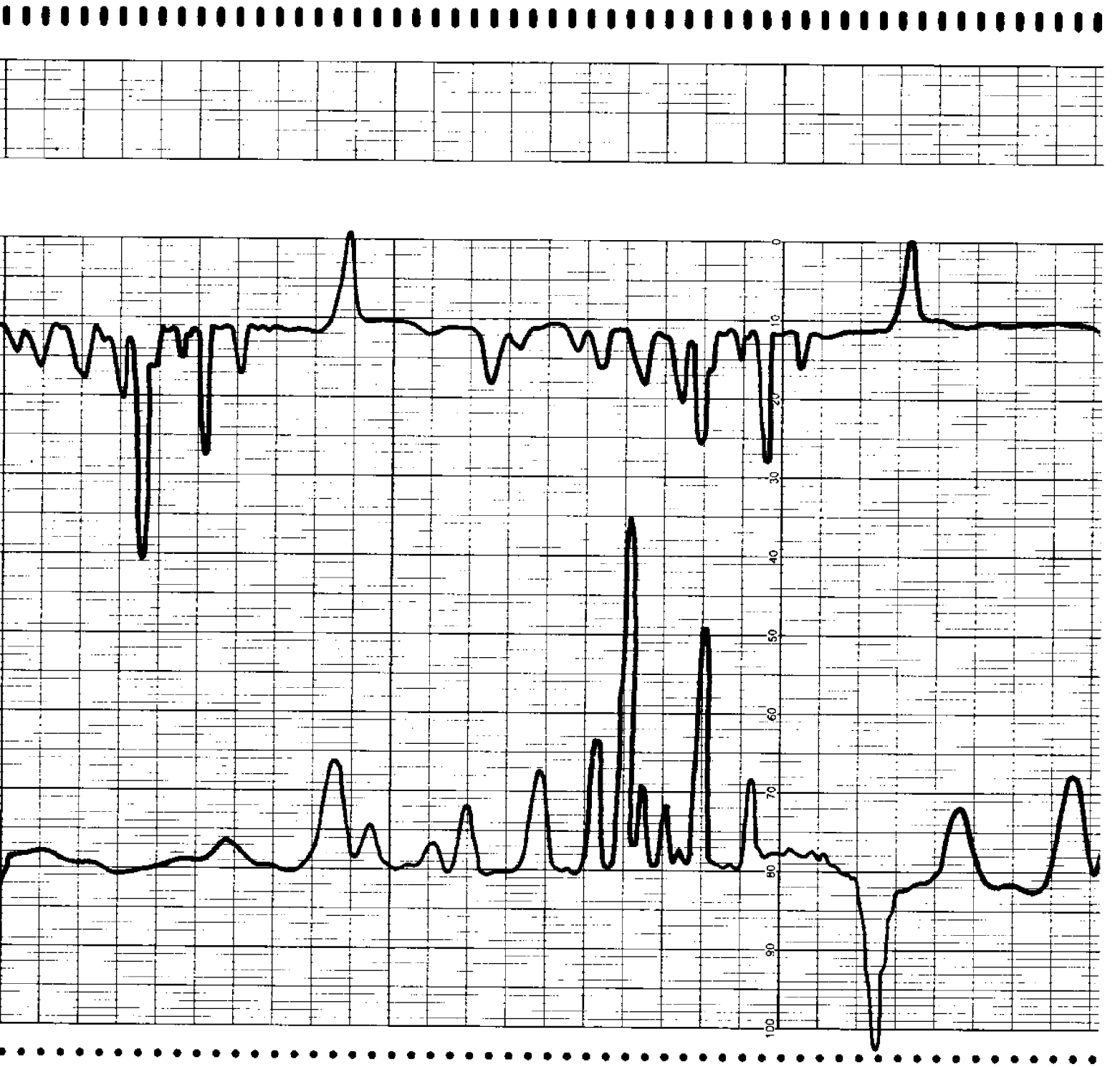
Methods for Analysis of Organic Compounds in the Great Lakes



Joseph J. Delfino, Editor — University of Wisconsin Sea Grant Institute

Methods for Analysis of Organic Compounds in the Great Lakes

Joseph J. Delfino, Editor

**The Proceedings of an Invitational Workshop
Held April 1-2, 1980, at the
University of Wisconsin Great Lakes Research Facility
Milwaukee, Wisconsin**

University of Wisconsin Sea Grant Institute

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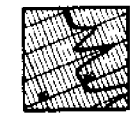

Preface

This report is a summary of an invitational workshop held April 1-2, 1980, in Milwaukee, Wis. The workshop was funded by the University of Wisconsin Sea Grant College Program and by a special research grant from the Great Lakes Research Facility, University of Wisconsin System.

The workshop culminated months of planning by many people. The efforts of the following individuals were critical to the success of the workshop -- Barbara Arnold, Lois Husebo, James Lubner and Helen Schopp of the UW Sea Grant's Advisory Services Division, and Donald Mraz, Abe Bowman, Arthur Brooks, Joan Flores and Delpfine Welch of the UW Great Lakes Research Facility.

Assistance was also provided by my staff at the University of Wisconsin-Madison; their names appear as the recorders of the various workgroup discussion sections. This report is based on their notes. Also identified in the discussion sections are the chairpersons of the workgroups, whose efforts are to be commended. Finally, the Sea Grant Communications staff was very helpful in the production of this report, particularly publications editor Stephen Wittman, production coordinator/designer Christine Kohler and Catherine Shinners.

To all of these individuals, and to the two funding organizations, I express my sincere thanks and appreciation.

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Introduction

Joseph Delfino, workshop coordinator, University of Wisconsin

The presence of anthropogenic compounds in the Great Lakes has been a significant regional environmental issue since the early 1960s. The ability of certain insecticides and industrial chemicals to bioaccumulate in Great Lakes organisms, due in part to their chemical structure and availability to the food chain, is a major reason for this concern. A number of fish species have accumulated polychlorinated biphenyls (PCBs), DDT and dieldrin at levels exceeding the maximum limits set by the U.S. Food and Drug Administration for human consumption. The study of micro-contaminants, especially organic compounds, has been a major part of the research program of the University of Wisconsin Sea Grant Institute.

The validity of standards like the FDA's human consumption limits is open to question. In the rush to collect data to support the development of such regulatory standards, a more fundamental need for data is seldom emphasized enough -- that is, precise and accurate analytical data are essential for enforcing existing regulations, assessing the economic impacts and determining the public health implications of the presence of organic compounds in Great Lakes water and fish.

The analysis of organic compounds like PCBs becomes increasingly difficult as the complexity of the environmental matrix increases. This complexity creates significant problems in the quantitative separation of the desired analyte(s) from the matrix, particularly from fish and sediment. Further confounding the issue is the fact that PCBs and other complex organic mixtures are "weathered" (i.e., partially degraded, metabolized or otherwise chemically rearranged) in and by the environment. Thus, the longer the materials are in the environment, the more difficult they become to identify.

These problems lead to ambiguity in qualitative identification as well as in determining the precise amount of contaminant present. In effect, each analyst has to exercise considerable independent judgement to resolve such complexities before completing an analysis. This may result in a lack of agreement among laboratories as different analysts use different criteria to analyze and quantitate organic contaminants.

One of the main analytical methods of choice for organic compounds is gas chromatography, using either one or more of the traditional detectors (e.g., flame ionization and electron capture). Frequently, gas chromatography is coupled with a mass spectrometer. Most, if not all, of the organics of interest can be extracted from environmental matrices into volatile solvents, derivatized if necessary, and then injected into a gas chromatograph. This field has become quite sophisticated in recent years, particularly in terms of the instrumentation available. The interpretation of the gas chromatograms and mass spectra, however, requires considerable experience and skill.

It is important for researchers involved in the analysis of Great Lakes matrices to use validated analytical protocols, or at least protocols that provide equivalent results in spite of actual step-by-step methodological differences.

The chief objective of this workshop was to determine the extent of agreement and disagreement among research scientists and analytical chemists dealing with organic compounds found in the Great Lakes. By inspecting gas chromatograms and mass spectra and discussing point-by-point the details of the methods used, we hoped to overcome the limitations of relying solely on published papers, where gas chromatograms and mass spectra are usually artistically stylized and methods sections are kept very brief. On the basis of the collective experience of workshop participants, we hoped to determine which analytical approaches are appropriate to the analysis of water, biota, sediment and atmospheric samples for organic compounds. And lastly, we attempted to establish, where possible, a consensus among the participants regarding the most useful and applicable analytical techniques for use in the Great Lakes.

What follows is a summary report reviewing the discussions held at the workshop and presenting certain options for those involved in Great Lakes analytical work. More than 60 U.S. and Canadian scientists participated in the workshop, which was divided into four workgroups -- polychlorinated biphenyls, pesticides, industrial chemicals,and polynuclear and aliphatic hydrocarbons. A number of papers either selected or supplied by workshop participants are reprinted in Appendix A as a source of additional, detailed information.

The objectives of this workshop were essentially fulfilled by the time it concluded. Differences among analysts will remain, however, since each analyst has developed methodology tailored to specific research and/or monitoring programs. Regulatory agency personnel will continue to apply mandated techniques, while others will utilize the best methods available in the literature, regardless of agency sanction.

Perhaps the most apparent difference among the analysts at the workshop involved the type of column used in gas chromatography. The traditional packed column technique is slowly giving way to capillary column technology. But until most analysts begin using capillary columns, there will continue to be differences in analytical reporting -- both in terms of the number of compounds found (more with capillary compounds due to better separation and resolution) and the means of quantification.

Perhaps the strongest consensus reached concerned the need for some organization to develop and maintain an active, round robin sample analysis program. To some extent, the U.S. Environmental Protection Agency and the International Joint Commission are already doing this for various programs, but the most significant need is in fish samples. Few "standard" fish have been circulated widely enough at this time to enable most interested laboratories to check their performance against others. When such a program is in effect on a regular basis, the quality of data on fish contaminants in the Great Lakes will be better known and be of more use to resource managers in the future.

Workgroup Reports

Methods for Analysis of Organic Compounds in the Great Lakes

Workgroup on Polychlorinated Biphenyls (PCBs)

M. D. Mullin, chairman, U.S. Environmental Protection Agency

T. A. Gibson, recorder, University of Wisconsin-Madison

Participants in the PCBs workgroup represented a wide variety of technical backgrounds. Interests ranged from routine regulatory agency monitoring activities to state-of-the-art method development and environmental and toxicological research. This broad perspective led to some interesting and stimulating discussions, with the effect that all workgroup members gained an appreciation for the extent of PCB-related activity in the Great Lakes Basin.

■ FEDERAL REGISTER METHOD FOR PCBs

The initial discussion centered on the methods proposed by the U.S. Environmental Protection Agency (EPA) for use in the National Pollutant Discharge Elimination System monitoring programs for PCBs in effluents. PCBs were included in the EPA method No. 608, which also includes organochlorine pesticides (Federal Register 44(233):69501, December 3, 1979).

Dwight Easty of the Institute of Paper Chemistry, Appleton, Wis., suggested certain revisions to the proposed EPA method that were particularly aimed at separating the steps for organochlorine pesticides from those to be used when only PCBs are of interest. The Federal Register cited above should be reviewed for a description of the complete method. In summary, Easty suggested the following:

1) modify the extraction solvent (use petroleum ether, 30°-60° cut, or hexane in lieu of the prescribed methylene chloride;

2) do not require a Kuderna-Danish solvent evaporation device but also allow techniques such as rotary evaporators, a beaker with a dry nitrogen stream or low-temperature heating if the solvent isn't taken to dryness;

3) do not require a pH adjustment or refrigeration of a sample unless authenticated experiments indicate the need for such precautions (Methylene chloride was mentioned as being effective for stabilizing water samples. It also helps to start the extraction sequence. Air space at the top of the collection bottle immediately above the sample should be minimized.);

4) do not require specific amounts of Florisil (Floridin Corp., Pittsburgh, Pa.), since this material is used for clean-up and not fractionation.

5) elute PCBs from Florisil with hexane alone because 6% ethyl ether in hexane elutes potential interferences in addition to the PCBs;

6) consider a quick sulfur-removal step -- add mercury dropwise to a test tube of the sample solvent extract and shake 10 to 20 minutes, continuing until a fresh dose of mercury ceases to turn black, thus indicating the absence of sulfur;

7) discuss the problem with PCB Aroclor standards (i.e., depending on the source of the PCB standards, various chromatographic patterns [fingerprints] can be observed due to differences among production lots of the various commercial Aroclors that were formerly produced);

8) expand the variety of liquid phases allowed for packed column chromatography.

Related comments concerning various steps in the previous EPA protocol for PCBs in industrial effluents, particularly those related to pulp and paper mills, appear in a paper by Delfino and Easty (1979) in Appendix A.

Workgroup participants commented on what appeared to be a philosophical conflict regarding publication of a method that must be adhered to rigorously unless an alternative protocol is approved by the EPA. The reluctance of those not affiliated with regulatory agencies to embrace verbatim a mandated method is understandable, though regulatory agency activities necessitate standardized, legally enforceable techniques -- a situation frequently encountered in the effluent monitoring program.

Despite the obvious advantage of having an EPA-sanctioned method for PCBs alone published in the Federal Register, thereby simplifying the number of steps involved, there was no clear workgroup consensus as to the type of action that should be taken to try to convince EPA to alter its position. Perhaps not enough of the attendees had occasion or are required to use Federal Register-mandated methods in their work and could therefore employ whatever available method desired as long as it was applicable to the specific needs at the time.

■ CAPILLARY COLUMN GAS CHROMATOGRAPHY

Discussion also focused on capillary column gas chromatography. Glass capillary columns have been used throughout the world for many years, particularly in Europe, but only recently have they taken hold in North America. The workgroup was about evenly divided between packed column and capillary users. Those using packed columns were often prevented from converting to capillary columns because of unadaptable instruments. Others felt that packed columns provided adequate separation efficiency for their purposes. Capillary column users, on the other hand, did not hesitate to proselytize their colleagues. In the words of one chemist, "once you have tried capillary columns, you'll never go back to packed columns." Apparently, few chemists disagree.

The packed versus capillary column discussion, while mainly in the PCBs workgroup, was a topic common to each workgroup.

Gas chromatography (GC) users are turning to capillary columns in increasing numbers. Some chromatographers feel that packed columns will be obsolete in the near future, while others see a continuing -- though reduced -- role for them in the future. It is probably a matter of the "haves" versus the "have-nots." Unless one has capillary column capability, the arguments are moot. If a laboratory does have such capability, individual experiences will determine whether the conversion to capillary systems is total, or if both technologies are maintained to meet various needs.

Probably of greater importance is the problem of dealing with the significantly increased number of peaks observed in capillary columns as compared to packed columns. When analyzing environmental sample extracts, this becomes a significant issue. Some problems can be avoided by taking precautions during the column chromatographic separation processes. Yet many residues will continue to co-elute with one another in the same solvent fraction. Thus, when doing PCB analyses -- despite the reasonably good separation of PCBs from most other organochlorine compounds (principally pesticides) -- there may still be a carry-over. A common example is the determination of PCBs in Lake Michigan fish, especially the salmonids. The p,p'-DDE residue is almost always co-extracted with PCBs in the 94%:6% hexane:ether eluate fraction passing through a Florisil column.

