a possible means of achieving soil anaerobiosis in the field, thereby accelerating the degradation of persistent pesticides to less harmful compounds and allowing a more rapid decontamination of soil.

An earlier paper by Willis *et al.* (*7*) reported a method and apparatus for monitoring atmospheric concentrations of pesticides after field application. Results showed that under some conditions volatilization could contribute significantly to a net loss of endrin observed after application to sugarcane. In view of the limited information relative to the atmospheric pollution potential of field-applied pesticides, it seemed appropriate to extend this earlier work to a study of DDT volatilization from soil after selected flooding treatments were imposed in an attempt to enhance its degradation. This paper reports the extent of volatilization and atmospheric concentration of soil-applied DDT and DDD as affected by different moisture regimes.

Materials and Methods

SOIL TREATMENT AND INSECTICIDES

A commercial formulation of DDT, labeled as 50% DDT wettable powder, was applied to three 12 x 12 foot uncropped plots of Commerce silt loam, an alluvial soil used principally for sugarcane production. Analysis by gas chromatography revealed that this particular pesticide formulation was a mixture of two insecticides, 42.3% DDT and 16.2% DDD (often referred to as TDE or Rhothane®*, which is a registered trade name of the Rohm and Haas Company).

The plots were treated with the pesticide mixture in two separate steps. First, the mixture was broadcast uniformly on each plot to deliver 67.6 and 26.0 lb/acre of DDT and DDD, respectively, and then incorporated and mixed with soil to a depth of 6 inches by rototilling. Assuming a uniform soil density of 2 x 10^6 lb/acre in the upper 6 inches, the resulting pesticide concentration would be 33.8 ppm of DDT and 13.0 ppm of DDD. In a second operation, the mixture was surface-applied on each plot to deliver 17.0 lb/acre of DDT and 6.4 lb/acre of DDD. If incorporated and mixed as before, the resulting concentrations would be an additional 8.5 and 3.2 ppm of DDT and DDD. Although this second application was not incorporated, the total average "concentration" of DDT and DDD was calculated to be equivalent to 42.3 and 16.2 ppm, respectively. The two modes of application (i.e., soil incorporation and surface application) were intended to simulate a condition in which a pesticide was surface-applied to a soil where such chemicals had already accumulated to high levels.

MOISTURE REGIMES

Immediately after application of the insecticides, different soil moisture regimes were imposed on the three plots:

(1) continuous flooding with water to a depth of 4 inches

(2) alternate flooding and draining for 7 days each—i.e., a 14-day cycle

(3) no water applied other than natural rainfall—hereafter referred to as the nonflooded plot.

AIR SAMPLING

After application of the insecticides and flooding of the first two plots, a vapor-collection apparatus described earlier (7) was activated above all three plots for continuous monitoring of the atmospheric concentrations of DDT and DDD during a 6-month period. In attempting to characterize concentration gradients, two booms were positioned over each plot at heights of 10 and 30 cm above the water or soil surface depending on the particular moisture regime.

The vapor traps (1-liter Erlenmeyer flasks) contained 500 ml of technical grade ethylene glycol that had been washed with *n*-hexane to remove impurities that might interfere with gas chromatographic analysis of DDT and DDD. Air was drawn through the portholes on each boom and into the trapping solvent at a rate of 1 liter/minute.

EXTRACTION AND ANALYSIS

Periodically, the vapor traps were replaced with identical units and removed for extraction and analysis. The ethylene glycol was extracted with 250 ml of *n*-hexane for 1 hour with a magnetic stirrer, after which the trapping solvent was discarded. The hexane layer was then washed with distilled water, dried with anhydrous Na_2SO_4, and concentrated by evaporation to an appropriate volume for gas chromatographic analysis. Extractant volumes were adjusted so that a 5-μl injection contained 1 to 5 ng, which was within the linear portion of the standard curve for both insecticides. Previous laboratory studies with DDT-spiked ethylene glycol (1000 ng in 100 ml) samples indicated that this extraction procedure yielded 94 to 95% recovery.

Soil samples were taken from the plots for pesticide residue analysis immediately after pesticide application and at different times during the study. Forty grams of soil was extracted for three 1-hour periods with 80 ml of 8:8:1 solution of *n*-hexane, acetone, and saturated sodium acetate, using a wrist action shaker. Studies with spiked samples indicated that this method yielded 93% recovery with DDT.

Aliquots (5 μl/injection) of hexane extracts from air and soil samples were assayed for *p,p'*-DDT and *p,p'*-DDD with a Micro-Tek Model 220 gas chromatograph equipped with a ^{63}Ni electron capture detector and Infotronics Digital Integrator Model CRS-100. Operating parameters were:

Column:	Glass, 180 cm x 6 mm, packed with 80/90 mesh Chromport XXX (acid and base washed, silanized) coated with 3% SE-30.	
Temperatures:	Oven	195 C
	Detector	295 C
	Inlet	215 C
Carrier Gas:	N_2 (prepurified) at 130 cc/minute.	

A 3% OV-1 on Chromosorb W column was used on some of the samples. No other confirmatory tests were used. High purity analytical standards of *p,p'*-DDT and *p,p'*-DDD were supplied by the Geigy Chemical Company and the Rohm and Haas Company, respectively.

* Mention of trade names or commercial materials is for the convenience of the reader and does not constitute any preferential endorsement by the U.S. Department of Agriculture over similar products available.

Results and Discussion

Results showed little difference in either atmospheric concentrations or cumulative recovery of DDT and DDD above the flooded plot compared with the one that was alternately flooded and drained on a 14-day cycle. The best explanation is that the latter plot remained essentially saturated for most of the 7 days allowed for draining, and behaved primarily as a flooded system. Thus, only the results for the flooded vs nonflooded plots will be reported.

Table 1 reports the levels of DDT and DDD found in the upper 6-inch layer of soil on the nonflooded plot. Based on the application rate of the pesticide mixture, the theoretical concentrations of DDT and DDD were calculated as 42.3 and 16.2 ppm, respectively. Although these values compare favorably with the levels actually detected some 6 to 8 hours after application (47.3 ppm of DDT and 12.4 ppm of DDD), we agree with Van Middelem (*6*) that "... it is very difficult to incorporate a predetermined, homogeneous mixture of a pesticide to a 6-inch depth in soil plots of the size normally employed in field experiments."

TABLE 1.—*Levels of DDT and DDD in soil (0- to 6-inch depth) of the nonflooded plot after initial application and during the experimental period*

Date	Residues in ppm [1]	
	p,p'-DDT	*p,p'*-DDD
	42.3 [2]	16.2 [2]
10/2/68	47.3	12.4
10/17/68	29.3	11.0
11/22/68	25.2	10.1
1/8/69	29.7	11.6
1/27/69	23.7	14.8

[1] Based on oven-dry weight of soil.
[2] Theoretical concentration assuming a uniform soil density of 2 x 10^6 lb/acre in 0- to 6-inch depth.

During the 4 months following application of the pesticides, the average concentration of DDT in soil was 27.0 ppm, considerably lower than the initial level detected on October 2, 1968. However, the average concentration of DDD for this period was 11.9 ppm, indicating little change from the initial value. The exact reason(s) for these results is not clear. Based on earlier work (*1,2,4,5*) a rapid rate of DDT degradation would not to be expected in the nonflooded, i.e., well-aerated, plot. Moreover, if extensive degradation of DDT had occurred, a concomitant increase in DDD would probably have resulted since the major degradative pathway for DDT by the soil microflora involves the reductive dechlorination to DDD (*1,4,5*). However, degradation to less complex polar compounds not detectable with gas chromatography by electron capture cannot be ruled out (*1,4*). The extent to which surface-applied DDT would undergo photochemical decomposition yielding DDD and other products is not known (*3*).

