International Mussel Watch

Coastal Chemical Contaminant
Monitoring Using Bivalves

The International Mussel Watch

A Global Assessment of Environmental Levels of Chemical Contaminants

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A Global Assessment of Environmental Levels of Chemical Contaminants

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The International Mussel Watch Project: Executive Summary

Background

The problem being addressed concerns the consequences of the continued, and in some cases, increasing use of biocides/pesticides in tropical and southern-hemispheric regions. The project looks specifically at the levels of organochlorine pesticides in the nearshore and coastal marine environment and the possible implication for human health, the use of marine resources and the changes in coastal ecosystems. Since the time scales of their persistence in the environment are of the order of tens of years, the present problem is urgent. A recent study by the World Health Organization (WHO) on DDT in mother's milk showed much higher levels in several developing countries than in European countries that controlled its usage decade ago.

The program has been triggered by: i.) the realization that the production and major use of persistent biocides/pesticides has shifted from northern hemispheric regions to tropical and southern hemispheric regions, and ii.) the knowledge gained from similar studies in the 1960's and 1970's in the Northern Hemisphere, which concluded that excessive use of persistent biocides/pesticides resulted in grave impacts on coastal marine ecosystems and on the health of the environment. The solution applied to the problem in most Northern Hemisphere countries was a ban or a regulation on the use and the production of selected biocides. This solution, however, may not be appropriate for the countries of the regions presently under discussion. Education in the controlled use of biocides, coupled with the introduction of alternative substances, may be a more practicable solution.

The project uses bivalves for monitoring the concentration of selected pollutants and as an indicator of bioavailability. Bivalves are chosen because of their worldwide distribution and ubiquitous abundance, their general ability to bioconcentrate most pollutants, and their sedentary habits.

Studies similar to this proposed program have been carried out in North America and in the North Atlantic (coordinated through ICES), and resulted in the identification of zones of high and low contamination levels which can serve as reference areas. Experience gained in the design of techniques for sampling, analysis, preservation, and evaluation from these studies have been taken into account in the present design. Lessons learned from the previous project include the need for stringent data quality control and quality assurance; agreed common methods of sampling, preservation and analysis; and, participation in intercalibration exercises by all participating laboratories.

International Mussel Watch Goals

The primary goal of the International Mussel Watch is to ascertain and assess the

levels of chlorinated hydrocarbon pesticide (CHP) and polychlorinated biphenyls (PCB) in bivalves collected from coastal marine waters throughout the world. The emphasis is on tropical and southern hemispheric locations where the use of these biocides continues and appears to be increasing. Increased use of these persistent toxic biocides may result in contamination of living coastal resources from whole ecosystems to specific food resources with consequent implication for human health and the integrity of marine communities.

Comparison of the measured values with those from the northern hemisphere of the 1960's and the 1970's (at which times morbidities and mortalities related to chlorinated hydrocarbon pollution were observed) will provide an assessment as to whether populations at upper trophic levels, the most susceptible parts of the ecosystem (e.g., mammals and birds), are at risk from these compounds.

Another goal for the International Mussel Watch Project will be to help develop a sustainable activity for observation and monitoring chemical contamination in especially susceptible regions of the world's oceans. Such a global scientific network will provide comparable and reliable data sets for environmental decision makers.

International Mussel Watch Objectives

* To establish on a global scale the levels of contamination of selected organochlorine pesticides and the polychlorinated biphenyls, in the coastal marine environment.
* To compare, where possible, present day levels of organochlorine compounds found in the tropics and the southern hemispheric locations with those found in the northern hemisphere during the 1960's and 1970's, where ecosystems disturbances at the upper trophic levels (fish, birds, cetaceans) were apparent.
* To establish an archive of samples to provide a basis for a time series comparison for both these compounds and as yet unidentified industrial and agricultural contaminants.
* To contribute to the global data base for the evaluation of the present oceans. Provide laboratories and regional organizations with baseline data against which to interpret to make future environmental management decisions.

Important Products of the Project

* Stimulation of an approach whereby regional specialized networks of laboratories employ the sentinel organism technique for surveillance and monitoring of contamination.
* A global network of sentinel organism data exchange between regional networks, with agreement on associated quality control, sample analysis, data exchange and data analysis procedures.
* A sustainable organization or mechanism capable of obtaining quality controlled data or priority contaminants on a global basis in the near-shore and coastal zone using tested methods of sampling and analysis,

either for baseline studies, "hot spot" monitoring or future trend monitoring.

* A data base on the distribution of organochlorine residues in sentinel bivalves on a global scale.

* Publications on the state of the marine environment with respect to these pesticides and industrial chemicals and a critical assessment of those contaminants in reference to finding published in the open scientific literature.

* Evaluations for use by decision-makers in governments.

* Increased national capabilities to assess environmental problems related to organochlorine pesticides and industrial chemicals and other contaminants in the broader context of a global baseline.

* A base for assessment of priorities for future research and monitoring in relation to the information gathered.

Follow-up Actions

In addition to the sample analysis and synthesis of acquired data, consideration will be given to: a) monitoring of additional sites in consultation with participating national laboratories, b) negotiating with scientists on the expanded use of the archival material for other pollutant classes, c) strengthening national capabilities to continue the monitoring effort, d) aquisition of national production and use data.

Project Benefits

The successful completion of the program will provide a format for future international activities whose goals are to maintain or improve the quality of the global environment. Although there have been regional programs in marine pollution (noteworthy are those of UNEP in their Regional Seas activities and those of the IOC through GIPME/MARPOLMON) very few (e.g. MARPOLMON) have been as widespread geographically and have been carried out under a non-governmental umbrella.

A major benefit will be the acquisition of an initial set of data for chemical contaminants in sentinel organisms to evaluate the extent and severity of chemical contaminants in coastal areas on a regional and global basis. This will be significant initial step in a continuing program that could comprise a sustained and expanded regional and global monitoring effort to assess chemical contamination in coastal areas. These data and their interpretation will provide a sound basis for formulation and implementation of policies for protection of human health and for wise management of coastal ecosystems.

We expect that this project will benefit from and integrate with existing national and regional efforts. In addition, we expect that the project will provide a basis for additional national and regional activities concerned with pollution of coastal areas.

An added benefit will be dissemination to the world community of the results of a

collaborative experience with reference to: sampling, sample storage, chemical analysis quality assurance procedures; and data interpretation strategies emanating from this program.

Required Resources

Costs for a global monitoring program were estimated in the original report at US$500,000 per year, for a regional approach that divided the sampling into four major global regions. This cost estimate presumed a 5-year program and did not include ongoing national and international efforts which would contribute significantly to the International Mussel Watch effort.

Costs for the initial implementation phase which is operational in 1991-92 are estimated separately.

*ACKNOWLEDGEMENT**

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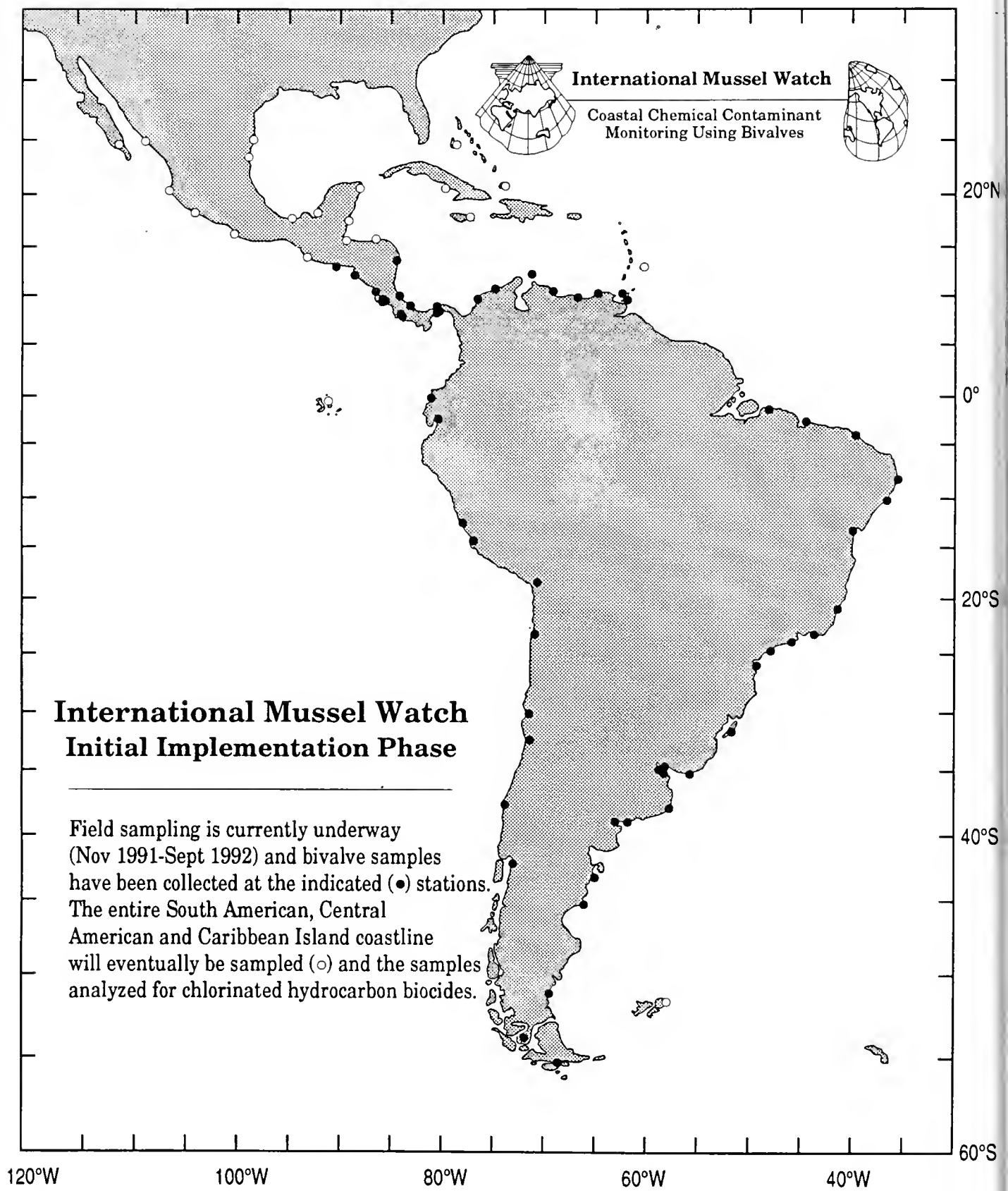
International Mussel Watch
Coastal Chemical Contaminant
Monitoring Using Bivalves
20°N
0°
20°S
40°S
60°S
120°W
100°W
80°W
60°W
40°W
International Mussel Watch
Initial Implementation Phase
Field sampling is currently underway
(Nov 1991-Sept 1992) and bivalve samples
have been collected at the indicated (•) stations.
The entire South American, Central
American and Caribbean Island coastline
will eventually be sampled (○) and the samples
analyzed for chlorinated hydrocarbon biocides.

The International Mussel Watch Program

The Environmental Levels of Chlorinated Hydrocarbons

We are concerned about the consequences of the continuing, and in some regions, increasing use of chlorinated pesticides and the release and persistence in the environment of industrial chemicals, e.g. polychlorinated biphenyls, especially in the tropical and southern hemispheric regions of the world. Our particular interest encompasses the levels of these organochlorine molecules in the coastal marine environment and the possible implications for human health, restriction in uses of marine resources and impacts upon coastal ecosystems.

There is strong evidence of an ever increasing usage of chlorinated hydrocarbon pesticides in the developing world, especially in the tropics and in the southern hemisphere, based upon analysis of the atmosphere, natural waters (Tanabe *et al.*, 1982) and human mother's milk (Slorach and Vaz, 1983), as well as examinations of the global production and use data of these biocides (Postel, 1987). The concern of environmental scientists relates to a possible recurrence of the ecodisasters that occurred in the northern hemisphere in the 1960's and 1970's as a consequence of heavy use of DDT and similar pesticides impacting upon non-target organisms (Goldberg, 1976). The production and use of organochlorine insecticides in the European OECD countries and North America reached a peak value in the early 1970's. At that time ecological effects were observed in top marine predator species, through egg-shell thinning in birds and reproductive failures in marine mammals.

In 1972, observations of the morbidities, mortalities and reproductive failures of higher trophic level organisms including fish-eating birds led the United States Environmental Protection Agency to ban the use of DDT in agriculture and public health activities (Goldberg, 1976). This action was repeated in many northern European countries. It must be emphasized that regulating the flows of toxic chemicals to the environment had previously been based upon insults of human health. This most sophisticated and advanced step of environmental action to maintain the integrity of communities of organisms has been followed in many other cases.

Following stringent environmental legislation, the use of chlorinated insecticides in the U.S. and OECD countries gradually decreased. However, complementary use increased in tropical areas and in the southern hemisphere. Subsequently, production and use shifted from north to south.

Following the decrease in use, levels of organochlorine pesticides in marine biota gradually diminished in North America, Northern Europe and Japan (Slorach and Vaz, 1983). In the Baltic, present levels are down to one-third of the levels of the early 1970's. Recently, however, the

International Mussel Watch Committee has received verbal reports of an upward trend in the Baltic Sea biota.

Industrial production and use data for these chlorinated hydrocarbon pesticides, which have a high toxicity and a long persistence in the environment, are difficult to obtain. Often countries and individual corporations categorize these data as proprietary information to protect their economic and political interests. However, despite uncertainty about the data, the presently available information suggests that the production rates of persistent chlorinated pesticides are not decreasing on a global basis. Where several hundred thousand tons of DDT each year were produced and used globally in the 1960's (Goldberg, 1976), annual world production of all persistent chlorinated pesticides today is probably around several million tons.

Some sense of the use of chlorinated hydrocarbons can be gained from the statistics of small and moderately sized countries. Chile in 1979 utilized 6500 tons of chlorinated hydrocarbon pesticides including 1288 tons of benzene hexachloride and 4400 tons of Aldrin (CPPS, 1981). Ecuador used in 1970 nearly 100 tons of benzene hexachloride and an equal amount of endrin (CPPS, 1981). These are small countries with a high per capita consumption of hard pesticides. Hexachlorocyclohexane has long been extensively manufactured in Japan and mainland China and present environmental levels show that it has clearly been dispersed about the tropics and southern hemisphere (Tanabe *et al.*, 1982). In India alone, the production of benzene hexachloride is around 30,000 tons per year (U.K. Venugopalan, personal communication, 1983).

These statistics are complemented by levels measured in the atmosphere and oceans. Hexachlorobenzene and hexachlorocyclohexane are usually the dominant halogenated hydrocarbon pesticides in atmospheric samples. Their concentrations over islands in the northern hemisphere (Eniwetok) and in the southern hemisphere (Samoa) are of the same order of magnitude, although somewhat less in Samoa (Atlas and Giam, 1989). Similar atmospheric distributions have been reported for a more extensive set of stations in the Pacific by Dr. S. Tanabe and co-workers (1982) of Ehime University of Japan who also have measured the pesticides in surface seawaters. Richardson and Waid (1982) have indicated a pattern of polychlorinated biphenyl contamination in Australia, similar to that of the Northern hemisphere.

In 1980-1982, the United Nations Environment Program and the World Health Organization collaborated with the Swedish National Food Administration and developed an assessment of the human exposure to selected organochlorine compounds in human mother's milk (Slorach and Vaz, 1983). This study provides a measure of the global distribution of these compounds in the biosphere and shows that the median levels of the p,p'-DDT reported in the fat

of human milk were higher in lesser developed countries like China, India and Mexico; whereas in countries that have prohibited or severely restricted the use of DDT, the levels were almost an order of magnitude lower.

The scientific team that conducted the study concluded that the high levels of DDT were due to continuing and extensive use of DDT as an insecticide in agriculture and in disease vector control. In these countries, the elevated levels of DDT-DDE in mothers milk indicate that the intake of the DDT family, by some or most breast-fed infants in the participating countries, exceed the acceptable daily intake (ADI) of 5µg/kg body weight established by the United Nations Food and Agriculture Organization and the World Health Organization. In the developing countries, the ADI is exceeded several fold by most infants. The same investigation showed that the levels of the industrial chemical polychlorinated biphenyls (PCB's) were higher in Japan and European countries and were almost undetectable in China, India and Mexico.

However, there is documented increased usage of electrical components which have PCB's as potting fluids in transformers and condensers. Recent reports of spills of these PCB's on the African continent and their potential movements in the coastal zone, exemplify the concerns of environmental scientists. These industrial chemicals have been linked to the declining mammal populations in the Baltic and North Seas and in California coastal waters. Are the concentrations of PCB's in any zones of the tropics and southern hemisphere attaining levels that might jeopardize marine organisms?

These data support the crucial need for global scale environmental monitoring of these persistent agriculturally- and industrially-based chlorinated hydrocarbons. The lack of scientific resources, instruments, laboratories and available scientists coupled with the high level of usage of chlorinated compounds in many regions, argues for a carefully conducted assessment to measure these compounds on a global basis. This problem underlines the need for reliable information on the distribution of these toxic chemicals in the biosphere such that appropriate regulatory policies can be considered at the national and international level.

Modern societies have benefited substantially from the production and use of synthetic chemicals as well as the increased mobilization of naturally occurring chemicals (e.g. mining and petroleum production). It has been estimated that at least 1000 of these chemicals, a small percentage of the hundreds of thousands produced, are of environmental concern (Butler, 1976; Sheehan *et al.*, 1984). The chlorinated hydrocarbons are among these.

Lessons learned from the problems with DDT and related persistent chlorinated pesticides resulted in development and use of other pesticides such as the thiophosphate and carbamate families of pesticides. These newer pesticides generally, but not in all cases, have less persistence

in the environment. Thus, even though the newer pesticides may account for an increasing proportion of pesticide use in certain regions of the world, the continued use of the more persistent chlorinated pesticides such as DDT is of continuing concern.

Furthermore, we cannot undertake simultaneous analyses for all possible chemicals of environmental concern in the initial phases of the program. The target analytes have been carefully chosen because of: 1) continuing concern with respect to their presence in the environment, 2) previous extensive data bases of their distribution in coastal ecosystems, especially bivalves, in several Northern Hemisphere countries, and 3) reasonably well established methods for analysis of bivalve tissues. There is no doubt that there will be a need for expansion of the effort in the future to include other analytes.

The Mussel Watch Approach

We propose that the most effective course of action initially to assess the global environmental levels of halogenated hydrocarbon compounds is to conduct a (single-shot) monitoring of sentinel organisms in the worlds coastal oceans. A "Mussel Watch" sampling and analysis program can provide this required information (Goldberg, 1976).

Our proposal is based on the utilization of sentinel organisms as an approach for monitoring the concentrations of selected pollutants, and as an indicator of their bioavailability and concentration in the biosphere. Several types of marine bivalves, especially mussels and oysters, have been found useful as indicator organisms, due to their worldwide distribution and ubiquitous abundance, their general ability to bioconcentrate most pollutants, and their sedentary habits (Farrington *et al.*, 1983; NRC, 1980; Phillips, 1980; INFERMER, 1983; Topping, 1983; US-NOAA, 1987, ICES, 1988).

Regional "Mussel Watch" monitoring programs have been carried out in North America and the North Atlantic, resulting in identification of zones of high levels of contamination as well as areas of low levels, which can serve as reference areas. Experience in sampling, analysis, preservation and evaluation from these studies have been taken into account for the present program design and will provide a springboard for the implementation of this proposed program.

In 1980, an international gathering of scientists in Barcelona, Spain (NRC, 1980; Appendix A), assessed the state of knowledge using bivalves as sentinel organisms for chemical contamination in coastal areas based mainly on results from localized, within country regional, and national efforts. They recommended planning begin for an International Mussel Watch effort, including a further assessment of the state of knowledge. A second gathering of international

scientists (Appendix A) under the auspices of the Scientific Committee for Problems of the Environment (S.C.O.P.E.) of the International Council of Scientific Unions (I.C.S.U.); UNEP, and IOC-UNESCO at the East-West Center, Hawaii, U.S.A. in November, 1983 provided further assessment and recommended planning for the implementation of an International Mussel Watch Program (Peterson and Tripp, 1984; Sivalingam, 1984). The deliberations and recommendations of these two meetings led to the design of the present International Mussel Watch Program.

Lessons learned from the previous Mussel Watch monitoring programs include the need for stringent data quality control and quality assurance practices, the need for common methods of sampling, preservation and analysis, and involvement in intercalibration exercises by all participating laboratories. The ability to carry out the collection, analysis and interpretation of the data in its inter-regional and global contexts, however, does not yet exist in some developing regions of the world.

The Present Problem

The continuous introduction of both old and new potentially harmful chemicals to the marine environment, through river discharges, effluent dumping releases, land run-off and atmospheric deposition, requires a sustainable capacity to obtain reliable relevant data for most regions in order to protect human health and manage wisely valuable living resources of the worlds coasts. This capacity will be developed through this proposed program.

The solution to the pesticide impact on ecosystems in most northern hemisphere countries was a ban or regulation of use and production of persistent biocides. This path may not be appropriate to many of the developing countries of the world for economic and public health reasons. Instead, a controlled use of these pesticides along with other pest control measures such as integrated pest management (IPM) is a rational course to follow. Education in the use of biocides, coupled with the introduction of complementary strategies, will be required. An important ingredient towards solution of this problem is the accumulation of substantial data on present day build-up of persistent biocides in the marine environment.

The importance and priority of this project has been recognized by two agencies within the United Nations. The Sixth Session of the Intergovernmental Oceanographic Commission's (IOC) Scientific Committee on the Global Investigation of Pollution in the Marine Environment (GIPME), (Paris, October, 1986) endorsed the proposal to establish a Global Monitoring Program for the detection of organochlorine pesticide residues in sentinel bivalves, i.e. the International "Mussel Watch" (IMW). Further, it recommended that a Steering Committee be convened to draw up an acceptable plan of action to conduct such a program and invited the United Nations

Environment Program (UNEP) to consider lending its support to this important activity through its Regional Seas Environmental Program. The Committee also recommended that the Program be given priority support by the Intergovernmental Oceanographic Commission (IOC) under the umbrella of GIPME. Intergovernmental Oceanographic Commission and the United Nations Environment Program have committed seed funds and support to initiate the International Mussel Watch Program. Both agencies have agreed that this important scientific program should move forward and will incorporate IMW into their existing environmental regional programs. Particularly encouraging is the agreement to include Mussel Watch activities in the United Nations Regional Seas protocols and treaty agreements. These regional environmental treaties and pacts provide the legal, political and scientific access to many countries around the world for participation in regional environmental programs.