■ PATTERN RECOGNITION

A corollary to the capillary column issue is the manner of identifying specific commercial Aroclor PCB residues and subsequently quantifying these residues. Some did not see any value in trying to relate residues to specific Aroclors; rather, with the power of the capillary column separation, it is now possible to identify most PCB isomerides individually (an isomeride is defined as one of a set of compounds that have similar structural groups but not necessarily that same number of atoms, according to Hackh's Chemical Dictionary [1969]. Many authors use the terms isomer or cogener to represent what might more appropriately be termed isomeride).

The problem with this approach is at least twofold. First, regulatory actions are based on source reconciliation, and the "matching" of Aroclor patterns from known sources with contaminated environmental samples serves a specific purpose in enforcement actions. Second, and of major significance is the lack of availability of all 209 PCB isomerides. Without individual compounds available for use as standards, the full power of the capillary column chromatographic separation has yet to be exploited. However, the EPA Large Lakes Research Station (Grosse Ile, Mich.) has contracted with Stephen Safe of the University of Guelph (Ontario, Canada) to synthesize a number of specific chlorinated biphenyls. These compounds will not be available in quantity, but the EPA will assess their gas chromatographic behavior on capillary columns and will provide information on their relative

retention times, using standardized conditions and various capillary column liquid coatings. In this manner, at least until all of the PCB isomerides are commercially available (if ever), gas chromatographers will be able to qualitatively identify many of the observed PCB peaks seen on capillary column chromatographs.

David Weininger of the EPA's Duluth, Minn., laboratory has developed a computer program (unpublished) to statistically reduce capillary column PCB data and to assign the most probable Aroclor formulation to a residue based on its capillary column "fingerprint." Until most or all of the individual PCBs are available commercially, this computer-surveyed capillary column "pattern recognition" approach (developed by Weininger and David Armstrong at the University of Wisconsin-Madison) offers promise as a suitable means of rationalizing the more than 50 peaks typically seen on capillary column chromatograms of PCB-contaminated Great Lakes matrices. A recent paper by Krupcik et al. (1980) contains information related to this discussion.

Denis Foerst of the EPA's Cincinnati, Ohio, laboratory discussed quantitation of Aroclor mixtures by gas chromatography/mass spectrometry (GC/MS). The technique searches various mass/charge ratios at the appropriate retention times for various PCBs, in effect doing selected ion monitoring for several molecular ions at specific GC retention times.

■ METHOD CALIBRATION

Standards. The question of adequate and available standard PCB materials is still unresolved. With PCBs no longer manufactured, analytical standards have to be obtained from those organizations that maintain repositories or inventories for laboratory purposes. The EPA and some commercial organizations are the principal suppliers, but the quantities available are limited or relatively expensive. Of some concern are lot-to-lot differences in the original commercial production runs that result in different chromatographic fingerprints and can affect interlaboratory comparison studies. This issue will not become easier to resolve, since U.S. production of PCBs ceased in the mid-1970s. The recommendation of the workgroup included securing PCB standards from the U.S. Food and Drug Administration (FDA) and then calibrating stock solutions with samples prepared by the EPA laboratory in Cincinnati.

The trend toward individual isomeride identification and quantitation will partially alleviate the Aroclor mixture problem, but until most or all of the individual PCBs are available, analysts will still have to overcome the problems inherent in residue analysis through fingerprint pattern recognition.

Quantitation. There was some discussion covering the methods of quantitation (e.g., peak area, peak height, etc.). The workgroup consensus indicated a preference for quantitation by peak areas, particularly when resolution is high. This can be particularly achieved with capillary columns. It was suggested that an effort be made to improve the consistency of sequential injections, which is possible with the automated GC injection systems now available.

The Webb and McCall (1973) and Sawyer (1978; see Appendix A) methods of quantitation were discussed. The discussion was stimulated by the question of what an analyst should do when interfering peaks appear. This is likely to be a greater problem with packed columns than with capillary columns. In Great Lakes fish, for example, p,p'-DDE generally is unresolvable from the PCB pattern on packed columns, and most preliminary clean-up and separation schemes fail to resolve the p,p'-DDE from PCB peaks, at least when using packed columns in the GC.

Reporting results for environmental samples can be problematical. Some participants believed that it is incorrect to report PCB data as specific Aroclors; it is better to say that the Aroclor pattern in the sample extract "resembles" a particular commercial mixture. The analyst can report that results are based on the Aroclor used for quantitation. Since "weathering" is a frequent problem, particularly with PCBs extracted from Great Lakes fish and sediments, the problem of quantitating the weathered pattern is significant. Again, analyzing a sample for specific isomerides (e.g., those which have been shown to be most toxic) may resolve some of the problem in the future.

Perchlorination was suggested as a solution to the pattern recognition problem. The goal is to convert all PCBs to decachlorobiphenyl and then quantitate them on the basis of this single compound. But research performed by Lawrence Burkhard and David Armstrong at the University of Wisconsin-Madison indicated that perchlorination does not yield quantitative results, despite earlier reports in the literature.

Further discussion focused on extending the Webb and McCall (1973) technique to capillary column systems. Since such a venture would involve considerable work, there were no immediate volunteers to initiate the study. If the pattern recognition approach continues to be used in conjunction with capillary columns, then it appears that a study extending the Webb and McCall (1973) approach to various capillary column coating materials is necessary, since many altered or "weathered" patterns are observed in extracts of Great Lakes sample matrices.

Carrier gases for capillary columns were briefly covered. Experimental evidence indicates that high-purity hydrogen provides the best selectivity and speed of analysis and is thus better than either helium or nitrogen.

■ ANALYSIS OF PCBS IN VARIOUS MATRICES

Many extraction procedures were discussed for each of the matrices likely to be analyzed in the Great Lakes Basin. Most are based on techniques in the Federal Register that are mandated for certain EPA programs and those listed in the FDA's Pesticides Analytical Manual. Also, methods developed by the Fish and Wildlife Service (FWS) of the U.S. Department of the Interior are widely employed. The references and Appendix A of this report should be consulted for further information.

Water and Wastewater. Methylene chloride appears to be the extraction solvent of choice by most analysts. Hexane may be suitable if PCBs alone are sought in any specific sample. Quality assurance recommendations are given in the Federal Register (1979) and should be followed.

Fish. FDA methods (Pesticides Analytical Manual) are generally applicable. Various means of extracting the residues from fish tissue can be used, including ether extraction in an explosion-proof blender. Soxhlet extraction after mixing with anhydrous Na_2SO_4 for eight hours or column chromatography percolation with 20% ether in hexane is often used.

Sediments. Many laboratories use a 1:1 acetone:hexane solution in conjunction with a Soxhlet apparatus. Steam distillation for eight hours is used at the University of Wisconsin-Madison and the EPA laboratory in Duluth, Minn. Pretreatment of the sample is important, including balancing the chemical oxygen demand prior to distillation. There is general agreement that samples should be wet prior to extraction; the EPA found 10% moisture by weight yielded good recovery (Bellar and Lichtenberg 1975). The 1:1 acetone:hexane mixture is the most common and apparently most appropriate solvent system to use. Chau et al. (1979) at the Canada Centre for Inland Waters (CCIW) (Burlington, Ontario) have developed a method for spiking sediments used in an International Joint Commission (IJC) round robin study in 1979-80. For details, contact Keijo Aspila at CCIW, or Robert White at the IJC in Windsor, Ontario, Canada.

Air. Various solid substrates have been used to collect PCBs in the atmosphere. Recently, XAD-2 resins have been reported to give quantitative recoveries (Doskey and Andren 1979; see Appendix A). The PCBs are extracted from the resin substrate using hexane in a Soxhlet extractor.

Clean-up Prior to Gas Chromatography. Various clean-up steps are used, among them alumina, silica gel or silicic acid, Florisil, high-pressure liquid chromatography, Sep-Paks (Florisil cartridges manufactured by Waters Associates) and gel permeation chromatography. Most workgroup members reported success with many of these materials. Apparently, there is no consensus, except that an analyst should try to apply one or more of these techniques to the samples to be analyzed and then determine the specific clean-up and interference removal steps that may be required.

Sulfur in sediments presents problems in gas chromatography and must be removed. The Federal Register (1979) suggests using mercury to eliminate sulfur, but many analysts use copper activated with dilute nitric acid to avoid the problems of handling and disposing of mercury in the laboratory. Some chemists place copper turnings at the base of a Florisil clean-up column and thus combine Florisil clean-up with sulfur removal.

■ SUMMARY

PCBs are probably the most studied class of organic contaminants in the Great Lakes today. This level of activity involves various groups, from regulatory agencies to universities. The result is that a variety of analytical techniques are employed to suit the needs of each laboratory. It appears that this approach will continue until standardized methodologies are generally accepted.

Workgroup on Pesticides

J. J. Lech, chairman, Medical College of Wisconsin

J. Allen, co-chairman, National Fishery Research Laboratory

D. J. Dube, recorder, Laboratory of Hygiene, University of Wisconsin

Contamination of the Great Lakes by persistent pesticides, especially the chlorinated hydrocarbons, has been known and documented since the 1960s. Considerable analytical effort has been expended to assess the scope of the problem, and extensive monitoring is now conducted by various state and federal agencies.

The initial discussion of this workgroup involved the identification of the major activities and interests of its members. State and federal agency representatives were concerned with screening various matrices, especially fish, for those pesticides that are regulated or are of interest to the FDA. These agencies monitor pesticide residue levels at the marketplace and accordingly have an impact on the activities of certain industries such as commercial fishing.

Other participants were interested in the environmental fate of pesticides, including the physical, chemical and biochemical interactions that affect the residues until they reach their ultimate sink in the Great Lakes system. This sink is typically the sediment of the lakes, though contaminated fish take their residues with them wherever they go, particularly as human or animal food.

■ CURRENT RESEARCH AND MONITORING ACTIVITIES

According to Robert Hesselberg, the FWS laboratory in Ann Arbor, Mich., has compiled a list of over 400 chemical contaminants that were found in lake trout and walleye from the Great Lakes. They used GC/MS to exhaustively study fish samples collected from one or more locations on each of the five Great Lakes.

Briefly, the method used composited and homogenized five adult fish (lake trout or walleye, depending on availability) from each sampling location. Contaminants were extracted by column chromatography using 10% diethyl ether in petroleum ether, followed by gel permeation clean-up. Contaminants in the sample extract were then separated into two fractions by silica gel chromatography, eluted with petroleum ether and 20% diethyl ether in petroleum ether. Each sample fraction was concentrated to 0.1 ml and 1 ul injected into a Finnigan 4000 GC/MS equipped with a 30 m capillary column coated with SE-30. The column was temperature programmed from 50°-260° as follows: 50°-145°C at 10°C/minute; 145°-215°C at 2°C/minute, and 215°-260°C at 4°C/minute. The MS was scanned from 60-460 amu

every two seconds, and data were collected and identified by the Incos data system containing the NIH/EPA/MSDC library. Over 400 different compounds in Great Lakes trout and walleye were identified by this technique.

The Provincial Pesticide Laboratory at Guelph, Ontario, Canada, represented at the workshop by Heinz Braun, monitors the common chlorinated hydrocarbons, specifically the DDT series (including DDE), dieldrin, the chlordanes, heptachlor epoxide and PCBs, among others. Michael Ribick of the FWS laboratory at Columbia, Mo., said that they are studying toxaphene-like residues in Great Lakes fish. That laboratory's approach is to use pattern recognition based on capillary column gas chromatography. Over 100 peaks can be identified by temperature programming. Some chemists expressed concern that toxaphene (a mixture of chlorinated camphenes) is routinely missed by analysts, even at levels approaching 5 mg/kg in fish. The ubiquitous presence of PCBs in Great Lakes fish may be part of the problem. However, if appropriate separation techniques are used, this problem can be diminished. The perchlorination approach to solving the toxaphene analytical problem was discussed, but no one had enough experience to discuss the results at that time.

The type of sample analyzed for pesticide residues in fish varies with the research or monitoring programs involved. The samples used by those laboratories represented at the workgroup include the whole fish, "edible tissue," fillets, eggs and bile. Research performed by John Lech and his colleagues at the Medical College of Wisconsin in Milwaukee indicates that most pesticide residues tend to accumulate in one anatomical site in the fish (e.g., the bile) -- a tendency which can bias the interpretation of results, depending on the type of sample chosen. In some fish species, perhaps 30%-35% of a pesticide residue can be found in the eggs.

Workgroup members were interested in learning which pesticides were being found most often and which ones also might be monitored on a routine basis that weren't presently being actively studied. The Wisconsin State Laboratory of Hygiene (SLH) at Madison, for example, in cooperation with the state's Department of Natural Resources and Coastal Zone Management Program, found during 1979-80 the following residues in Lake Michigan fish: dieldrin (25-500 ug/kg); p,p'-DDE (50-5,500 ug/kg); p,p'-DDD (50 to ca. 900 ug/kg); p,p'-DDT (50-1,100 ug/kg), and 50-500 ug/kg of cis-chlordane and cis- and trans-nonachlor. Small amounts of hexachlorobenzene and alpha- and gamma-BHC (hexachlorocyclohexane), generally not exceeding 50 ug/kg, have been detected. These levels most often appear in northern pike and the salmonids, particularly the lake trout in Lake Michigan. Of particular interest was the report that dacthal (dimethyl 2,3,5,6-tetrachloroterephthalate) was found by the SLH at a level of 1 mg/kg in a carp taken from the Pike River, a tributary to Lake Michigan.

Some investigators expressed interest in hearing of any findings of chlorinated dioxins and chlorinated dibenzofurans in Great Lakes matrices. The FWS, Canada Centre for Inland Waters and the Pesticide Research Center at Michigan State University -- represented at the workshop by Fumio Matsumura and his co-workers -- all have research interests in these compounds, though few knew of any definitive data available at the time.

Sample clean-up periodically poses problems, particularly with fish with a high lipid content. The tissues of some Great Lakes fish extracted for residue analysis contain more than 20% fat. Various steps are used for clean-up, including gel permeation, chromatography, Florisil, alumina and silica gel. High-pressure liquid chromatography (HPLC) is becoming an alternative for those laboratories having this capability. Carbon foam is being used at the Columbia National Fishery Laboratory to concentrate residues.

There did not appear to be much interest or activity regarding herbicides. The Provincial Pesticide Laboratory (Guelph) has been monitoring atrazine washed into the Great Lakes from Ontario watersheds, but there was little discussion of other herbicide monitoring efforts.

■ SELECTION OF ANALYTICAL METHODS

The main methodological sources used by the workgroup members include:

1) Pesticides Analytical Manual (FDA), an important reference for those analyzing residues in fish under regulatory agency programs and a good starting point for anyone beginning residue analyses. Appropriate column packing materials, detectors, solvents, etc., are discussed;

2) Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples (J. F. Thompson, ed., EPA, Research Triangle Park, N.C.);

3) Official Methods of Analysis of the Association of Official Analytical Chemists (W. Horwitz, ed., 1980); and

4) methods used by the Columbia National Fisheries Laboratory and other FWS laboratories in their Great Lakes fishery monitoring program.