It is also possible that these results (Table 1) could have been due to (a) adsorption of DDT by organic and inorganic soil colloids and (b) intracellular binding of the pesticide by microorganisms, both of which could lead to incomplete extraction. Direct volatilization losses to the atmosphere, the subject of this report, could also contribute significantly to these results.

Climatological data for the experimental area from October 1968 through April 1969 are summarized in Table 2. October was an extremely dry month with only 0.11 inches of rainfall reported. Moreover, an unusually dry period began during the first week in January and continued through mid-February. With these exceptions, the data are considered to be near normal.

TABLE 2.—*Climatological data for the experimental area from October 1968 through April 1969*

Month	Temperature (F)		Rainfall (inches)	Evaporation (inches)	Average Wind (miles/day)
	Average Maximum	Average Minimum			
October	82.8	56.3	0.11	5.47	44
November	68.5	44.3	6.74	2.81	68
December	62.5	38.3	4.99	2.12	70
January	62.8	42.0	1.63	2.81	87 [1]
February	62.3	43.4	6.52	2.31	73
March	62.6	43.0	4.72	3.60	72
April	77.8	58.6	9.58	5.29	59

[1] Based on measurements for 19 days during the month.

Atmospheric concentrations of DDT and DDD monitored at 10 and 30 cm above the water or soil surface of flooded and nonflooded plots during a 6-month period are presented in Table 3. Concentrations of both pesticides were highest for all treatments and sampling heights during the 24-hour period following application. The higher levels of DDT compared with DDD are indicative of the relative amounts applied in the original mixture (42% DDT and 16% DDD). By the second day, concentration of DDT at the 10-cm height had dropped markedly from 1977 to 58 ng/m^3 above the flooded plot, and from 2041 to 100 ng/m^3 above the nonflooded plot. The corresponding levels of DDD had decreased from 405 to 30 ng/m^3 and from 575 to 92 ng/m^3, respectively.

Why the concentration of DDD (Table 3) above the nonflooded plot reached temporary minima of 44 and 17 ng/m^3 at 10 and 30 cm, 4 days after application, is unknown. A similar phenomenon for DDT occurred 2 days later, with low values of 25 and 14 ng/m^3, respectively, above the nonflooded plot. Concentrations of each pesticide then increased markedly, remaining rather high for several months. It is also noteworthy that the concentration of DDD above the nonflooded plot was actually higher than DDT from 4 to 34 days

after application, suggesting differences in either the relative volatility of the two pesticides under these conditions, or in the relative amounts of each pesticide at or near the soil surface due to photochemical or biological conversion of DDT to DDD.

TABLE 3.—*Atmospheric concentrations of DDT and DDD monitored at 10 and 30 cm above the water or soil surface of flooded and nonflooded plots from October 2, 1968, to March 21, 1969*

Date	Elapsed days	Atmospheric Concentration—ng/m³							
		DDT				DDD			
		Flooded		Non-flooded		Flooded		Non-flooded	
		10 cm	30 cm	10 cm	30 cm	10 cm	30 cm	10 cm	30 cm
10/2/68	1	1977	651	2041	1523	405	152	575	405
10/3/68	2	58	54	100	55	30	37	92	64
10/5/68	4	—	—	36	25	—	—	44	17
10/7/68	6	—	—	25	14	—	—	70	12
10/17/68	16	14	9	93	50	10	8	102	37
11/4/68	34	3	3	112	41	2	3	99	41
11/22/68	52	4	5	95	72	4	2	49	36
12/20/68	80	5	1	14	10	4	2	35	27
1/3/69	94	2	2	65	56	2	1	58	34
2/13/69	135	2	2	157	118	3	2	79	32
2/27/69	149	13	8	75	62	3	1	52	17
3/21/69	172	9	5	49	5	2	1	48	2

NOTE: — equals no sample.

It is evident that flooding effectively retarded the volatilization of the pesticides. This was noted during the first several days of the study and became more pronounced with time. The presence of concentration gradients of each pesticide throughout the study, as well as retardation of the volatilization process due to flooding, are illustrated in Fig. 1 and 2.

The cumulative recovery of DDT (Fig. 1), after 172 days, at 10 and 30 cm above the nonflooded plot was 20,335 and 13,520 ng. Corresponding values for the flooded plot were 4,960 and 2,639 ng, respectively. Cumulative recovery of DDD (Fig. 2) at this time, 10 and 30 cm above the nonflooded plot was 15,985 and 7,090 ng, while a total of 1,520 and 1,050 ng was recovered above the water surface of the flooded plot.

Several major changes in the atmospheric concentrations of both pesticides above the nonflooded plot (Table 3) are apparently related to certain climatological factors (Table 1). These relationships are suggested by separating Table 3 into three parts (shown by the broken lines). First, following the initial maxima during the first 24 hours after application, and the aforementioned minima 4 to 6 days later, the atmospheric concentration of each pesticide remained relatively high for approximately 45 to 50 days, which corresponded to a period of extremely low rainfall and a rather high rate of evaporation. Increased precipitation during the next 30 days was related to the concentration minima reached after 80 days (12/20/68).

FIGURE 1.—*Cumulative recovery of DDT at 10 and 30 cm above the water or soil surface of uncropped, flooded and nonflooded plots from October 1968 to April 1969*

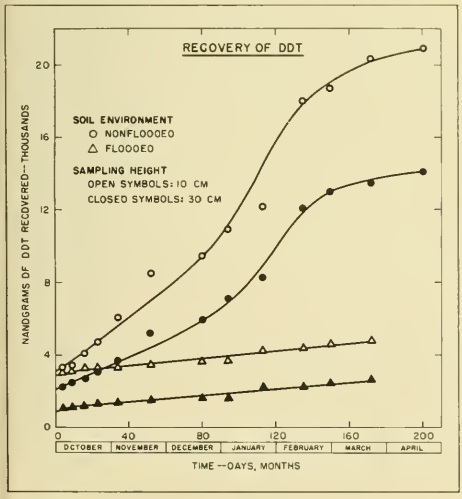
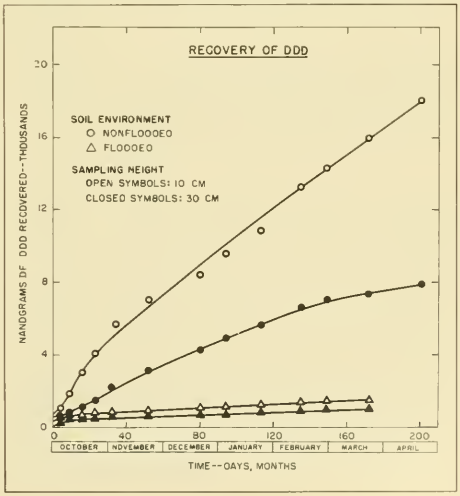


FIGURE 2.—*Cumulative recovery of DDD at 10 and 30 cm above the water or soil surface of uncropped, flooded and nonflooded plots from October 1968 to April 1969*



A second relationship between pesticide concentration and certain climatological factors appears during the first 40 days of 1969. The marked increase in pesticide concentration, reaching a maxima after 135 days (2/13/69), was associated with a period of unusually low rainfall and the greatest amount of wind. Finally, a decrease in the atmospheric concentration of each pesticide is shown from mid-February to the end of the experiment and is related to a period of abundant precipitation.