In 1987 United Nations agencies provided $12,000 of support for organizing activities and in 1988 they contributed $60,000 for IMW scientific, technical and program development. This support continued in 1989 with a contribution of $40,000 and these agencies have agreed to cooperatively fund national and regional meetings to organize and implement the program. Full implementation of the global program will require an estimated 2.5 million dollars in 1989 dollars to fund implementation, and the data assessment and synthesis activities of this global program.

Initial implementation for the South American and Caribbean region has been partially funded for program organization, sampling, chemical analyses, and data assessment. A combination of United Nations Agencies (UNEP and IOC/UNESCO) and the U.S. National Oceanic and Atmospheric Administration have jointly contributed to create a Project Secretariat and to begin the sampling and analysis of field-collected samples. A budget cooperatively funded by U.S. NOAA and IOC/UNESCO has been committed to support the coordination activities of the Project Secretariat, chemical analyses of chlorinated hydrocarbon contaminants at two central laboratories and the field sampling program. This funding has permitted implementation of scaled-down program in the Western Hemisphere for one year.

International Mussel Watch Goals

The primary initial goal of the International Mussel Watch is to ascertain and to assess the levels of chlorinated hydrocarbon pesticides (CHP) and polychlorinated biphenyls in bivalves collected from coastal marine waters throughout the world. The emphasis is on tropical and southern hemispheric locations where the use of these biocides continues and appears to be increasing. Increased use or continued use at present rates of these persistent toxic biocides may

result in contamination of living coastal resources from whole ecosystems to specific food resources with consequent implications for human health and the integrity of marine communities.

Comparison of the measured values with those from the northern hemisphere of the 1960's and the 1970's (at which times morbidities and mortalities related to chlorinated hydrocarbons pollution were observed) will provide an assessment as to whether populations at upper trophic levels, the most susceptible parts of the ecosystem (e.g., mammals and birds), are at risk from these compounds.

Another goal for the International Mussel Watch Program will be to help develop a sustainable activity for observation and monitoring chemical contamination in especially susceptible regions of the world's oceans. Such a global network will provide comparable and reliable data sets for environmental decision makers.

International Mussel Watch Objectives

* To establish on a global scale the levels of contamination of selected organochlorine pesticides and the polychlorinated biphenyls, in the coastal marine environment.

* To compare, where possible, present day levels of organochlorine compounds found in the tropics and the southern hemispheric locations with those found in the northern hemisphere during the 1960's and 1970's, where ecosystems disturbances at the upper trophic levels (fish, birds, cetaceans) were apparent.

* To establish an archive of samples to provide a basis for a time series comparison for both these compounds and as yet unidentified industrial and agricultural contaminants.

* To contribute to the global data base for the evaluation of the present and future state of the health of the oceans. Provide laboratories and regional organizations with baseline data against which to interpret trends in the global environment and to make future environmental management decisions.

Important Products of this Program

* Stimulation of an approach whereby regional specialized networks of laboratories employ the sentinel organism technique for surveillance and monitoring of contamination.

* A global network of sentinel organism data exchange between regional networks, with agreement on associated quality control, sample analysis, data exchange and data analysis procedures.

* A sustainable organization or mechanism capable of obtaining high quality data of priority contaminants on a global basis in the near-shore and coastal zone using tested methods of sampling and analysis, for baseline studies, highly polluted "hot spot" regional monitoring programs and periodic surveillances.

* A data base on the distribution of organochlorine residues in sentinel bivalves on a global scale.

* Publications on the state of the marine environment with respect to these pesticides and industrial chemicals and a critical assessment of those contaminants in reference to findings published in the open scientific literature.

* Evaluations for use by decision-makers in governments.

* Increased national capabilities to assess environmental problems related to organochlorine pesticides and industrial chemicals and other contaminants in the broader context of a global baseline.

* A base for assessment of priorities for future research and monitoring in relation to the information gathered.

Follow-up Actions

In addition to sample analysis and synthesis of acquired data, consideration will be given to: a) monitoring of additional sites in consultation with participating national laboratories, b) negotiating with scientists on the expanded use of the archival material for other pollutant classes, c) strengthening national capabilities to continue the monitoring effort and d) acquisition of national production and use data.

Program Benefits

The successful completion of the program will provide a format for future international activities whose goals are to maintain or improve the quality of the global environment. Although there have been regional programs in marine pollution (noteworthy are those of UNEP in their Regional Seas activities and those of the IOC through GIPME/MARPOLMON) very few (e.g. MARPOLMON) have been as widespread geographically and have been carried out under a non-governmental umbrella.

A major benefit will be the acquisition of an initial set of data for chemical contaminants in sentinel organisms to evaluate the extent and severity of chemical contaminant in coastal areas on a regional and global basis including the Southern Hemisphere. This will be significant step in a programme that could comprise a sustained and expanded regional and global monitoring effort to assess chemical contamination in coastal areas. These data and their interpretation will provide a sound basis for formulation and implementation of policies for protection of human health and for wise management of coastal ecosystems.

We expect that this program will benefit from, and integrate with, existing national and regional efforts. In addition, we expect that the program will provide a basis for additional national and regional activities concerned with pollution of coastal areas.

An added benefit will be dissemination to the world community of the result of a collaborative experience with reference to: sampling, sample storage, chemical analysis, quality assurance procedures; and data interpretation strategies emanating from this program.

The International Mussel Watch Program Plan

The International Mussel Watch Program will ascertain whether the increasing use of chlorinated pesticides and polychlorinated biphenyls, in the developing world and primarily in the tropics and in the southern hemisphere, is resulting in concentrations in coastal marine waters that could jeopardize the health of organisms as happened in the northern hemisphere in the 1960's and 1970's. For the entire sampling period, during the 1990's, bivalves, primarily mussels and oysters, will be collected from the coastal waters of about one hundred countries both in the northern and southern hemispheres. These materials will be analyzed at selected international laboratories as well as in some of the countries where the samples are collected. Sampling during the Initial Implementation Phase will be restricted to approximately 80 sites in the South America, Central America and the Caribbean region (see map, p. 6).

Selection of sampling locations includes contaminated (industrial, urban or agriculture run-off) and non-contaminated (pristine) sites, and covering estuarine and open coastline parts of the sub-littoral zone. One site covers a linear distance of about 200 meters; the identification of sites using these criteria should be made by local scientists familiar with the area in concert with a representative of the International Mussel Watch Committee.

We estimate that about 1000 samples (replicates from 330 sites) will be taken and selected subsamples analyzed for a variety of chlorinated pesticides and chlorinated biphenyls:

Aldrin	Heptachlor
Endrin	Heptachlor epoxide
Dieldrin	Hexachlorobenzene (HCB)
Chlordanes	a-Hexachlorocyclohexane (a- HCH)
	ß-Hexachlorocyclohexane (ß- HCH)
o,p'-DDD	Lindane (γ-HCH)
p,p'-DDD	Trans-nonachlor
o,p'-DDE	Methoxychlor
p,p'-DDE	
o,p'-DDT	
p,p'-DDT	

Mirex and Kelthane may be added to the list in the future, depending on available financial resources for analyses. A final common set of individual chlorobiphenyls (PCB's) will be chosen following the assessment of the results of the first round of intercalibration exercises of IOC/ICES/JMG.

Analytical Centers

Sample analysis during the Initial Implementation Phase will be performed by two contract laboratories. Selection of these analytical facilities for analyses of field-collected samples from the regions will be based on the following criteria:

(i) prior experience in chemical analyses for organochlorine compounds using capillary gas chromatography with confirmatory gas chromatographic mass spectrometric (GC MS) techniques.

(ii) proven capability to produce high quality data for organochlorine analyses in marine tissue samples. This should include glass or fused silica capillary GC and access to glass or fused silica capillary GC MS back up.

(iii) commitment of supervisory scientists in the laboratory for the direction of analysts in the project, quality assurance checks, and assessment of data.

(iv) reputation and acceptability to international-regional groups of scientists, their governments and international bodies.

(v) ability to carry out the program within the designated time period.

Selection of the laboratories has been the responsibility of the International Mussel Watch Committee taking into account recommendations of the Joint IOC-UNEP Group of Experts on Methods, Standards and Intercalibration (GEMSI).

The first two Analytical Centers selected were the IAEA International Laboratory for Marine Radioactivity (ILMR), Monaco, and the Geochemical and Environmental Research Group (GERG), Texas A&M University, College Station, Texas, U.S.A. These laboratories will analyze samples and participate in data interpretation from the Caribbean and Central America-South America region phase of the International Mussel Watch Program.

Program Schedule: Progress to Date

The International Mussel Watch Committee under the Chairmanship of Professor E. Goldberg was established in 1989 along with a small coordinating office at the University of

Maryland, which was overseen by Committee Member R. Dawson. The committee, supported by IOC and UNEP has been instrumental in organizing meetings of experts in order to seek the best scientific advise in designing the master plan, and in designing and testing analytical protocols and sampling and preservation strategies. The committee has also been instrumental in the dissemination of information concerning the programme and in advertising its goals to the wider scientific community as well as investigating avenues for funding and support from governmental and non-governmental sources.

In May, 1991 members of the International Mussel Watch Committee and representatives of three regional monitoring programs met at the University of Costa Rica to finalize the Initial Implementation Phase of International Mussel Watch. At that meeting, sampling sites and participating national scientists were selected. A Project Secretariat has been established at the Coastal Research Center, Woods Hole Oceanographic Institution to coordinate the work and the two central analytical facilities, International Laboratory for Marine Radioactivity (ILMR) in Monaco and Geochemical and Environmental Research Group (GERG) at Texas A&M University, will analyze the collected samples for organochlorine contaminants. Tissue samples and extracts will be archived for later analysis of other contaminants if funding is available. ILMR will also supervise the Field Scientist responsible for sample collection. The International Mussel Watch Project will complement regional monitoring programs where they are established, thus linking the existing programs and increasing their effectiveness. These existing regional programs provide a base on which to build an international program and their support and collaboration is critical to the success of the international program. Discussions in Costa Rica led to a fine-tuning of the International Mussel Watch program outlined above. A total of 80 potential sampling areas were selected and host-country scientists have been invited to collaborate in the program. In the Initial Implementation Phase, samples will be collected throughout the region with the assistance of host-country scientists and relevant national institutions. These scientists will form the nucleus of an international marine monitoring network through which the results of the project will be disseminated. Some of these scientists will also analyze collected tissue samples and will participate in the data interpretation of analytical results with the Project Secretariat and Analytical Centers.

Host-Country scientists and IMW sampling sites will be coordinated by the Woods Hole-based Project Secretariat, working with the Field Scientific Officer. All sampling and sample logistics will be supervised by the Field Scientific Officer and the Host-Country scientists will work directly with him. The field sampling is currently underway. Samples will be analyzed at the two contract laboratories, which will also participate in data review and interpretation. Initial data

interpretation will be carried out by the Project Secretariat and all data will be made available to participating Host-Country scientists. The Project Secretariat and the Field Scientific Officer will provide technical support to Host-Country scientists as resources permit. The International Mussel Watch Committee, in concert with the Project Secretariat, the Field Scientific Officer, and the contract laboratories will provide final data interpretation, taking into account comments from Host-Country scientists. For those scientists with analytical expertise, tissue samples will be available for in-country analysis and inter-laboratory comparison. Host-Country scientists will be asked to assemble production and use data from available sources in their respective countries. The workplan and timetable for the initial implementation phase follows:

International Mussel Watch
Workplan and Timetable
Initial Implementation Phase

ACTIVITY	TIME FRAME	RESPONSIBLE AGENCY
Contract with WHOI and ILMR for support of the work of IMWC. Create Project Secretariat.	Aug.1991	IOC-UNESCO
Selection and appointment of Executive Officer and data manager	Aug. 1991	WHOI, with advice from IMWC
Selection and appointment of Field Scientific Officer	Aug.1991	ILMR, with advice from IMWC
Contract with the two analytical laboratories, ILMR and GERG	Sept. 1991	IOC, and NOAA in consultation with IMWC through Project Secretariat
Establish the network of national institutions participating in the Project	Aug.-Oct. 1991	Project Secretariat jointly with ILMR, in consultation with IOC and IMWC

IMW Workplan and Timetable (cont.)

Purchase of equipment; purchase and distribution of reference standards to host country participants	Sept.1991- May, 1992	Project Secretariat and analytical laboratories
Collection of field samples from Caribbean, Pacific and Atlantic coastal stations and shipment to analytical laboratories	Nov.1991- Sept. 1992	Host country scientists under the direction of the IMW Field Scientist and in consultation with the Project Secretariat
Analysis of organochlorines in field samples including QA/QC by analytical laboratories and participating national laboratories	Mar.1992- Nov. 1992	Analytical labs (ILMR and GERG) under guidance of IMWC through Project Secretariat
Data quality control, analysis of data and preparation of reports evaluating and interpreting results	Nov.1991- Nov. 1992	Project Secretariat with advice of IMWC and in cooperation with analytical laboratories and the Field Scientific Officer
Meeting of IMWC and representatives of IOC and NOAA to review progress and plans for draft final report of phase one of the Project	Feb. 1992	IMWC, with support from Project Secretariat and in cooperation with IOC, NOAA and UNEP
Draft Final report, ready for review by IMWC reviewers and participating regional scientists	Jan. 1993	IMWC and Field Scientific Officer, through Project Secretariat and analytical laboratories and in cooperation with IOC and UNEP
Schedule international meeting of IMWC, GERG, ILMR, and Host-Country scientists to review and discuss IMW data and the draft final report	July-Dec. 1992	Project Secretariat, with Field Scientist in consultation with IOC and analytical labs

IMW Workplan and Timetable (cont.)

Hold international data review meeting in the region	Mar. 1993	Project Secretariat, with local host in the region (e.g., CIMAR or other university) and in collaboration with the Field Scientist, IMWC and the analytical labs
Incorporate comments of reviewers into final report, issue final report	May 1993	Project Secretariat, with IMWC, Field Scientist, analytical labs
Provide technical support to western hemisphere IMW scientific network, support expansion of IMW project to Pacific Rim.	Jan.-Dec. 1993	Project Secretariat

This Initial Implementation Phase will:

-generate high quality data on chlorinated pesticides and estimate PCB concentrations in the Central-South America coastal region

-serve as a "field-test" of a large-scale international marine monitoring program for chemical contaminants

-create an international network of coastal environmental scientists

-provide a forum for training and for discussion of analytical results

-create the institutional structure for a global scale coastal monitoring program

Continuation (and expansion to other global areas) of this project will be considered when the program is assessed at the conclusion of the Initial Implementation Phase. Host-Countries will benefit from the scientific results generated during this initial phase and will have an opportunity to expand local monitoring activities with technical support from the Project. The Project will assist to integrate these activities into regional and global-scale programs.

Selection of Sampling Locations

The appended list of suggested collection sites (Appendix B-1) is a product of suggestions received at one of the IMW organizational meeting of experts (Solomons, MD, USA, July, 1988). These are based, in part, on personal knowledge of the various countries and recommendations of international colleagues. Actual sites may thus vary depending on the recommendations of host-

country scientists with whom we will collaborate in the individual regions. The global coverage (ca. 325 stations), together with other areas covered by current monitoring programs (e.g., O'Connor and Ehler, 1991) would be within the scope of the proposed global effort. This master list was used as a basis for discussion at the organizational meeting for the first implementation phase (San Jose, Costa Rica, May 1991). Sampling sites and indigenous species to be collected were further refined (Appendix B-2) with added local knowledge supplied by participants in regional monitoring programs. Final selection of sample sites and species collected will be determined in-country by the Field Scientist and his in-country hosts.

Preparation of Manuals

Considerable attention has been given to the production of manuals for both chemical analysis for organochlorine pesticides and chlorobiphenyls and biological and ancillary parameter recording and sampling procedures. These manuals are appended in this document and are provided to guide the participating national laboratories in their sampling and analyses. These appendices could be updated at the end of the program to draw on experiences gained and will form the experiential base for updating United Nations manuals. They will be highly appropriate for national laboratories as they develop a monitoring strategy and will supplement reports published by national and international agencies.

Sampling, Preservation and Shipment Strategy

Unlike national mussel watch programs, an international venture poses some unique problems. First of all, contact with scientists for sampling and analyses requires initial approval of sovereign nations. We propose to utilize, where possible, members of the existing frameworks of the United Nations Environment Program and the Intergovernmental Oceanographic Commission. Through a variety of mechanisms, including all of the meetings referred to above, a list of researchers who might be willing to participate in the International Mussel Watch Program has been assembled. To initiate the first phase, the IOC Secretariat contacted the official heads of agencies and international organizations in the region, while the Project Secretariat in Woods Hole was simultaneously contacting research scientists directly (Appendix E). This "double-ended" strategy seems to have worked well to disseminate IMW information. Following a contact by the Project Secretariat to introduce the Program and to explain Program needs, the Host-Country scientists who confirmed their interest in participation were contacted by the Field Scientist. The Field Scientist works closely with the Host-Country scientists on field trip logistics and sampling.

Support by participating national laboratories is essential for an efficient sampling program as it includes technician assistance, the identification of sampling sites and local support (e.g. local travel, housing, etc.) of the Field Scientist.

Also, the sampling tactics in the field must be markedly revised for an international program. Whereas in national programs, samples could be shipped immediately to analyzing laboratories or frozen prior to shipment, this strategy probably cannot be used in all cases in the southern hemisphere and in tropical countries where trans-national shipments must be made. The first implementation phase will attempt to handle frozen samples but this may not be feasible in all locations.

One special technique has been developed for the shipment of unfrozen samples. The methodology developed utilizes a "mussel sand." The sample is made through the treatment of homogenized mussel or oyster tissue with anhydrous sodium sulfate. The removal of water from the organic tissue results in a dry-solid suitable for shipment in a stable form. The validity of this technique has been tested (Dr. J. Farrington, Woods Hole Oceanographic Institution) and we are optimistic that this will be feasible (see Appendix D).

Quality Assurance and Intercalibrations

Dr. J. Farrington, Woods Hole Oceanographic Institution, has agreed to provide oversight of quality assurance procedures and Dr. J. Duinker, University of Kiel, has agreed to act as confirmation laboratory for the program. An inter-laboratory comparison exercise will be conducted by the Project Secretariat for the two analytical laboratories during the Initial Implementation Phase. Both Analytical Centers will also participate in an ongoing U.S. NOAA Status and Trends intercalibration exercise. Intercalibration exercise for Host-Country analysts will be supported by the Project as resources permit.

Establishment of reliable quality control and assurance procedures require the following components:

(i) internal analytical quality control by analyses of Standard Reference Materials, when available;

(ii) periodic intercalibrations between the Analytical Centers, as well as the participating national laboratories;

(iii) double blind comparisons between the Analytical Centers and evaluations by reference laboratories.

(iv) split samples will be analyzed both by the participating national laboratories and by the laboratories responsible for analyses of samples from a particular region (Analytical Centers).

(v) provision for one or two confirmatory laboratories (laboratories with access and routine operation of higher level confirmatory analytical procedures) which would undertake to identify unknown, interfering or suspected co-analyzed components, or samples of unusual composition at high levels of precision and confidence.

Future Proposed Actions

The implementation of the Mussel Watch program involves three successive steps:

(1) the establishment of an Project Secretariat, including an Executive Officer to coordinate field collections and maintain a communications network with identified participating laboratories and their scientists in up to 100 countries where samples will be collected. The Secretariat will also establish a data center and maintain close contact with the Analytical Centers and the participating national labs. The central analytical laboratories, in collaboration with two designated confirmatory laboratories (i.e. Drs. Farrington and Duinker) will intercalibrate, establish the quality control procedures specified in the chemical manual (Appendix D), and coordinate their laboratories for receipt and timely analysis of samples from the field.

(2) the sampling and analyses of samples from ea. 80 stations in Central and South America has begun (1992);

(3) data assessment and publication of the initial results by late 1993. Efforts to collect data on production and use of pesticides in the participating countries will be accomplished as an ancillary task by Host-Country scientists during the Initial Implementation Phase. Each of these steps will be developed in detail as the Project progresses.

Such an international venture will require the assistance of international agencies to establish collaborations with communities of the participating countries. Support from the United Nations Environment Program and from the Intergovernmental Oceanographic Commission of UNESCO has been sought and granted. Clearly, seed money is inadequate to carry out a substantial and definitive program to reach the above stated goal and Member State support, in excess of international seed monies, is required.

Tasks Ahead: Identification of Participating Scientists and Laboratories

Close cooperation between academic scientists and international agencies is needed to identify participants from the regions. A stimulus towards establishing a communicating network could feasibly be provided by a meeting of the scientists on a region-by-region basis capitalizing on planned regional activities and meetings (e.g. IOCARIBE, WESTPAC, CPPS, etc.). This would provide a series of opportunities for all potential participating laboratories to consider the goals, sampling strategies, site selection criteria, intercalibration exercises, and any assemblage of production and use data that can obtained through discussions at such regional meetings. Such a meeting was successfully held in Costa Rica in May 1991 in conjunction with a CEPPOL training workshop. A directory of all participating laboratories and associated scientists will then be assembled and at such meetings, the participating scientists will meet the IMW representatives to set the stage for the field work. These meetings will also develop a list of needs of each country of the region in the way of transportation costs to the collection sites, minor expenses in preparation for the sample collection, and related activities.

This approach may suggest, therefore, that implementation of the global program may be staggered until these contacts have been established in each global region (i.e. implementation is conducted on a sequential region by region basis). Further, during a sampling year, it is intended to send to each country a representative of the International Mussel Watch Program to assist in the sampling such that uniformity in techniques are achieved.