Sampling. Water samples from the Great Lakes might have to be collected with a large-volume sampling device, perhaps one like that developed by R. W. Risebrough (University of California, Bodega Bay Marine Laboratory) that can collect up to 40 L. Smaller sample volumes often do not provide enough sensitivity because of low residue concentrations in open waters.

Some laboratories use XAD-2 resins (Rohm and Haas Corp.) for residue concentration from water. There are some problems with background bleed from the resins, which must be removed by extraction before use in sampling. Water Associates Sep-paks (C-18) have been used as a preliminary clean-up for HPLC, but workgroup members did not have sufficient experience with them to develop any detailed comments.

Extraction. The preparation of fish tissue for extraction varies among laboratories. Among the techniques used are a Soxhlet extractor with acetone-hexane solvent mixture and column chromatography whereby 10 g of fish are combined with Na_2SO_4, the latter having been pre-ignited (ca. 500°C)

in a muffle furnace. Various solvents are used to extract the fish after the column has been prepared. A household food processor has been used successfully to homogenize fish tissue prior to extraction.

Sediment extraction and analysis procedures generally follow accepted approaches, and typical techniques are described in some of the papers in Appendix A. Extraction protocols vary among the organizations represented at the workshop. Some laboratories air-dry the sediment, while others use field-moist conditions after pouring off any supernatant liquid. One recommendation was to adjust the moisture level to ca. 10% of the sample weight as is done for PCBs. An acetone-hexane extraction mixture (1:1) is popular. Sulfur removal is accomplished with mercury or copper, as is also done in PCB methodology.

Clean-up. The Columbia National Fisheries Laboratory uses gel permeation chromatography extensively as an initial clean-up step. It also uses cesium hydroxide to clean up samples prior to analysis of acidic compounds, such as phenols. Some participants were interested in clean-up steps for mirex and its subsequent analytical determination, but there wasn't much experience with this compound, as its Great Lakes distribution appears to be focused mainly in Lake Ontario (Kaiser 1978).

Spiking and Recovery. Various techniques are used to assess precision and accuracy in pesticide analyses, with no apparent standard approach taken. Some analysts add residue "spikes" and hold the sample anywhere from one to 30 days before extraction and analysis. The question of the extent of binding of the residue to tissues is still unresolved. Most workgroup members agreed that results should be reported as found and not corrected for independent recovery data. However, a separate list of recovery experiment data should be provided with each data report to help the person interpreting the data.

There was general interest in having some organization periodically provide homogenized samples, especially fish tissue, for round robin testing for residue parameters. This is apparently done informally now among certain individuals and laboratories. It might be desirable for the IJC, EPA or FDA to formally develop such a program, perhaps operating it similarly to the water and sediment analysis studies that now exist.

■ CHROMATOGRAPHY

The capillary column technology for GC is expanding rapidly, as was noted in the discussion in the PCB workgroup. Fused silica capillary columns were the topic of some discussion as well. It would be imprudent, however, to make specific recommendations regarding column coatings and construction materials, since the field is changing very quickly. The best recommendation is to review the current literature and catalogs of chromatography suppliers and develop expertise based on the instrumentation available and the types of samples that will be handled. Good overview information has been published by Onuska (1979), and Onuska and Comba (1978), Grob (1979) and Grob and Grob (1979), among many authors. The topic was also reviewed in a book by Jennings (1978), which provides the basics of the technique. However, many improvements have occurred in the past two years.

HPLC wasn't frequently used by workgroup members, but it appeared to be appropriate for selected applications.

■ DETECTORS

Pulsed electron capture detectors using ^{63}Ni appear to be most popular for chlorinated hydrocarbons, though the Coulson detector and the element-specific (halogen, nitrogen and sulfur) Hall Electrolytic Conductivity Detector (Tracor Co.) are gaining acceptance as laboratories upgrade the capabilities of their instruments.

■ CONFIRMATION AND QUANTITATION

Confirmation of a residue can be made by using two different columns, matching retention times to known standards, or using a single GC column followed by mass spectrometric confirmation. GC coupled with infrared spectroscopy is attracting interest now and may become popular in the future.

Quantitation using computerized data handling systems is becoming more popular as the equipment becomes more widely available. Electronic integrating recorders are employed extensively. Sharp, early eluting peaks that are well resolved can be quantitated by peak height.

■ QUALITY CONTROL

Quality control programs vary according to the mission of a given laboratory. The monitoring-oriented laboratories usually run duplicates at fixed percentages of the workload. The Columbia National Fisheries Laboratory uses ^{14}C spikes to trace recoveries through the various extraction and separation steps. Scrupulous attention must be paid to glassware cleaning, solvent quality, etc. Some laboratories bake glassware in a muffle furnace at temperatures up to 400°C, though the Federal Register (1979) cautions against doing this for volumetrically calibrated glassware. There is obviously a trade-off between possible low-level residue contamination carry-over and the alteration of volumetric calibration.

■ SUMMARY

The general consensus is that good analytical methods exist for most of the pesticides likely to be found in the Great Lakes. There are still problems with specific clean-up techniques because of difficult matrices, but most can be resolved with additional work. Probably the main follow-up activity for residue analysts is to keep up with new developments in capillary column technology and instrumentation, as these areas are improving rapidly. Also, chromatographers should remain alert for the appearance of residues that haven't been detected or recognized previously. The report of dacthal in a Pike River fish and the "classic" mirex case in Lake Ontario in the mid-1970s illustrate this point.

Workgroup on Industrial Chemicals

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The analysis of Great Lakes matrices for industrial chemicals (excluding PCBs, for the purposes of this workgroup) has begun to take on added importance as analytical techniques uncover a myriad of previously unreported compounds. In the 1960s and 1970s, considerable emphasis was placed on the analysis of chlorinated pesticides and PCBs; these were known contaminants, and analytical techniques (based predominantly on the use of GC) were relatively well established. With the increased use of GC/MS, many new compounds have been identified that previously were relegated to the status of "unknown" peaks that appeared on GC recorder charts.

The Great Lakes Basin is a highly industrialized region that supports about 15% of the population of the United States and nearly 40% of the Canadian populace. For many years, industrial effluents were discharged directly into the Great Lakes following varying degrees of treatment. This led to a build-up of certain chemicals in the biota and sediments of the lakes. As environmental legislation matures and the influence of the International Joint Commission (IJC) expands, a substantial decrease in the direct discharge of these chemicals into the lakes is anticipated. There are very few comprehensive summaries available that provide quantitative estimates of the chemicals reported in the Great Lakes. A recent report by Konasewich et al. (1978) is most likely the best accumulation of data currently available. Mila Simmons, Clifford Rice and William Sonzogni are currently expanding this report to include an intepretative narrative.

The Workgroup on Industrial Chemicals brought together representatives from university research groups, state and federal agencies, and a private laboratory specializing in contaminant analyses. The interests were varied. Some participants were interested in specific compounds or classes of compounds, while others were interested in the more general question of what kinds of organic compounds are present in the various matrices, without regard for any particular chemical grouping.

■ ISOLATION AND EXTRACTION OF ORGANIC COMPOUNDS

Water. The most common technique used for water and wastewater samples is liquid-liquid extraction. The most popular solvent is methylene chloride, which seems to possess almost ideal extraction qualities and presents few of the usual hazards (i.e., flammability, toxicity, carcinogenicity) that plague most other organic extractants. Today, few analysts use chloroform, and diethyl ether is beginning to lose popularity.

Depending on the anticipated concentration of organic contaminants, various sample volumes are used, ranging from the typical 1-2 L to 40 L, which is not uncommon for the studies performed by Ronald Hites at Indiana University. His group adds methylene chloride directly to the sample container and, in the presence of a magnetic stirring bar, begins the extraction soon after collection without the necessity of transferring the sample to another container.

When so-called base-neutral compounds are desired in addition to the acidic compounds, various approaches are taken. Some analysts collect a large sample and split it prior to any extraction. Hites' group lowers the pH to 2 or less, extracts the neutral and acidic groups first, and then increases the pH to 10-12 and extracts the basic compounds. Other laboratories perform the extractions in reverse sequence, particularly if emulsions are expected. Emulsions occur frequently when large concentrations of fatty or resin acids are present, such as in paper mill effluents or in waters receiving paper mill wastes.

A column of anhydrous Na_2SO_4 can be used to minimize the effect of the emulsions, though it is possible that some compounds of interest might be removed with the emulsion materials.

Emilio Sturino of the EPA laboratory at Chicago felt additional effort is needed to improve the accuracy, precision and sensitivity of methodology for organic compounds in Great Lakes open waters. The lack of relatively easy methods is probably responsible for the paucity of activity in this area. The Risebrough sampler, discussed in the pesticides workgroup section, may be one solution to this problem.

Bottles used for water samples should be rinsed beforehand and extracted prior to being filled with sample. Indiana University's Bertha Proctor extracts HCl with methylene chloride, then rinses the bottles three times with the HCl, then with methylene chloride, and bakes them in an oven at 450°C. The bottles are then sealed until use. Bottle caps have to be used with caution, since phthalates may leach from the caps even if they have teflon liners. Phthalate contamination seems to occur in most laboratories due to the wide use of these materials in floor and ceiling tiles, laboratory tubing and containers.

Besides liquid-liquid extraction, adsorption methods using XAD resins, polyurethane foam plugs and Sep-pak cartridges are used under various circumstances.

The XAD resins (chiefly XAD-2) appear to have potential application to Great Lakes open waters, where very low concentrations of most organic compounds may exist. However, the extraction efficiencies of the resins must be determined prior to use, and the resins must be pre-extracted with the solvents of choice to assure that no unwanted background materials will be leached from the resins during the sample extraction steps. Sometimes more than one solvent is used for pre-extraction, usually covering a range of polarities.

Polyurethane foam plugs have been used successfully by Mila Simmons at the University of Michigan, especially when looking for PCBs. University of

Michigan researcher Clifford Rice has also had success with these materials. Again, the plugs must be pre-extracted. Large volumes (in the range of 100 L) have been passed through the plugs, but the volume is limited by the amount of suspended materials present in the water. The plugs are typically extracted with methylene chloride in a Soxhlet device. Emilio Sturino suggests keeping the plugs wet after pre-extraction and using them as soon as possible following the pre-treatment, because they readily pick up ambient contaminants when exposed. There is still uncertainty over the quantitative recovery of many compounds, especially when large volumes are passed through the plugs, and the make-up of the organic compound mixtures is unknown until the initial GC/MS screening is completed.

The Sep-paks marketed by Waters Associates have seen some use, particularly when specific compounds are being studied. James Wiersma at UW-Green Bay is evaluating them for use in a chlorophenol project, but the best results with Sep-paks appear to be limited to the higher chlorinated compounds. The same concerns over pre-extraction were expressed as with the XAD resins and polyurethane foam plugs. Also discussed were steam distillation techniques for semi-volatile organic compounds in water.

An excellent review of the possible solvents of choice in liquid-liquid extraction was published by the late Ronald Webb (1978). Additional papers dealing with industrial organic chemicals in river systems appear in Appendix A and provide specific details about extraction procedures.

The Master Analytical Scheme being developed by the EPA's Athens, Ga., laboratory was described by John Pope. The Federal Register (1979) was also cited as a compendium of methods useful for determining EPA priority pollutants in water.

Sediment. Organic chemicals in sediments seem to be extracted according to two main methods: Soxhlet extraction and steam distillation. This is consistent with the approaches taken by the PCBs workgroup.

Solvent systems used in Soxhlet devices include acetone-hexane (particularly with wet sediments), isopropanol-methylene chloride and benzene-methanol. Discussions similar to those of the PCB group were held concerning the appropriate manner of handling the sediments prior to Soxhlet extraction. Air-dried sediments are easier to handle and can more simply be homogenized and split among different laboratories for collaborative studies. However, drying may cause changes in the surface-active sites, allowing some organic compounds to be irreversibly bound to sediment surfaces. Ronald Hites prefers to extract sediments in their field wet condition, abiding by the philosophy that minimum alteration of the sample is the best course to follow. Depending on the moisture content and solvent mixture chosen, the extract could be dried through a Na_2SO_4 column to ensure removal of traces of water.

James Kinsinger's Raltech laboratory uses steam distillation and various other extraction devices. Edward Leonard said the EPA laboratory in Duluth, Minn., also uses steam distillation, but he noted that varying success has been experienced with this method, depending on the polar nature of the organic chemicals present.

Techniques involving the use of carbon disulfide, developed by Grob (1973), Grob and Grob (1974) and Grob et al. (1975), were mentioned briefly, but no one professed any experience with these methods.

A quality control point was made about the common use of aluminum foil as a bottle sealer. Some analysts said they use the foil "dull side down" (i.e., having the dull side in contact with the sample) because it may have less surface contamination than the "shiny" side, but no data were available.

The removal of sulfur from sediment extracts can be performed in a manner similar to that explained in the PCB section. Both mercury and copper have been found satisfactory.

Fish. The removal of organic chemical contaminants from fish tissue is accomplished by column chromatography extraction, steam distillation and Soxhlet extraction.

In one column chromatography approach, a fish sample is ground with dry ice in a blender and then placed in a freezer to allow the dry ice to sublime away. The tissue is then mixed with Na_2SO_4 and extracted in a column with various solvent mixtures (hexane, hexane combined with diethyl ether or acetone, or methylene chloride). Further clean-up is then applied as required, including Florisil, silica gel, alumina, etc.

Steam distillation of contaminants from fish tissue seems satisfactory in some of the EPA laboratories.

The Columbia National Fisheries Laboratory uses Soxhlet extraction followed by gel permeation chromatography clean-up to separate out fish oils. Some analysts have used purge-and-trap techniques to free fish tissues of volatile organics.

■ SPIKING AND RECOVERY

Among the ways of measuring analytical performance, the use of radiotracer spiking samples was discussed and recommended as an ideal way of evaluating the accuracy of a method. The lack of available radioisotopic counting equipment is one deterrent to widespread application of this technique.

Specific spiking approaches include the use of corn oil as a carrier for volatile and semi-volatile organic compounds. The manner of spiking substrates like fish and sediment must be carefully designed to assure homogeneity when aliquots are taken for replicate analyses. Chau et al. (1979) have discussed ways to improve spiking of sediments for industrial organic chemicals, using PCBs as model compounds. A broader perspective, involving the selection of reference organic compounds to assess analytical capability, was published by Larry Keith (1979), who is representing the American Chemical Society in an international effort to intercalibrate the analysis of organic water pollutants.

The specific point at which to add a spiked reference compound in the analytical protocol is still under discussion. Ronald Hites prefers not to add an internal standard reference compound until the sample has been initially screened by GC/MS. Some workgroup members suggested using compounds that would not likely be present in the sample (e.g., p,p'-dibromobiphenyl), which would give good GC and GC/MS response.