The plot size (12 x 12 feet) used in these studies may have been somewhat small to ensure a complete mixing equilibrium for a pesticide with air at the heights where concentrations were monitored. Such an equilibrium is essential to the application of an aerodynamic approach in calculating pesticide volatilization rates under field conditions. The use of larger plots (50 x 75 feet) with this particular objective in mind will be the subject of a future report. Nevertheless, these data indicate that different climatic variables, and interactions thereof, can significantly influence the extent of volatilization of two field-applied pesticides.

See Appendix for chemical names of compounds mentioned in this paper.

LITERATURE CITED

(1) *Guenzi, W. D. and W. E. Beard. 1968.* Anaerobic conversion of DDT to DDD and aerobic stability of DDT in soil. Soil Sci. Soc. Amer. Proc. 32:522-524.

(2) *Hill, D. W. and P. L. McCarty. 1967.* Anaerobic degradation of selected chlorinated hydrocarbon pesticides. J. Water Pollut. Contr. Fed. 39:1259-1277.

(3) *Mosier, A. R., W. D. Guenzi, and L. L. Miller. 1969.* Photochemical decomposition of DDT by a free radical mechanism. Science 164:1083-1085.

(4) *Parr, J. F., G. H. Willis, and S. Smith. 1970.* Soil anaerobiosis: II. Effect of selected environments and energy sources on the degradation of DDT. Soil Sci. (in press).

(5) *Spencer, D. A. 1967.* Problems in monitoring DDT and its metabolites in the environment. Pesticides Monit. J. 1(2):54-57.

(6) *Van Middelem, C. H. 1969.* Cooperative study on uptake of DDT, dieldrin, and endrin by peanuts, soybeans, tobacco, turnip greens, and turnip roots. General summary and conclusions. Pesticides Monit. J. 3(2):100-101.

(7) *Willis, G. H., J. F. Parr, R. I. Papendick, S. Smith. 1969.* A system for monitoring atmospheric concentrations of field-applied pesticides. Pesticides Monit. J. 3(3):172-176.

PESTICIDES IN SOIL

Soil Persistence of Fungicides—Experimental Design, Sampling, Chemical Analysis, and Statistical Evaluation[1]

W. J. Polzin, I. F. Brown, Jr., J. A. Manthey, and G. W. Probst

ABSTRACT

Soil persistence of a candidate foliar fungicide, parinol α,α-bis(p-chlorophenyl)-3-pyridinemethanol, was studied under practical field conditions. Included is an experimental design, method of sampling, and sampling device devised to improve the reliability of soil persistence data. Variability present in chemical analysis is considered, and a technique for statistical analysis is presented for examining persistence when repetitive applications of chemicals are made during the growing season. Employing a linear additive model, levels of compound remaining in the soil at the end of a growing season were predicted, and small differences in fungicide persistence between cultivated and uncultivated plots were detected.

Introduction

With the general use of pesticides in agriculture, effective techniques for determining soil persistence of these compounds is of interest. Predicting soil accumulations of chemicals applied to crops during a growing season may be uncertain for a number of reasons: difficulties may be encountered in recovering the test chemical from soil on a per-application basis; sampling at intervals may lack consistency; and chemical analyses performed at different times may vary considerably. Inadequate consideration of these factors and their interactions may result in heterogeneous data so erratic as to be inconclusive or data that appear reasonable but are misleading.

The purpose of this paper is to describe and evaluate a design and sampling technique devised to improve the reliability of data on the persistence of pesticides in soil by minimizing the effect of the above factors.

The study relates to liquid foliar fungicides. Residues from these materials accumulate beneath foliage drip lines when orchards, vineyards, and row crops are sprayed to "run-off" with high-volume applications. With either low- or high-volume sprays, these soil areas receive accumulations from the washing action of condensate processes and rainfall.

Edwards (*2,3*) has shown that a number of factors can affect the rate of disappearance of pesticides from soil; also, that the larger the dose of the chemical applied to the soil the less disappears in terms of percentage of the original application in a given time. In the study reported here, the gradual buildup of fungicide was simulated by repeated applications during a growing season.

Cultivation for weed control serves to mix surface chemicals in the soil. Since the distribution of a chemical through the soil profile may affect its persistence (*4,6,7*), this factor was examined. The chemical was applied to the soil surface and intermittently incorporated in the soil by cultivation.

Because our laboratory experience showed that individual soil samples varied widely, numerous subsamples were used to provide composited samples that would reliably represent the overall average for field plots. In earlier studies variability due to gas chromatographic analysis was assessed; within-day and between-day components of variation each proved to be approximately 25% of the mean. Their combined effect was estimated to result in a coefficient of variation of 40%. This suggested that a sufficient number of composited samples be taken at each point in time to permit scheduling analyses in a manner that would reduce the effects of these sources of variation.

[1] From Agricultural Research, Eli Lilly and Company, Greenfield, Ind. 46140.

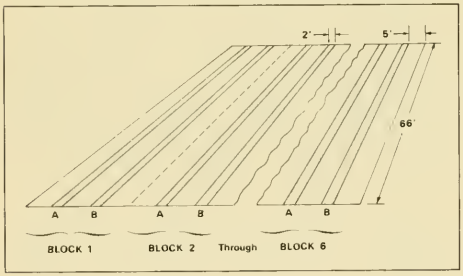
Materials and Methods

Parinol, α,α-bis(*p*-chlorophenyl)-3-pyridinemethanol (coded as EL-241), a powdery mildew fungicide *(8)* was selected as the test compound since, in earlier trials *(Unpublished greenhouse and field tests conducted at Eli Lilly and Company, Greenfield, Ind.),* this compound appeared to resist leaching and degradation in the soil.

DESIGN

A randomized block design was employed in which both uncultivated and cultivated plots (A and B), were replicated six times (Fig. 1). A site on level ground was selected to minimize lateral washing of the compound by rainfall. The soil type was a Brookston silty clay loam. Each plot, 2 x 66 feet, was separated by 5-foot border strips to avoid cross-contamination. To increase uniformity of applications and subsequent sampling, the plots were not cropped. The uncultivated plots were treated with trifluralin at 1.0 lb/acre to control weeds before the initial parinol application. Two shallow, 3-inch, cultivations were made to the B plots with a power-driven rotary hoe 1 and 2 months following the initial application to determine the effect of soil mixing on persistence.

FIGURE 1.—*Randomized block design; each block containing 2 plots, 2 x 66 feet in size; each plot separated by strips 5 feet wide (A = uncultivated plot, B = cultivated plot)*



APPLICATIONS

Soil plots were surface sprayed six times at biweekly intervals beginning June 30 and extending through September 14 with 35 ppm of parinol using 4% EC + 1% Sponto 206 and 6% Sponto 217 as emulsifying agents, diluted with water, and applied at a rate of 125 gal/acre (16.5 g/acre).

The application was equivalent to a concentration of 0.350 ppm in the top 1 inch of soil. Applications were made with a tandem of three spray nozzles set at a height of approximately 6 inches. Low pressure (15 psi) Monarch Whirl Chamber type nozzles designated as 49 x 49, 120° angle (*5*), were employed to further increase uniformity of application. To minimize spray drift, plots were treated early in the morning when air movement was minimal.

SAMPLING

Samples were collected and analyzed immediately after the initial application to establish a percent recovery value. Three samplings were made at monthly intervals during the growing season on July 30, August 30, and September 30; two additional samplings were made, during the winter and the following spring (Table 1).