As described above, this regional approach is being used for the first implementation phase. At the organizational meeting in Costa Rica working scientists who were attending a CEPPOL training workshop, together with IMW Committee members and participants from other South American regional monitoring programs (i.e., CASO, South-west Atlantic region and CPPS, eastern Pacific region) discussed the implementation of an international contamination monitoring program. The overall Program structure has been shaped by previous meetings and the discussions in Costa Rica focused on the implementation of a field program within this structure (Fig.1).

INTERNATIONAL MUSSEL WATCH

Initial Implementation Phase Caribbean, Central America and South America

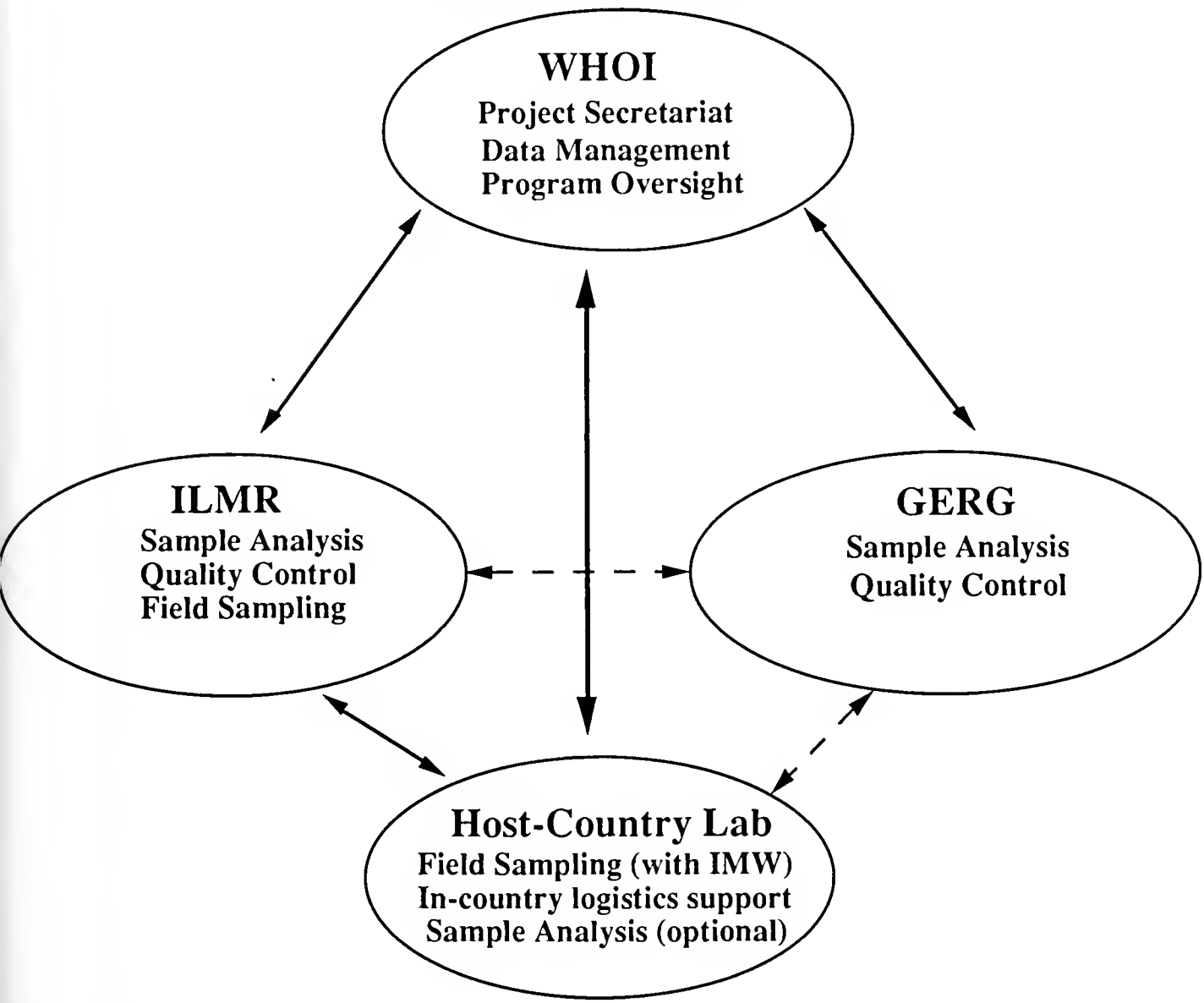

Fig. 1

Using the local knowledge available, participants at the Costa Rica organizational meeting made decisions concerning such issues as:

- location of field sampling sites
- criteria for selecting sample sites
- criteria to select bivalve organisms to sample
- schedule for a field program
- required support by host scientists
- program support of national efforts
- selection of participating national scientists

It has become apparent to all concerned that close collaboration with national scientists and with existing regional monitoring programs will be essential to the success of the international program.

Sampling has been initiated in the South American - Central American - Caribbean region. Following initial contacts as described above, the Field Scientist has begun working with host-country scientists to select sample sites and to sample bivalve organisms from these sites. Frozen tissue samples will be distributed to the Analytical Centers as they are collected. Distribution of samples for analysis and interpretation of analytical results will be coordinated by the Project Secretariat.

Analyses

The Project Secretariat will maintain contact with all laboratories to ensure the analyses are being carried out on a reasonable time scale and will enter the results to the central data bank as they are received. The Project Secretariat will also support analyses by national laboratories as resources permit by supplying technical information and quality assurance materials.

Confirmation laboratories will be called upon to interpret anomalous findings and establish quality assurance controls. Analytical Centers will be required to use the same primary standards and will be sent inter-comparison samples routinely throughout the analytical period in a doubly blind way (i.e., the laboratories will not be able to distinguish inter-comparison samples from field samples).

As only chlorinated hydrocarbons will be analyzed during the Initial Implementation Phase, tissue samples and extracts will be archived for later analysis. We presume that not all collected tissues will be extracted and analyzed within the current budgets.

Sample Bank

Splits of the samples from all of the stations will be maintained in a sample bank for future utilization. Long term specimen banking requires dedicated facilities having experienced

personnel. Therefore, storage will be at an IOC/UNEP Member State facility based on offers of assistance in response to requests from IOC and UNEP requesting such assistance. Funding for the archiving of samples from the initial implementation phase has not yet been secured. Requests for access to the archived samples should be made through the IMW Committee.

Production and Use Data

The gathering of this information will be an on-going effort from the outset of the program and will rely heavily on collaboration with International Agencies and participating member states laboratories and professionals. During the Initial Implementation Phase, this task will be done voluntarily by Host-Country scientists as their individual resources permit.

The Results

The results of all of the analyses will be distributed in draft form to all participating Host-Country scientists, along with the production and use data for the chlorinated hydrocarbon pesticides. We suspect that there will be some discrepancies or some unusual results that will require some further sampling and analyses. This latter activity should be carried out and completed during the first months of the year following the field program in each region. If funding can be secured, an international meeting will be organized by the Project Secretariat to discuss the analytical results from the Initial Implementation Phase.

The Final Product

The final document will be produced by the International Mussel Watch Committee. Herein the results will be assessed and related to potential problems for coastal environmental quality concerns with a local, regional, and global contexts and will include all data and interpretations. A rather rapid publication of the volume is envisaged (i.e. after each region(s) is completed). The volume will be distributed to all of the participating laboratories, relevant government agencies and to the scientists themselves. The results of scientific interpretations will be also published in the peer-reviewed literature after the final report is issued.

Budget Overview

Clearly, in such a complex undertaking as this, all of the impediments to success have not been recognized. Further, the time involved in carrying the project to a successful completion most probably has been underestimated. Yet we must proceed because the problems addressed are

important to protecting human health and the world's coastal ecosystems. Optimism is warranted since national and even a few regional sentinel organism monitoring programs have been successful, providing data and interpretations that have already significantly influenced policy and management related to coastal ecosystems. The following budget is presented in two formats: (1) Full implementation of a global three year exercise and (2), implementation stepwise by sub-components, representing a regional approach, certainly taking longer than 3 years to complete.

The International Mussel Watch Budget

Option 1: A 3-Year Budget For Implementation of a Global Program

Analyses

One thousand samples analyzed for about 15 selected organic pesticides and their residues, some selected chlorobiphenyls and screening for toxaphene at $650/sample with quality control parameters, analyses of primary standards, blanks and inter-laboratory comparisons.

$650,000

Sample Collection

Salaries for 4 teams of two people for one year of sample collection activity in 25 countries/team. Salaries of $35,000/person and 30% fringe benefits

$365,000

Travel at $1000/month for 8 collectors for ten months.

$80,000

Per Diem for 8 collectors at $100/day for 300 days.

$240,000

Shipping of 1000 samples @ $50/sample

$50,000

Collection supplies, including glassware, chemicals, etc. (@ $50/sample for 1000 samples)

$50,000

International Communication with Project Secretariat and in-country expenses

$15,000

$800,000

Management

Operations Manger, $40,000 per year with 30% fringe benefits for three years.

$155,000

Secretary, $20,000/year with 30% fringe benefits for three years, and Data Manager (half time) at $30,000/yr. with 30% fringe benefits for three years

$135,000

Administrative Costs -- telephones, telex, xerox, mail (@ $15,000 a year for three years)

$45,000

Travel, in support of Project management

$20,000

$355,000

Scientific Direction

Consultants and scientists, all part time. (1.0 man year @ $60,000/year for 3 years with 25% fringe benefits)

$250,000

Regional meetings associated with other international meetings. Additional costs of Mussel Watch representatives and participant. Estimated costs for four to six meetings

$100,000

Three annual meetings of the International Mussel Watch Committee (@ $25,000/meeting)

$75,000

$425,000

Overhead (@ 7%) **$150,000**

Estimated Total Global Program ***$1,730,000***

Option 2:
Sequential Regional Implementation

*Regional Budget: Central America, Caribbean, South America**
(IMW Initial Implementation Phase)

Projected Sample Sites: 80 in 25 Countries

1. Programme Management and Coordination	Per Year
Coordinating Unit	
Executive Officer (1/3 time), Data Base assistant (1/2 time), Secretary (1/3 time) Total Salary & Benefits	US $49,000
Administrative Costs	35,000
Scientific Director	
Consultants and Scientists	$20,000
Annual Meeting IMW Committee	15,000
Supplements to Regional Meetings	
Travel and Per Diem South Atlantic	$15,000
IOCARIBE	10,000
CPPS	10,000
Travel for Program Coordination	
Domestic U.S.	3,000
International	10,000
Equipment	
Personal Computer & Peripherals	6,000
Other Direct Costs	
e.g. Expendable Lab Supplies (National Lab Support) Computer Supplies, Software, Photo Copying, E-Mail, Graphic Arts Services, Shipping (reference materials, etc.), Printing (reports)	17,000
Total Programme Management and Coordination Costs	**$190,000**

* *NOTE:* This estimated budget is not fully funded at the initiation of the field sampling program.

2. Sample Collection (80 Sample Sites)

Field Scientific Officer Salary & Benefits (1/2 time)	40,000
Travel - 25 countries	20,000
Per Diem 300 days at $100 per day	60,000
Sample Shipping $50 per sample	8,000
Collection Supplies $50 per sample (x 80)	4,000
Local (in-country) Sampling Expenses	5,000
Communication	3,000
Support for Base Site	10,000
Total Collection	**$150,000**

3. Sample Analysis

80 Samples (@$650 per sample) plus replicates and QA/QC analyses	$150,000

Estimated Total Regional Program Costs ***US $490,000***
(estimated from Initial Implementation Phase)

References

ATLAS, E. and GIAM, C.S. 1988. Ambient Concentration and Precipitation Scavenging of Atmospheric Organic Pollutants. Water, Air and Soil Pollution. 38:19-36.

ATLAS, E. and GIAM, C.S. 1989. Sea-Air Exchange of High Molecular Weight Synthetic Organic Compounds: Results from the SEAREX Program. In: Chemical Oceanography, SEAREX: The Sea/Air Exchange Program. J.P. Riley, R. Chester and R.A. Duce (eds.) Academic Press Limited, 10:339-378.

BUTLER, G.C. 1976. (ed.) Principles of Ecotoxicology. SCOPE 13, New York, John Wiley and Sons. 350 pp.

CPPS. 1981. Fuentes, Niveles y Efectos de la Contamination Marina en El Pacific Sudeste. CPPS, Serie Seminarios y Estudios, No. 2 (1981).

FARRINGTON, J.W., GOLDBERG, E.D., RISEBROUGH, R.W., MARTIN, J.H. AND BOWEN, V.T. 1983. U.S. "Musselwatch" 1976-1978: An overview of the trace metal, DDE, PCB, Hydrocarbon and artificial radionuclide data. Environ. Sci. Technol. 17, 490-496.

GOLDBERG, E.D. 1976. The Health of the Oceans. UNEC Press, Paris. 172 pp.

ICES. 1988. Results of the 1985 Baseline Study of Contaminants in Fish and Shellfish. Cooperative Research Report No. 151. ICES Copenhagen, Denmark.

IFREMER. 1983. Reseau national d' observation de la qualite du milieu marin. Institut Francais de Recherche pour l Exploration de la Mer. Departement Milieu et Resources, B.P. 1049, 44037 Nantes Cedex, France.

NRC. 1980. The International Mussel Watch, Report of a Workshop. National Research Council, Publications Office, National Academies Press, National Academy of Science, Washington, D.C.

O'CONNOR, T.P. AND EHLER C.N. 1991. Results from the NOAA National Status and Trends Program on Distribution and Effects of Chemical Contamination in the Coastal and Estuarine United States. Environmental Monitoring and Assessment 17:33-49.

PETERSON, S. AND TRIPP B. 1984. Mussel Watch II: chemical changes in the coastal zone. Marine Policy.

PHILLIPS, D.J.H. 1980. Quantitative Aquatic Biological Indicators. Applied Science Publishers, Ltd. London, U.K.

POSTEL, S. 1987. Defusing a mood of uncertainty. Worldwatch Paper 79. Worldwatch Institute, Washington, D.C. 69 pp.

RICHARDSON, B.J. and WAID, J.S. 1982. Polychlorinated biphenyls (PCB'S): An Australian viewpoint on global pollution. Search 13, 17-25.

SHEEHAN, P., MILLER, N., BUTLER, G.C., BORDEAUX, P. (eds.) 1984. Effects of Pollutants at the Ecosystems Level. SCOPE 23. John Wiley and Sons, New York.

SIVALINGAM, P.M. 1984. Chemical Changes in the Coastal Zone. Mar. Pollut. Bull. 15(3):86.

SLORACH, S.A. and VAZ, R. 1983. The Assessment of Human Exposure to Selected Oroganochlorine Compounds thru Biological Monitoring, prepared by UNEP and WHO by the Swedish National Food Administration, Upsala Sweden.

TANABE, S., TATSUKAWA, R., KAWANO, M. and HIDAKA, H. 1982. Global distribution and atmospheric transport of chlorinated hydrocarbons: HCH (BHC) isomers and DDT compounds in the Western Pacific, Eastern Indian and Antarctic Oceans. J. Ocean. Soc. Japan 38, 137-148.

TOPPING, G. 1983. Guidelines for the Use of Biological Material in First Order Pollution Assessment and Trend Monitoring. Department of Agriculture and Fisheries for Scotland. Scottish Fisheries Report No. 28, 1983 The Director Marine Laboratory, MAFF, Aberdeen, Scotland.

US-NOAA. 1987. A Summary of Selected Data on Chemical Contaminants in Tissues Collected During 1984, 1985 and 1986. NOAA Technical Memorandum NOSOMA 38, Rockville, Maryland, USA.

International Mussel Watch Workshop
Barcelona, Spain
4-7 December 1978

Convened by: U.S. National Research Council

Chair: Edward D. Goldberg
Scripps Institution of Oceanography
La Jolla, CA, USA

The workshop had five major goals:

1. to assess the use of bivalves in determining environmental concentrations of chemical pollutants and pathogens;

2. to evaluate current data with respect to distinguishing natural from pollutant concentrations for heavy metals and hydrocarbons and determining changes in the amount of pollution as a function of time;

3. to formulate effective biological monitoring strategies to complement the measurement of pollutant levels;

4. to appraise existing techniques for analysis and to comparisons and standards for all collectives of pollutants; and

5. to consider the expansion of the U.S. Mussel Watch program to a worldwide basis as a continuous monitor of the health of the coastal waters.

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Mussel Watch II: Chemical Changes in the Coastal Zone
Honolulu, Hawaii
7-11 November 1983

Convened by: Scientific Committee on Problems of the Environment (SCOPE)
International Federation of Institutes for Advance Study (IFIAS)
East-West Center Environment and Policy Institute
Woods Hole Oceanographic Institution, Coastal Research Center

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The Mussel Watch II workshop goals included:

1. assess the concepts and methodologies used to detect changes in chemical contaminant concentrations in the coastal environment;

2. promote discussion concerning the significance of these changes;

3. review the monitoring data produced by national biogeochemical cycles and related processes;

4. consider the implementation of the "mussel watch" concept in coastal monitoring programs in the Southern hemisphere;

5. strengthen the process of international communication and collaboration already began.

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*Appendix B-1:**

Suggested Collection Sites for the International Mussel Watch

m = mussels o = oysters c=clams

Canada

1)	Cape Bathurst	(Beaufort Sea)	N. W. Territory	(m)
2)	Mackenzie Bay	(Beaufort Sea)	N. W. Territory	(m)
3)	Nelson River	(Hudson Bay)	Manitoba	(m)
4)	Port Harrison	(Hudson Bay)	Quebec	(m)
5)	Gaspe Peninsula	(Gulf of St. Lawrence)	New Brunswick	(m)
6)	Port Burwell	(Labrador Sea)	Newfoundland	(m)
7)	St. Johns	(Atlantic Ocean)	Newfoundland	(m)
8)	Vancouver Island	(Pacific Ocean)	British Columbia	(m)
9)	Str. of Juan de Fuca	(Pacific exit of Puget Sound)	British Columbia	(m)
10)	Prince Edward Island	(Atlantic Ocean)	Nova Scotia	(m)
11)	St. Andrews	(Bay of Fundy)	New Brunswick	(m)

USA (not covered by US Mussel Watch)

11)	Near Islands	(Aleutians, Bering Sea)	Alaska	(m)
12)	Pribilov Islands	(Aleutians, Bering Sea)	Alaska	(m)
13)	St. Lawrence Island	(Aleutians, Bering Sea)	Alaska	(m)

Mexico

14)	Bahia San Quintin	(Pacific Ocean)	Baja Califomia	(m)
15)	Bahia Madalena	(Pacific Ocean)	Baja Californa	(m)
16)	San Felipe	(Gulf of Californa)	Sonora	(o)
17)	Topolobampo	(Gulf of Californa)	Sinaloa	(o)
18)	Mazatlan	(Pacific Ocean)	Sinaloa	(o)

* Appendix B-1 is a list of collection sites as printed in the original (1987) version of the International Mussel Watch Manual. This list was generated as a result of discussions at several international conferences with the intention of identifying coastal sampling sites. Every coastal country is not necessarily included. Countries and territories highlighted in **bold type** are being sampled during the initial implementation phase (1991-92). These countries are listed separately, with more detail in appendix B-2 (arranged alphabetically, by country).