■ FRACTIONATION AND CLEAN-UP

This topic was discussed to some extent, with silica gel receiving the most attention. Important details included using a column length 10 times the diameter of the column and adjusting the amount of silica gel used to the sample size. An excellent review of the column chromatographic separation of anthropogenic organic chemicals has been prepared by Stalling et al.(1979), reprinted in Appendix A.

■ ANALYTICAL METHODS

Methods Other Than GC/MS. HPLC is an area of active instrument development and research interest at this time. HPLC is being used for cleanup and, more importantly, for identification and quantification of compounds that are less amenable to GC analysis. The current literature is well represented by HPLC articles, and those interested in this area should consult the chromatography journals. There did not appear to be a significant amount of HPLC usage among members of the workgroup, but future use of the method was indicated by those who recently have purchased HPLC instruments or were considering such a purchase.

Gas Chromatography. Discussion centered around packed columns versus capillary column GC. This discussion was similar to that reported in the PCB workgroup section but was not as intensely debated. Traditional detectors -- such as electron capture and flame ionization -- were mentioned, and increased use of the Hall Electrolytic Conductivity detector was indicated. The latter's popularity should increase due to its inclusion in the proposed Federal Register methods for some of the EPA priority organic pollutants. Capillary columns, especially flexible fused-silica columns, have found acceptance in many laboratories. Good separation, ease of use and ready interfacing with GC/MS systems are the major reasons for their recent popularity.

Column performance must be checked regularly. A multicomponent standard is recommended; internal standards should be run frequently. Tailing of a peak whose resolution is normally sharp indicates that column or injection port conditions are deteriorating.

The use of hydrogen as a carrier gas with capillary columns seems to be most popular, particularly because its flow rate is higher than helium. Column longevity varies with the types of samples and columns used. There was no consensus on how long a capillary column might be expected to last.

Gas Chromatography/Mass Spectrometry. The Federal Register (1979) gives specific instructions for calibration of GC/MS instruments that involves

the use of decafluorotriphenylphosphine. Some participants expressed concern that the specifications might be a little too rigid, though others claimed they were able to meet them with the instruments they had. Most agreed that the methodology proposed in the Federal Register should be more flexible than it is presently. Some calibration discrepancies apparently involve operational differences between quadrapole and magnetic focusing GC/MS systems.

The manner of acquiring mass spectra (i.e., electron impact versus chemical ionization) was discussed. The EI approach is still the most commonly used technique, though failure to obtain a molecular ion by EI is one justification for using CI with certain classes of molecules. New instruments come with multiple capabilities, and it is suggested that they be purchased with as many options as possible -- including negative ion capability -- to allow maximum future flexibility. Many workgroup members use GC/MS for identification and then use GC with specific detectors for a more facile quantification. The interfacing of more sophisticated computers with the GC/MS systems, as well as rigorous control over the GC/MS operational parameters, will improve quantitative determinations.

Mass Spectra Interpretation. There are many routes available for interpreting mass spectra. Library searches involving GC/MS computers are common, including telephone hook-ups with the EPA-NIH mass spectral search system. The EPA-NIH spectra (the NBS library) are also available in bound volumes (Heller and Milne 1978). The first edition is now out of print, but an updated edition is expected in the future. Use of the "Eight-Peak Index" (Mass Spectrometry Data Centre 1974) is also popular. The group was unanimous, however, in its caution against accepting unequivocally the results of computer library searches. There are errors in the systems that may cause incorrect identifications. Those acquiring mass spectra of unknowns that are not in the MSSS and that can be verified with standards should submit the spectra and details to the MSSS program managers for possible inclusion in the next revision.

GC/MS users should always try to rationalize the spectra once the computer library search has tentatively identified the compound. Texts are available for this purpose and should be consulted (e.g., McLafferty 1973). Short courses, like those offered by the American Chemical Society and the Finnigan Institute and other instrument manufacturers are helpful, as is the annual summer session offered by Ronald Hites at Indiana University. Everyone agreed that the more that is known about a sample, the better the identification is likely to be. Such data include sample location, possible sources of contamination (industries, etc.), extraction and clean-up protocol followed, and GC/MS conditions.

■ TYPICAL INDUSTRIAL ORGANIC CHEMICAL DATA

The development of chemical inventories is one aid to analysts seeking industrial organic contaminants in waterways. Projects conducted by Ronald Hites and his associates at Indiana University, and the Lower Fox River study in Wisconsin by Joseph Delfino and his co-workers were discussed as examples. The IJC is getting involved in expanded inventory assessment

studies, and sources like the Stanford Research Institute Directory of Chemical Producers and the EPA discharge permit files are heavily used. The Information System for Hazardous Organics in Water (ISHOW) is being developed at the University of Minnesota-Duluth under IJC and EPA contracts.

Data should be reported with due attention to the number of significant figures. Considerable discussion centered on when to report "below detection limits," "not detected" and other combinations and possibilities. The IJC has an interest in learning of compounds that might be below the usual reportable detection limit but are likely to be present. The existence and validity of these trace levels is still an area that needs further work. Many analysts simply do not want to report data that are near or just below the "detection limit," while program managers want to know what is happening in aquatic systems regardless of firm statistical evidence. Despite this uncertainity, everyone agreed that data reports should be accompanied by all pertinent analytical information so those interpreting or otherwise using the data will have an appreciation of what the numbers actually represent.

■ CASE STUDIES

The workgroup's discussion concluded with summaries of typical case studies with which its members were involved. Some of these have been published (see Appendix A). Others in progress include the Niagara River, N.Y., study being performed by Ronald Hites, Bertha Proctor and Vincent Elder at Indiana University; various organic contaminant studies (e.g., of Curene 442) under the direction of James Bedford, and the Lower Fox River study mentioned above, which is investigating the impact of pulp and paper industry discharges in that area.

The group agreed that some organization (IJC or EPA) should act as a repository for unknown mass spectra that might someday be interpreted or recognized by another group working elsewhere. Such a transfer of information might help alleviate the "pollutant of the month" problem by allowing the possibility of more rapid identification of compounds that remain unknown to one discoverer but might already be known to someone else for a different reason in a different place. Also, the prediction of industrial process reaction products that don't appear on chemical inventories (which are inadequately known in the Great Lakes region anyway), remains a major puzzle to be solved in the coming years.

Workgroup on Polynuclear and Aliphatic Hydrocarbons

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Of the four workgroups organized during the workshop, this category -- covering the polynuclear aromatic hydrocarbons (PNAs or PAHs) and petroleum-derived hydrocarbons -- attracted the smallest number of participants. Perhaps this was inevitable, since there is an abundance of activity in the area of PCBs and pesticides and a rapidly expanding interest in industrial chemicals. Nonetheless, a considerable amount of research on these chemicals is being conducted at a few laboratories and in Sea Grant-funded projects directed by Anders Andren and David Armstrong at the University of Wisconsin-Madison. But until regulatory agencies begin a broad-based monitoring effort in these areas, the visibility of these classes of organic compounds will probably remain rather low. A major oil spill on one of the Great Lakes or the discovery of significant tainting of biota from normal shipping activities may attract more attention to these compounds. It seems that similarly strong stimuli were required to generate interest and funding among oceanographers and the U.S. Coast Guard, who appear to be the most active PNA and petroleum researchers at this time.

■ GENERAL COMMENTS--SAMPLE COLLECTION, PRESERVATION AND EXTRACTION

There is a potential for constituent loss during sampling, and precautions must be taken to avoid this. Samples should be extracted as soon as possible after collection to avoid hydrocarbon adsorption onto the sample container surface. In situ sampling and concentration devices (resins, foams, etc.) are recommended for surface waters and treated drinking waters. All concentration steps should be performed with Kuderna-Danish evaporative condensers.

For storage, the best approach appears to be to keep samples in the dark at 4°C. Sediment samples have remained "stable" as long as four weeks under these conditions. Analytical standards should be similarly stored. If possible, use yellow lab lights to prevent photolytic degradation of the compounds in standard solutions and extracts.

The matrix of interest determines the collection and extraction procedure to be used, as is discussed below. Sediment is the customary matrix for aliphatic hydrocarbon analyses, while the PNAs are generally sought in the sediments, effluents, the water column and atmospheric samples. As a general rule, sediments appear to be the matrix of choice, since reports in the literature indicate a possible threefold to fourfold concentration of PNAs in sediments as compared to water-column concentrations.

■ METHODS FOR EFFLUENTS AND SPILLS

Collection, Preservation and Extraction. Point-source effluents should be collected in amber glass bottles (ca. 1 L) that contain several milliliters of methylene chloride. The bottle should be filled, avoiding any headspace volume. In the laboratory, the sample should be filtered through a pre-extracted glass fiber filter, and the filtrate extracted with methylene chloride. If desired, the filter itself can be extracted to determine the hydrocarbon components present in the particulates. A general acid-base-neutral fractionation scheme is available from Vassilis Stamoudis at the Argonne National Laboratory.

Clean-up and Fractionation. The number of clean-up steps required depends on the background interferences present and the range of compounds of interest. Samples collected on glass fibers can be extracted sequentially with pentane, cyclohexane and methylene chloride. This solvent sequence is particularly useful to fractionate parent PNAs, alkylated PNAs, heterocyclic PNAs and aliphatic hydrocarbons when using silica gel chromatography. Workgroup members recommended silica gel in lieu of alumina. Warner (1976) described a fractionation scheme for saturated and aromatic hydrocarbons, while Severson et al. (1980) used gel permeation chromatography with Bio-Beads SX-12 to separate aliphatics, parent PNAs and alkylated PNAs. Use caution in working with some of the n-alkanes, which apparently irreversibly adsorb onto silica gel.

■ METHODS FOR SURFACE AND POTABLE WATERS

Collection, Preservation and Extraction. PNAs exhibit relatively low solubility in water (in the nanograms per liter range), so routine monitoring is not recommended in the absence of a known incident of contamination. Monitoring of streams is similarly not recommended, though stream sediments might yield relatively interesting data. Large volumes of water (ca. 50 L) should be extracted to attain the necessary analytical sensitivity for analysis by GC-FID or GC/MS. Smaller volumes (less than 1 L) can be used for analysis by HPLC-fluorescence, but the analysis is then restricted to known compounds whose standards are available. Filtration and concentration *in situ* are highly recommended. For PNAs, the XAD-2 or XAD-4 Amberlite resins or polyurethane foam are suitable. The XAD-7 or XAD-8 resins and polyurethane foams appear to be satisfactory for aliphatc organic compounds, with the foams particularly helpful with compounds of higher molecular weight. The flow rate is very important when using *in situ* devices, and care must be taken to avoid overloading them, especially the XAD resins. The *in situ* concentration of aliphatic organics on polyurethane plugs is described by Lappe et al. (1980).

Extraction of the resins or foam should be performed using methylene chloride, while the pentane-cyclohexane-methylene chloride sequence is adequate for fractionation. To assess the accuracy of extraction, a water sample can be spiked with a low concentration of PNA by coating a column of glass beads with the desired materials and then eluting exhaustively with water. The spiked water is then extracted as discussed above.

Clean-up and Fractionation. If needed, the extract can be cleaned up and fractionated in a fashion similar to that discussed in the section for effluents.

■ METHODS FOR SEDIMENTS

Collection, Preservation and Extraction. Sediments are best collected with a box core sampler, a device widely used by oceanographers and one that has become popular for Great Lakes sampling. It was noted that liquid nitrogen has been injected into the box core device to freeze the sample *in situ* to avoid the loss of fine sediment particles as the box core is returned to the boat. It is not known how widely this modification is employed.

Box cores generally do not collect sufficient sediment to allow detection of PNAs at the low levels at which they are typically found. Consequently, grab samples using various dredges (Ponar, etc.) are often taken. PNAs apparently concentrate in the upper 4-5 cm of the sediment profile, so dilution might occur if a too-great sediment profile is retrieved by the grab sampler. Stream sediments should be collected near the mouth, in harbors or in slow-moving stretches. Areas of active sediment movement should be avoided. A pill-box mechanical sampler should be used to collect about 200 g of sediment from the uppermost 2-3 cm.

Field moist sediments should be stored frozen and in darkness. Some individuals have freeze-dried sediments prior to storage, but the impact of this on constituent concentrations is not fully known. Extraction of the sediments should be performed in a Soxhlet apparatus using a toluene-methanol solvent system. There are reports that starting with wet or dry sediments can affect the recovery of certain PNAs and aliphatic hydrocarbons. Depending on the sediment substrate encountered, analysts should determine the extraction behavior of samples of varying moisture contents in their own laboratories.

Clean-up and Fractionation. Clean-up of the sediments prior to analysis for PNAs and aliphatic compounds is likely to be an extensive process as compared to samples from other matrices. The protocol given in the effluents section is pertinent here, but additional modifications might be necessary if the quality of the gas chromatograms indicates further work.

■ METHODS FOR BIOTA

Collection, Preservation and Extraction. Routine monitoring of PNAs and aliphatic hydrocarbons in biological samples is not recommended because their levels in tissue are usually very low. However, "hotspot" analyses might be indicated for samples collected at the site of an oil spill. Collect appropriate specimens in a manner consistent with the activity and habitat of the genera involved. Manually scrub from the surface of the organism any adhering oil or sediment. Store frozen pending analysis.

To prepare biological samples for analysis, homogenize the sample and saponify with potassium hydroxide and methanol; extract with methylene

chloride (Bieri and Stamoudis 1976). Additional details are given by Warner (1976), who recovered aliphatic and aromatic hydrocarbons from marine organisms using aqueous alkaline digestion, ether extraction, silica gel chromatography and finally gas chromatography (see section on qualitative analysis).

The use of fish as biomonitors has been suggested, since these organisms concentrate PNAs and their metabolites in the bile (Melancon and Lech 1979; Statham et al. 1976, Appendix A). It should be noted that some of the PNAs in fish muscle and other tissues are present as metabolites, so analytical methods must take this into consideration.

Clean-up and Fractionation. Aside from sediments, biotic tissues can present significant clean-up challenges. Generally, the protocol presented in the effluents methods section is sufficient, but unsuitable gas chromatograms might dictate additional steps in certain cases.

■ METHODS FOR AIR PARTICULATES (PNAs ONLY)

Collection, Preservation and Extraction. PNAs are generally adsorbed onto particulate matter in the air, so collecting them follows the approaches used for air particulates. The particulates are collected on glass fiber filters in either a Hi-Vol sampler or cascade impactor. Volatile components can be trapped by pulling large volumes of air through XAD resins. The filters should be stored in darkness at 4°C. Extraction methods have been published by the National Institute of Occupational Safety and Health (NIOSH, Cincinnati, Ohio, 1977) and should be consulted for specific details.

Clean-up and Fractionation. The procedures specified by NIOSH (1977) appear to be satisfactory, at least to the relatively small group of people performing these measurements in the Great Lakes region. Chemical ionization has also been found useful to confirm interpretation of mass spectra of substituted PNAs (including nitrogen, oxygen and halogens) for which standards are not available.