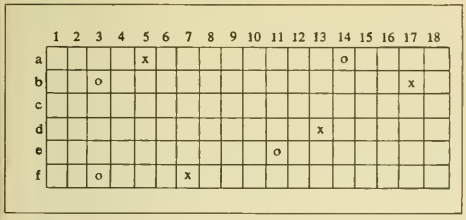
At each sampling time, subsamples were taken from each plot and composited into two main samples. Each composite sample consisted of 44 subsamples, weighing approximately 25 g each, taken from a uniform soil surface area to a depth of approximately 1 inch. Subsamples were taken with the aid of a 2 x 6 foot aluminum frame which was divided into 108 4-inch squares, each square having an alpha numeric identity. The grid was laid over one end of the plot, then sampled from and moved to successive 6-foot areas until the entire plot was sampled. Stakes placed in the plot insured identical placement of the grid for each sampling. With each placement of the frame, samples were taken from

TABLE 1.—*Level of compound theoretically recoverable in the cultivated and uncultivated plots at different times during the study*

	Soil Depth (inches)	Time of Application/Sampling								
		6/30	7/14	7/30	8/14	8/30	9/14	9/30	12/30	3/30
Application Number		1	2	3	4	5	6			
Sampling Number		1		2		3		4	5	6
		LEVELS IN PPM								
A Plots—uncultivated (Theory)	1	.255	.510	.765	1.020	1.275	1.530	1.530	1.530	1.530
B Plots—cultivated (Theory)	1	.255	.510	.255	.510	.425	.680	.680	.680	.680
	2	0	0	.255	.255	.425	.425	.425	.425	.425
	3	0	0	.255	.255	.425	.425	.425	.425	.425
				.765/3		1.275/3				

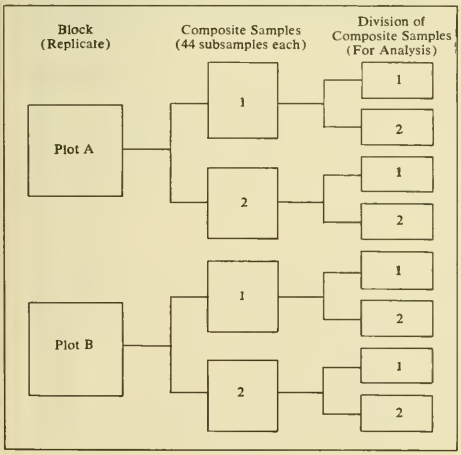
eight specified squares. Four of these were pooled in composite sample No. 1 and the other four in composite sample No. 2. Eleven frame placements covered the plot length and provided the 88 subsamples making up the two composite samples. All plots were sampled with a common grid pattern at a given sampling time. By employing a different pattern at successive sampling times, previously sampled soil was avoided. A single plot was sampled in less than 5 minutes; the entire trial in less than 1 hour.

FIGURE 2.—*Metal grid for taking subsamples, 2 x 6 feet; the grid consists of 108 4-inch squares* (o = subsamples for composite sample No. 1, x = subsamples for composite sample No. 2)



Immediately after sampling, the individual bags each containing 44 pooled subsamples were shaken and kneaded to insure complete mixing. Each composite sample was then divided into two portions for analysis (Fig. 3).

FIGURE 3.—*Diagram of sampling procedures*



CHEMICAL ANALYSIS

The test compound, parinol, was extracted from the soil samples with methanol-acetone (1:4). Extracts were analyzed by gas-liquid chromatography (sensitivity 0.005 ppm) and monitored by thin layer chromatography following procedures described in detail by Day *et al.*, (*1*).

SCHEDULING OF ANALYSES

To avoid confusing day-of-analysis effects with level of persistence, sample sets representing blocks were confounded with day-of-analysis at each sampling date. Confounding was accomplished by analyzing the eight portions representing the two plots of a given block during one day—so that sets representing different blocks were analyzed on separate days.

This arrangement provided an estimation of the parinol residue of each plot type by a total of 24 analyses at each sampling time (two analyses on each of two samples conducted on six different days). Randomizing the order of analyzing the eight samples in each test permitted valid comparisons of analyses within samples, samples within plots, and plot types within the blocks. The eight samples were a convenient number for the laboratory to process in a single day.

EFFECT OF STORAGE ON SAMPLES

After each collection, samples awaiting analysis were kept refrigerated (0 C) to minimize the effect of storage. However, to account for any sample storage effects that might occur within the time period of 6 working days required to process each set of 48, a 6 x 6 latin square of day-of-analysis sequence was superimposed on the six blocks of samples over the six sampling times (Fig. 4).

FIGURE 4.—*Latin square design employed to examine the effect of storage on samples awaiting assay (Cells contain block identity)*

Period of Sample Storage Divided Into 6 Intervals

Sampling Dates	1	2	3	4	5	6
1 July	B_1	B_2	B_3	B_4	B_5	B_6
2 Aug.	B_6	B_5	B_4	B_3	B_2	B_1
3 Sept.	B_4	B_1	B_6	B_5	B_3	B_2
4 Oct.	B_5	B_3	B_1	B_2	B_6	B_4
5 Dec.	B_2	B_4	B_5	B_6	B_1	B_3
6 Mar.	B_3	B_6	B_2	B_1	B_4	B_5

Results

RECOVERABLE THEORY

Statistical analysis of analytical results from samples taken immediately after the initial application of parinol indicated that the presence of the herbicide trifluralin did not interfere with the fungicide analysis (Table 2). This finding permitted all 48 assay values, the total from both plot types, to be averaged together to estimate an amount recoverable following each application. The overall mean amounted to 73% recovery of the quantity of compound applied (0.255 ppm vs 0.350 ppm) which agreed well with the recovery efficiency of 75% found routinely with standard laboratory reference controls.

Exclusion of confounding between plot types and day-of-analysis increased the credibility of the conclusion that the herbicide did not interfere with analysis. Additional findings from the statistical analysis (Table 2) indicated: (a) an absence of plot by block interaction, signifying that plot type differences were measured equally in each block, (b) a significant block effect caused by either plot location or day-of-analysis or a combination of the two, and (c) a significant sampling effect suggesting the two main samples obtained from each plot were of value in estimating the true level of each plot. Table 3 presents the plot means from the first sampling in the order they were analyzed. A comparison of these plot means indicates that differences between cultivated and uncultivated plots within blocks were random in nature and not related to the presence of herbicide. A comparison of block means also appears random indicating no decrease in compound levels associated with length of sample storage.

EFFECT OF SAMPLE STORAGE WITHIN DIFFERENT SAMPLING DATES

After completing analyses for the six sampling dates, the overall effect of the sequence of analyses within dates was examined. To facilitate this, the six sequences of analytical mean values (day 1 through day 6) are summed over the six dates in Table 4. Inspection of the pooled means indicated no loss trend associated with length of sample storage. The effect of sample storage was not detectable with the test system employed; and it, therefore, was considered as one of the components of random error in the model. Analyses of variance of the latin squares confirmed this interpretation.

TABLE 2.—*Analysis of variance of soil analyses made on samples taken immediately after the initial applications*

Source of Variation	Degrees of Freedom	Mean Squares	F Ratio
Plot (Herbicide Presence)	1	.0027	
Block	5	.0418	12.3 [1]
Plot/Block	5	.0085	2.5
Sample/Plot/Block	12	.0034	2.3 [2]
Assay/Sample/Plot/Block	24	.00146	

[1] $P < .05$
[2] $P < .01$

TABLE 3.—*Percent of compound (applied at 0.35 ppm) found in samples taken immediately after the initial application*

	No. of Samples	Blocks in order analyzed												Overall Mean
		Percent												
		1		2		3		4		5		6		
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		
		A	B	A	B	A	B	A	B	A	B	A	B	
Plot Means	4	75	84	61	59	61	50	50	72	121	102	57	85	73
Block Means	8	79		60		55		61		111		71		73

NOTE: A = uncultivated plots; B = cultivated plots.