19)	Punta Mangrove	(Balsas River)	Mexcala (Michoacan)	(m)
20)	Punta Maldonado	(Rio Grande River)	Guerrero-Oaxaca	(m)
21)	Puerto Madero	(Pacific Ocean)	Chiapas	(m)
22)	Laguna Madre	(Gulf of Mexico)	Tamaulipas	(o)
23)	Laguna de Tamiahua	(Gulf of Mexico)	Veracruz	(o)
24)	Ciudad del Carmen	(Gulf of Mexico)	Campeche	(o)
25)	Cancun	(Caribbean Sea)	Quintana Roo	(o)
Guatemala				
26)	Puerto Barrios	(Caribbean Sea)		(o)
Belize				
27)	Belize City	(Caribbean Sea)		(o)
Honduras				
El Salvador				
Nicaragua				
28)	Golfo de Fonseca	(Pacific Ocean)		(m)
Nicaragua				
29)	Bluefields	(Caribbean Sea)		(o)
Costa Rica				
30)	Golfo de Nicoya	(Pacific Ocean)	Puntarenas	(m)
31)	Limon	(Caribbean Sea)		(o)
Panama				
32)	Portobelo	(Caribbean Sea)		(o)
33)	Puenta Chame	(Golfo de Panama)		(m)
Colombia				
34)	Golfo de Uraba	(Caribbean Sea)		(o)
35)	Nuqui	(Pacific Ocean)		(m)
36)	Tumaco	(Pacific Ocean)		(m)

Ecuador

37)	Guayaquil	(Pacific Ocean)	(m)
38)	Esmeraldas	(Pacific Ocean)	(m)
39)	Galapagos Islands	(Pacific Ocean)	(m)

Peru

40)	Puerto de Eten	(Pacific Ocean)	(m)
41)	Pisco	(Pacific Ocean)	(m)

Cuba

42)	Santa Cruz del Sur	(Caribbean Sea)	(o)

Turks and Caicos

43)	Grand Caicos	(Caribbean Sea)	(o)

Jamaica

44)	Morant Bay	(Caribbean Sea)	(o)

Aruba

45)	Aruba	(Caribbean Sea)	(o)

Puerto Rico (sampled by US Mussel Watch)

46)	Mayaguez	(Caribbean Sea)	(o)
47)	San Juan	(Caribbean Sea)	(o)

Martinique

48)	Martinique	(Caribbean Sea)	(o)

Trinidad and Tobago

49)	Tobago	(Caribbean Sea)	(o)

Venezuela

50)	Maracaibo	(Caribbean Sea)	(m/o)
51)	Isla de Margarita	(Caribbean Sea)	(m/o)

52)	Curiapo	(Orinoco River)	(m/o)

Chile

53)	Arica	(Pacific Ocean)	(m)
54)	Antofagasta	(Pacific Ocean)	(m)
55)	La Serena	(Pacific Ocean)	(m)
56)	Valdivia	(Pacific Ocean)	(m)
57)	Strait of Magellan	(Pacific Ocean)	(m)
58)	Punta Arenas	(Pacific Ocean)	(m)
59)	Easter Island	(Pacific Ocean)	(m)

Argentina

60)	Rio Gallegos	(Atlantic Ocean)	(m)
61)	Puerto Deseado	(Atlantic Ocean)	(m)
62)	Rawson	(Atlantic Ocean)	(m)
63)	Bahia Blanca	(Atlantic Ocean)	(m)
64)	Mar del Plata	(Atlantic Ocean)	(m)
65)	La Plata	(River Plata, Atlantic Ocean)	(m)

Falkland Islands (Islas Malvinas)

66)	Eagle Passage	(Atlantic Ocean)	(m)

South Georgia Island

67)	Grytviken	(Atlantic Ocean)	(m)

Uruguay

68)	Punta del Este	(Parana River, Atlantic Ocean)	(m)

Brazil

69)	Lagoa dos Patos	(Atlantic Ocean)	(m)
70)	Florianopolis	(Atlantic Ocean)	(m)
71)	Paranagua	(Atlantic Ocean)	(o)
72)	Vitoria	(Atlantic Ocean)	(o)
73)	Salvador	(Atlantic Ocean)	(o)

74)	Aracaju	(Rio Sao Francisco)	(o)
75)	Recife	(Atlantic Ocean)	(o)
76)	Fortaleza	(Atlantic Ocean)	(o)
77)	Belem	(Atlantic Ocean)	(o)
78)	Isla Caviana	(Amazon River)	(o)

Surinam
French Guiana

79)	St. Laurent	(Marowijne River)	(o)
80)	Nieuw Nickerie	(Atlantic Ocean)	(o)

Guyana

81)	Georgetown	(Atlantic Ocean)	(o)

Bahamas

82)	Andros Island	(Atlantic Ocean)	(o)

Bermuda

83)	St. Georges Island	(mid N. Atlantic Ocean)	(c)

Azores

84)	Sao Mateus	(mid W. Atlantic Ocean)	(m)

Cape Verde Islands

85)	Sao Vicente	(mid W. Atlantic Ocean)	(m)

Ascension Islands

86)	Georgetown	(mid S. Atlantic Ocean)	(m)

Morocco

87)	Essaouira	(Atlantic Ocean)	(m)
88)	Rabat	(Atlantic Ocean)	(m)
89)	Tetouan	(Mediterranean Sea)	(m)

Mauritania

90) Nouakchott	(Atlantic Ocean)	(o)

Senegal

91) Dakar	(Atlantic Ocean)	(o)

Gambia

92) Bathurst	(Gambia River, Atlantic Ocean)	(o)

Guinea-Bissau

93) Bissau	(Atlantic Ocean)	(o)

Sierra Leone

94) Freetown	(Atlantic Ocean)	(o)

Liberia

95) Monrovia	(Atlantic Ocean)	(o)
96) Harper	(Atlantic Ocean)	(o)

Ivory Coast

97) Grand Bassam	(Atlantic Ocean)	(o)

Ghana

98) Axim	(Atlantic Ocean)	(o)
99) Keta	(Atlantic Ocean)	(o)

Benin

100) Cotonou	(Atlantic Ocean)	(o)

Nigeria

101) Mahin	(Atlantic Ocean)	(o)
102) Niger Delta	(Atlantic Ocean)	(o)

Cameroon

103)	Victoria	(Atlantic Ocean)	(o)

Gabon

104)	Port Gentil	(Atlantic Ocean)	(o)

Congo

105)	Pointe-Noire	(Atlantic Ocean)	(o)

Zaire

106)	Moanda	(Congo River, Atlantic Ocean)	(o)

Angola

107)	Luanda	(Atlantic Ocean)	(o)

Namibia

108)	Walvis Bay	(Atlantic Ocean)	(m)

South Africa

109)	Cape Columbine	(Atlantic Ocean)	(m)
110)	Port Elizabeth	(Indian Ocean)	(m)
111)	Durbin	(Indian Ocean)	(m)

Mozambique

112)	Villa de Loao Belo	(Limpopo River)	(o)
113)	Chinde	(Zambezi River)	(o)

Madagascar

114)	Androka	(Indian Ocean)	(o)
115)	Besalampy	(Mozambique Channel, Indian Ocean)	(o)
116)	Diego Suarez	(Indian Ocean)	(o)
117)	Tamatave	(Indian Ocean)	(o)

Reunion Island

118) Etang Sale	(Indian Ocean)	(o)

Mauritius

119) Mahebourg	(Indian Ocean)	(o)

Seychelles

120) Anse Boileau	(Indian Ocean)	(o)

Tanzania

121) Mtwara	(Indian Ocean)	(o)
122) Mafia Island	(Indian Ocean)	(o)
123) Zanzibar	(Indian Ocean)	(o)
124) Tanga	(Indian Ocean)	(o)

Kenya

125) Shimoni	(Indian Ocean)	(o)
126) Malindi	(Sabaki River, Indian Ocean)	(o)
127) Kapini	(Tana River, Indian Ocean)	(o)

Somalia

128) Kismayu	(Indian Ocean)	(o)
129) Mogadishu	(Indian Ocean)	(o)
130) Bangal	(Indian Ocean)	(o)
131) Berbera	(Gulf of Aden)	(o)

Djibouti

132) Tadjoura	(Gulf of Aden)	(o)

Ethiopia

133) Dahlak Archipelago	(Red Sea)	(o)

Sudan

134)	Port Sudan	(Red Sea)	(o)

Egypt

135)	Marsa al 'Alam	(Red Sea)	(o)
136)	Ra's Muhammad	(Red Sea)	(o)
137)	Alexandria	(Mediterranean Sea)	(m)

Libya

138)	Benghazi	(Mediterranean Sea)	(m)
139)	Tripoli	(Mediterranean Sea)	(m)

Tunisia

140)	Sfax	(Mediterranean Sea)	(m)
141)	Bizerte	(Mediterranean Sea)	(m)

Algeria

142)	Annaba	(Mediterranean Sea)	(m)
143)	Mostaganem	(Mediterranean Sea)	(m)

Malta

144)	Valetta	(Mediterranean Sea)	(m)

Sicily

145)	Messina	(Mediterranean Sea)	(m)

Crete

146)	Iraklion	(Mediterranean Sea)	(m)

Turkey

147)	Mersin	(Mediterranean Sea)	(m)
148)	Izmir	(Mediterranean Sea)	(m)
149)	Istanbul	(Black Sea)	(m)
150)	Samsun	(Black Sea)	(m)

Greece

151)	Crete, south side	(Mediterranean Sea)	(m)
152)	Korfu	(Aegean Sea)	(m)
153)	Thessaloniki	(Aegean Sea)	(m)

Romania

154)	Sulina	(Danube Estuary, Black Sea)	(m)

Slovenia
Croatia

155)	Dubrovnik	(Adriatic Sea)	(m)
156)	Leniski Canal	(Adriatic Sea)	(m)
157)	Sibenik	(Adriatic Sea)	(m)

Italy

158)	Portogruaro	(Gulf of Trieste)	(m)
159)	Isola di Lipari	(Tyrrhenian Sea)	(m)
160)	Napoli	(Tyrrhenian Sea)	(m)
161)	Savona	(Gulf of Genoa)	(m)

Sardinia

162)	La Maddalena	(Tyrrhenian Sea)	(m)

Spain

163)	Tarragona	(Mediterranean Sea)	(m)
164)	Cartagena	(Mediterranean Sea)	(m)
165)	San Fernando	(Atlantic Ocean)	(m)

Saudi Arabia

166)	Jeddah	(Red Sea)	(o)

Yemen

167)	Birk	(Red Sea)	(o)

168)	Aden	(Gulf of Aden)	(o)
Oman			
169)	Mushsail	(Arabian Sea)	(o)
170)	Masira Island	(Arabian Sea)	(o)
171)	Lima	(Arabian Sea)	(o)
172)	Bukha	(Arabian Gulf)	(o)
Bahrain			
173)	Bahrain	(Persian Gulf)	(o)
Kuwait			
174)	Bubiyan	(Persian Gulf)	(o)
Pakistan			
175)	Gwadar	(Arabian Sea)	(o)
176)	Ormara	(Arabian Sea)	(o)
177)	Indus Delta	(Arabian Sea)	(o)
India			
178)	Jamnagar	(Gulf of Kutch)	(o)
179)	Bhavnagar	(Gulf of Cambay)	(o)
180)	Bankot	(Arabian Sea)	(o)
181)	Karwar	(Arabian Sea)	o)
182)	Cochin	(Indian Ocean)	m/o)
183)	Cape Comorin	(Indian Ocean)	(m/o)
184)	Kilakarai	(Indian Ocean)	(m/o)
185)	Karikal	(Indian Ocean)	(m/o)
186)	Masulipatnam	(Indian Ocean)	(m/o)
187)	Vishakhapatnam	(Indian Ocean)	(m/o)
188)	Paradip	(Indian Ocean)	(m/o)
189)	Hooghly River	(Indian Ocean)	(m/o)

Bangladesh

190)	Ganges River mouth	(Indian Ocean)	(o)

Sri Lanka

191)	Dondra Head	(Indian Ocean)	(o)
192)	Negombo	(Indian Ocean)	(o)
193)	Batticaloa	(Bay of Bengal)	(m/o)

Laccadive Islands (India)

194)	Kavarati	(Arabian Sea)	(o)

Maldives

195)	Male	(Indian Ocean)	(o)

Andaman Islands (India)

196)	Port Blair	(Andaman Sea)	(m/o)

Nicobar Islands (India)

197)	Parsons Point	(Andaman Sea)	(m/o)

Kerguelen Island

198)	Kerguelen Island	(South Indian Ocean)	(m)

Myarmar (Burma)

199)	Irrawaddy Estuary	(Bay of Bengal)	(o)
200)	Palaw	(Andaman Sea)	(o)

Thailand

201)	Phuket	(Andaman Sea)	(o)
202)	Narathiwat	(Gulf of Thailand)	(m)
203)	Songkla	(Gulf of Thailand)	(m/o)
204)	Pak Phanang	(Gulf of Thailand)	(m/o)
205)	Surat Thani	(Gulf of Thailand)	(m/o)

206)	Chonburi	(Gulf of Thailand)	(m)
207)	Chantaburi	(Gulf of Thailand)	(m)

Indonesia

Sumatra

210)	Medan	(Malacca Strait)	(o)
211)	Tambilahan	(Malacca Strait)	(o)
212)	Bengkulu	(Indian Ocean)	(m/o)
213)	Kepulauan Batu	(Indian Ocean)	(m/o)

Java

214)	Palabuhan Ratu	(Indian Ocean)	(o)
215)	Nusa Kambangan	(Indian Ocean)	(o)
216)	Surabaja	(Java Sea)	(o)
217)	Jepara	(Java Sea)	(o)

Bali

218)	Benoa	(Indian Ocean)	(o)

Lesser Sunda Islands

219)	Waingapu	(Savu Sea)	(o)

Timor

220)	Baun	(Timor Sea)	(o)

Christmas Island

221)	Christmas Island	(Indian Ocean)	(o)

Malaysia

208)	Penang, west side	(Malacca Strait)	(m/o)
209)	Kuantan	(South China Sea)	(o)
222)	Tawau, Sabah	(Celebes Sea)	(o)
223)	Kota Kinabalu, Sabah	(Celebes Sea)	(o)

224)	Miri, Sarawak	(Celebes Sea)	(o)
225)	Kuching, Sarawak	(Celebes Sea)	(o)
226)	Pontianak, Kalimantan	(Celebes Sea)	(o)
227)	Tanjung Keluang	(Java Sea)	(o)
228)	Tanjung Selatan	(Java Sea)	(o)
229)	Tanjung Bajor	(Makasar Strait)	(o)
230)	Tarakan	(Celebes Sea)	(o)

Sulawesi

231)	Ujung Padang	(Flores Sea)	(o)
232)	Manado	(Celebes Sea)	(o)

Kampuchea

233)	Ream	(Gulf of Thailand)	(m/o)

Vietnam

234)	Ba Dong, Mekong Estuary	(South China Sea)	(o)
235)	Danang	(South China Sea)	(o)
236)	Haiphong	(Gulf of Tonkin)	(o)

China (Peoples Republic)

237)	Wan-ning, Hainan I.	(South China Sea)	(o)
238)	Chin-Chou	(Gulf of Tonkin)	(o)
239)	Macao Port	(Sui Hsi River)	(m)
240)	Xiamen	(Formosa Strait)	(m)
241)	Fu-Chou	(Formosa Strait)	(m)
242)	Hang-Chou	(East China Sea)	(m)
243)	Nan-t'ung	(Yellow Sea)	(m)
244)	Tsingtao	(Yellow Sea)	(m)
245)	Tietsin	(Gulf of Chihli)	(m)
246)	Ying-k'ou	(Gulf of Chihli)	(m)

Commonwealth of Independent States

247)	Batumi	(Black Sea)	(m)
248)	Odessa	(Black Sea)	(m)
249)	Ayan	(Sea of Okhotsk)	(m)
250)	Magadan	(Sea of Okhotsk)	(m)
251)	Petropavlovsk-Kamchatskiy	(Bering Sea)	(m)
252)	Olyutorskiy	(Bering Sea)	(m)
253)	Anadyr	(Bering Sea)	(m)
254)	Provideniya	(Bering Strait)	(m)

Peoples Democratic Republic of Korea

255)	Yongamp'o-ri	(Korea Bay)	(m)
256)	Ch'ongjin	(Sea of Japan)	(m)
257)	Wonsan	(Sea of Japan)	(m)

China (Taiwan)

258)	Chi-lung	(East China Sea)	(m)
259)	Hsi-lo	(Formosa Strait)	(m)
260)	Kao-hsiung	(South China Sea)	(m)

South Korea

261)	Pusan	(Sea of Japan)	(m)
262)	Mokpo	(Yellow Sea)	(m)
263)	Inch-on	(Yellow Sea)	(m)

Philippines

264)	Aparri	(Luzon Strait)	(m/o)
265)	Lingayen	(Lingayen Gulf, South China Sea)	(m/o)
266)	Batangas	(Batangas Bay)	(m/o)
267)	Balabac I., Palawan	(Balabac Strait)	(m/o)
268)	Batan, Capiz	(Batan Bay)	(m/o)
269)	Dapitan	(Mindanao Sea)	(m/o)
270)	Babak	(Davao Gulf)	(m/o)

271)	Oras, Samar	(Philippine Sea)	(m/o)
272)	Legaspi	(Albay Gulf)	(m/o)

Papua New Guinea

273)	Weam	(Coral Sea)	(o)
274)	Gaima	(Fly River Estuary)	(o)
275)	Port Moresby	(Coral Sea)	(o)
276)	Alotau	(Coral Sea)	(o)
277)	Sepik River mouth	(Bismarck Sea)	(o)
278)	Brussels Univ.	(North Bismarck Sea)	(o)

SOUTH PACIFIC OCEAN

Guam

279)	Pago Bay	(North Pacific Ocean)	(o)

Yap

280)	Yap	(North Pacific Ocean)	(o)

Palau

281)	Koror	(North Pacific Ocean)	(o)

Truk

282)	Lemotol Bay	(North Pacific Ocean)	(o)

Wake Island

283)	Peale	(North Pacific Ocean)	(o)

Marshall Islands

284)	Bikini	(North Pacific Ocean)	(o)

Nauru

285)	Boe	(Pacific Ocean)	(o)

Solomon Islands

286)	Choiseul	(Pacific Ocean)	(o)

Kiribati

287)	Tarawa	(Pacific Ocean)	(o)
288)	Phoenix	(Pacific Ocean)	(o)

Vanuatu

289)	Malo	(Pacific Ocean)	(o)

New Caledonia

290)	Noumea	(Pacific Ocean)	(o)

Fiji

291)	Suva	(Pacific Ocean)	(o)

Tonga

292)	Nukualofa	(Pacific Ocean)	(o)

American Samoa

293)	Pago Pago	(Pacific Ocean)	(o)

Cook Islands

294)	Muri, Rarotonga	(Pacific Ocean)	(o)

Kiritimati

295)	Tuba Island, Christmas Atoll	(Pacific Ocean)	(o)

French Polynesia

296)	Hiva Oa (Marquesas Islands)	(Pacific Ocean)	(o)
297)	Isthme de Taravao (Tahiti)	(Pacific Ocean)	(o)

Pitcairn Island

298)	Adamstown	(Pacific Ocean)	(o)

Australia

299)	Darwin	(Timor Sea)	(o)
300)	Cambridge Gulf	(Indian Ocean)	(o)
301)	King Sound	(Indian Ocean)	(o)
302)	Dampier Archipelago	(Indian Ocean)	(o)
303)	Denham Sound	(Indian Ocean)	(m/o)
304)	Fremantle	(Indian Ocean)	(m)
305)	Cape Leeuwin	(Indian Ocean)	(m)
306)	Pt. D'Entrecasteaux	(Indian Ocean)	(m)
307)	Esperance	(Great Australian Bight)	(m)
308)	Gulf of St. Vincent	(Great Australian Bight)	(m)
309)	Coorong, Murray R.	(Great Australian Bight)	(m)
310)	Westernport Bay, Phillip I.	(Bass Strait)	(m)
311)	Davenport, Tasmania	(Bass Strait)	(m)
312)	South Bruny	(Tasman Sea)	(o)
313)	Manly	(Tasman Sea)	(o)
314)	Kempsey	(Tasman Sea)	(o)
315)	Brisbane	(Tasman Sea)	(o)
316)	Gladstone	(Coral Sea)	(o)
317)	Bowen	(Coral Sea)	(o)
318)	Cairns	(Coral Sea)	(o)
319)	S. Wellesly Island	(Gulf of Carpentaria)	(o)

New Zealand

320)	New Plymouth	(Tasman Sea)	(m)
321)	Milford Sound	(Tasman Sea)	(m)
322)	Bluff	(Foveaux Strait)	(o)
323)	Banks Peninsula	(South Pacific Ocean)	(m)
324)	Marlborough Sounds	(Cook Strait)	(m)
325)	Hawke Bay	(South Pacific Ocean)	(m)

*Appendix B-2:**

Suggested Collection Sites for the International Mussel Watch Initial Implementation Phase

Argentina	*Ocean/Bay*	*Notes*	*Land Use*
Arroyo Parejas	Atlantic	(Odd sample)	
Bahia Blanca	Atlantic	IADO lab facilities, good local data base; no data south of here	Wheat, Navy base
Mar del Plata	Atlantic	INIDEP lab facilities, off shore stations. Local red tide research project.	Fisheries, potatoes, Navy base, Subtidal mussel pop., open ocean site
Pehuen-co	Atlantic	Different spp. small pop. and small org.	
Punta Loyola	Atlantic	A control site, strong tidal cycle	Shrimp, sheep, petroleum production
Rio Gallegos	Atlantic	A control site, strong tidal cycle	Shrimp, sheep, petroleum production
Rio Negro	Atlantic		Fruit Production
Puerto Deseado	Atlantic/G. San Jorge	No scientific contact, difficult access	Petroleum, fishing
Rawson	Atlantic/R. Chubut	River sample	Fruit production
Atalaya	Atlantic/R. Plata		Navy base, industries
Hudson	Atlantic/R. Plata		Closest to Buenos Aires (land fill) urban
Punta Piedras	Atlantic/R. Plata		

* Appendix B-2 is a list of proposed sampling sites which originates with Appendix B-1 but was refined at the organizational meeting for the IMW Initial Implementation Phase held in Costa Rica in May 1991. This list will be further refined during the sampling period, Nov. 1991-Sept. 1992, based on the input of participating Host-Country Scientists.

Argentina continued			
R. de la Plata	Atlantic/R. Plata	A very important site, SE of Buenos Aires. SHN lab facilities. Good estuarine mixing regime, strong tides affect sampling.	Fruit production, paper pulp, urban.
Ushuaia	Patagonia/Atlantic	Area similar to P. Deseado, originally identified for sampling.	Navy base
Bahia Camarones	Puerto Madryn/Atlantic		Isolated, no agriculture, good control, rocky shoreline
Aruba			
Commander's Bay	Caribbean	Same spp. as B. del Toro	Oil refinery
Bahamas			
Andros Island	Atlantic	•	
Belize			
Belize City	Caribbean Sea	Sample from Mexico	
Brazil			
Cabo Frio	Atlantic	Control site	
Fortaleza	Atlantic		Industrial-urban-port.
Guanbura Bay	Atlantic	Remote site, tourism	Petroleum. Cities of Rio, Niteroi
Vitoria	Atlantic		Shipping, iron ore
Lagoa dos Patos	Atlantic / R. G. do Sul.	Much data available	Industrial, agriculture
Bragança	Atlantic/R. Amazonas		100 Km north of Belem
Santos	Atlantic/Sao Paulo	Chemical data available, 3 stations along ship channel	Industrial-urban.