■ QUALITATIVE AND QUANTITATIVE ANALYSIS

The complexity of PNA mixtures and the similarity of their physical and chemical properties present analytical problems that are compounded by the low levels of PNAs found in typical Great Lakes matrices. The instrumentation for the general analytical approach involves gas chromatography coupled with either a flame ionization detector or a mass spectrometer. HPLC has been found to be sensitive for PNAs. With very few exceptions, capillary columns must be used in GC work to separate PNAs from their alkylated analogs and related molecules containing nitrogen, oxygen and sulfur atoms.

For screening purposes, PNAs can be analyzed by GC with the flame ionization detector, but GC/MS or HPLC with the fluorescence detector are nec-

essary for rigorous quantitation. The combination of electron impact and chemical ionization techniques allows greater selectivity for GC/MS. Negative and positive chemical ionization GC/MS for hydrocarbons has been described by Harrison (1980). Chemical ionization has also been found useful to confirm interpretation of spectra of substituted PNAs (including nitrogen, oxygen and halogens) for which standards are not available.

The HPLC method with fluorescence detector is very sensitive for PNAs and appears in the Federal Register (1979) as the method of choice for this class of compounds, at least when they are suspected to be present in effluents. Larger sample volumes are needed when analyzing surface waters and potable water. Additional optimization of excitation emission wavelengths in HPLC systems can be accomplished. Ogan et al. (1979) discuss liquid chromatography methods for a number of EPA priority-pollutant PNAs.

■ SUMMARY AND RECOMMENDATIONS

There is no specific detector for either the PNAs or the aliphatic hydrocarbons in the same sense as the electron capture or Hall Electrolytic Conductivity detectors are used for chlorinated organic compounds. This presents boundary conditions on the achievable detection limits. Developments in the area of more specific detection are needed (e.g., refinement of photoplate GC/MS) to reduce the need for large sample sizes. Test mixtures of commercial capillary columns do not ordinarily include PNAs with high molecular weights. This would be more valuable than current evaluations based on aliphatic hydrocarbons and occasional PNAs with low molecular weights.

Pure standard materials are needed, particularly for the heterocyclic and oxygenated analogs. If sufficient standards were available, aza-arenes could be useful indicators of anthropogenic pollution in natural waters.

PNA and aliphatic hydrocarbon methodology is not yet sufficiently refined to justify interlaboratory studies, but additional efforts should be made so qualititative or semi-quantitative information can be developed. Extraction efficiencies and spike recovery data must be documented, especially for sediment and water samples. This will aid in judging the acceptability and consistency of each method. Ultrasonic extraction methods warrant additional research. Sample preservation, especially for sediments, must be further refined and documented, particularly the comparison of extraction using wet versus freeze-dried sediments.

Despite its relatively small number of participants, this workgroup approached its topic with vigor and provided an excellent summary of the status of analytical methodology for the PNAs and aliphatic organic compounds in Great Lakes samples.

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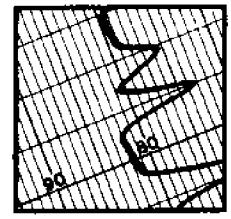
Appendix A
Related Journal Articles

Toxic Substances in the Great Lakes

J. J. Delfino



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Toxic substances in the Great Lakes

Here is an overview of the origin, distribution, and fate of some heavy metals and organics, and some suggestions as to what may be done about them

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Concern over the presence of chemicals in the Great Lakes has increased in recent years. Alfred Beeton reviewed historical trends of many common anions and cations, and found that their concentrations increased slowly but continuously in the past half century (*Eutrophication*, National Academy of Sciences, 1969). These changes were accompanied by changes in the biota, particularly the algae, as populations evolved from so-called clean-water species to those indicating eutrophic symptoms. Beeton hypothesized that man's activities were influencing the limnology of the Great Lakes, resulting in decreased water quality. These conditions were noted particularly in Lakes Erie and Ontario and in the southern basin and Green Bay in Lake Michigan.

An international conference, held in Madison, Wis., in 1967, focused on eutrophication and spurred greater research interest. Numerous additional conferences have been held on this topic in the past 12 years. However, environmental issues rarely remain simple, and in the early 1970's the eutrophication problem was confounded by concern over the potential effects of thermal pollution in the Great Lakes, a topic reviewed some years ago by Arthur Levin and others (*ES&T*, March 1972, p 224). For instance, as demands for electric power increased, especially in the Midwest, many power plants were constructed on Great Lakes shorelines.

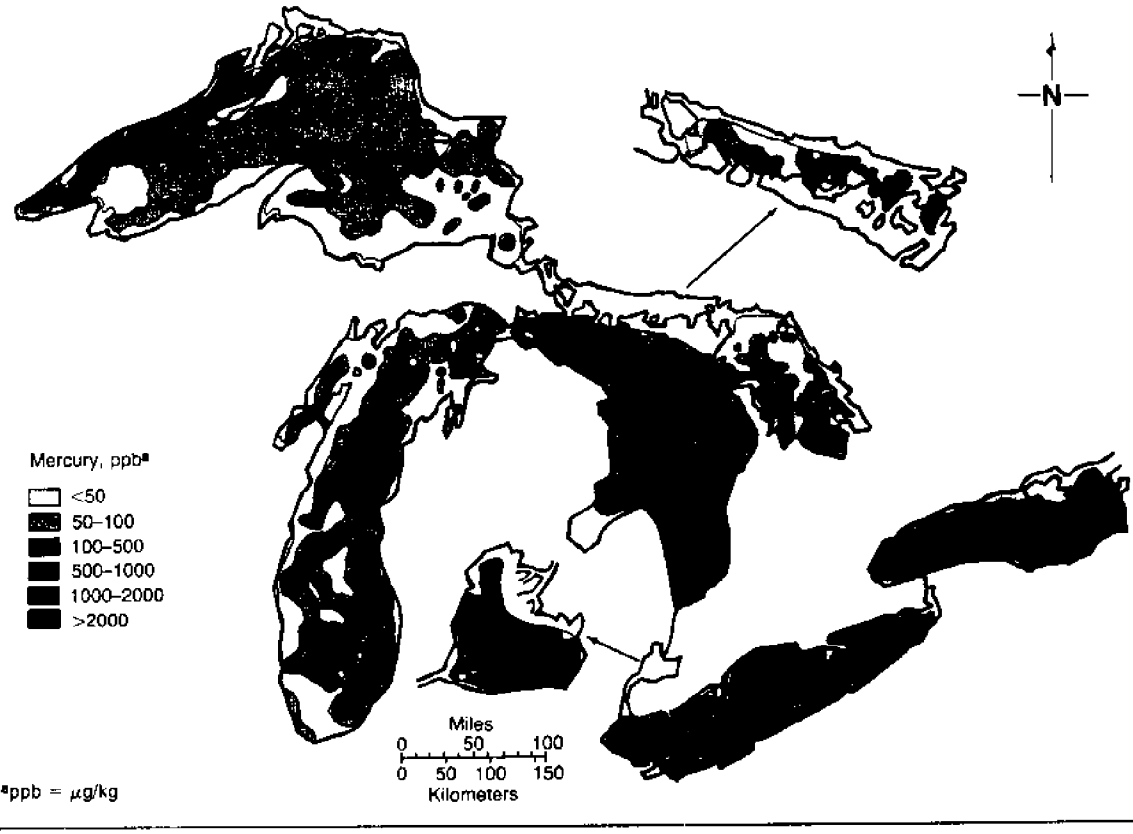
Citizens and regulatory agencies, already alarmed by the specter of eutrophication-influenced water quality changes, attempted to restrict the discharge of power-plant cooling water into the lakes. By 1972, these two issues had created sufficient attention that an interstate enforcement conference on Lake Michigan pollution was convened. Also in 1972, the Great Lakes Water Quality Agreement between the United States and Canada was first signed. It established water-quality objectives and timetables, and espoused a nondegradation philosophy.

While these environmental issues in the early 1970's originally emphasized phosphorus loading and thermal pollution in the Great Lakes, the toxic-chemical problem was also developing. Although the first serious alert concerning toxic chemicals, particularly pesticides, had been sounded by Rachel Carson in *The Silent Spring* in 1962, it was not until the late 1960's and early 1970's that the combination of increased environmental awareness and laboratory analytical capability led to the discovery of pesticides throughout the Great Lakes Basin. The chemical stability of many pesticides, coupled with their capacity to bioaccumulate, resulted in high concentrations of compounds such as DDT and dieldrin in fish. Fish that exceeded U.S. Food and Drug Administration (FDA) tolerance levels for pesticides could not be sold commercially.

Feature articles in ES&T *have by-lines, represent the views of the authors, and are edited by the Washington staff. If you are interested in contributing an article, contact the managing editor.*

FIGURE 1

Mercury concentrations in surface sediments of the Great Lakes



During the time that extensive effort was mounted to document DDT levels in Great Lakes fish, chemists noted that additional compounds were also present. In fact, some of these other substances created significant problems in the analysis of DDT. It was soon recognized that these troublesome compounds were polychlorinated biphenyls (PCB's), and that they were also present at levels as high as 25 μg/g. These historical levels have been documented by scientists from the U.S. Fish and Wildlife Service (FWS), the U.S. Environmental Protection Agency (EPA), and particularly by Gilman Veith at EPA's Duluth, Minn., laboratory. Thus, what was first a problem involving nondegradable pesticides, such as DDT, soon blossomed into a major issue of contamination by all types of toxic chemical substances.

To be sure, there is still concern about the input of phosphorus and thermal discharges into the Great Lakes. But, according to the Pollution from Land Use Activity Reference Group (PLUARG) of the International Joint Commission (IJC), toxic chemicals have now become the predominant water-quality issue in the Great Lakes Basin. This concern is also shared at the national level by the President's Council on Environmental Quality.

What are toxic substances?

Webster's *Seventh New Collegiate Dictionary* defines toxic as something "of, relating to or caused by a poison or toxin", where a poison is "a substance that through its chemical action usually kills, injures, or impairs an organism." The Congress of the United States defined toxic substances in October 1976, when it enacted the Toxic Substances Control Act (Public Law 94-469). That law defines toxic substances as those chemical substances "whose manufacture, processing, distribution in commerce, use, or disposal may present an unreasonable risk of injury to health or the environment." More specific definitions of a toxic substance and a hazardous polluting substance were included in the Great Lakes Water Quality Agreement of 1978, signed by the U.S. and Canada on Nov. 22, 1978.

Numerous industrial chemicals, such as PCB's, have been entering the environment for many years and, like DDT, have accumulated in living organisms including Great Lakes fish and in the people that consume them. PCB's have been identified essentially everywhere in the world, despite the fact that they were produced for uses that should not have resulted in such widespread distribution. These uses included capacitors, transformers, and hydraulic fluids. Their careless use and disposal have led to ubiquitous contamination of the environment.

Details about certain toxic chemicals, including lists of the so-called "priority pollutants", were published recently by Larry Keith and William Telliard (*ES&T*, April 1979, p 416). These should be consulted for additional information.

Distribution in water

The extent of toxic-substances distribution in the water, sediments, and fish of the Great Lakes Basin is only now beginning to be understood. For example, a comprehensive inventory of information on the distribution of or-

ganic and trace-metal contaminants in the basins of all of the Great Lakes, except Lake Ontario, has been prepared for a committee of the IJC's Water Quality Board by Dennis Konasewich and his co-workers. This inventory will serve as a basis for an assessment that will determine those contaminants that may present a hazard to human health and the environment. Data were obtained from municipal, state, provincial and federal agencies in the U.S. and Canada, as well as from reports and papers published by university researchers, government scientists and consulting firms. Among those groups that have performed considerable work and compiled extensive data on the Great Lakes are the Canada Centre for Inland Waters, the Ontario Ministry of the Environment, various EPA laboratories, the National Oceanic and Atmospheric Administration, and the natural resources departments of the Great Lakes states.

Chemicals in Lake Michigan

The most complete interpretive compilation of historical data for chemicals in Lake Michigan was published by Marguerite Torrey in 1976. No similarly thorough treatise for the other Great Lakes has been prepared.

At one time, organochlorine pesticides were widely used in the Lake Michigan Basin and were applied to forest and agricultural lands, and to noncrop lands. Pesticides were also used in industry and for household-pest control. According to a 1972 EPA report, the extensive use of DDT and dieldrin in Wisconsin led to the transport of these chemicals to Lake Michigan via tributaries, land runoff, and wastewater-treatment plants. Atmospheric precipitation was also implicated because of the wind-borne transport of these pesticides following aerial spraying.

The concentrations of pesticides in the waters of the Great Lakes is relatively low, due partly to their low aqueous solubility. As reported by Torrey, typical concentrations were generally below 1 μg/L, and often near 1 ng/L. In addition to DDT and dieldrin, numerous other compounds were detected by gas chromatography, including PCB's, lindane, heptachlor, heptachlor epoxide, endrin, aldrin, and methoxychlor. Much of the early DDT and dieldrin data from Lake Michigan and other waters, prior to the late 1960's, are now considered only approximate because of the likely interference of PCB's in the analysis of these pesticides.

Torrey demonstrated that the concentrations of the main toxic inorganic materials (Hg, Pb, As) are quite low in Lake Michigan. There, inorganic substances do not appear to pose significant problems, except for As in the Green Bay area (currently being studied by Marc Anderson and associates at the University of Wisconsin-Madison), and Pb in the southern basin.

Distribution in sediments

Great Lakes sediments are the ultimate sink for many, but not all, of the toxic contaminants that enter the lakes. Some of the contaminants, such as mercury, can be converted to more biologically active forms. Also, compounds such as PCB's are taken up by bottom organisms that either live or feed in the sediments, and which are subsequently eaten by larger carnivores. This results in an eventual increase in the concentration of this contaminant in carnivorous fish. Thus, despite the fact that the sediments are many meters below the water's surface, they do not always act as the ultimate sink for toxic substances.

A toxic substance means "a substance which can cause death, disease, behavioral abnormalities, cancer, genetic mutations, physiological or reproductive malfunctions or physical deformities in any organism or its offspring, or which can become poisonous after concentration in the food chain or in combination with other substances." A hazardous polluting substance is defined to mean "any element or compound which, if discharged in any quantity into or upon receiving waters or adjoining shorelines, would present an imminent and substantial danger to public health or welfare. For this purpose, public health or welfare encompasses all factors affecting the health and welfare of man including but not limited to human health, and the conservation and protection of flora and fauna, public and private property, shoreline and beaches."

Source: Great Lakes Water Quality Agreement of 1978, International Joint Commission

The presence of many contaminants in the Great Lakes sediments was summarized in the 1978 PLUARG Final Report to the IJC. Of particular concern were Hg, Pb, and PCB's. A contour map prepared by PLUARG, showing the approximate distribution of mercury (Hg) in Great Lakes sediments, is reproduced as Figure 1.

The highest Hg concentrations generally appear in Lakes Erie, Ontario, and St. Clair. Previous industrial discharges, apparently current atmospheric deposition into the Great Lakes, and runoff from the land surface appear to be sources of Hg.

Prior to 1970, major inputs into the lakes' system were discharges from the St. Clair and Detroit Rivers. On the basis of the sediment patterns, Lake St. Clair appears to be a continuing source of Hg to Lake Erie, despite the elimination of the chloralkali-plant source.