TABLE 4.—*Day-of-analysis mean values, summed over all six sampling dates (the columns of the latin squares)*

	Sequence of Analysis						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Overall
A Plots (ppm)	.306	.338	.249	.261	.387	.307	.308
Uncultivated (%)	(99)	(110)	(81)	(85)	(126)	(100)	(100)
B Plots (ppm)	.149	.139	.151	.189	.177	.143	.158
Cultivated (%)	(94)	(88)	(96)	(120)	(112)	(91)	(100)
Both Plots (ppm)	.228	.239	.200	.225	.282	.225	.233
Total (%)	(98)	(103)	(86)	(97)	(121)	(97)	(100)

COMPOUND PERSISTENCE

Percentages of compound theoretically recoverable at each sampling time are presented in Table 5. The baselines for these values were obtained by computing recoverable theories at each sampling date using the recovery values obtained from the initial sampling (0.255 ppm) as a per application constant. For the uncultivated A plots, theoretical quantities expected at each point in time are the summed increments of the recoverable portions of the applications that were made. For the cultivated B plots, recoverable theory consisted of this same additivity, plus a 1:3 soil dilution factor adjustment to account for cultivations made after the third and fifth applications (Table 1).

To provide an equation that would describe changes in the amount of compound remaining over time, individual data were adjusted for cumulative increases in theory by transforming to the proportion of recoverable theory remaining at each sampling date. A log transformation of these proportions was found to normalize the variation, as determined by half-normal plotting of the residual variations, and to provide a linear response over time. These properties of the transformed data permitted the exponential model $y = e^{Bt+r}$ to describe persistence, where $y =$ proportion of theory remaining; $t =$ time in months; $B =$ slope of persistence curve (unknown parameter); and $r =$ replicated plots. In the regression analysis, neither block effect nor the interaction between the covariate sampling time and blocks was significant, so the degradation model became simply $t = e^{Bt}$.

The persistence slopes of the uncultivated and cultivated plot curves were, —0.3808 and —0.3071, respectively. These values are significantly different ($P<.01$) indicating greater loss when the compound was surface-applied than when surface-applied and intermittently soil-incorporated (Fig. 5).

Under the trial conditions (with the effects of location, weather, etc.) the prediction equation indicates that compound residues of 0.1% or less would remain in the undisturbed plots after 18 months and the same level or less after 21 months in cultivated plots (Table 6). Thus, each plot type showed sizable degradation in less than 1 year, but small differences in rate of degradation between the cultivated and uncultivated plots were detectable.

FIGURE 5.—*Persistence curves of compound in soil from cultivated and uncultivated plots*

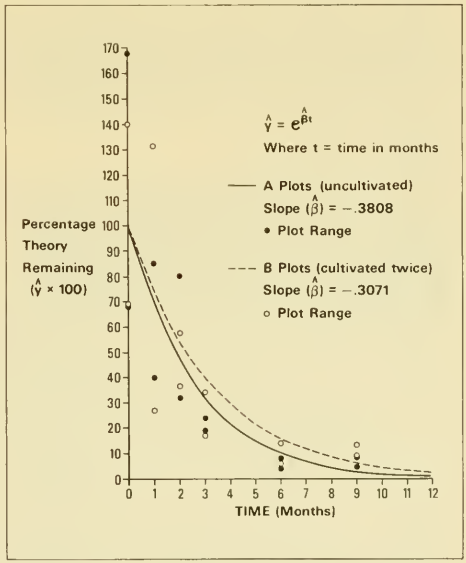


TABLE 5.—*Percent of compound, theoretically recoverable, found in cultivated and uncultivated plots of Blocks 1 through 6*

Block Number	Sampling Time (months)	Date Sampled	Cumulative Appl.	Recov. Theory (ppm)	Found/Recoverable Theory × 100—Percent						
					Block Number						Mean
					1	2	3	4	5	6	
A PLOTS (UNCULTIVATED)											
1	0	6/30	1X	.255	103	85	83	68	168	78	97
2	1	7/30	3X	.765	65	85	44	52	40	46	55
3	2	8/30	5X	1.275	80	51	59	52	42	32	53
4	3	9/30	6X	1.530	21	24	19	20	19	20	21
5	6	12/30	6X	1.530	8	8	5	8	4	4	6
6	9	3/30	6X	1.530	4	5	8	5	6	5	6
B PLOTS (CULTIVATED) [1]											
1	0	6/30	1X	.255	115	80	69	99	140	116	103
2	1	7/30	[1] 3X	.255	40	56	131	34	31	27	53
3	2	8/30	[1] 5X	.425	40	36	48	50	57	53	47
4	3	9/30	6X	.680	28	30	34	24	17	31	27
5	6	12/30	6X	.680	10	14	9	11	8	6	10
6	9	3/30	6X	.680	9	10	13	11	13	9	11

[1] Cultivations on 7/30 and 8/30 immediately after applications; samples taken immediately after cultivating.
NOTE: Samplings were made immediately after the applications on the dates indicated.

Discussion

PERSISTENCE MODEL

The linear additive model employed in this study to measure the persistence in cultivated and uncultivated plots could be used to evaluate the effects of any number of factors (F), such as moisture, soil type, cropping, fertility, etc., where the exponential model:

$$y = e^{Bt+F_1+F_2+ \ldots +F_n+b_1F_1+b_2F_2+ \ldots +b_nF_n+ \text{error}}$$

becomes linear when log transformed values are analyzed:

$$\log y = Bt+F_1+F_2+ \ldots +F_n+b_1F_1+b_2F_2+ \ldots +b_nF_n + \text{error}$$

Data in this form are amenable to conventional analysis of covariance procedures. With this technique slope by factor interactions (b_1F_1, b_2F_2, etc.,) can be examined for detection of significant factor-associated changes in persistence slopes.

SPECIFIC CONSIDERATIONS

Assurance that leaching was not a factor was checked by a single set of 44 core samples taken during the eighth month from one of the uncultivated plots. One-inch sections to a depth of 9 inches were composited and analyzed. Over 92% of the total compound was found in the top 1-inch layer of soil, and none was detected below 4 inches (Table 7), confirming the original premise. Where leaching is a factor in the degradation model, cores should be taken. No attempt was made to evaluate the persistence of the compound at depths below 1 inch as occurred in the case of the cultivated plots. This could have been investigated by taking cores instead of surface samples.

TEST SYSTEM VARIATIONS

The plot and block values found at different sampling times (Tables 3 and 5) indicate the sizable amount of variation encountered in the study. Plot differences can be attributed to a combination of (1) lack of uniformity of applications, (2) inadequacy and inconsistency in sampling, and (3) analytical variation. Because of the care exercised in applying the compound, and the extensive subsampling, the largest contributor to plot variation is postulated to have resulted from analytical variability. Components-of-variance estimates indicate only 18% of the total variability encountered within a given date of sampling could be assigned to within-day analytical variation. This together with the changing pattern of differences between blocks over the six sampling dates (Table 5) suggests that differences observed may be attributable to a day-of-analysis effect.