Brazil continued			
Salvador	B. de Todos os Santes	Chemical data available, Multiple habitats, 3 spp.	Urban, cane, paper, tourism.
São Luis	L. da Jensen		
Maceió	L. Mundaio		Mussel aquaculture. Domestic effluents.
Paranagua	Laranjeiras Bay	Control site	Mangrove, shrimp, fish. Control.
Recife	Pina Bay		Cane, heavily urbanized, multiple river discharge
Aracaju	R. Sao Francisco		
Chile			
Arauco Gulf	Bio-Bio R.		Industry-agriculture
Antofagasta	Pacific		Desert, copper, control.
Arica	Pacific		Industry, mines, domestic effluent
Concepcion	Pacific		Domestic-industrial-paper.
La Serena	Pacific		Control
Meijeillones	Pacific	Control site	
Punta Arenas	Pacific	Control site	Domestic effuent
Valpariso	Pacific		Urban-industrial
Puerto Montt	Pacific		Domestic effluent, paper, fisheries
Columbia			
Bahia de Cartagena	Caribbean Sea	CIOH local lab, heavy fishing of oysters, inside and outside bay, oysters	pesticides, industry
Cienaga Grande	Caribbean Sea / R. Magdelena	(Santa Marta) 3 stations	Cotton, oysters, hard bottom (1) and mangrove (2)

Columbia continued			
Buenaventura Bay	Pacific	Not sampled in 1992	Pesticides
Bahia Tumaco	Pacific	Ecuador border, CCP Lab, 3 stations	Jungle
Costa Rica			
Tortuguero	Caribbean	Replace Limon	Suspended sediments
Golfito	G. Dulce	Several spp.	Mangrove
Punta Zancudo	G. Dulce	Several spp.,	Mangrove
Estero Cocoroca	G. Nicoya		
Isla Paloma	G. Nicoya		
Limon (Cahuita)	Caribbean Sea	Suspended sediments from 1991 earthquake	Banana
Estero Jicaral	G. Nicoya	Same spp. different sites	
Golfo de Nicoya	Pacific	• 3 sample stations, same spp., different sites	Aquaculture, agriculture
Golfo Dulce	Pacific	Anoxic basin; 2 stations, several spp.	Palm oil, banana, mangrove, mangrove
Cuba			
Santa Cruz del Sur	Caribbean Sea		
Ecuador			
Galapagos Islands	Pacific		Marine sanctuary, open ocean control site
Paita	Pacific	Control site, Replace Puerto de Etan, Peru	Desert, aquaculture
Guayaquil	Pacific/R. Guayan		Principle port
Bahia de Caraquez	R. Chone		Shrimp aquaculture. Agriculture

El Salvador			
Golfo de Fonseca	Pacific	2 sampling stations	Very high pesticide use
Guatemala			
Puerto Barrios	Caribbean Sea		
Guyana			
Georgetown	Atlantic		Malaria
Honduras			
Trujillo	Caribbean Sea / Lempa R.		Banana
Islas Malvinas/Falkland Islands			
Eagle Passage	Atlantic	Open ocean control site	
Jamaica			
Morant Bay	Caribbean Sea	2 sample stations	
Martinique/Barbados			
Martinique	Caribbean Sea		
Mexico			
Punta Mangrove	Balsas River		
Cancun	Caribbean Sea	Control site	
San Felipe	Gulf of California		
Topolobamo	Gulf of California		
Ciudad del Carmen	Gulf of Mexico		
Coatzacoacols	Gulf of Mexico		
Laguan de Tamaihua	Gulf of Mexico		
Laguna Madre	Gulf of Mexico		
Bahia Madalena	Pacific		
Bahia San Quintin	Pacific		

Mexico continued			
Mazatlan	Pacific	Alternate: Altata	
Puerto Madero	Pacific		
Punta Maldona	Rio Grand River		
Nicaragua			
Bluefields	Caribbean Sea	Control site; CIRA & IRENA assistance	(Remote, must fly in)
Islade Aserradores	Pacific	CIRA lab facilities	Cotton, banana, sugar
Ostional	Pacific		Mangrove coastline
Corinto	Pacific Ocean	Not sampled in 1992	cotton
Panama			
Puerto Almirante			Domestic effluent. cholera.
Portobelo	Caribbean Sea	A control site?	Small oyster population, mangrove
Bocas del Toro	Caribbean/Puerto Almirante	A CEPPOL pilot study site, sample from Costa Rica	Banana
Punta Chame	Gulf de Panama/Pacific	2 sample stations, difficult access, several spp.	Mangrove, same area as P. Bique
Playa Bique	Gulf de Panama/Pacific	No obvious contam.	
Peru			
Callao	Pacific		Industrial, urban
Pisco/Paraeus	Pacific		Grapes
Puerto Rico			
Sample through U.S. Mussel Watch			

Surinam/French			
Paramaribo	Caribbean Sea		
Trinidad and Tobago			
Tobago	Caribbean/Orinoco R.	Replace Curiapo	
Port of Spain	Caribbean/Caroni	Replace Curiapo, IMA local lab. Recent mussel die off.	High pesticide use, agriculture
Caroni Swamp			Agriculture
Southern Range			Orinoco R., v. small bivalve
Trinidad and Tobago continued			
Grand Caicos	Caribbean Sea	Control site	
Uruguay			
Punta del Este	Parana River / Atlantic ocean	Local red tide research project, INEPA Lab, include offshore Gorritti Is.	Uruguay-Montevideo
Santa Lucia	R. de la Plata		River sample, agriculture
Ilha de Fernando de Noroha		Control	
Venezuela			
Morrocoy			Remote, undeveloped
Cumana	Caribbean Sea	No local contact	
Maracaibo	Caribbean Sea	No bivalve population in 1992	
Curiapo	Orinoco River	No local contact, sample in Tobago	
Paparo	Tuy R.	Local lab, Simon Bolivar U.	Urban (Caracas)

Appendix C:

International Mussel Watch

Methods I

Manual on
Specimen Collection Methods
and Data Recording

Prepared in Cooperation with IOC and UNEP
(Draft January, 1990-Final Version January, 1992)

1. Collection Methods

1.1 Sampling Sites

Samples will be obtained from pristine areas away from any obvious sources of local contamination, from agricultural region, and from the outer estuaries of major river systems which may drain regions receiving inputs from both agriculture and industry. Sampling from estuarine areas undergoing major salinity variations will be avoided.

1.2 Seasonality

It is recognized that seasonal changes in bivalve physiology can lead to variations in chlorinated hydrocarbon content and concentration. However, in cases where these effects have been studied (Phillips, 1980), chlorinated hydrocarbon concentration rarely varies more than a factor of 2 to 5. Since order of magnitude differences in chlorinated hydrocarbon concentrations are sought for, the spatial trend analysis, seasonality effects are considered to be of minor importance. Therefore, we do not feel it necessary to sample during any particular season, particularly in the tropical and subtropical regions where trickle spawning is common.

1.3 Depth

At bivalve sites, depth to bottom will be estimated.

1.4 Position on shoreline

Bivalve samples should be collected as low on the tidal horizon as possible. Time of collection, time of high or low water for the day, and the tidal reference location are recorded on the Bivalve Sampling Position or Bivalve Observations Logs.

The tidal horizon is estimated most accurately at the time of low water for that day.

1.5 Temperature

Bottom water temperature will be measured to 0.1 degree at each bivalve sampling site. Temperature may be measured with a portable digital thermometer or calibrated glass mercury thermometers.

1.6 Salinity

Surface salinity will be determined at each bivalve site using a surface-deployed probe or a refractometer.

1.7 Bivalve Sampling Methods

In sampling for bivalves, the primary objective is to obtain a pooled sample of approximately 100 individuals representative of the site. Obtaining a pooled site sample ("composite") will generally occur when collection is made by dredge, or under other subtidal collecting methods. Depending on the station depth and bivalve species, as well as environmental conditions at a site, several different collecting techniques may be employed to obtain bivalves. The methods used must be indicated on the Bivalve Observation Log. Polyethylene, or other non-contaminating gloves must be worn when handling bivalves. Clean polyethylene buckets with reclosable lids should be used for temporary storage of bivalves during collection and/or prior to packaging. Buckets should be maintained only for bivalve storage and should be cleaned prior to each use. Collecting methods are described below.

1.7.1 Sampling Fork and Rake

In water depths of less than 1 m, mussels may be collected using a stainless steel pitch pork or stainless steel quahog rake. Clusters of mussels, attached to each other and debris by byssal threads, can be pried apart using the tines of the fork or rake and transferred to a culling table or tub for identification, sizing and cleaning.

1.7.2 Tongs

In water depths of 2 to 2.5 m, and where the bottom is relatively soft, both oysters and mussels may be sampled with stainless steel tongs. The depth limitation of the tongs (maximum of 12 feet) relates to the length of the tong handles. Tong handles (toothed baskets generally 18 to 20 inches wide) are dug into the bottom with a down-jabbing motion. The bivalves are brought to the surface by squeezing and lifting the tong handles.

1.7.3 Hand Collection

Mussels may be collected from rocks or other non-contaminating substrates by hand. Wearing polyethylene or other non-contaminating gloves, the mussels will be removed from the substrates, if possible, by cutting the byssal threads with a clean knife.

In areas where rock oysters are used, the outer shell which is not fixed to the rocky substrate can be removed by placing a pre-cleaned stainless steel diving knife or other sharp object near the base of the hinge and tapping with a hammer.

1.7.4 Identification and Sorting of Bivalve Specimens

Following collection of bivalves using the above technique, the specimens will be carefully separated from one another; if clumped, place on a non-contaminating sorting tray and wash of mud and debris with site water. A plastic bristle brush may be used to scrub debris and algae from bivalves. Polyethylene gloves should be worn while handling samples. Bivalve species will be identified according to keys and guides found in the field manual. The size of the specimens collected at a site should only be from the same upper 1/3 of the population. All bivalve samples should be processed, packaged and properly stored as soon as possible following collection. Although both oyster and mussel species will be collected for this program, only one species will be collected for the sample at any one site. At selected sites, however, where overlap in oysters and mussels occur, a pooled sample of both species will also be prepared. Shell length of a representative number of individuals should be noted for each species sampled.

1.7.5 Subsampling of bivalves

For each station sample, approximately 300 g wet weight of oyster or mussel tissues will be required for the organics sample.

Wearing polyethylene gloves, double wrap 30 mussels or 20 oysters in aluminium foil.

Place foil-wrapped samples inside plastic freezer bags and seal.

Label the bags appropriately and place inside another freezer bag.

Transfer the samples to an ice chest containing dry ice or regular ice + salt if unavailable.

Check off the "Bivalve Organics" box under "Samples Collected" on the Bivalve Observations Log.

For some species, such as rock oysters, it is preferable to remove the top shell while the oyster is attached to the rock and carefully collect the soft parts using pre-cleaned scalpels.

1.7.6 Bivalve Samples for Analysis and the Gonadal Index Sample

In addition at each station, at least 10 bivalve specimens should be collected for the gonadal index sample. Oyster will be shucked and mussels shells will be split open prior to storing in preservative. The procedures for preparing each type of bivalve are described below.

1.7.6.1 Mussel Sample

To split the shells, the tip of an oyster or clam knife should be carefully inserted between the shells immediately posterior to the joint where the byssus emerges, and rotated to pry the shells apart. A 90-degree rotation of the knife blade should cause the blade to be wedged firmly in place, such that a scalpel blade can easily be inserted to sever the posterior adductor muscle. This procedure should allow the shells to open with little or no additional force. If the mussel is small, rotation of the knife blade could tear the adductor muscle and no further severance would be required.

Place the drained soft parts of 100 individuals in a mortar, add a known quantity of sodium sulfate and macerate the tissues with pre-cleaned stainless steel knife and a pestle. This constitutes the desiccated sample for chlorinated pesticide analysis. It should then be stored in a pre-cleaned container for shipment.

Place the additional sample of 10 mussels from each station or each split in a 0.5 l wide-mouth plastic jar prefilled with Dietrich's fixative.

Seal the jar securely, label it, and store at ambient temperature.

Mark the "Bivalve -Gonadal Index" box under "Samples Collected" on the Bivalve Observations Log.

Check jars periodically for excess CO_2 buildup caused by acid degeneration of the shells. Jars may require opening and resealing to relieve pressure.

1.7.6.2 Oyster Sample

To remove the oyster tissue, the oyster should be pressed with a flattened hand on a firm, flat surface.

In the case of rock oysters, the top shell should be knocked off the fixed bottom shell be carefully topping a pre-cleaned pointed object (e. g., diving knife) placed at the hinge.

An oyster knife (or other round-tipped blade) should be inserted between the shells at the ligament and firmly twisted to dislocate the hinge, slightly separating the shells.

The shells can then be held apart firmly while the knife blade is carefully inserted between the mantle and upper shell in the area of the adductor muscle.

With the blade pressed as closely as possible against the inner shell surface, the adductor muscle should be carefully cut.

Once the adductor muscle is cut, the upper shell can be lifted off the oyster bode and discarded (now have oyster-on-the-half-shell).

The oyster tissue is removed from the lower shell by carefully inserting the knife blade between the mantle and lower shell, cutting the adductor muscle and gently prying the oyster from the shell.

Place the removed, drained soft parts of about 100 individuals in a mortar; add a known quantity of sodium sulfate and macerate the tissues with a pre-cleaned stainless steel knife and a pestle. This constitutes the desiccated sample for chlorinated pesticide analysis. It should then be stored in a pre-cleaned container for shipment.

Place the 10 oyster bodies from each station into a 0.5 l wide-mouth plastic jar prefilled with Dietrich's fixative.

Securely seal the jar, label it and store at ambient temperature.

Check off the "Bivalve - Gonadal Index" box under "Samples Collected" on the Bivalve Observations Log.

1.7.7 Partitioning the Sample

The bivalve sample should eventually be divided into three aliquots as follows:

100 individuals (ca. 300 g wet wt.)

Regional Lab	Participating Lab	Archives
100 g wet wt	100 g wet wt	100 g wet wt
10 g dry wt	10 g dry wt	10 g dry wt
2.5 g (1/4 of sample) for analysis	2.5 g	2.5 g
75 mg lipid	75 mg lipid	75 mg lipid

1.7.8 Site Position Log

The Site Position Log (Fig. 1) is the form on which the coordinates and other site location information is recorded. This form is to be completed for all sites sampled or where sampling was attempted.

1.7.9 Site Observations Log

The Site Observations Log (Fig. 2) is to be used to record field observations, measurements and types of site composite samples collected. This form is to be completed for all sites sampled or where sampling was attempted.

Weather and sea state are recorded via the Beaufort Sea scale as follows:

Weather Codes

0 = Clear, no clouds at any level.
1 = Partly cloudy, scattered.
2 = Continuous layer(s) of clouds.
3 = Blowing snow, sandstorm.
4 = Fog or haze.
5 = Drizzle.
6 = Rain.
7 = Snow, or snow mixed with rain.
8 = Shower(s).
9 = Thunderstorms.

International Mussel Watch Program
Site Position Log

Site Identification

Site Number _____ Code _____

Site Name _____ _____
General Location Specific Name

Date ____ ____ ____ Time ____ ____ (24 hr clock)
DD MM YY HH MM

Site Type: Mussel_____Oyster _____Other Bivalve_____

Site Location

Name _____

_____

Corrected Site Center
TD1_____TD2_____Lat_____N Lon_____W
(xxxxx.x) (xxxxx.x) DD MM MM DDD MM . MM

Bearings: _____degrees to _____
(xxx) (object)
_____degrees to _____
(xxx) (object)

Comments _____
_____
_____
_____

Recorder
Name _____ ID No. _____

International Mussel Watch Program
Site Observations Log

Site Identification

Site Number _____ Code _____ Sample No. _____

Field Observations

Weather _____ (code) Sea State _____ (code) Wind Spd/Dir _____ (xx.xx) kts _____ (xxx) deg

Bottom Depth _____ (xxxx) m Water Temp. _____ (xx.x) °C Salinity _____ (xx.x) ppt

Additional Comments _____

Recorder

Name _____ ID No. _____

1.7.10 Bivalve Sampling Position Log

Under "Sampling Location", sections are provided for documentation of three types of sampling techniques: subtidal use of fork, tongs or rake; subtidal dredge sampling and intertidal sampling. If only one sampling location is applicable to the bivalves collected for the station(s) referenced, the number 1 is entered on the line following "Sampling Location". If more than one location is sampled for the station(s) referenced, additional Bivalve Sampling Position Logs should be completed and numbered accordingly.

1.7.11 Bivalve Observations Log

Field observations and types of bivalve samples collected are recorded on the Bivalve Observations Log. Where possible LORAN coordinates are preferable for site location recordings. Otherwise, accurate long/lats records with compass bearings of prominent land marks will be acceptable.

Due to variation in propagating conditions, losses and irregularities over the signal path and internal receiver conditions, LORAN position resolution and accuracy degrades with increasing distance from transmitting stations. Distance from the transmitter generally affects LORAN accuracies according to the following relationship.

Distance from Transmitter	LORAN Offset
200 mi (370 km)	15 - 90 m
500 mi (975 km)	60 - 210 m
750 mi (1390 km)	90 - 340 m
1000 mi (1850 km)	150 - 520 m

Precision is greatly influenced by the quality, condition and calibration of the receiver. LORAN-C generally provides precision repeatability of a recorded fix taken several times at a known location within 15 - 91 m (Maloney, 1978).

Positioning accuracy is ensured through the daily calibration or position offset compensation of the LORAN receiver relative to charted landmarks of Coast Guard aids to navigation. Corrected LORAN time differences and latitude/longitude conversions are to be recorded at all site centers.

International Mussel Watch Program
Bivalve Sampling Position Log

Site Identification

Site Number _____ IMWP Code _____

Site Name _____ _____
General Location Specific Name

Station No. _____ Date ___ ___ ___ Time ___:___(24 hr clock)
(x/x-x) DD MM YY HH MM

Sampling Location

Subtidal Fork, Tongs, Rake Sampling
TD1 _____ TD2 _____ Lat _____ N Lon _____ W
(xxxxx.x) (xxxxx.x) DD MM . MM DDD MM . MM

Bearings: _____ degrees to _____
(xxx) (object)
_____ degrees to _____
(xxx) (object)

Bottom Depth _____m Mean Wire Out _____ No. Bivalves _____
(xxx) (xxx) (xxx)

If additional locations are sampled, check (x) here ☐ and fill out another *Bivalve Sampling Positions Log*.

Intertidal Sampling—Transect Center
TD1 _____ TD2 _____ Lat _____ N Lon _____ W
(xxxxx.x) (xxxxx.x) DD MM . MM DDD MM . MM

Bearings: _____ degrees to _____
(xxx) (object)
_____ degrees to _____
(xxx) (object)

Distance of Station from Transect Center ____m Direction _____
(xx)

Recorder

Name _____ ID No. _____

1.7.12 Visual Positioning

In addition to LORAN navigation, techniques of visual positioning are used to accurately and precisely document site center locations. Lines of position will be established using all of the following practical methods:

Relative bearing to fixed, charted landmarks (e. g., towers, monuments, stacks, etc.) using hand compass or equivalent.

Relative bearing to charted Coast Guard aids to navigation (e. g., buoys, flashers, etc.).

Relative bearing to fixed uncharted landmarks (e. g., buildings, poles, etc.) using hand compass or equivalent.

1.7.13 Photodocumentation

Photographic documentation of intertidal collection sites will be carried out where appropriate. Bivalve transects and site centers may be photographed with fixed reference points for future relocation of a site. A photographic log should be kept with date, time, frame number, film roll number and location information of any photographs taken for documentation purposes.

Appendix D:

International Mussel Watch:

Methods II

Determination of Selected Chlorinated Hydrocarbon Pesticides and CB Congeners by Capillary Gas Chromatography/Electron Capture Detection

Prepared in Cooperation with IOC and UNEP
(Draft January, 1990-Final version June, 1992)

Forward

Several methods and variations of these methods have been published in the scientific literature. These may be used for analyses of chlorinated hydrocarbon pesticides and PCB's; especially for the extraction and initial separations of the classes of analytes of interest. The methods described in this Manual are intended as guides for analysts in laboratories in participating countries. Local circumstances including available equipment, chemicals, and solvents, and analytical requirements for other programs in a given laboratory will govern methods used by each laboratory.

Ultimately, when a full scale, more routine monitoring program is in place, there should be agreement on a few common methods of analyses which have been carefully evaluated as giving comparable results via a rigorous quality control and quality assurance program. Until that time, a program of inter-laboratory comparison exercises is necessary in order to compare data generated by participating laboratories (UNEP 1990).

The methods set forth in this manual, or equivalent methods, will be used by the central laboratories during the Initial Implementation Phase for the analysis of chlorinated pesticides and specific PCB congeners. There is a large scientific literature on the analysis of pesticides and we refer participating scientists to that body of knowledge. Greater detail on the analysis of specific PCB congeners is provided here because this information is comparatively new.

Regional scientists who retain field-collected samples for analysis during the Initial Implementation Phase will be provided with several Standard Reference Materials (SRMs) and with a freeze-dried tissue homogenate for use in an inter-laboratory comparison exercise.

Notes:

1. Preservation of Total Lipids

The preservation of total lipids in samples is very important because all data for chlorinated pesticide concentration need to be reported on a lipid normalized basis as well as wet weight and dry weight basis to facilitate comparisons of temporal and spatial trends, especially when comparing data for different bivalve species. The efficacy of lipid concentrations was more of a concern than the preservation of chlorinated pesticides in non-frozen samples because of the inherent lability of several lipid classes compared to chlorinated pesticides.

Data clearly illustrate that total extractable lipid amounts are not changed appreciably by storage at room temperatures between 22 to 29^{o}C over three months in sealed glass jars with either 1:7 (weight/weight) wet tissue homogenate anhydrous sodium sulfate: or 1:3:8 wet tissue

homogenate: anhydrous sodium sulfate: dichloromethane compared to frozen tissue. The two methods of room temperature storage and shipment will work from the perspective of preserving total extractable lipids.

2. *Chlorinated Pesticides*

Fused silica column capillary-gas chromatography analyses demonstrate that three months storage in solvent at room temperature does not appreciably alter the concentrations of major chlorinated pesticide residues of interest.

1. Introduction

This method deals with the determination of selected chlorinated pesticides and PCB's in marine environmental samples using high resolution gas chromatography. Several other halogenated pesticides and other electron capturing organic compounds may be present in samples and many of these may also be determined by this method. Prior to using the method for contaminants other than the compounds described here the analyst must test his/her own recovery and analytical reproducibility for every residue quantified. Not all electron capturing residues will be resistant to all of the clean up procedures described here for the analysis of DDT's and PCB's. Therefore, additional information on the stability of some common pesticides using this methodology is also provided.

The high separation power of open tubular ("capillary") columns allows the identification and quantification of many compounds in the complex mixtures occuring in environmental samples. This manual provides information on the theoretical and practical aspects of the use of these high resolution columns for the analysis of DDT's and PCB's in environmental samples.

The qualitative and quantitative method can be applied to any sample type (aerosol/vapor, water, particulates, biota, etc.) provided that suitable cleaned-up extracts dissolved in n-hexane are available for injection into the GC systems.