The transport of Hg-enriched sediments from Lake St. Clair, through the Detroit River, and into western Lake Erie, continues to be a problem. The resuspension of Hg-contaminated sediments from western Lake Erie, and subsequent in-lake transport, has carried Hg along the southern shoreline and led to its ultimate deposition in the eastern basin. The western basin of Lake Erie is an active area of sediment resuspension because of wind-induced wave action.

In Lake Ontario, Hg appears to be carried by the Niagara River, with eventual dispersal throughout the eastern basin. The sharp decrease in point-source inputs of Hg to the lakes should result in a gradual decrease in Hg content of the surface sediments, according to PLUARG.

The concentrations of most organic contaminants in Great Lakes sediments have not been well mapped. PCB contours, however, were compiled by PLUARG for Lakes Huron, Erie, and Ontario. These indicate a general occurrence of PCB's in the sediments, with higher loadings in the western and southern areas of Lake Erie and in the south central area of Lake Ontario. The PLUARG Report estimated that nonpoint sources, including atmospheric inputs, account for the major reflux of PCB's to the lakes.

Distribution in fish

Data for PCB's in Great Lakes fish were compiled in the PLUARG Report with additional data provided by the State of Wisconsin. These appear in Table 1. The most significant PCB contamination occurs in Lake Michigan, according to data now available.

One interesting aspect of PCB, DDT, and dieldrin contamination of fish in Lake Michigan is the slightly decreasing trend in concentrations that has developed over the past five to eight years. Data from the FWS and EPA indicate such a trend, but further analysis and monitoring will be required in the future to verify the trend. The PCB concentrations in fish can

vary from one season to another, as well as during spawning periods. Thus, fish monitoring programs must be conducted in a consistent manner to avoid bias in the interpretation of the data.

One reason that PCB's continue to appear in relatively high concentrations in Lake Michigan fish is that the chemical is easily bioaccumulated; that is, passed through the food chain as smaller organisms are consumed by larger ones, ultimately reaching the primary carnivores in the lake (salmonids) which are highly prized sport fishery species. This process is illustrated in Figure 2.

Mercury concentrations in Great Lakes fish are not too much of a concern, except for those caught in Lake Erie. Concentrations from fish in that lake indicate that the FDA consumption guideline of 1.0 μg/g is still being exceeded, although trends of decreasing concentrations have been noted. The PLUARG Report noted that lead (Pb) concentrations are nowhere close to the recommended consumption-advisory limit of 10 μg/g in any of the Great Lakes.

Effects of toxic substances

One effect on citizens in the Great Lakes Basin, particularly those who consume relatively large amounts of locally caught fish, is contamination from certain species by chemicals, especially PCB's. Most of the species that have sport fishery importance, particularly the salmonids, consistently exceed the proposed FDA tolerance guideline of 2 μg/g in Lake Michigan. However, sportsmen are not prevented from consuming their catch, and many of these people are unwilling to abandon their sport (which involves considerable financial investment), particularly since toxicological studies have not conclusively linked the dietary intake of low levels of PCB's with human health problems.

Despite the lack of firm human health evidence, there is ample documentation that the intake of PCB's near the FDA guideline significantly affects rhesus monkeys. James Allen and co-workers at the University of Wisconsin-Madison have shown that infants born to monkeys that were fed 2.5–5.0 μg/g of PCB's developed facial acne and edema, swelling of the eyelids, loss of facial hair including eyelashes, and hyperpigmentation of the skin. Half of the PCB-exposed infant monkeys died within eight months following birth, assumedly from PCB intoxication. Surviving infant monkeys proved to be hyperactive in locomotor tests, and to be slow in learning how to

Toxic substances: classes and sources

According to the Great Lakes Basin Commission, the general classes of toxic substances that are presently of concern in the Great Lakes Basin are:

- halogenated organic compounds (PCB's, PBB's, chlorophenols),
- radioactive substances,
- pesticides,
- heavy metals and toxic nonmetals (Hg, Pb, As), and
- petroleum products.

There are many sources for the toxic substances that enter the Great Lakes. The Great Lakes Basin Commission and others have itemized these as:

- point sources (municipal- and industrial-effluent discharges),
- nonpoint sources (land application of toxic materials followed by runoff; the atmosphere),
- urban runoff (Pb from automobile exhausts; grease and oil; metals; household pesticides),
- toxic and hazardous waste disposal sites or "phantom" disposal activities (legal or illegal waste disposal that results in the pollution of the Great Lakes; transportation accidents),
- the atmosphere (*ES&T*, November 1979, p 1337) (can be considered a nonpoint source but represents a major point of entry of PCB's, Pb and other toxic substances to the lakes),
- agricultural runoff (nonpoint source but emanates from specific useage of chemicals in agriculture).

TABLE 1
PCB concentrations in Great Lakes fish.

Lake	Sampling period	Mean PCB concentrations[a] µg/g	Range µg/g
Superior	1968–1975	0.61	<0.1–3.7
Michigan	1972–1974	10.2	2.1–18.9
	1974–1978	NC[b]	1.2–37[c]
Huron	1968–1976	0.82	<0.1–7.0
Erie	1968–1976	0.88	<0.1–9.3
Ontario	1972–1977	2.37	<0.1–21.1

[a] The FDA tolerance level is 2 µg/g
[b] Not calculated
[c] Based on the analysis of 35 fish consisting of chubs, lake trout and coho salmon. Data from the State of Wisconsin Department of Natural Resources
Source: PLUARG, 1978

The PLUARG Report

The 1978 PLUARG Report summed up the toxic substances control problem very well and the following points are excerpted from that document. In order to control toxic substances and reduce their inputs to the Great Lakes:

- Toxic substances must be controlled at their source.
- Closer cooperation between the U.S. and Canada in implementing toxic substances control legislation must occur.
- Proper management control, and the means of ultimate disposal of toxic substances presently in use, must be developed.
- Identification and monitoring of historic and existing solid waste disposal sites (where there is an exisiting or potential discharge of toxic substances) must be done, and control programs at those sites must be executed as needed.
- Joint U.S.-Canadian expansion of efforts to assess the cumulative and synergistic effects of increasing loads of toxic contaminants on environmental health must be given high priority.
- The rapid translation of these assessments into revised water-quality objectives must be made; moreover, for certain toxic substances, a zero loading to the Great Lakes must be a requirement.

solve various types of discrimination problems, when compared to control animals.

The linkage of rhesus monkey health effects to potential human health problems is still not firm. However, the animal studies suggest that humans should limit their consumption of PCB-contaminated fish taken from the Great Lakes, especially Lake Michigan, until epidemiological research determines the extent, if any, of low-level dietary intake effects of PCB's on humans.

The most serious effect of toxic substances in the Great Lakes seems to be the diminished value of the Great Lakes as a natural resource, particularly in the production of fish that are suitable for human consumption. Commercial fishing has already been impaired. Sportsmen now enjoy their pastime with the caveat that their catch is tainted, and that their fish intake should be limited to no more than one meal per week. Pregnant or nursing women have been advised to eliminate the PCB-contaminated fish entirely from their diets.

Problems in the Lake Michigan basin

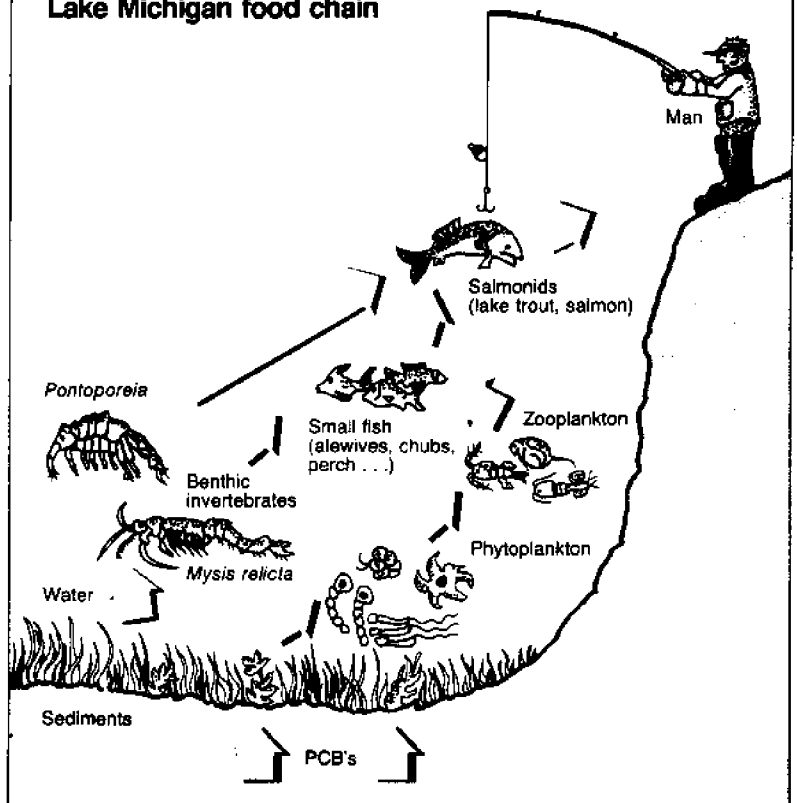
In recent years, numerous toxic and potentially hazardous organic compounds have been identified in the Wisconsin waters of the Lake Michigan basin. The findings in Wisconsin are representative of experiences in the other Great Lakes states, and will be discussed further here to provide an overview of some current problems.

Perhaps the best studied of the toxic organic compounds are the PCB's. A National Conference held in Chicago, Ill., in 1975 (*ES&T*, February 1976, p 122) addressed this industrial contaminant in detail. The unusual stability of the PCB's in the environment, and their capability to bioaccumulate, have contributed to the fishery problems mentioned above.

Contamination of Lake Michigan waters by PCB's has been a matter of concern since the late 1960's when the Wisconsin Department of Natural Resources (DNR) found that PCB's in fish collected from the lake exceeded the FDA guidelines (which were set at 5 μg/g at that time). PCB sources were found to be industrial and municipal effluents; but also, perhaps more significantly, the atmosphere was implicated as a possible major contributor of PCB's to the lake. Indeed, as the point-source contributions of PCB's are eventually closed off, nonpoint sources, such as the atmosphere, will take on a more important role.

Nevertheless, according to Stanton Kleinert of the Wisconsin DNR, the PCB problem in Wisconsin is chiefly a fishery problem, in terms of natural-resources management. Public water supplies in the state, for example, do not contain detectable amounts of PCB's, while nearshore waters of Lake Michigan and its tributaries contain only small concentrations of the contaminant.

FIGURE 2
How PCB's are passed through the Lake Michigan food chain



Source: University of Wisconsin Sea Grant Program. Published by permission of the copyright owner.

PCB's in the Sheboygan River

In March 1978, very high concentrations of PCB's were reported in fish collected in the Sheboygan River, a Lake Michigan tributary in northeastern Wisconsin. The fish, originally collected in late 1977, showed PCB concentrations ranging from 26 to almost 1000 μg/g. Recall that the FDA guideline is 5 μg/g; lowering it to 2 μg/g is proposed.

Because of these very high concentrations, an intensive sampling program was begun. The results verified the earlier findings that the PCB concentrations in the Sheboygan River fish were significantly elevated above the values normally found in the Lake Michigan basin.

These findings indicated the probable existence of a previously unidentified source of PCB's. Investigative work by the Wisconsin DNR quickly identified a metal-casting plant as the PCB source. The plant had been disposing PCB-contaminated wastes behind its building and along a dike adjacent to the Sheboygan River. High water, runoff, and erosion deposited the PCB's in the river sediments, and through food-chain accumulation, contaminated numerous fish species.

The PCB-contaminated shoreline material has now been isolated and placed in sealed drums, and awaits final disposal. The sediments, however, are still loaded with PCB's and continue to contaminate fish. They also are washed out into Lake Michigan through normal stream sediment transport processes. The question of dredging the sediments has not been resolved, since dredging might mobilize the PCB's, and lead to more contamination of the nearshore Lake Michigan area. Also, an adequate dredge spoil disposal site would have to be developed, and such sites can be placed only on land or in contained shoreline areas. The disposal of con-

taminated dredge spoils in Lake Michigan waters is unlawful, posing the dilemma that both action and inaction will result in continued PCB pollution problems. This case is an example of the type of toxic substance control problem that is likely to arise again in the future.

The company involved in the Sheboygan River case has changed ownership since the period when PCB's were used in the hydraulic systems of the casting machinery. PCB's are no longer in use, but their careless disposal in the past haunts the present owners, as well as the environment near the casting plant. A similar case is now the object of considerable research and regulatory interest in the Waukegan, Ill., harbor area.

Wastewater: the Lower Fox River

Probably the most industrialized drainage basin entering into Lake Michigan is the Lower Fox River, a tributary to the waters of Green Bay in northeastern Wisconsin. Leading from Lake Winnebago to Green Bay, it receives wastewaters from many pulp and paper mills, and other industrial and municipal wastewater treatment facilities.

A study initiated in 1976 sought to identify the sources of PCB's and other chlorinated organic compounds in the river. Many organic and chlorinated organic compounds were identified in the wastewaters, river water, sediment, and fish collected throughout the watershed. Included in these results were 20 of the "priority pollutants" that were discussed by Keith and Telliard (*ES&T*, April 1979, p 416).

Representative data from this study appear in Table 2. They indicate that the Lower Fox River system receives considerable loadings of chemicals from a variety of sources. The heavy use of chlorine as a bleaching agent in pulp-mill operations, the recycling of paper products contaminated with PCB's, and chlorination of municipal treatment plant effluents are sources of many of the chlorinated organic compounds.

The environmental significance and ultimate fate of these materials are not fully known at present, except of course, for PCB's which are found in high concentrations in fish taken from the Lower Fox River. Research sponsored by state and federal organizations, including the University of Wisconsin Sea Grant Program, is underway and will help to resolve many of the current uncertainties.

TABLE 2
Selected organic compounds identified in the Lower Fox River watershed in Wisconsin[a]

Compound	Concentration ranges		
	Wastewaters (μg/L)	Sediments (μg/g)	Fish (μg/g)
Anisole,			
Tetrachloro-	0.04–0.08	—[b]	—
Pentachloro-	0.65–0.38	—	0.005–0.06
Benzothiazole	10–30	—	—
Hydroxy-	10–30	—	—
Methylthio-	10–40	—	—
Dehydroabietic acid	100–8500	2.7	—
Dieldrin[c]	—	—	0.008–0.022
Guaiacol,			
Trichloro-	10–60	—	—
Tetrachloro-	10–50	—	—
Hexachlorocyclohexane[c]	0.04	—	—
Phenol(s),			
Dichloro-[c]	15–40	—	—
Trichloro-[c]	5–100	—	—
Tetrachloro-	2–20	—	—
Pentachloro-[c]	0.1–40	0.22–0.28	—
Polychlorinated biphenyls (PCB's)[c] (Aroclors 1242, 1248 and 1254)	0.1–56	0.05–61	0.5–90
Polycyclic aromatic Hydrocarbons (PAH's)[c]	0.5–10	—	—

[a] Source: Wisconsin Department of Natural Resources, 1978
[b] Not determined or not detected
[c] Compound on EPA priority pollutant list

PCB's in lake trout

A recently completed study has added substantially to knowledge of how PCB's are taken up by salmonid species, particularly lake trout in Lake Michigan. This research was performed by David Weininger, under the direction of David Armstrong, at the University of Wisconsin-Madison. In his Ph.D. thesis, Weininger developed a bioenergetic model to account for the accumulation of PCB's by Lake Michigan lake trout. With some modification, the model might apply to related species in other lakes that have similar feeding habits and metabolic processes.