If plot differences between blocks are largely due to day-of-analysis effects, several approaches could diminish this effect:

TABLE 6.—*Percent of compound remaining estimated from curves*

	Predicted Levels—Percent										
	Months										
	0	1	2	3	6	9	12	15	18	21	24
A PLOTS (UNCULTIVATED)											
$y = e^{Bt}$	100	69	47	32	10	4	1	.3	.1	<.1	
Found	97	55	53	21	6	6					
B PLOTS (CULTIVATED)											
$y = e^{Bt}$	100	73	54	40	16	6	3	1	.5	.1	<.1
Found	103	53	47	27	10	11					

TABLE 7.—*Distribution of parinol in soil profile at eighth month in the uncultivated (A) plot of Block 1*

Depth (inches)	Chromatographic Observations μG/G			
	Nonhydrolyzed Soil [1]		Acid-Hydrolyzed Soil	
	Gas	Thin layer [2]	Gas	Thin layer [2]
1	.073	+++	.08	++
2	.006	±	.03	±
3	.004	±	NEG	±
4	NEG	0	NEG	0
5	NEG	0	NEG	0
6	NEG	0	NEG	0
7	NEG	0	NEG	0
8 and 9	NEG	0	NEG	0
Check	NEG	0	NEG	0

[1] No treatment of soil prior to extraction.
[2] Relative intensity of zones observed indicated by + signs.
NOTE: NEG = negative.

Refinement of analytical techniques. Historically, this has taken place many times with some major increases in precision occurring in recent years. However, despite these considerable increases in analytical precision, the day effect has remained relatively constant which somewhat limits the value of this approach.

Confounding blocks with day-of-analysis. Components-of-variance estimates from this study indicate that single composited plot samples from eight blocks instead of six, each analyzed once, on a different day, would have required one-third fewer samplings (96 vs 144) and two-thirds fewer analyses (96 vs 288) and would have provided precision equivalent to that obtained. This paradox exists, because the major source of variation was associated with the confounded block and day-of-analysis component. In retrospect, the confounding employed was sound, and a more complete commitment to it would have lead to increased precision per analysis.

Blocking directly on the day-of-analysis. This could be accomplished by collecting and storing sets of persistence samples taken over time. Accumulating complete sets of samples would permit employing an analytical scheduling design that would more effectively remove the day-of-analysis effect.

It would be necessary to analyze samples taken immediately after the initial application, over several days, to establish a recovery per application baseline. However, samples taken subsequently for persistence determinations could be stored to await analysis until all were collected.

If portions of the soil sampled initially were stored under similar conditions with those taken later, they would be available to be analyzed concurrently to permit the effect of sample storage to be assessed. The procedure would not require that the samples be completely stable under the conditions of storage, but only that the storage conditions for all samples be uniform.

The design and techniques followed in this study were more than adequate to evaluate the persistence of the model compound which degraded to less than 10% of the total applied within less than 1 year. For compounds which exhibit properties of greater persistence, such as the chlorinated hydrocarbon insecticides, the techniques are appropriate, but the design modifications suggested for increasing efficiency would be of value.

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LITERATURE CITED

(1) *Day, E. W., O. D. Decker, J. R. Koons, and J. F. Holzer. 1970.* Analytical methods for fungicide α, α- bis(*p*-chlorophenyl)-3-pyridinemethanol (parinol). J. Ass. Offic. Anal. Chem. 53(4):747-755.
(2) *Edwards, C. A. 1964.* Factors affecting the persistence of insecticides in soil. Soil and Fert. XXVII p. 461.
(3) *Edwards, C. A. 1966.* Insecticide residues in soils. Residue Rev. 13:113.
(4) *Klingman, G. C. 1961.* Weed control: as a science. John Wiley and Sons, Inc., New York, N.Y. p. 65.
(5) *Klingham, G. C. 1964.* Whirl chamber nozzles compared to other herbicide nozzles. Weeds 12:10.
(6) *Nash, R. G. and E. A. Woolson. 1965.* Persistence of chlorinated hydrocarbon insecticides in soils. Science 157:924-927.
(7) *Sheets, J. J. and L. L. Danielson. 1960.* Herbicides in soils. USDA ARS-20-9, p. 172.
(8) *Thayer, P. L., D. H. Ford, and H. R. Hall. 1967.* Bis-(*p*-chlorophenyl)-3-pyridinemethanol (EL-241): a new fungicide for the control of powdery mildew. Phytopathology 57:833, abstract.

PESTICIDES IN WATER

Chlorinated Hydrocarbon Pesticides in Iowa Rivers[1]

Lauren G. Johnson and Robert L. Morris

ABSTRACT

The routine monitoring of a number of Iowa rivers for chlorinated hydrocarbon pesticides over a 3-year period has shown the presence of dieldrin, DDT, or DDE in the majority of the samples taken. Dieldrin has occurred more frequently and in higher concentrations than either of the other residues, and this is attributed to the amount of agricultural activity in the watersheds involved and to the amount of surface water runoff.

Introduction

Chlorinated hydrocarbon insecticides have been widely used in Iowa for many years. The greatest use has been by the agricultural industry although municipalities have also applied significant amounts of these chemicals. DDT and chlordane were widely used in corn insect control, but they have been largely discontinued in the past 5 years. Heptachlor is still used to some extent; but aldrin has been used more than any of the other chlorinated hydrocarbon insecticides in Iowa for a number of years.

Because of this widespread use, the State Hygienic Laboratory has maintained a pesticide residue monitoring program for several years. Residues of various chlorinated hydrocarbon insecticides have been detected in soil, water, fish, and wildlife samples collected throughout the State *(3,5)*.

To assess the amount of various chlorinated hydrocarbon pesticides carried into the rivers, a number of Iowa streams have been monitored for pesticides on a monthly basis. This paper reports the results of the 1968, 1969, and 1970 river water surveys.

[1] From the State Hygienic Laboratory, University of Iowa, Iowa City, Iowa 52240.

Materials and Methods

Six sampling sites were included in the 1968 survey; the 1969 and 1970 surveys included these six, plus an additional four. The municipal water plants at the sampling sites were supplied with clean quart jars with teflon lid liners. Samples of the water plants' raw river water intake were collected in these jars and mailed to the State Hygienic Laboratory in expanded polystyrene mailing cartons during the first week of each month. The samples were extracted as received with petroleum ether and the extracts cleaned up on a 1-g silica gel column following a procedure developed at the State Hygienic Laboratory *(2)*. Identification and quantitation were done on an F & M model 400 gas chromatograph equipped with an electron capture detector. The temperatures of the injector, oven, and detector, respectively, were 200 C, 175 C, and 200 C.

A mixed column (6% QF-1 and 4% OV-1 liquid phase) was used for quantitation, and a 3% OV-1 column was used for further confirmation of the identities of the individual pesticides.

Glass columns 4 feet long and 3mm i.d. were used for the analysis. A 6%:4% mixed QF-1:OV-1 liquid phase column was used for quantitation and a 3% OV-1 column was used for further confirmation of the identities of the individual pesticides.

Recovery tests performed by spiking distilled water with dieldrin, DDT, and DDE at concentrations ranging from 0.025 to 0.250 μg/liter gave average recoveries of 92%, 102%, and 105%, respectively. Extraction efficiencies decrease as the turbidity of river water samples increases, and the values reported on turbid samples may be somewhat less than the total dieldrin content. Turbidities were run on the 1970 samples, as received, using a Hach DC-DR colorimeter.

Results and Discussion

Tables 1, 2, and 3 show the pesticide content of the rivers sampled in 1968, 1969, and 1970, respectively. Dieldrin was found in 40% of 179 samples analyzed; DDT was detected in 19%; and DDE, in 14.5%.