Many of the 209 possible CB (chlorobiphenyl) congeners can now be separated from interfering compounds and thus be determined as individual compounds, using one capillary column only (Ballschmiter and Zell, 1980; Zell and Ballschmiter, 1978; Zell and Ballschmiter, II, 1980; Zell and Ballschmiter, III, 1980). SE-54 is the coating material of choice, because the retention behavior of all 209 congeners has been determined for this column material. Therefore, as well as determining whether CB's are present in measurable quantities and whether hot-spots can be identified (similar to the possibilities offered by the use of packed column GC) additional

valuable information is gained on the composition of the usually complex mixture of congeners representing "total PCB". This information is the basis for understanding the sources, distribution, transport pathways, sinks, degradation mechanisms and effects on organisms of these individual congeners and groups of congeners. It eliminates most of the ambiguities associated with the use of packed columns.

2. Sample Preparation in the Field

For ancillary data to be recorded prior to initiating the following procedures see The Manual on Specimen Collection Methods and Data Recording (Appendix C).

All implements should be cleaned thoroughly with detergent obtained locally and rinsed with copious amounts of fresh water, or clear seawater not visibly contaminated with particles, oil slicks, etc. Jars and caps should not be cleaned on the spot as these have been precleaned.

The following sample preparation procedure should be applied prior to shipment. A sample of sufficient live mussels or oysters to provide 300 grams of wet tissue (less tissue wet weight may have to be accepted for less numerous populations or smaller individual organisms) is scrubbed clean with a nylon brush and shucked. The contents are allowed to drain in a metal collander until no further drops of shell fluids drain off (about 15 min.). The tissue is transferred with a metal spoon and ground in a metal hand-operated meat grinder with a fine (1 mm) screen into a stainless steel bowl. Divide the sample into three aliquots and transfer to three pre-cleaned sampling jars to be frozen. Remove a small quantity (1-2 grams), place it in a scintillation vial of known weight for wet/dry weight determination, and carefully record the weight of vial and tissue.

If transport of frozen samples is not possible, then the following procedure is recommended. Transfer about 100 grams of the minced tissue into each three precleaned and preweighed sample jars and reweigh and record the weight of wet tissue in each jar. Add about 700 grams of sodium sulfate to each jar, reweigh and record the weights. Transfer the wet tissue to a mortar and add 700 grams of sodium sulfate in aliquot, while grinding the tissue with pestle. Care must be taken not to loose sample from mortar. Transfer the sodium sulfate plus tissue to the sample jar. Seal the jars and record details according to instructions.

As soon as practical, the dry weight of the sample should be determined using supplied portable oven, by drying at 105 degrees centigrade to constant weight with storage in a desiccator when cooling. Record wet and dry weights.

Before leaving the site, a picture of the sampling location should be taken, other ancillary observations that are specified elsewhere should be recorded and the note book should be checked for completeness.

Two of the jars from each sampling location will be transported to the appropriate regional analytical laboratory. The other jar will remain in the country at the laboratory assigned by the country.

3. Sample Extraction and Clean-up

3.1 Principles

Lipids are extracted from an aliquot of a sample by solvent extraction, fractionated into classes by adsorption chromatography prepared according to guidelines in UNEP (1991) using hexane or petroleum ether as solvent. Extracts may also be treated with concentrated sulphuric acid to destroy some of the interfering lipids and then further cleaned and fractionated into classes of chlorinated hydrocarbons by silica gel adsorption chromatography using known reference substances for identification.

3.2 Reagents

All reagents, including the distilled water should be of demonstrated analytical quality. Their use must result in adequate signal-to-noise ratio with the electron capture detection. All reagents must be checked for their ECD response individually and by analysing complete procedural blanks. If contaminants are detected the solid reagents must be cleaned by extracting them with pure solvents and/or evaporating the chlorinated hydrocarbons by heating overnight at 260°C to 300°C. All solvents should be "distilled in glass" quality and pre-tested for their suitability for pesticide analysis. The solvent should be kept in sealed ampoules (about 100 ml each). Alternatively, it can be kept in Sovirel (teflon-lined caps, pyrex glass) glass bottles (200 ml), and kept at low and constant temperature (10°C). They may require redistillation in the laboratory on a routine basis. Each bottle of solvent must be checked routinely.

a. Demineralized distilled water produced by distillation over potassium permanganate (0.1 g $KMnO_4$ l^{-1}) or equivalent quality, demonstrated free from interfering substances.

b. Ethanol, 96%.

c. Hexane, methylene chloride acetone, iso-octane, and methanol (89°C) (all "distilled in glass" quality).

d. Detergent.

e. Fuming sulphuric acid ($^{d}20^{o}C$ = 1.84 g ml^{-1}), if required for confirmation.

f. Nitrosulphuric acid made of concentrated sulphuric acid and sodium nitrate.

g. Anhydrous sodium sulfate, precombusted at 400°C in muffle furnace.

h. Alumina

i. PCB reference solutions - Prepare stock solutions of Aroclor 1242 and Aroclor 1254 by dissolving 100 mg Aroclor in 100 ml iso-octane. Store stock solutions in sealed glass ampoules.

j. CB isomer reference solutions: DDT reference solutions - Prepare a stock solution of the DDT series (p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD) by dissolving 100 mg of each compound in iso-octane. Store stock solution in sealed glass ampoules.

k. Other reference solutions must be prepared for all other residues to be quantified in these procedures. The suggested list of organochlorine pesticides for International Mussel Watch is as follows:

Aldrin	Heptachlor
Endrin	Heptachlor epoxide
Dieldrin	Hexachlorobenzene (HCB)
Chlordanes	a-Hexachlorocyclohexane (a-HCH)
	ß-Hexachlorocyclohexane (ß-HCH)
o,p'-DDD	Lindane (γ - HCH)
p,p'-DDD	Trans-nonachlor
o,p'-DDE	Methoxychlor
p,p'-DDE	Mirex
o,p'-DDT	Kelthane
p,p'-DDT	

In addition to a common set of selected individual chlorobiphenyls - PCB I (IOC-Kiel) and standards for semi-quantitative screening for toxaphene residues should be prepared.

l. Potassium hydroxide pellets.

m. 2,5,2',6' tetra-chlorobiphenyl and 1,1 dichloro-2,2-diphenylethylene

Note: Working solutions from the stock reference solutions should be prepared on a regular basis and stored in clean glass vials tightly capped with non-contaminating materials such as teflon or glass. Extreme care must be taken to ensure that standards have not changed their concentrations through solvent evaporation. This can be done efficiently by weighing solutions on an electronic balance with the weight recorded on the vial.

3.3 Apparatus

a. High purity carrier gas for the gas chromatograph including molecular traps to remove trace contaminants and moisture.

b. Rotary evaporator.

c. Kuderna-Danish (or similar) concentrator and heater.

d. Soxhlet and/or tissue miser (e.g., Bransonic).

e. Glassware including boiling flasks, ground glass stoppers, beakers, Erlenmeyer flasks, gas chromatography columns (length 1.8 m, 0.4 cm I. D.), separatory funnels, centrifuge tubes, weighing bottles, pipettes, syringes, tissue grinders.

f. Drying oven (temperature range up to at least 300°C) for determining sample dry weights, baking of contaminant residues from glassware and reagents.

A muffle furnace is required for baking materials, such as Na_2SO_4, at greater than 300°C, if required.

g. Centrifuge and tubes (at least 600 x g).

h. Porcelain mortar and pestle.

i. Analytical balance with a precision of 0.0001 g and an electrobalance with a precision of at least 1 µg.

j. Stainless steel tweezers and spatulas.

k. Glass wool.

l. Supply of clean, dry nitrogen.

m. HPLC with normal phase silica column (high-resolution silica, Petrick, *et al.*, 1988).

n. Gas chromatograph with electron capture detector and appropriate silica capillary column (see 5.3).

o. Vacuum pump (water-jet air pump).

4. Preparative Analytical Procedures

4.1 Cleaning of glassware

Scrub all glassware vigorously with brushes in hot water and detergent. Rinse five times with tap water and twice with distilled water. Bake overnight in an oven at 300°C. All glassware should be stored in dust-free cabinets and tightly sealed with precleaned aluminium foil when not in use. Ideally, glassware should be cleaned just before use. If necessary, to remove tough organic residues, the glassware must be soaked for at least two hours in the nitrosulphuric acid cleaning solution, thoroughly rinsed with tap and distilled water and then put into the drying oven.

4.2 Extraction procedure

Select approximately 1/4 of the sample which has been previously ground in Na_2SO_4 and subsample into three aliquots. Aim to end up with approximately 75 mg lipid. Transfer the mixture to a precleaned glass extraction thimble, add internal standards and extract with about 200 ml hexane/pentane or hexane:acetone (90:10) in N_2-atmosphere for 8 hours in the Soxhlet apparatus cycling 4 to 5 times per hour. Extract the same amount of sodium sulfate for a procedural blank.

Alternatively, the Na_2SO_4 -sample may be extracted with solvents in a Tissumizer using CH_2Cl_2 and 8 or 9:1 $Na_2SO_4$to wet tissue ratio. The procedure is described in detail in MacLeod *et al.*, 1985.

Sample Extraction (adapted from: MacLeod *et al.*, 1985)

1. Using a spatula, and being careful to place the sample on the bottom and not the sides, weigh 3 ± 0.5 g of sample to the nearest 0.01 g into the 50 ml screw cap centrifuge tube. (Set aside ca. 1 g for Dry Weight Determination)

2. To each tissue sample in the centrifuge tube add: (a) 35 ml of CH_2CL_2, (b) Standard solution. Make certain that the solutions are placed into the CH_2CL_2.
3. For each set of samples prepare a spiked blank ("reagent spike") by adding to a centrifuge tube containing 35 ml of CH_2CL_2 and Standard solution.
4. If the sample set requires a field blank ("tissue blank"), prepare this by washing down the empty sample container 3 times with 10 ml of CH_2CL_2 each time and adding the combined washings to an empty centrifuge tube. Add 5 ml more of CH_2CL_2 to the tube, and proceed as in the next step starting at (b).
5. For each set of samples prepare a blank ("reagent blank") by adding to an empty centrifuge tube: (a) 35 ml of CH_2CL_2, (b) Standard solution.
6. Prepare 2 AH/PES analyte-calibration solutions.
7. Add 25 g Na_2SO_4 to each tube from steps 2-5. Macerate/extract the sample in the tube with the Tissumizer. Avoid spattering the tissue.
8. Wash down the probe with CH_2CL_2, collecting the washings in the centrifuge tube. Centrifuge the sample.
9. Decant the extract into a labeled flask.
10. Add 35 ml of CH_2CL_2 to the tube. Repeat steps 7-9 once.
11. Wash the Na_2SO_4/sample mass by adding 10 ml of CH_2CL_2 to the tube, and mixing on the Vortex Genie for 5-10 seconds at setting 5-6.
12. Repeat steps 8-9 once.

4.3 Concentration of extract

Initial concentration to small volume should be performed by rotary evaporation. For both extraction procedures the final extracts are concentrated in a Kuderna-Danish concentrator. Concentrate extract to near 1 ml with the concentrator and adjust extract volume to exactly 1 ml by a gentle stream of clean dry nitrogen. Record the volume accurately. This can be done by weight.

Caution: too strong a N_2 stream may remove the compounds to be analyzed.

4.4 Clean-up by Alumina and High Performance Liquid Chromatography

Prior to GC-ECD, fats and other interfering substances are removed and the compounds of interest are separated into different fractions. This is accomplished in a two step operation: one involving Alumina solid-liquid adsorption chromatography which is followed by clean-up and separation using normal phase HPLC.

4.4.1. Alumina clean-up

Activated alumina, activated at 800°C and 5% deactivated is slurried into a precleaned Pastuer pipette (0.5 cm i. d. with a glass wool plug) to a height of 4 cm and kept under n-hexane. The tissue extract which has been concentrated to below 1 ml in a Kuderna-Danish concentrator or Rotavap is transferred to the column and eluted with n-hexane (10 ml). The eluate received in a Kuderna-Danish concentrator vial is reduced immediately in volume to below 1 ml and reduced further under a a mild stream of nitrogen to about 100 µl ready for injection unto the HPLC column.

Caution: temperature must be controlled. As an example, concentrate from 1 ml to 100 µl at <10°C in 20 minutes.

4.4.2. HPLC separation

The details of the HPLC procedures are described in Petrick, *et al.*, (1988) and are summarized here. The equipment consists of an isocratic HPLC system equipped with a Rheodyne (or equivalent) injector with 200 µl loop capacity, a guard column with a back flush valve and a 20 cm x 0.4 cm, normal phase, 5 um Nucleosil (or equivalent) column. The total volume of extract is transferred with washings. The total volume is kept as small as possible. The organochlorine compounds of interest are eluted with 11 ml of n-pentane at a flow rate of 0.5 ml per minute, 4 ml of 20% dichloromethane in n-pentane and finally 10 ml 100% dichloromethane followed by a 5 minute backflush and a 5 minute equilibration with the first eluant.

The following classes of compounds can be separated consecutively (Petrick, *et al.*, 1988):

Fraction:	ml
1. n-alkanes and n-alkenes	0.5 - 2.0
2. HCB, PCB's and alkylbenzenes	2.0 - 4.5
3. PAH's and toxaphene	4.5 - 11.0
4. pesticides, and toxaphene	11.0 - 15.0
5. acids, etc. (polar compounds)	15.0 - 25.0

The actual elution volumes have to be determined for any individual set-up. It is essential that the suite of standards are available to verify the actual fractions and elution volumes. For the purpose of the Mussel Watch Programme, fractions 2 and 4 are to be analyzed by GC-ECD according to the ensuing methods, and fraction 3 may be used for the screening for toxaphene.

Sources of Contamination

Frequent sources of CHC's are solvents and adsorbents used for column chromatographic clean-up. Even nanograde solvents occasionally contain surprising concentrations of PCB's. It is not sufficient to test the untreated solvent for purity, because in the analytical procedure it will be concentrated by a factor of approximately 500. Thus, for testing the purity of solvents, they must be concentrated at least by the same factor before injection into the gas chromatograph.

Alumina is often contaminated, not infrequently with PCB's. It should be noted that alumina, when activated in a drying cabinet, becomes a very efficient sorbant for all vapors in the oven atmosphere. Thus, Soxhlet extraction and subsequent reactivation in a less than perfectly clean oven may result in a silica gel that is even more contaminated than prior to the intended purification. Drying and reactivation under vacuum are recommended. Store in sealed glass ampoules 2-3 g Al_2O_3.

4.5 E.O.M. determination

Solvent extractable organic matter (E.O.M.) is determined in the following manner. On the weighing pan of an electro-balance evaporate a known amount of the extract prior to clean-up (5 to 10 µl) and weigh the residue to ±1 µg.

The quantity of E. O. M. is:

$$\text{E.O.M.} = \frac{\text{mg}}{\text{g}} = \frac{\text{weight of residue (mg x volume of extract (ml) x } 10^3}{\text{amount evaporated (µl) x quantity of sample extracted (g)}}$$

Note that extreme care must be taken to ensure balance and pans are clean, dry and stable to obtain accurate readings of ±1 µg. A small hot plate is used to warm pans and forceps and thus keep these instruments dry after solvent cleaning. If no electro-balance is available, a known volume of the extract can be transferred into a clean preweighed vial. The solvent is evaporated with dry nitrogen gas until a constant weight of ±5mg is reached. Calculate the amount of "lipids" in the sample taking into account the volume of the lipid extract which was dried.

4.6 Determination of Fresh Weight/Dry Weight (FW/DW) Ratio

The constant weight of a weighing bottle is determined by repeated weighing. A tissue subsample of 1-2 g is introduced into the bottle and heated in an oven at 105°C for 24 hours. The flask is then transferred to a desiccator to cool and then weighed again. The procedure is repeated until a constant weight is reached. The (FW/DW) ratio is calculated.

5. Gas Chromatographic Determinations

5.1 Principles

After clean-up of the extracts of marine samples by adsorption chromatography, fractions are analyzed by high resolution gas liquid chromatography (HRGC) with electron capture detection (ECD). As many PCB congeners and pesticide compounds are separated and quantified as individual peaks within feasible and practical limits. The method relies on splitless or on-column injection of up to 50 ng per compound on a small-bore SE-54 coated, 25 m open tubular fused-silica column under optimum conditions of carrier gas type, flow and temperature conditions. Standards of well defined composition are used for peak identification and quantification and to establish optimum chromatographic performance. Confirmation and quantification of the DDT series is accomplished by a dehydrochlorination procedure where the p,p'-DDE peak is measured before and after oxidation with concentrated H_2SO_4/SO_3 (fuming sulphuric acid). Internal standards are used where required for accuracy assurance (see section 8).

5.2 Reagents

All reagents, including distilled water, should be of well defined analytical quality. Their use must result in an adequate signal to noise ratio with the electron capture detector. All reagents must be checked for their ECD response individually and by determining complete procedural blanks. If contaminants are detected, the solid reagents must be cleaned by extracting them with pure solvents and/or by heating overnight at 260°C to 300°C. All solvents should be "distilled in glass" quality and pre-tested for their suitability for organochlorine residue analysis. They may require redistillation in the laboratory on a routine basis.

a. n-hexane

b. Fuming sulphuric acid, sufficiently concentrated to remove the p,p'-DDE peak.

Reference solutions of individual chlorobiphenyl congeners and the necessary pesticides will be distributed to participating laboratories. Follow the enclosed instructions in diluting them with isooctane up to working solutions.

5.3 Apparatus

5.3.1 Narrow-bore (0.25 mm internal diameter), 25 m fused-silica open tubular column, coated with SE-54 (0.15 um film thickness, preferably chemically bonded) with sufficient resolution to separate the relevant peaks in the standards provided for PCB analysis.

5.3.2 Gas chromatography with a split/splitless or on-column injection system, multiramp temperature programming facilities (preferably microprocessor controlled), electron capture detector interfaced with the column with electronic control unit and pulsed mode facilities. An integrator with a short response time (less than 0.25) is essential.

5.3.3 Carrier gas should be high purity H_2. If this is not available, high purity He may be used. Gas purification traps should be used with molecular sieves to remove oxygen, moisture and other interfering substances.

5.3.4 Ar/5%CH_4 high purity gas as ECD make up gas is preferable, however, high purity N_2 (99.99+%) may also be used but will result in a loss of sensitivity.

5.4 Gas Chromatography and Electron Capture Detection

5.4.1 GC Column

5.4.1.1 Column characteristics: Fused-silica columns are the columns of choice for their inertness and durability (they are extremely flexible). They are made of material that is stable up to 360°C. The 1% vinyl 5% phenyl methylsilicone gum (SE-54) liquid phase, is present as a thin (o.15 um) uniform film which can tolerate temperature up to 300°C. SE-54 is relatively resistant to detrimental effects of solvents, oxygen and water at least at low temperatures. These columns are even more resistant and durable if the liquid phase is chemically bonded by the manufacturer. This column was chosen because at present it is the only column which allows unambiguous identification of the most CB congeners.

5.4.1.2 Column installation: The flexible fused-silica columns can be conveniently connected directly to the inlet and outlet systems without the transfer lines used in conventional

glass capillary chromatography which often leads to increased dead volume. Low bleed graphite (vespel) ferrules provide a good seal.

5.4.1.3 Column conditioning: The presence of extraneous peaks and elevated base-line drift will result in poor detector performance. This can be caused by components which elute from the column, such as residual solvents and low molecular weight liquid phase fractions on new columns and a build-up of later eluting compounds on old columns. Conditioning is a necessary step to remove these contaminants. New columns are connected to the inlet while left unconnected to the detector. *CAUTION: IF H_2 IS USED AS A CARRIER GAS, POSITION THE COLUMN END OUTSIDE OF THE OVEN),* flushed with carrier gas at low temperature for 15 minutes to remove oxygen from the column, heated at 70-100°C for 30 min. and finally in cyclic temperature programmed runs, with 250°C as end temperature overnight. The column can then be connected to the detector. Old columns can be heated directly at elevated temperatures overnight. The final temperature is selected as a compromise between time required to develop a stable baseline and expected column life. Thus, it may be necessary for older columns to be heated to the maximum temperature of the liquid phase resulting in shorter column life. The temperature of the ECD, when connected to the column, should always be at least 50°C higher than the column, in order to avoid condensation of material onto the detector foil. It is essential that carrier gas flows through the column at all times when at elevated temperatures. Even short exposure of the column to higher temperature without sufficient flow will ruin the column.

5.4.1.4 Column test: Column performance tests should be carried out at regular intervals and a continuous record kept in a log book.

5.4.1.5 Column maintenance: The column remains in optimum condition as long as the liquid phase exists as a thin, uniform film. This requires that the interactive forces between the film and the inside wall of the capillary column dominate over the cohesive interaction within the liquid phase. Conditions which cause the deterioration of the film are: 1. exposure to high temperatures; 2. insufficient gas flow at elevated temperatures; and, 3. sample components which have stronger attractive interaction with the column wall than the liquid phase. The quality of the film at the inlet side may suffer from repeated splitless injections. Decreased column quality may be remedied by the removal of parts of the column (0.1-1.0 m) at the inlet side and/or by heating to the upper limit of liquid phase stability. One should be aware of selective adsorption that can happen with contamination of the column at the injector end. This can be remedied by removal of a

small portion of the column at the injector end. Chemically bonded liquid phases require less maintenance.

5.4.1.6 Inlet and injection: The inlet/injection system should allow introduction of the sample onto the column with minimum change in composition (discrimination). In the split mode, only a small, preset fraction (typically in the order of 1%) of the total amount injected reaches the column. This is an appropriate method for the analysis of major components of mixtures. The low boiling fraction may be lost; this includes components with boiling points up to C_{15}. However, for the determination of components that are present at trace concentrations, it is essential that the entire amount injected is transferred to the column. This is accomplished in the splitless mode, using essentially the same injector with a few modifications. In the splitless injection technique, 0.5 - 3.0 µl sample is introduced into a glass liner in the injector, where flash vaporization takes place at 200 - 250°C. The glass liner must have been deactivated. Such liners are also available commercially. (The temperature should be high enough to allow rapid evaporation of solvent and solutes but it should be low enough to minimize septum bleed and the destruction of labile compounds such as p,p'-DDT.) The solutes are concentrated on the column as a small band at the inlet, which is kept at a temperature 10 - 30°C below the boiling point of the solvent. This creates an area of increased solvent concentration, focuses the sample and results in increased resolution, known as the "solvent effect". If pentane is the solvent the initial temperature should be lower than 60°C (see 5.4.1.8).