The primary source of PCB's accumulated in the lake trout is adult alewives. Furthermore, for an average adult lake trout, the PCB concentration is more related to its age than its size, signifying that accumulation is more a function of time or exposure than of diet alone.

Of major importance in Weininger's work is the prediction that greater than 50% of the PCB's in adult lake trout have cycled through Lake Michigan sediments. This emphasizes food-chain processes, since benthic invertebrates are eaten by adult alewives which, in turn, are consumed by adult lake trout.

To reduce future concentrations in lake trout, the PCB content of the alewives must be reduced. Weininger speculated that if the alewife population could be reduced, then lake trout might switch to smelt as a primary food source. Smelt have much lower PCB concentrations; thus, lake trout PCB levels should decrease rapidly. This might result in lake trout eventually meeting the FDA tolerance limit in the future.

Weininger's hypothesis is intriguing, and fish resource managers have now been challenged to take innovative steps to reduce the PCB content of prized Lake Michigan stocked species (salmonids). The alternative is to abandon salmonid stocking for many years until PCB's in the sediments are buried beyond those levels at which they would be available to invertebrate organisms.

Control of contamination

Resource-management strategies in the Great Lakes Basin must contend with the toxic-substances issue. This

involves human and environmental health, thereby elevating itself to a position of prominence above some of the earlier Great Lakes problems, such as eutrophication and thermal pollution. The task will not be easy because it requires resource managers to adopt nontraditional concepts. However, progress can and must be made.

A toxic substances control plan has been developed by the Great Lakes Basin Commission. It draws on numerous sources for its background information including publications and policies of the IJC, PLUARG and EPA. Some of the important cornerstones of the Basin Commission's plan are the many existing environmental statutes, including the following (*ES&T*, February 1978, p 154):

- Toxic Substances Control Act,
- Federal Insecticide, Fungicide and Rodenticide Act,
- Federal Water Pollution Control Act Amendments,
- Resource Conservation and Recovery Act,
- Clean Air Act,
- Hazardous Materials Transportation Act,
- Ports and Waterways Safety Act,
- Safe Drinking Water Act,
- Atomic Energy Act,
- Marine Protection, Research and Sanctuaries Act, and
- Food, Drug and Cosmetic Act.

Despite the laws that have been enacted to control pollution, serious contamination incidents still occur in the Great Lakes Basin. These problems indicate the need for continued concern and cooperation on the part of those who produce, use, and discharge toxic substances. Regulatory surveillance and enforcement must continue, and will remain a major stimulus in controling the release of toxic substances in the basin.

In addition to surveillance, increased emphasis must also be placed on research. During the course of the analysis of environmental samples, numerous unknown compounds still appear as recorder traces on gas chromatographs. Resources must be made available to government and university scientists so that the number of these "unknowns" can be reduced. Expanded research efforts should result in a decrease in the number of incidents that were at one time sarcastically referred to as "pollutants of the month".

It is known that many toxic substances are transported to the Great Lakes Basin from other areas of the U.S. and Canada. This emphasizes the need for environmental-protection personnel to learn more about atmospheric chemical transport and reaction phenomena. Atmospheric chemistry is now receiving attention in a number of Great Lakes area universities. A symposium on atmospheric chemistry at the ACS meeting in September (*ES&T*, November 1979, p 1337) highlighted much of the current research in this area.

Why controls would be difficult

- **A number of toxic substances are already present in the Great Lakes.**
- **Some toxic pollutants in the Great Lakes are derived from sources well removed from the basin and are carried hundreds of kilometers by atmospheric processes, illustrating the need for national and international planning.**
- **The national goal of zero discharge of toxic substances into natural waters must be vigorously pursued, particularly in the Great Lakes where residence times can be long.**
- **Methods must be sought for the attenuation or removal of those toxic substances already present in the Great Lakes Basin.**
- **Existing laws must be enforced to reduce and ultimately eliminate the discharge of toxic substances to the aquatic environment.**

The Great Lakes states and the EPA should ultimately agree on a common list of priority pollutants that will receive primary emphasis for monitoring, research, and eventually control in the basin. The Great Lakes Basin Commission has stated that federal and state cooperation is critical in solving toxic-substances problems. And, importantly, research is also needed to determine the human health implications and environmental significance of the toxic substances that have been found distributed throughout the area.

Another item involves the analytical detection of lower and lower concentrations of chemicals which were not previously anticipated in the Great Lakes Basin. Should government regulate these substances "just because they are there", or should they await appropriate toxicity testing to determine their potential environmental and human health impacts? It is an important question that may require the wisdom of Solomon to answer to everyone's satisfaction.

In summary, it should be clear that there are toxic-substances problems in the Great Lakes Basin, and that steps are now being taken to resolve them. This process will take considerable time and money, but for the more than 15% of the U.S. population that lives in the Great Lakes area, these investments will provide long-term dividends in terms of improved water quality and enhanced utilization of the Great Lakes as a natural resource.

Note: This article is a condensed version of a report that was originally prepared for a course on water-quality issues entitled, "Decisions for Lake Michigan", funded by the U.S. Office of Education as part of the Interstate Water Quality Training Program, and coordinated by the Purdue University Calumet Campus and the Lake Michigan Federation.

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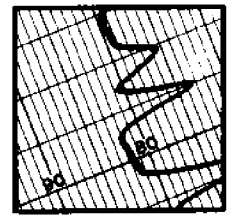
Some Factors Affecting the Recovery of Polychlorinated Biphenyls from Water and Bottom Samples

T. A. Bellar and J. J. Lichtenberg



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T. A. Bellar[1] *and J. J. Lichtenberg*[1]

Some Factors Affecting the Recovery of Polychlorinated Biphenyls (PCB's) from Water and Bottom Samples

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ABSTRACT: During studies on analytical methods for PCB's in water and bottom samples, variable recoveries from dosed samples and apparent decreasing recoveries with the age of the sample were observed. Losses from dosed river waters as high as 10 percent in one day and greater than 40 percent in one week were common. Recoveries from bottom samples varied widely with the sample pretreatment and the method of extraction. In an effort to define some of the factors affecting the recovery of PCB's from water, the following parameters and conditions were studied: sample container, extremes of pH, aging under ambient conditions, and several preservation techniques. Several methods of sample preparation and extraction of bottom samples were studied. Environmentally contaminated water and lake bottom samples and dosed natural samples were studied.

The separatory funnel liquid-liquid extraction method was found to be the most efficient for the extraction of PCB's from natural waters. The air-dried, moisture-added soxhlet extraction procedure proved to be the most efficient for recovery of PBC's from environmentally contaminated lake bottom samples. Formaldehyde was found to be effective for preserving PCB's in dosed natural waters for at least two weeks.

KEY WORDS: water quality, water pollution, pesticides, toxicity, recovery, aquatic animals, polychlorinated biphenyls (PCB's)

Polychlorinated biphenyls (PCB's) have been recognized for several years as ubiquitous environmental pollutants. These complex mixtures of variably chlorinated biphenyls have occurred in nearly every environmental media [*1,2*].[2] Their presence in water, sediment, biota, fish, birds, and animals have been reported [*3-5*]. While the PCB's are reported to be less toxic than the chlorinated hydrocarbon pesticides, acute toxic effects to aquatic life have been demonstrated [*3,6*]. Since they do exhibit acute

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[2] The italic numbers in brackets refer to the list of references appended to this paper.

toxic effects and, like the chlorinated pesticides, they are biologically magnified [*7*], it is important to maintain surveillance of the PCB levels occurring in the environment.

The PCB's are chemically similar to many of the organochlorine pesticides; therefore, the same analytical procedures are applied to both. Being complex mixtures, the PCB's present a serious interference problem when determining many pesticides. Therefore, special separation and cleanup procedures are required for analyses when both occur in a given sample. Much work has been published on the separation and the determinative aspects of the analysis [*8-10*]. However, little has been reported on factors that might affect the extraction and recovery of PCB's from environmental samples.

During our studies on water, we noted variable recoveries from dosed samples and an apparent decreasing recovery with the age of the sample. In an effort to define some of the factors affecting the recovery, the following parameters were investigated: the sample container, extremes of pH, aging under ambient conditions, and several preservation techniques. Liquid-liquid extraction and extraction with urethane foam plugs were studied. During our studies on recovery of PCB's from bottom samples, several methods for preparation and extraction of bottom samples were compared. We were particularly interested in the newer column extraction procedures since they appear to be becoming very popular. Environmentally contaminated samples and dosed natural samples were studied.

Experimental

Apparatus—The high frequency mechanical dispersion instrument used in one phase of the bottom sample extraction study was the Super Dispax Tissumizer, Model STD 182N (Tekmar Company, Cincinnati, Ohio).[3]

Reagents—Reference Standard PCB's Aroclors 1242, 1254, 1260, and 1016 (Monsanto Chemical Company, St. Louis, Mo.) prepared in acetone solution. Formaldehyde—37% aqueous solution.

Procedure—The samples were collected in 1-qt to 6-gal glass containers at various times over a period of one year.

The primary liquid-liquid extraction procedure employed for water samples was that recommended by the U.S. Environmental Protection Agency (EPA) for organochlorine pesticides [*11*]. In addition, the semi-automatic magnetic stirring (Vortex) system of Kawahara et al [*12*] was tested. The urethane foam procedure for extraction of PCB's from water was that published by Gesser et al [*13*]. A modification of Gesser's procedure, that of soaking the foam plugs in the sample with occasional shaking, was also tested.

[3]Mention of products and manufacturers is for identification only and does not imply endorsement by the Methods Development and Quality Assurance Research Laboratory of the U.S. Environmental Protection Agency.

Several methods for extraction of bottom materials were investigated. The soxhlet extraction procedure of Breidenbach et al [*14*], column elution procedures of Hesselberg and Johnson [*15*] and Southeast Environmental Research Laboratory [*16*], the high frequency dispersion procedure of Johnson and Starr [*17*], the mechanical shaking procedure of Goerlitz and Brown [*18*] and Shell Chemical Company [*19*], and the blender extraction method of Muth [*20*] were tested.

All bottom sample extracts were cleaned up in the same manner. The standard Florisil column procedure [*11*] and the semi-micro silica gel procedure of Leoni [*9*] were employed. When necessary, elemental sulfur was removed from the extracts using mercury according to the method of Goerlitz and Law [*21*]. Table 1 lists the conditions for preparation and extraction of bottom samples.

TABLE 1—*Extraction procedures tested for recovery of PCB's from bottom samples.*

Method	Ref	Preliminary Drying	Dry Matter, %	Water Added	Extracting Solvent
Soxhlet	[*14*]	air dry at room temperature, grind—mortar and pestle	>95	10%	hexane-acetone (9:1)
Shake I	[*19*]	air dry at room temperature, grind—mortar and pestle	>95	slurry	hexane-isopropyl alcohol (3:1)
Shake II	[*18*]	none	...	none	acetone with hexane added later
Blender	[*20*]	Na_2SO_4 added	...	none	acetonitrile-acetone (1:1)
Column I	[*15*]	partial air dry at room temperature, mix with Na_2SO_4 and grind	~70	none	hexane
Column II	[*16*]	partial air dry at room temperature, mix with Na_2SO_4 and grind	~50-70	none	hexane-acetone (1:1)
High frequency dispersion	[*17*]	none	...	none	acetone

Dosing Procedures—Water Samples (880 ml) in 1-qt sample bottles were individually dosed by pipetting the PCB's in acetone solution (1 μg/ml) and shaking vigorously to achieve uniform mixing.

Bottom Samples—Approximately 400 g of an air dried sediment (98.7 percent dry matter) was ground with a mortar and pestle and passed through a 20 mesh screen. The sediment was then placed in a 1-qt wide mouth glass bottle. Distilled water (200 ml) was added and mixed to form a slurry. Aroclor 1242 (50.0 μg) and Aroclor 1254 (50.0 μg) in 1 μg/μl

acetone solution was added to the slurry with continual mixing. The bottle was sealed and allowed to equilibrate for 20 h with occasional shaking. Appropriate aliquots of the dosed sample were taken and treated as required by the extraction methods to be tested. The high frequency dispersion device, Column II, and soxhlet extraction were selected for testing recovery on dosed bottom samples.

Quantitation Techniques—Two methods were used to reduce the electron capture gas chromatographic data:

(*a*) When a dosed or environmentally occurring PCB equivalent to or closely resembling a standard Aroclor was present, a simple calculation was made by summing all the peak heights of the unknown and comparing it to the sum of the peak heights of the reference Aroclor.

(*b*) When mixtures of PCB's, not representing a single Aroclor were present, the method of Webb [*10*] was used to determine the PCB concentrations and the most likely Aroclor represented.

All results for bottom samples were calcualted on a dry weight basis. The dry weight was obtained by oven drying an aliquot of the sample at approximately 105°C overnight.

Gas chromatography and mass spectrometry techniques were used to confirm the identity of the PCB's in the environmentally contaminated samples.

Results and Discussion

Water Samples

Initial studies to determine the cause of low and variable recoveries of PCB's from dosed natural waters centered around the bottle cap liners. Teflon lined caps which are used routinely are considered the best choice when collecting samples for organic analysis. It was thought that the Teflon may be sorbing some of the PCB's. However, no PCB's were recovered from Teflon liners after soaking in hexane for 1 h. Subsequently, a comparison of recoveries using Teflon lined caps and aluminum lined caps was made. The samples were stored under ambient conditions in the laboratory, up to 11 days. Figure 1 shows that there is a tendency toward higher recoveries when using aluminum lined caps. In each case, samples stored with aluminum cap liners yielded greater recoveries than those stored with Teflon lined caps.