Three distinct trends can be seen from these data. The overall pesticide concentration varies from year to year and from season to season, and the levels and particular pesticides present vary from river to river. These variations are related to the amount of surface runoff water entering the river, the annual spring application of insecticides by agricultural industry, and the location of the river with reference to nearby heavy row-crop agriculture.

A study was conducted in Iowa in 1967 *(4)* on the pesticide content of runoff water from fields where aldrin had been applied in the spring for corn insect control.

TABLE 1.—*Pesticides in Iowa rivers, 1968*

Location	Pesticide	Residues in PPB (μg/liter)						
		April	May	June	July	August	September	October
Cedar River	Dieldrin	0.004		0.005		0.010		
Cedar Rapids	DDT	0.012	0.010					
	DDE			0.004			0.012	
Iowa River	Dieldrin	0.006	0.004	0.008	0.006		0.007	
Iowa City	DDT			0.012				
	DDE							
Mississippi River	Dieldrin						0.004	
Davenport	DDT					0.004		
	DDE			0.003				
Mississippi River	Dieldrin	0.003						
Dubuque	DDT				0.005			
	DDE		0.005	0.002				
Missouri River	Dieldrin				0.002			
Council Bluffs	DDT							0.004
	DDE							
Raccoon River	Dieldrin	0.003		0.004				0.005
Des Moines	DDT	0.006	0.005	0.004		0.005	0.004	
	DDE							

TABLE 2.—*Pesticides in Iowa rivers, 1969*

Location	Pesticide	Residues in PPB (μg/liter)							
		April	May	June	July	August	September	October	November
Cedar River	Dieldrin			0.007	0.012	0.009			
Cedar Rapids	DDT	0.009							
	DDE		0.006						0.003
Iowa River	Dieldrin	0.007		0.051	0.047	0.022	0.014	0.015	0.005
Iowa City	DDT								
	DDE		0.004						
Little Sioux River	Dieldrin								
Cherokee	DDT		0.005	0.007	0.005	0.016	0.009	0.011	
	DDE								0.003
Mississippi River	Dieldrin			0.004	0.011	0.006			
Davenport	DDT								
	DDE		0.006						
Mississippi River	Dieldrin								
Dubuque	DDT							0.004	
	DDE	0.004				0.005	0.010		
Missouri River	Dieldrin				0.014				
Council Bluffs	DDT								
	DDE	0.006							
Nishnabotna River [1]	Dieldrin	0.004	0.014	0.021	0.063	0.054		0.039	0.040
Hamburg	DDT								
	DDE								
Raccoon River	Dieldrin	No	0.007	0.007		0.041	0.006	0.010	
Des Moines	DDT	Sample	0.009		0.015				
	DDE	Received							
Skunk River	Dieldrin	No	0.010	0.027	0.029	0.019	0.019		0.006
Oskaloosa	DDT	Sample							
	DDE	Received							
Upper Iowa River	Dieldrin					0.006			
Decorah	DDT				0.007				
	DDE								

[1] Residues of heptachlor (0.600 ppb) found in samples taken in September.

TABLE 3.—*Pesticides in Iowa rivers, 1970*

[( ) = turbidities in Jackson turbidity units determined on Hach DC-DR colorimeter]

Location	Pesticide	Residues in PPB (μg/liter)					
		March	April	May	June	July	August
Cedar River Cedar Rapids	Dieldrin	0.013 (35)	(17)	(50)	0.019 (80)	0.015 (55)	(45)
	DDT						0.009
	DDE			0.006			
Iowa River Iowa City	Dieldrin	0.016 (60)	0.008 (70)	0.012 (90)	0.036 (75)	0.030 (75)	0.021 (60)
	DDT						
	DDE						
Little Sioux River Cherokee	Dieldrin	(85)	(500)	(85)	(225)	(105)	(80)
	DDT	0.009	0.020	0.009	0.013	0.011	
	DDE						0.006
Mississippi River Davenport	Dieldrin	(15)	(65)	(100)	0.012 (300)	0.010 (75)	(30)
	DDT			0.006			
	DDE		0.004				0.009
Mississippi River Dubuque	Dieldrin		(50)	(50)	(90)	(70)	(30)
	DDT						
	DDE						
Missouri River Council Bluffs	Dieldrin	(75)	(125)	(70)	(90)	(65)	(40)
	DDT		0.023	0.004			
	DDE				0.008	0.005	
Nishnabotna River Hamburg	Dieldrin	0.065 (500)	0.012 (105)	(30)	0.058 (150)	No Sample	0.023 (60)
	DDT	0.014					
	DDE			0.017			0.012
Raccoon River Des Moines	Dieldrin	(190)	(70)	(350)	0.011 (180)	0.013 (55)	0.020 (500)
	DDT	0.023	0.012				
	DDE			0.019			
Skunk River Oskaloosa	Dieldrin	0.005 (95)	0.015 (350)	0.008 (42)	0.021 (115)	0.019 (40)	0.024 (280)
	DDT						
	DDE						
Upper Iowa River Decorah	Dieldrin	(15)	(5)	(12)	0.006 (300)	(105)	(25)
	DDT						
	DDE						0.007

Samples of surface water draining from the fields were taken immediately after a heavy rain, which was 1 month after the application of aldrin on these fields. The principal residue found was dieldrin, and in these highly turbid samples about 50% of the total dieldrin was adsorbed on the soil particles which settled in the sample bottles during a 24-hour holding period. The dieldrin content in the water draining directly from the fields was 10 to 20 times that found in the Iowa River.

Because a significant portion of the pesticide residues was carried by the suspended matter in the water, turbidity was also recorded for the 1970 samples. It is anticipated that as sufficient data are accumulated, a seasonal correlation will be developed between turbidity and the pesticide content of the individual rivers.

Table 4 shows the flow in the Iowa River for 1968, 1969, and 1970. These are the discharge rates of the river at Marengo, Iowa, as measured by the U.S. Geological Survey *(7, 8* and *9)*. Marengo was chosen as the best location for an index of the variation of flow in the Iowa River, because it is only 40 miles upstream from the Iowa City sampling station. The table shows that in 1969 the flow in the Iowa River averaged 5 to 10 times more than in 1968, with the 1970 values averaging somewhat less than those of 1969. The concentration of dieldrin in Tables 1, 2, and 3 follow this same pattern indicating that as the surface runoff into the river increases significantly, so does the dieldrin level.

The dieldrin levels also follow a strong seasonal pattern. Both 1969 and 1970 show large increases in the dieldrin concentration in June and July followed by decreasing concentration in later months.

Aldrin is normally applied for corn insect control during May in Iowa and is rapidly converted to dieldrin in the environment. This seasonal usage coupled with high surface water runoff apparently accounts for the high concentration of dieldrin found in the Iowa River in June and July of 1969 and 1970.

The levels found in the Mississippi River and the Missouri River are similar to those reported by Breidenbach *et al. (1)* in their survey of major river basins for 1957 to 1965. However, those smaller internal rivers in Iowa which drain highly cultivated portions of the State show significantly higher levels of dieldrin, especially during years of high surface water runoff.

Those internal rivers in Iowa which do not drain highly cultivated areas, such as the Upper Iowa River in northeastern Iowa, consistently have low pesticide concentrations.

Monitoring the chlorinated hydrocarbon pesticides in a selected group of Iowa rivers over a 3-year period has shown several consistent trends which directly relate the dieldrin concentration in the rivers to the agricultural application of aldrin.