The movement of solvent and solutes from the injector to the column inlet is carried out under low carrier gas flow rate in order to minimize dilution of solute with carrier gas. For the same reason, injected volumes should not be under 0.5 µl. After transfer of essentially the entire amount of solutes to the column (*usually 20 - 60 seconds, to be operationally determined for optimum results*), the injector is flushed with increased carrier gas flow, which removes the remaining solvent. In this way, tailing of the early eluting compounds is avoided. The loss of components of interest is negligible by this technique. In order to minimize contamination of the column by products derived from septa, the inlet is purged continuously except during injection. The column temperature can then be increased at a rate, selected as a compromise between efficiency and length of time required for a given separation. (Note: the concentration effect at the column inlet can also be affected by a condensation technique which involves keeping the column inlet at a sufficiently low temperature. This technique is effective for compounds with boiling points at least 150°C above the column temperature. Compounds with lower boiling points need a "solvent effect" for concentration at the column inlet). Laboratories equipped with on-column

injection devices are encouraged to use them. This may eliminate many of the problems mentioned above.

5.4.1.7 Carrier gas: Suitable carrier gases for electron capture detection are nitrogen, hydrogen and helium. The maximum separation efficiency is slightly higher for N_2 than for H_2 and He, but the corresponding average linear gas velocity is considerably lower for N_2. Similar resolution can be obtained when H_2 or He are used resulting in much shorter GC runs. As the change in resolution with variations in gas velocity is lowest for H_2, it is the preferred carrier gas. Care must be taken to prevent H_2 from entering the GC oven at high flow rates, therefore, tests for leaks are a mandatory part of operation. This can be done by including a flow controller in the carrier gas supply immediately after the regulator. Non-zero gas flow, with carrier gas supply at the GC being closed, indicates a leak somewhere in the system. The use of electronic flow rate controllers to check carrier gas flow and emergency shut-off valves are highly recommended.

The quality of the carrier gas can be checked by keeping the column at room temperature at normal flow rates overnight and carrying out a normal temperature programmed run. The presence of contaminants in the carrier gas will show up in the form of extraneous peaks and/or unstable and increased baseline. The problem may be resolved by regeneration and/or replacement of the external and internal (GC mounted) molecular sieve gas traps.

5.4.1.8 GC conditions and optimization: Resolution and time required for a chromatographic run depend essentially on the interaction of six parameters: column internal diameter, O; film thickness, d; column length, L; carrier gas type and average velocity, V and temperature, T.

A smaller O results in increased capacity ratios, and thus, in better separations at the cost of longer analysis times. Alternatively, time can be unchanged with a corresponding smaller L and/or higher V and T. The selection of column properties specified in section 5.4.1.1 represents a useful compromise between resolution and analysis time. Changes in O and d have considerably more effects on separation efficiency than column length (the opposite is true with packed columns). With O, d and L fixed by column selection, and H_2 selected as the carrier gas, V and T have to be optimised for the separation problem at hand. There are many variations of temperature programming rates, etc. for each type of compound to be analyzed. Appropriate chromatographic conditions for the analysis of pesticide CB's are the following:

a. H_2 or He carrier gas at inlet pressure of 0.5 to 1.0 kg cm^{-2} to achieve a flow rate of 1.0 to 2.0 ml min^{-1}

b. make up gas Ar/CH_4 30 ml min^{-1}

c. ECD temperature 320°C (For ECD designed to be heated to that temperature only-check detector manufactures specifications. CAUTION: DO NOT HEAT A ^3H DETECTOR TO THIS TEMPERATURE).

d. injector 230°C

e. septum purge 3 ml min^{-1}

f. injector purge 20 ml min^{-1}

g. temperature program 60°C hold 5 min the 4°C min^{-1} to 260°C followed by a 15 min hold at 260°C. A problem arises when using pentane (after HPLC clean up) as the temperature programme should start well below the boiling point (i. e., 36°C). This may be difficult to achieve. In this case, split/splitless injection is not recommended. Replacement of pentane by hexane or heptane may be useful.

5.4.1.9 Column test: The degree to which closely eluting CB congeners are separated by the column is used as a criterion for column performance. Suitable pairs of congeners are present in standards to be supplied and may be used for this purpose.

5.4.1.10 Possible contamination effects should be checked by blank determinations involving the entire procedure without using the sample. If significant ECD signals are observed, the contamination problem should be traced and eliminated (e. g., re-cleaning of glassware, adsorption materials, solvents, syringes, etc.).

5.4.2 Electron Capture Detector

The electron capture detector is an extremely sensitive tool for analysis of organochlorine compounds, about five orders of magnitude more sensitive than for hydrocarbons. An equilibrium concentration of thermal electrons is supplied by repeated collisions of high-energy electrons

emitted by a radioactive source within the detector (^{63}Ni)* with carrier gas molecules. The thermal electrons can be captured by sample molecules. The resulting reduction in cell current provides the signal. The range of sample concentrations where the response of an ECD is linear with concentrations of electron captive compounds is extended considerably in the constant current, pulsed voltage mode. The constant current results from the modification of the frequency of polarizing pulses to the cell electrodes. The optimum flow for an ECD (30 ml min^{-1}) is much higher than carrier gas flow through the column of one or two ml min^{-1}. Thus, additional detector purge flow is necessary (Ar/CH_4 or N_2). Once leaving the outlet of the column the compounds have to be taken up into an increased gas flow in order to avoid extra-volume band-broadening within the detector. Thus, the detector purge flow also serves as sweep gas. As the ECD is a concentration dependent detector, the detector flow must compromise between low band broadening and high concentration of compounds within the detector.

High boiling organic compounds eluting from the column may be deposited in the detector. The contamination results in lower sensitivity. The effects are less serious at higher detector temperatures. Periodic heating to 350°C overnight assists in maintaining detector performance. The ^{63}Ni ECD can be used routinely at 320°C with relatively limited contamination. Oxygen, even at trace levels, has a detrimental effect on ECD performance. This is especially true at higher temperatures. The standing electrode current must be checked regularly according to the manufacturers manual and performance data should be recorded in a log book to maintain a permanent record.

The linearity of the ECD must be established at regular intervals by injecting several concentrations of standards. Sample determinations should be made only in the linear range.

Phthalates are common in the atmosphere and in plastic tubing, they may, therefore, be artifacts when detected in natural samples. Their source should be identified and their presence should be eliminated.

5.5 Identification, Verification and Confirmation

The most widely used information for identification of a peak is its retention time, or its relative time (i. e., the adjusted retention time relative to that of a selected reference compound).

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* *NOTE:* The use of a tritium detector is not recommended. If its use cannot be avoided, be sure to ventilate the gas stream to the outside atmosphere. Under no circumstances should a ^{63}Ni or ^{3}H detector be serviced in the laboratory. It should be serviced by a specialized and authorized laboratory only.

Retention behavior is temperature dependent and comparison of retention times obtained at two or more temperatures may aid in determining a peak's identity. However, retention times are not specific and despite the high resolution offered by capillary columns, two compounds of interest in the same sample may have identical retention times.

Strong additional evidence is obtained from comparison of retention times obtained on two columns of different polarities, and from application of chemical reactions such as saponification, treatment with Raney nickel, dechlorination reactions, treatment with basic ethanol or oxidation with fuming sulphuric acid. The effects of the latter two methods on composition are summarized in Table D.1.

Confirmation of p,p'-DDT and p,p'-DDD involves dehydrochlorination with ethanolic KOH solution. The p,p'-DDT is transformed to p,p'-DDE and p,p'-DDD is converted into p,p'-DDMU. After GC/ECD analysis of the appropriate HPLC fraction, add 1 ml of ethanol and one pellet of KOH to an aliquot of the extract in a ground glass concentrator tube equipped with a Snyder column. Heat for 30 minutes. Allow the mixture to cool, remove the Snyder column, add 10 ml of clean distilled water and 1 ml iso-octane. Put a ground glass stopper on and extract by shaking vigorously for 5 minutes. Centrifuge to separate the phases. Remove the iso-octane and dry the extract by passing it through a Pasteur pipette plugged with glass wool which contains a few grams of purified Na_2SO_4. Concentrate the extracts to an appropriate volume for GC analysis. The volume required to obtain sufficient response may be as low as 100 µl. The final reduction in volume can be facilitated by using a gentle stream of high purity N_2 over the solvent (if this is not available, dry purified air may be substituted).

P,p'-DDE is usually present at appreciably higher concentrations than the other members of the DDT family. Its contribution can be distinguished qualitatively and quantitatively from that of PCB components eluting at the same time in the HPLC fraction, by using fuming sulphuric acid as an oxidising agent. This removes p,p'-DDE but does not affect the PCB's. After ECD/GC analysis of this fraction, 1 ml of this oxidising agent added to an aliquot of the sample extract. The two phases are shaken vigorously in a stoppered glass tube for 2 minutes. The phases are allowed to separate and the upper layer is removed for injection into the GC. The decrease in the peak at the appropriate retention time is a measure of the concentration of p,p'-DDE originally present in the extract.

Additional techniques, such as automated two-dimensional gas chromatography or negative chemical ionisation GC/MS will be available for further confirmations as needed in the confirmatory and reference laboratory of Professor Jan Duinker Insitut fur Meereskunde, Kiel, Germany.

Generally, the quantitative composition of any sample matrix in a particular geographic region does not vary dramatically even over relatively long periods unless the region is subject to an acute input of a particular contaminant. Once the composition of each peak has been determined unambiguously with the techniques indicated above, identification in routine analyses of a series of such samples can usually be made on the basis of retention time data performed with only the one column type.

Table D.1. Stability of different compounds to different treatments.

Compound	Treatment H_2SO_4	Treatment (KOH/EtOH)	Transformation product
HCB	+	+	
1,1-dichloro-2,2-diphenyl-ethene (internal standard)	+	+	
gamma-HCH lindane	+	-	
2,5,2',6'-tetrachlorobi-phenyl (internal standard)	+	+	
alpha-HCH	+	-	
Aldrin	+	-	
p,p'-DDE	+	+	
heptaclor epoxide	-	-	
dieldrin	-	+	
o,p'-DDE	+	+	
o,p'-DDT	+	-	o,p'-DDE
p,p'-DDD	+	-	p,p'-DDMU
o,p'-DDD	+	-	
p,p'-DDT	+	-	p,p'-DDE
PCB's	+	+	
Toxaphene	+	-	
Phthalate esters	-	-	

+ = Stable against treatment
– = Not stable against treatment

Retention time determinations with modern GC equipment, including electronic integrators with fast response times (250 msec) can be carried out with a precision of 6-18 msec. Identification of a considerable number of compounds can be carried out by comparison of retention times in chromatograms of the sample and one or more mixtures of the compounds of interest. Some peaks eluting in the HPLC fraction may originate from CB congeners which are not present in the standards supplied. Comparisons with chromatograms of one of the Arochlor mixtures run under identical chromatographic conditions assists in their identification.

5.6 Quantification

The principle methods for quantification of compounds use both internal and external standards. In the external standard method, absolute response factors are determined by independent chromatographic runs of standards in known concentrations. These response factors are used to calculate the concentrations of compounds in the sample extract. The external standard method can be used when peak areas or heights are highly reproducible. Internal standards are added to the samples, preferably in a concentration range to produce a similar response on the ECD as the contaminants present in the samples. Preliminary "range finding" analyses are necessary to determine the amount of internal standard to be added to the samples. The addition of standard has to take place in a highly reproducible way. The internal standard method is calibrated in terms of response ratios, involving standards that do not occur in the environmental samples, do not interfere with the compounds of interest, are stable to all procedures applied to the samples and behave similarly to the compounds of interest. The recovery of the internal standard is used to more accurately assess the fraction of total sample injected.

The reproducibility of area counts from the integrator using the splitless injection technique can be as good as a few percent relative standard deviation (RSD). Under such conditions, the external standard method is reliable. Several injections should be made of standard solutions containing a range of concentrations, allowing the determination of the linear range of detector response and the response factor of each compound. The calibration mixture must be analyzed under the same instrumental conditions used for the samples. Differences in sensitivity of the detector for different compounds are accounted for by using the individual response factors. The amount injected, therefore, has to be highly reproducible. This system has to be calibrated frequently in order to minimize instrumental (column) drift.

After comparison of the retention times of peaks in the sample chromatogram to those of a corresponding standard, peak heights (or areas) are measured. The concentration of a particular compound is then calculated using the following formulas. As an example, the concentration of a compound in biological tissue, calculated as ng g^{-1} dry weight or lipid weight as follows:

First, the response of the ECD to each compound to be quantified is calculated from injections of standards. The response factors (RF's) are calculated as:

$$RF = C_{st} \times V_{st} \times 1/h_{st}$$

where h_{st} = peak height of the compound in the standard (mm)
V_{st} = volume of the standard injected (µl)

C_{st} = concentration of the standard (ng μl^{-1})

The RF's are, thus, tabulated as ng mm^{-1}; or if an area is measured h_{st} is replaced by a_{st} and the RF's are tabulated as ng per unit area.

Second, from a known aliquot of the sample injected the response of the ECD to each peak to be quantified is measured (peak height in mm, h_{sam}). Note that integrator areas are usually reported independently of the chart attenuation. But if peak heights or areas are measured by hand, then the chart attenuation must be the same between standard and sample injections or an appropriate correction in the calculations.

Third, perform the following calculation for each peak:

$$C_{sam} = h_{sam} \times RF \times \frac{A}{V_{sam}} \times \frac{1}{M}$$

where h_{sam} = peak height of the compound in the sample (mm)
V_{sam} = volume of the sample extract injected (μl)
M = dry wt. or lipid wt. of the sample (g)
A = total volume of the sample extract (μl)
C_{sam} = concentration of the compound in the sample (ng g^{-1} dry wt., or ng g^{-1}lipid)

Again, h_{sam} can be replaced with a_{sam} and the RF expressed in area units applied.

The quantity (A/V_{sam}) is the inverse fraction of the total sample analyzed and is termed the dilution factor.

An internal standard can be used to correct for losses of analyte throughout the analytical procedure and for errors in volume measurement (which increase as sample volume is decreased) and for slight variations in detector responses. The response factor for the internal standard (RF_{IS}) is determined as in equation 1 from several injections of the IS under identical chromatographic conditions as for samples. Sufficient amount of the internal standard is added to the sample before extraction to result in a measurable peak in the final chromatogram of the extract. The peak height of the internal standard measured in the sample chromatogram (h_{is}) is then used to compute a corrected dilution factor as follows:

$$IS \times 1/RF_{is} \times 1/h_{is} = XF_{is}$$

where IS is the total amount of internal standard that was added to the sample (in ng). This corrected dilution factor (dimensionless, ng ng^{-1}) replaces XF (dimensionless, vol vol^{-1}) in the calculations applied to sample peaks:

$C_{sam} = h_{sam} \times RF \times (IS \times 1/RF_{is} \times 1/h_{is}) \times 1/M$ (ng g^{-1} wet wt)

Under ideal conditions, $XF = XF_{is}$.

The construction of an analysis data reporting format for PCB's is not without problems. Background information for the selection of the reporting criteria used here is given below.

The number of PCB congeners which can be analyzed accurately under present conditions is restricted to those that are well separated from interfering compounds on the SE-54 column. In addition, the list is limited to those congeners that are available as reference compounds in sufficiently pure forms. The congeners that satisfy these requirements and that have been identified in environmental samples and commercial mixtures and that are in available standards are listed in Table D.4.

These components should be identified and evaluated quantitatively. The concentrations of these compounds in the sample matrix are calculated according to the formulae given in this section. Although a list of concentrations of the major PCB congeners present in environmental samples represents, both qualitatively and quantitatively, the most detailed and accurate information on PCB's in the particular sample, this does not result in the most convenient form for comparison purposes. For qualitative purposes, e. g., when comparing the composition of PCB mixtures from different samples, it would be more convenient to have data on the relative contribution of each PCB congener in the mixture. This information can be readily obtained by summing the concentrations of the individual congeners (PCB_{sum}) and calculating the percentage of each component in the mixture. The percentage contributions, are not dependent on the way the absolute concentration (e.g., in $\mu g\ g^{-1}$) have been expressed nor whether on a wet, dry or lipid weight basis. Though the absolute concentration may differ widely, qualitative comparisons between PCB mixtures in different samples is facilitated. For example, when evaluating the differences in composition of PCB's in organisms, the dependence on age, sex, season, etc. may play an important role in the distribution of individual congeners. Thus, in addition to the total PCB concentration, the relative contribution of each individual congener to PCB_{sum} will provide important information on the behavior of the individual congeners in the sample matrix.

PCB_{sum} represents the sum of concentrations of the congeners, accurately quantified individually. PCB_{sum}, therefore, approximates the sum of all congeners present in the sample (defined as total PCB) and it can serve as the basis for quantitative comparisons of total PCB concentrations in different samples. Therefore, in addition to concentrations of individual congeners, the reporting format requires their sum (PCB_{sum}) and their percentage contributions to PCB_{sum}.

The principles of this method are illustrated by a simple example, involving two samples and four congeners only.

	Sample 1		Sample 2	
	Conc. $\mu g\ g^{-1}$	Contrib. to PCB_{sum} %	Conc. $\mu g\ g^{-1}$	Contrib. to PCB_{sum} %
Congener A	3	30	5	25
Congener B	2	20	4	20
Congener C	4	40	9	45
Congener D	1	10	2	10
PCB_{sum}	$10\mu g\ g^{-1}$		$20\mu g\ g^{-1}$	

PCB_{sum} is an underestimation of total PCB concentration. The difference may depend on sample matrix and geographic region, and may typically be in the 10-30% range. It should also be appreciated that the same value of PCB_{sum} (and of course of total PCB) can represent widely different PCB compositions. This is the inherent difficulty of representing the content of PCB with one number.

Therefore, PCB_{sum} or total PCB values can only be compared reliably between samples if the corresponding mixtures are the same. This may be the case for mussels in different regions, or for different tissues of the same organisms, etc., but it may not apply for water and mussel tissue even in the same region.

Other congeners may be considered for evaluation as well. For this purpose, the PCB CBL 1 standards can be used.

6. Quality Assurance

During the Initial Implementation Phase, field-collected samples will be analyzed by two central laboratories. These laboratories will participate in Quality Assurance/Quality Control (QA/QC) exercises which are organized by the Project.

The need for quality control and intercalibration of analyses for chemical contaminants in environmental samples has been documented numerous times during the past two decades (e.g., UNEP, 1990). Some advantages of inter-comparison exercises include:

- create a frame of reference so that data from multiple labs can be used in a more comprehensive, regional assessments.
- introduce and evaluate advanced analytical methods
- permit self-evaluation by participating laboratories and assist with training new staff
- impose an external incentive to maintain internal quality control programs
- identify variation between laboratories and common sources of error which lead to this variation.

A goal of inter-comparison exercises is to reduce the inter-laboratory variation in analytical results. Such exercises are a mutual learning experience and are not a "test" to determine how close any particular analyst comes to the "correct" answer. A step-wise intercalibration exercise should sequentially include: a) analysis of standard solutions, b) check of participants ability to prepare quantitative standards mixtures, c) analysis of cleaned extracts, d) analysis of whole extracts (participant clean-up), and finally e) analysis of environmental samples.

In addition to analysis by the two central laboratories, Host-Country scientists participating in the Initial Implementation Phase will be encouraged to analyze a subsample of the organisms collected at their site and to participate in an inter-laboratory comparison exercise. Standard Reference Materials (SRMs) and a homogenized bivalve tissue sample will be distributed to participating analytical laboratories. This material should be used to establish the precision of the laboratory by performing an initial analysis of three aliquots of the reference material. Levels of the designated chlorinated hydrocarbons should be tabulated on both a dry weight and a lipid weight basis. Means and standard deviations are then computed. These data will establish the expected precision of replicate analysis for each compound analyzed. The laboratory is requested to submit to the Project Secretariat results of the initial replicate analysis, and the field-collected tissue as well as their quality control charts for inter-comparison with other participating and reference laboratories.

Quality control charts are constructed by reanalyzing further aliquots of a reference material on a regular basis. If a subsequent analysis deviates by more than one standard deviation of the expected mean value, then the analyst should reassess the precision of the analytical procedure and correct errors. Plot the results of the repeated analyses of SRMs on a simple chart, which contains

guidelines to indicate the quality of the data. This chart is known as an analytical quality control chart (Fig. D.2).

Analysts are reminded that before a method is used routinely for samples it must have been rigorously assessed to ensure that it will provide data of the required quality. The following procedure is used to construct an AQCC:

(i) Select the SRM to be analysed with samples on a regular basis.

(ii) Analyse the SRM at least 10 times for the analyte(s) under examination. These analyses should not be done on the same day but spread out over a period of time in an attempt to ensure that the full range of random errors within and between batch analyses are covered.

(iii) Calculate the mean value (X), and the standard deviation (sd) and then plot the following values on a blank control chart: X, X + 2sd (UWL), X + 3sd (UCL), X - 2sd (LWL) and X - 3sd (LCL).

Assuming that the analytical measurements for SRMs follow a normal distribution, 95% of them (19 in every 20) should fall within the area between UWL (upper warning limit) and LWL (lower warning limit). Similarly 99.7% of the results should fall within the area between UCL (upper control limit) and LCL (lower control limit).

The analyst should regularly analyze SRMs and plot the results of the analysis of the SRMs after each batch of analyses to check where the data lie in relation to these limits, as shown in the example of a plot given in Fig. D.2.

The following guidelines can be used to assess whether the data for the SRMs and consequently the data for the samples are of acceptable quality:

(a) A single result which falls outside the warning limits need not require the analyst to doubt the results or take any action provided that the next result falls within the warning limits.