TABLE 4.—*Mean monthly discharge of the Iowa River (Gaged at Marengo, Iowa)*

Month	Mean Discharge in C.F.S.		
	1968	1969	1970
March	358	5,217	2.917
April	920	4,643	1,563
May	588	2,725	4,238
June	460	4,469	1,180
July	1,286	11,340	
August	859	2,453	
September	295	783	

The significant increase of dieldrin in the Iowa River from 1968 to 1969 and 1970 is a result of the increased surface water runoff noted in Table 4. The significant increase in the summer months follows the spring application of aldrin for corn insect control. These variations are not seen in rivers such as the Upper Iowa which drains an essentially nonagricultural part of the State.

The Report of the National Technical Advisory Committee on Water Quality Criteria (6) lists the permissible level for dieldrin in public water supplies as 0.017 mg/liter. It also states in the criteria for fresh-water organisms that, since any addition of a persistent chlorinated hydrocarbon insecticide is likely to result in permanent damage to aquatic populations, their use should be avoided. The levels of pesticides in Iowa rivers are well below the listed permissible criteria for public water supplies. However, the agricultural use of aldrin has resulted in dieldrin concentration levels alleged to be significant to aquatic life in some of Iowa's rivers through the mechanism of surface runoff water from heavily cultivated areas.

Research already completed and being prepared for publication shows dieldrin concentrations several times greater than the Food and Drug Administration action guideline in the edible portion of catfish taken from rivers listed in this report.

LITERATURE CITED

(1) *Breidenbach, A. W., C. G. Gunnerson, F. W. Kawahara, J. J. Lichtenberg, and R. S. Green. 1967.* Chlorinated hydrocarbon pesticides in major river basins, 1957-65. Public Health Rep., U. S. Dep. Health, Educ., Welfare, 82(2):139-156.

(2) *Johnson, L. G. 1970.* Analysis of pesticides in water using silica gel column clean-up. Bull. Environ. Contamination Toxicol. (in press).

(3) *Johnson, L. G., H. Harrison, and R. L. Morris. 1970.* Preliminary study of pesticides levels in the eggs of Iowa pheasants, blue wing teal and coots. Bull. Environ. Contamination Toxicol. (in press).

(4) *Morris, R. L. and L. G. Johnson.* Pollution problems in Iowa. In: Water resources of Iowa, eidted by P. J. Horick, 1970, available from the Iowa Academy of Science, Univ. of Northern Iowa, Cedar Falls, Iowa, p. 89-109.

(5) *Morris, R. L. and L. G. Johnson. 1969.* Some aspects of pesticides in the Iowa environment. Rep. No. 70-10, State Hygienic Laboratory, Univ. of Iowa, Iowa City, Iowa.

(6) *National Technical Advisory Committee to the Secretary of the Interior. 1968.* Water quality criteria. Fed. Water Pollut. Contr. Admin., Washington, D. C., p. 20-63.

(7) *U. S. Geological Survey. 1967.* Water Resources Data for Iowa, p. 35-40.

(8) *Ibid. 1969.* p. 48-53.

(9) *Ibid. 1970.* (in press).

APPENDIX

Chemical Names of Compounds Mentioned in This Issue

ALDRIN	Not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
CARBARYL	1-naphthyl methylcarbamate
DDD (TDE)	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; technical DDD contains some *o,p'*-isomer also.
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
DDT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; technical DDT consists of a mixture of the *p,p'*-isomer and the *o,p'*-isomer (in a ratio of about 3 or 4 to 1)
DIELDRIN	Not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethano=naphthalene
ENDRIN	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
HEPTACHLOR	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
HEPTACHLOR EPOXIDE	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
LINDANE	1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer
TOXAPHENE	chlorinated camphene containing 67-69% chlorine
PROPANIL	*N*-(3,4-dichlorophenyl)propionamide
SILVEX	2-(2,4,5-trichlorophenoxy)propionic acid
2,4-D	2,4-dichlorophenoxyacetic acid
BEE 2,4-D	———————, butoxyethanol ester
DMA 2,4-D	———————, dimethylamine salt
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
TRIFLURALIN	α,α,α-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine

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The PESTICIDES MONITORING JOURNAL welcomes from all sources qualified data and interpretive information which contribute to the understanding and evaluation of pesticides and their residues in relation to man and his environment.

The publication is distributed principally to scientists and technicians associated with pesticide monitoring, research, and other programs concerned with the fate of pesticides following their application. Additional circulation is maintained for persons with related interests, notably those in the agricultural, chemical manufacturing, and food processing industries; medical and public health workers; and conservationists. Authors are responsible for the accuracy and validity of their data and interpretations, including tables, charts, and references. Accuracy, reliability, and limitations of the sampling and analytical methods employed must be clearly demonstrated through the use of appropriate procedures, such as recovery experiments at appropriate levels, confirmatory tests, internal standards, and interlaboratory checks. The procedure employed should be referenced or outlined in brief form, and crucial points or modifications should be noted. Check or control samples should be employed where possible, and the sensitivity of the method should be given, particularly when very low levels of pesticides are being reported. Specific note should be made regarding correction of data for percent recoveries.

- Preparation of manuscripts should be in conformance to the STYLE MANUAL FOR BIOLOGICAL JOURNALS, American Institute of Biological Sciences, Washington, D. C., and/or the STYLE MANUAL of the United States Government Printing Office.
- An abstract (not to exceed 200 words) should accompany each manuscript submitted.
- All material should be submitted in duplicate (original and one carbon) and sent by first-class mail in flat form—not folded or rolled.
- Manuscripts should be typed on 8½ x 11 inch paper with generous margins on all sides, and each page should end with a completed paragraph.
- All copy, including tables and references, should be double spaced, and all pages should be numbered. The first page of the manuscript must contain authors' full names listed under the title, with affiliations, and addresses footnoted below.
- Charts, illustrations, and tables, properly titled, should be appended at the end of the article with a notation in text to show where they should be inserted.
- Charts should be drawn so the numbers and texts will be legible when considerably reduced for publication. All drawings should be done in black ink on plain white paper.
- Photographs should be made on glossy paper. Details should be clear, but size is not important.
- The "number system" should be used for literature citations in the text. List references alphabetically, giving name of author/s/, year, full title of article, exact name of periodical, volume, and inclusive pages.

The Journal also welcomes "brief" papers reporting monitoring data of a preliminary nature or studies of limited scope. A section entitled *Briefs* will be included, as necessary, to provide space for papers of this type to present timely and informative data. These papers must be limited in length to two Journal pages (850 words) and should conform to the format for regular papers accepted by the Journal.

Pesticides ordinarily should be identified by common or generic names approved by national scientific societies. The first reference to a particular pesticide should be followed by the chemical or scientific name in parentheses—assigned in accordance with CHEMICAL ABSTRACTS nomenclature. Structural chemical formulas should be used when appropriate. Published data and information require prior approval by the Editorial Advisory Board; however, endorsement of published information by any specific Federal agency is not intended or to be implied. Authors of accepted manuscripts will receive edited typescripts for approval before type is set. After publication, senior authors will be provided with 100 reprints.

Manuscripts are received and reviewed with the understanding that they previously have not been accepted for technical publication elsewhere. If a paper has been given or is intended for presentation at a meeting, or if a significant portion of its contents has been published or submitted for publication elsewhere, notation of such should be provided.

Correspondence on editorial matters or circulation matters relating to official subscriptions should be addressed to: *Mrs. Sylvia P. O'Rear,* Editorial Manager, PESTICIDES MONITORING JOURNAL, Division of Pesticide Community Studies, Pesticides Office, Environmental Protection Agency, 4770 Buford Highway, Bldg. 29, Chamblee, Ga. 30341.