(b) If the results fall outside the warning limits often, particularly if the same warning limit has been crossed more than once on consecutive results, then the analyst needs to assess the source of this systematic error.

(c) If the results on more that 10 successive occasions fall on the same side of the X line (either between X and UWL or X and LWL) then the analyst needs to check the analytical procedure to determine the cause of this error.

(d) If the result falls outside the UCL or LCL lines then the analyst should check the analytical procedure to determine the cause of this source of error.

If any of the above cases occur the analyst should reject the results of the analysis of the particular batch of samples and should not carry out any further analysis of samples until the source(s) of the errors have been identified and he/she is satisfied that the results of future analyses will be within acceptable error limits.

The accuracy of a method can only be checked with a SRM for which the mean values and standard deviations are well documented (i.e. "certified"). Analysts who choose to use their own specially prepared reference material (RM) for quality control purposes should note that they are primarily checking the precision of measurements and not their accuracy. These internal RMs are very convenient, for analyses where large quantities of materials are required for each determination (e.g., analyses for organic contaminants) where the cost of SRMs for QC charts would be prohibitive or for sample types and analytes for which no certified SRM is available.

More detailed discussion of QA/QC practices is available in the published literature (see e.g., Taylor, 1985 and UNEP, 1990 and references sited therein).

7. Reporting of Results

A example format for reporting analytical results for PCBs, and DDT, DDD and DDE is given in Table D.3. Full details should be recorded for every entry.

8. Sources and Description of Certified Mixtures

Standards are commercially available through the National Institute of Standards and Technology (formerly National Bureau of Standards (U. S. A.) and from chemical suppliers who have agreed to prepare certified SRMs for the U.S. Environmental Protection Agency. Selected chlorinated pesticide SRMs will be shipped by the Project to participating analysts during the Initial Implementation Phase.

The IOC reference solution of PCB congeners PCB 1 (IOC-KIEL) in iso-octane contains 16 congeners at concentrations of about 1 ng μl^{-1}. Requests for a 500 µl vial should be directed to Head of Marine Pollution and Monitoring Unit, IOC UNESCO, 7, Place de Fontenoy, 75700 Paris, FRANCE.

A set of four mixtures (CLB 1-A, -B, -C, -D) in iso-octane can be purchased from the National Research Council, Atlantic Research Laboratory, 1411 Oxford Street, Halifax, Nova Scotia B3H 3ZI, CANADA.

Table D.4 presents the different PCB congeners present in two available (PCB 1 and CLB 1) standards.

Table D.3. Result reporting format.

Analytical Report

1. Sample Code _____

2. Determination of dry weight or moisture content in oven
   2.1 Duration of drying: _____ hours
   2.2 Date of drying (day, month, year): _____
   2.3 DW/WW ratio: _____ or moisture content _____

3. Analytical result
   3.1 Date (day, month, year): _____
   3.2 Result:

	p,p'-DDT	p,p'-DDD	p,p'-DDE	PCB_{sum}
$\mu g\ kg^{-1}$ WW of individual subsamples				
$\mu g\ kg^{-1}$ lipid of individual subsamples				
$\mu g\ kg^{-1}$ WW mean				
stand. dev				
coef. var.				
$\mu g\ kg^{-1}$ lipid mean				
stand. dev.				
coef. var.				

D.3 (continued) Composition of PCB mixture

Congener	ng g^{-1} individual subsamples				mean	std.dev. %	% contribution of ave. conc. of individual congeners in ave. (PCB_{sum})
18	____	____	____	____	____	____	_____
26	____	____	____	____	____	____	_____
52	____	____	____	____	____	____	_____
49	____	____	____	____	____	____	_____
44	____	____	____	____	____	____	_____
101	____	____	____	____	____	____	_____
151	____	____	____	____	____	____	_____
149	____	____	____	____	____	____	_____
118	____	____	____	____	____	____	_____
138	____	____	____	____	____	____	_____
187	____	____	____	____	____	____	_____
183	____	____	____	____	____	____	_____
128	____	____	____	____	____	____	_____
180	____	____	____	____	____	____	_____
170	____	____	____	____	____	____	_____
194	____	____	____	____	____	____	_____
PCB_{sum} $\mu g\ kg^{-1}$ lipid	____	____	____	____	____	____	_____

D.3 (continued) Other results:

	µg kg^{-1} subsamples				Mean	std. dev. %
p,p'-DDT	____	____	____	____	_____	_____
p,p'-DDD	____	____	____	____	_____	_____
p,p'-DDE	____	____	____	____	_____	_____

5. Estimation of accuracy
   - 5.1 Date (day, month, year): _____
   - 5.2 Type of certified standards used: _____
   - 5.3 Internal or external standard method used: _____

6. Anomalies observed during test and other remarks relevant to the interpretation of results:

_____
_____
_____
_____
_____

7. Quality control intercalibration (give details): _____

_____
_____
_____
_____

8. Full address of the institution which carried out the analyses:

_____
_____
_____
_____

9. Name(s) and signature(s) of the person(s) who carried out the analyses:

_____
_____
_____

Date: _____

Attachment: Sampling and sample preparation protocol relevant to the analyzed samples (IOC, UNEP, IMW, 1988).

Table D.4 PCB congeners present in the PCB 1 and CLB 1 standards. Identification of congeners by numbers (Ballschmiter and Zell, 1980).

PCB 1	CLB 1-A	CLB 1-B	CLB 1-C	CLB 1-D
18	18	15	15	15
26	54	52	114	101
52	31	103	153	151
49	49	60	137	118
44	44	154	129	153
101	40	143	183	141
151	121	105	185	138
149	86	182	171	187
118	87	128	200	180
138	77	202	191	170
187	153	173	201	201
183	159	208	203	196
128	156	207	189	195
180	209	205	206	194
170		209	209	209
194				

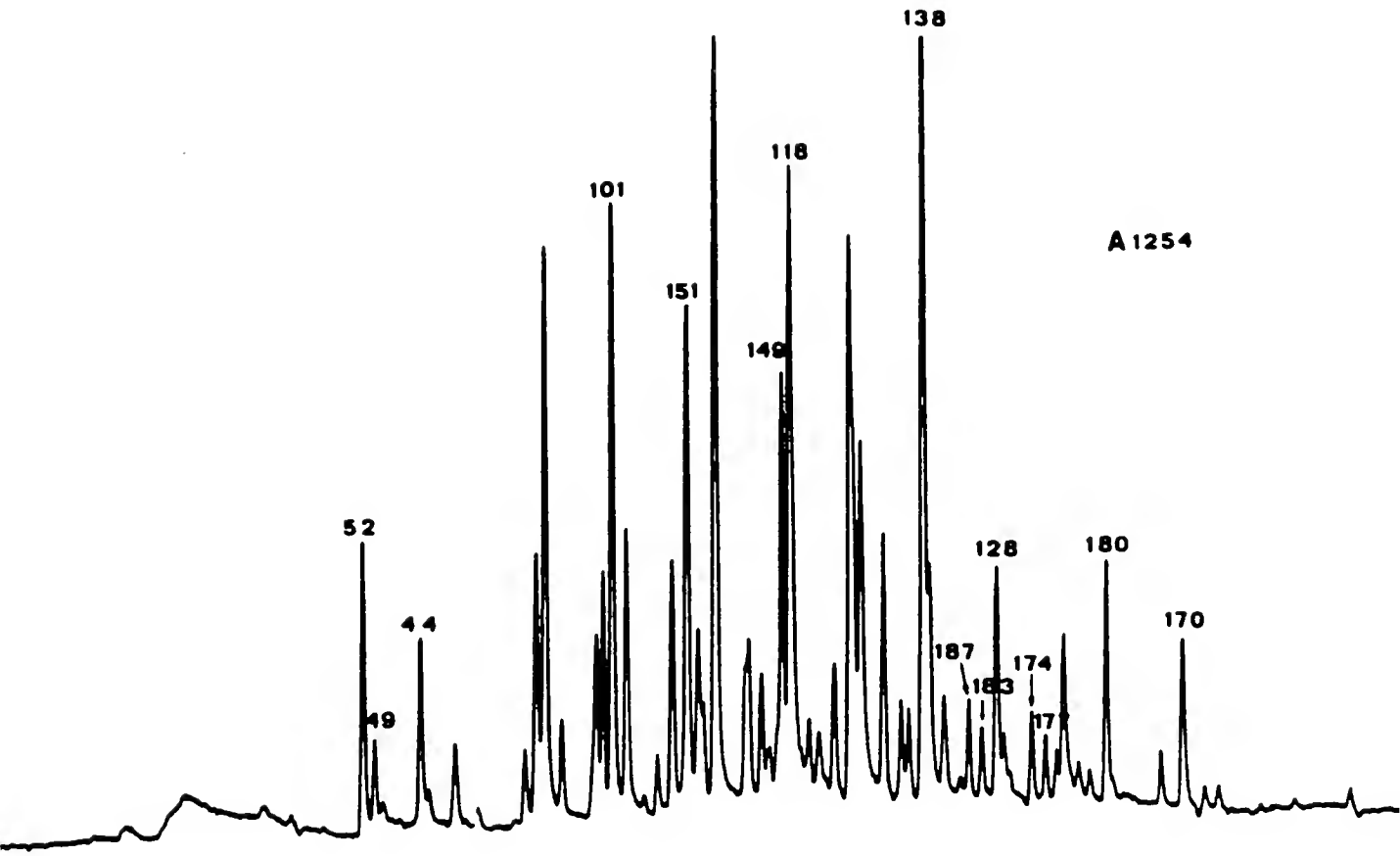
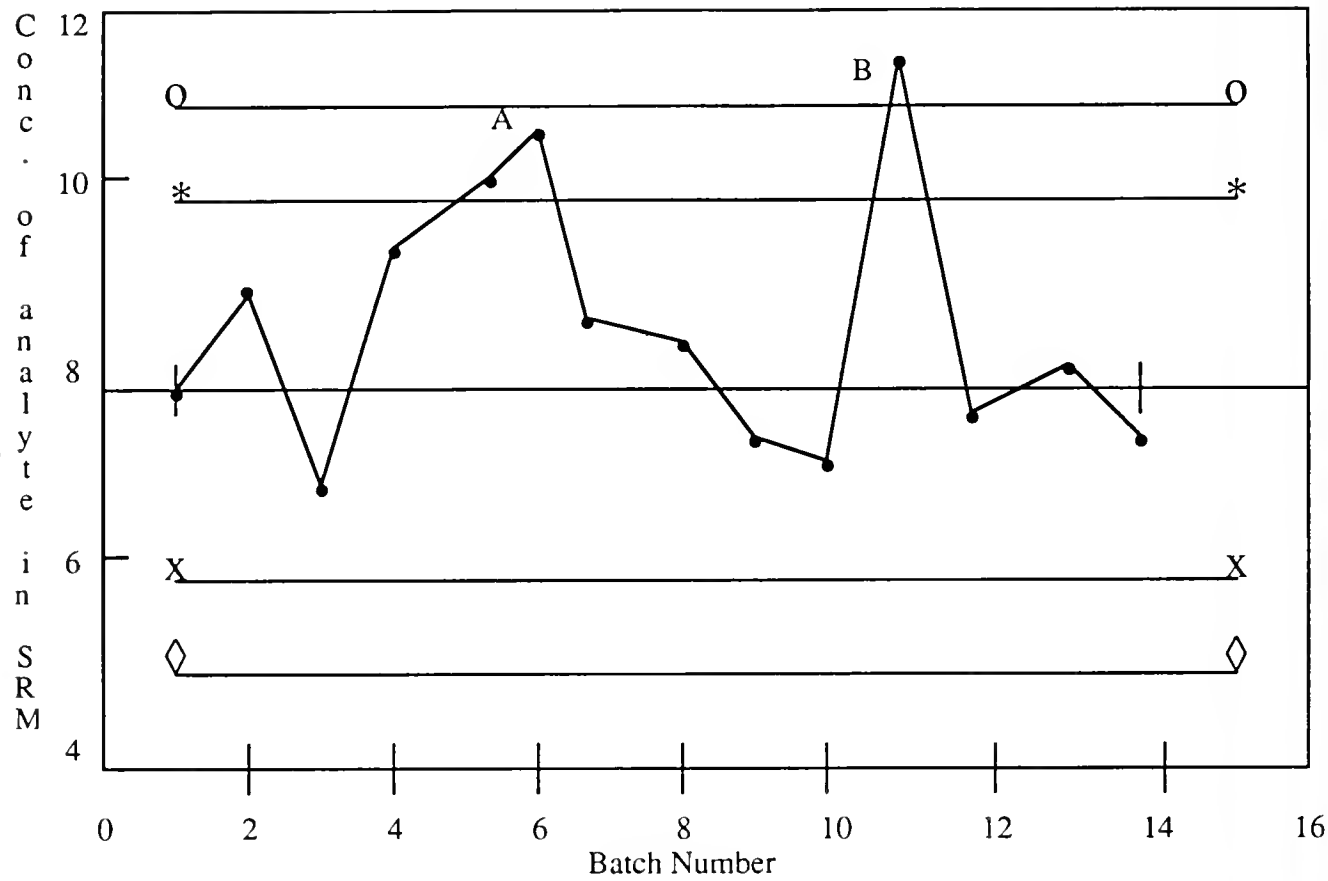


FIG. D.1: GC-ECD of Aroclor 1254, obtained from IAEA/ILMR, Monaco. Note the separation of peaks 149 and 118, indicating that the quality of the column is good. The position of the congeners (identified by numbers according to Ballschmiter and Zell, 1980) is indicated at the apex of the peak. All congeners which are contained in the PCB 1 (IOC-KIEL) standard solution are indicated, except for Nos.18 and 26 because they do not appear in Aroclor 1254. For their positions, as well as those of other less well resolved peaks, see e. g., Duinker and Hillebrand (1983) and UNEP (1988).



Symbols:

* upper warning limit

O upper control limit

X lower warning limit

◊ lower control limit

Fig. D.2. An example of Quality Assurance Control Chart, from UNEP (1990). A "consensus value" is established by repeated analyses of an SRM. Upper and lower limits are determined statistically from the standard deviation (sd) of the measurements made. Routine measurements of the SRM should be kept within the warning limits (i.e. between UWL and LWL). When apprent contamination occurs (i.e. at points A and B), the source of contamination must be found (e.g. reagents, dirty glassware) and corrected. Until the problem is resolved, analyses must be discontinued. Data from the affected samples are rejected.

References

BALLSCHMITER, K. and ZELL, M. 1980. Baseline Studies of the Global Pollution, I. Occurrence of Organohalogens in Pristine European and Antarctic Aquatic Environments. J. Environ. Anal. Chem. 8:15-35.

DUINKER, J.C., HILLEBRAND, M.T.J. and BOON, J.P. 1983. Organochlorines in the Benthic Invertebrates and Sediments from the Dutch Wadden Sea; Identification of Individual PCB Components. Netherlands Journal of Sea Research. 17(1):19-38.

MACLEOD, W.D., JR., BROWN, D.W., FRIEDMAN, A.J., BURROWS, D.G., MAYNES, O., PEARCE, R.W., WIGREN, C.A. AND BOGER, R.G. 1985. Standard Analytical Procedures of the NOAA National Analytical Facility, 1985-1986. Extractable Toxic Organic Compounds, Second Edition. National Oceanic Atmospheric Administration, NOAA Technical Memorandum NMFS F/NWC-92.

PETRICK, G., SCHULZ, D.E. and DUIKER, J.C. 1988. Clean-up of environment samples by high-performance liquid chromatography for analysis of organochlorine compounds by gas chromatography with electron-capture detection. J. Chromatogr. 435(1):241-248.

TAYLOR, J.K. 1985. Standard Reference Materials: Handbook for SRM Users. National Bureau of Standards Special Publication No. 260-100. U.S. Dept. of Commerce.

UNEP 1988. Determination of DDTs and PCBs by capillary gas chromatography and electron capture detection. Ref. Methods for Marine Pollution Studies No. 40.

UNEP 1990. Contaminant monitoring programmes using marine organisms: Quality Assurance and Good Laboratory Practice. Ref. Methods for Marine Pollution Studies No. 57.

UNEP 1991. Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. Ref. Methods for Marine Pollution Studies No. 12 Rev. 2.

UNESCO 1990. Standard and Reference Materials for Marine Science. Manuals & Guides # 21.

ZELL, M. and BALLSCHMITER, K. 1978. Single Component Analysis of Polychlorinated Biphenyl (PCB)- and Chlorinated Pesticide Residues in Marine Fish Samples, Identification by High Resolution Glass Capillary Gas Chromatography with an Electron Capture Detector (ECD). Fresenius Z. Anal. Chem. 292:97-107.

ZELL, M. and BALLSCHMITER, K. 1980. Baseline Studies of the Global Pollution, II. Global Occurrence of Hexachlorobenzene (HCB) and Polyuchlorcamphenes (Toxaphene) (PCC) in Biological Samples. Fresenius Z. Anal. Chem. 300:387-402.

ZELL, M. and BALLSCHMITER, K. 1980. Baseline Studies of the Global Pollution, III. Trace Analysis of Polychlorinated Biphenyls (PCB by ECD Glass Capillary Gas Chromatography in Environmental Samples of Different Trophic Levels. Fresenius Z. Anal. Chem. 304:337-349.

Appendix E:

International Mussel Watch:

Questionnaire

Questionnaire used to solicit participation and assistance of Host-Country scientists

INTERNATIONAL MUSSEL WATCH

BACKGROUND INFORMATION/QUESTIONAIRE
HOST-COUNTRY SCIENTISTS

The International Mussel Watch Project will assess coastal contamination by organochlorine chemicals, primarily biocides and PCBs, using bivalve molluscs in the South-Central America and Caribbean region over the next year. This project may be extended to other parts of the globe and to other chemical contaminants in the future. To the greatest extent possible, International Mussel Watch will collaborate with existing national and regional monitoring programs. In order to be successful, the Project will need the active participation of working scientists in the region. These scientists will be expected to support the Field Scientific Officer in planning and carrying out the sampling. Participating scientists will benefit from technical assistance (e.g., data reports, methods manuals, etc.) and from access to Project data. For instance, we will assemble technical information for distribution to Host-Country Scientists and will supply chemical standard reference materials to those who are now analyzing organochlorines in their own laboratories. Additionally, the Field Scientific Officer will be a technical resource for participants and will be prepared to present a seminar to your colleagues during the sampling visit. Host-Country Scientists may participate in the International Mussel Watch Project at any level they choose:

1. Sampling Assistance only
   Make local facilities available to support the sampling; participate in the data-interpretation following the analyses; receive all technical informational materials distributed by the Project.
2. Sampling plus Analysis
   Assist with the sampling, as described above plus retain some sample material for analysis. Receive Standard Reference Materials and participate in inter-laboratory comparison discussions as well as data interpretation.
3. Informational only
   Will not participate directly, but will receive progress reports on the Project at intervals.

With this questionaire, we are seeking your confirmation that you will participate in this international program. Your response will help Project staff make a final determination of sites to be sampled and to develop a final list of IMW participants.

INTERNATIONAL MUSSEL WATCH

QUESTIONNAIRE
Initial Implementation Phase

Name

Title

Address _____

_____

_____

Telephone _____

Telefax _____

E-Mail _____

Telex _____

I. Please review the enclosed suggested sampling sites for your country and respond to the questions for the site (or sites, use seperate sheets) for which you have personal knowledge.

Site Name _____

Does this site contain adequate bivalve population for repeated sampling? Yes ____ No ____

What species predominate? _____

_____

Does the site drainage basin include agricultural areas? Yes ____ No ____

Is access to the site available? Yes ____ No ____

Auto _____

Boat _____

Would you recommend other sampling sites, not included in our list? Please specify._____

II. Local support is essential for the Project as local knowledge and institutional facilities will be required to assist the Field Scientific Officer. The Field Scientist will supply his own sampling equipment but will need your assistance and your institutions' resources to accomplish the sampling. We may not be able to sample a site if local support is not available.

Will you be able to supply overnight accommodations for the Field Scientific Officer? Yes ____ No ____

Will you arrange for local ground transport?

To and from the airport? Yes ____ No ____

To the field site? Yes ____ No ____

Will you arrange for local permits and access to the site prior to the sampling visit? Yes ____ No ____

Will you or your technician be available to assist in the field and in sample preparation? Yes ____ No ____

Do you have adequate freezer space to temporarily store field samples in transit with the Field Scientist? Yes ____ No ____

Please indicate dates which will be most convenient for you to host a visit from the Field Scientific Officer (Jan 1992 - May 1992).

_____

III. Existing historic data, produced by your institution or by other academic and government agencies will useful as we interpret the data generated by the analyses of Project samples. We will seek the assistance of Host-Country scientists to identify and acquire such data.

Do you have your own (or your Institutions') data sets/data reports on:

Organochlorines?	Yes _____	No _____
Other organics?	Yes _____	No _____
Nutrients?	Yes _____	No _____
Metals?	Yes _____	No _____

Please include copies of data and technical reports with this questionnaire, if possible. Please include an inventory list if copies are not available.

Does your Institution have a collection of technical reports? Yes _____ No _____

Will you obtain production and use data and regional land use data prior to the arrival to the Field Scientist? Yes _____ No _____

IV. Where local analytical facilities are established, the Project will support these efforts with information and materials. We will distribute Standard Reference Materials and Methods and QA/QC documentation. We will assist with data interpretation of locally analyzed samples as well.

Do you now analyze for organochlorines

in sediment? Yes_____ No_____

in tissue? Yes_____ No_____

Do you now analyze for other chemical contaminants

other organics? _____

metals? _____

Do you use a specific quality control program for these analyses?	Yes _____	No _____
Have you participated in inter-laboratory intercomparison exercises?	Yes _____	No _____
Do you need Standard Reference Materials?	Yes _____	No _____
Will you participate in the analysis of Project samples?	Yes _____	No _____

Please include copies of organochlorine analytical methods currently being used in your lab, including quality control practices and intercomparison reports with this questionnaire.

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