Sample Preservation

Figure 2 shows the results of aging on recovery of PCB's from dosed natural river waters. Samples held at ambient conditions in the light showed variable and often low recoveries with a general decrease in

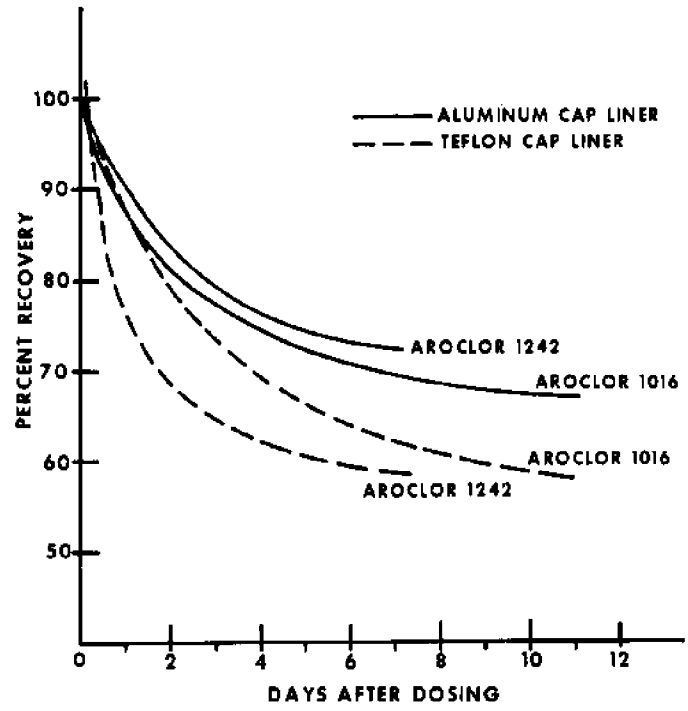

FIG. 1—*Effect of cap liner on recovery of PCB's from dosed river water (dose of 1 μg per sample).*

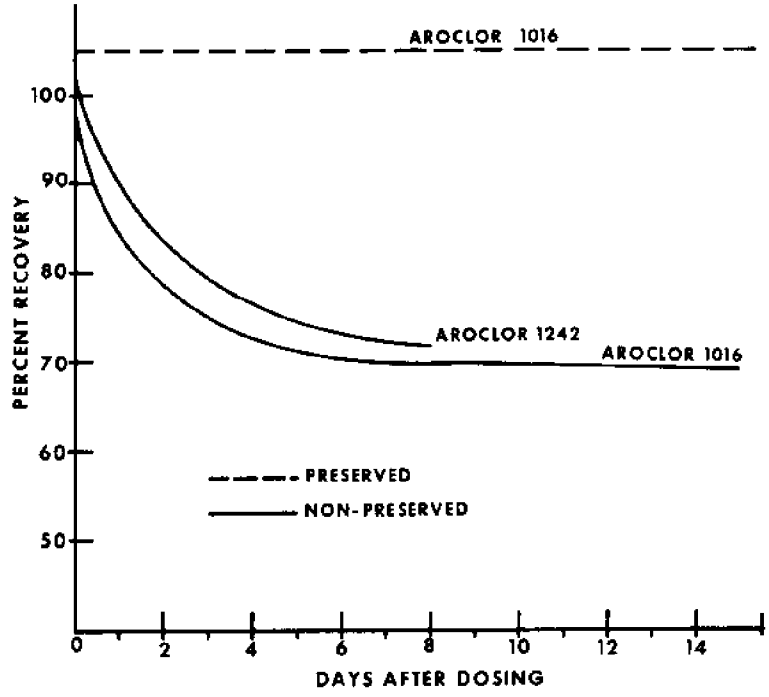

FIG. 2—*Effect of aging on recovery of PCB's from dosed river water at ambient temperature, preserved versus non-preserved (dose of 1 μg per sample).*

recovery with time. Recoveries ranged from 102 percent to a low of 67 percent over a period of 15 days. The recoveries from samples preserved with formaldehyde were quantitative over the time period, averaging 105 percent and ranging from 92 to 116 percent. Figure 3 depicts typical losses of the isomers of Aroclor 1016 that occur after aging a dosed sample for 15 days. The greater losses occur in the isomers that contain three or fewer chlorine atoms. The greatest losses occur in peaks 37 and 40 which represent PCB's containing three chlorine atoms. All natural water samples tested showed a trend toward reduced recovery with the age of the sample. The rate of loss appeared to be affected by the variation in physical, chemical, and biological characteristics of the samples.

Table 2 compares the recoveries of Aroclor 1016 obtained from dosed river waters that have been preserved with formaldehyde up to 15 days to the recoveries obtained at time zero (<8 h after dosing). A statistical

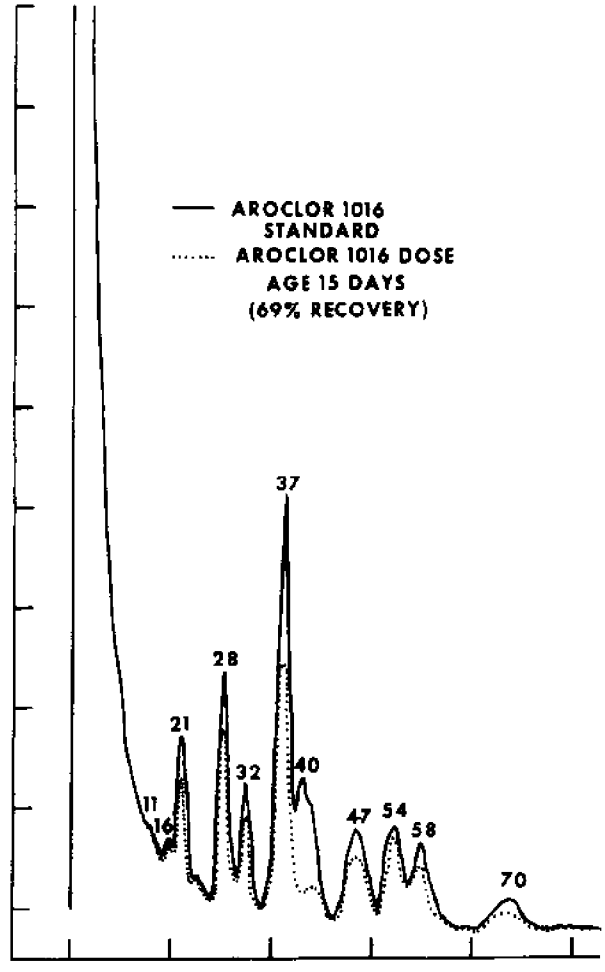

FIG. 3—*Electron capture gas chromatogram of reference standard and recovered Aroclor 1016.*

TABLE 2—*Recovery of Aroclor 1016 from river waters at time zero versus samples preserved and aged* (dose, *1 μg/sample*).

	Recovery, %	
	Time Zero [a]	Preserved [b]
	109	92
	107	107
	107	110
	98	111
	107	109
	116	93
	111	116
	102	111
	92	104
	109	105
	111	108
	100	111
	99	95
	105	...
Mean	105.4	105.5
No. of samples	14	13
Standard deviation	6.4	7.6
Variance	40.4	57.8
Coefficient of variation	6.0	7.2
Blank value (average)	6.0	6.0
Corrected mean	99.4	99.5

[a] Time zero = <8 h after dosing.
[b] Preserved with formaldehyde (15 ml of 37% solution).

comparison of the data shows very good agreement between the time zero and preserved samples.

The pH of the sample had no effect on the extraction efficiency from dosed samples over the range of 2.7 to 10.5. That is, the average recovery at time zero for the pH levels listed in Table 3 was quantitative. The accuracy and precision was essentially equivalent to that obtained for the untreated samples shown in Table 2. The limited data obtained suggest an apparent effect on the recovery as the sample ages at pH 5.3 and 7.3. Recoveries at these pH levels decreased with time although the decrease was not as great as noted in other studies. Recoveries at pH 2.7, 8.8, and 10.5 were near quantitative throughout the time period studied.

The effect of storing samples at ambient temperatures in the light, dark, and under refrigeration was studied. The results are listed in Fig. 4 and compared to results obtained for samples preserved with formaldehyde. Samples stored in the dark yielded higher recoveries throughout the period of study than those stored in the light. Refrigerated samples gave slightly better recoveries than those stored at ambient temperature in

TABLE 3—*Recovery of Aroclor 1016 from dosed river water at various pH levels (dose, 1 μg/sample).*

pH	Age, days	Recovery, %
2.7	2	98
2.7	6	102
2.7	14	91
5.3	2	107
5.3	6	89
5.3	14	86
7.3	2	109
7.3	6	87
7.3	14	84
8.8	2	107
8.8	6	106
8.8	14	95
10.5	2	107
10.5	6	105
10.5	14	100

NOTE—
Blank value = 7%.
Day zero value (mean recovery) = 107%.

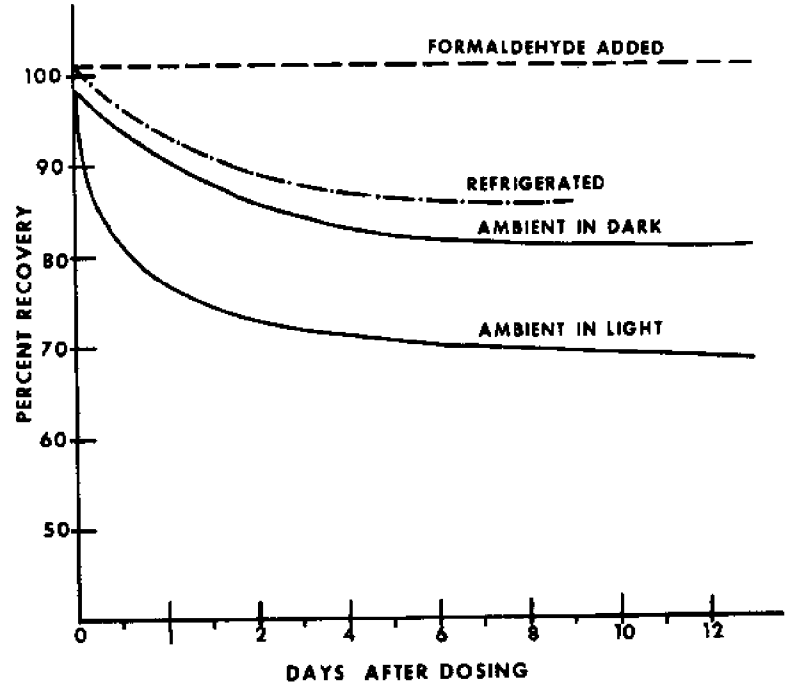

FIG. 4—*Recovery of Aroclor 1016 from dosed river water when stored at ambient temperature (in light and the dark), under refrigeration, and preserved with formaldehyde (dose of 1 μg per sample).*

the dark. However, the trend of decreasing recovery was evident under all three conditions. Samples preserved with formaldehyde gave the highest and least variable recoveries and showed no decreasing trend in recovery with the age of the sample.

The use of the semi-automatic liquid-liquid extraction system of Kawahara et al [*12*] was considered. A preliminary evaluation showed it to be inferior to the separatory funnel shake out. Therefore, it was not tested further. At time zero, the mean recovery using this system was 84 percent with a range of of 77 to 92 percent.

Polyurethane Foams

The use of urethane foam plugs as reported by Gesser et al [*13*] appeared to show promise as a means for concentrating and isolating PCB's. Thus, we evaluated the technique on dosed distilled and natural river water. Table 4 lists the data obtained using the column procedure of Gesser and by soaking the plugs in the sample. The column method worked well on distilled water and was more efficient than the soaking procedure. However, the column method was not at all suitable for use on

TABLE 4—*Recovery of Aroclor 1016 from distilled water using polyurethane foam plugs (dose, 1 μg/sample).*

	Recovery, %	
	Column Elution	Soaking
	84	61
	79	89
	100	77
	95	58
	91	77
	71	95
	109	106
	91	109
	100	86
	86	50
	98	77
	76	50
	91	97
	88	60
	94	75
	95	114
	84	66
	99	98
	114	99
	...	100
Mean	91.8	82.2
No. of samples	19	20
Standard deviation	10.7	20.0
Variance	114.6	399.0
Coefficient of variation	11.6	24.3

natural waters. Suspended material in the samples plugged the column. We were unable to pass the total volume of a 1-liter natural river water sample through the column without adding external pressure, in which case channeling occurred. Soaking the plugs in dosed river water worked, however, the accuracy and precision was poorer than that obtained with distilled water. Coating the plugs with SE-30 did not improve the efficiency. These results coupled with the high background material from the foam which had significant adverse effects on the gas chromatographic (GC) column and the detector were the bases for rejecting the method.

As a result of the foregoing studies, the separatory funnel liquid-liquid extraction technique was shown to be the most efficient for recovery of PCB's from natural waters. Therefore, this method was used for analyzing all wastewater samples described next.

Wastewater samples

Several industrial wastewater samples were collected and analyzed for environmental levels of PCB's. In one case, additional aliquots of the sample were then dosed with 5 μg/liter of Aroclor 1242 and again analyzed. Table 5 presents the results and statistical data derived from the

TABLE 5—*Recovery of PCB's from environmentally contaminated industrial waste samples, concentration of Aroclor 1242* (μg/liter).

Replicates	870	870 [a]	832	833
1	2.17	7.99	58.7	13.7
2	1.92	7.74	63.0	13.8
3	1.86	7.42	64.8	...
4	2.27	6.97	66.9	...
5	1.86	6.42	60.3	...
6	1.70	6.79	66.3	...
7	1.73	6.75	61.7	...
Mean	1.93	7.15	63.1	
No. of samples	7	7	7	
Standard deviation	0.214	0.575	3.07	
Variance	0.046	0.330	9.46	
Coefficient of variation	11.11	8.03	4.87	

[a] Dosed with 5 μg/liter of Aroclor 1242.

samples. The results show good precision for two levels of environmental contamination. The precision for the dosed samples was equally good. The recovery for the dosed sample averaged 103 percent.

Bottom Samples

Bottom samples collected from a large man-made lake in Ohio were found to be contaminated with Aroclor 1242 and 1254. Samples collected from three locations in the lake were selected in order to evaluate seven

extraction procedures. The total quantity of sample available to us limited the numbers of analyses that could be made. Nevertheless, useful information was derived from the results. Table 6 lists the results of our study on the lake bottom samples. The mechanical dispersion apparatus was not available at the time and no comparison of this technique could be made

TABLE 6—*Recovery of PCB's from environmentally contaminated lake bottom samples.*

Method	Ref	Sample Area [a]	Dry Matter, %	Aroclor 1242, μg/kg	Recovery, [b] %	Aroclor 1254, μg/kg	Recovery [b] %
Soxhlet	[14]	3	96.7 [c]	26.2	100	12.5	100
Shake I	[19]	3	96.7	15.3	58.4	7.4	59.2
Blender	[20]	3	37.3	5.6	21.4	9.4	75.2
Shake II	[18]	3	37.3	1.0	3.8	2.7	21.6
Soxhlet	[14]	5	96.4 [c]	54.3	100	24.7	100
Column I	[15]	5	76.9	6.4	11.8	8.9	36.0
Soxhlet	[14]	8	98.4 [c]	0	...	403	100
Shake I	[19]	8	98.4	0	...	377	93.5
Column I	[15]	8	98.4	0	...	343	85.1
Column I	[15]	8	70.2	0	...	318	78.9
Blender	[20]	8	70.2	0	...	343	85.1
Blender	[20]	8	56.0	0	...	246	61.0
Shake II	[18]	8	70.2	0	...	321	79.7
Shake II	[18]	8	56.0	0	...	258	64.0

[a] Samples from Areas 3 and 5 were muck; samples from Area 8 were sandy muck.
[b] Recovery for soxhlet procedure considered to be 100%.
[c] 10% water added prior to extraction.

on these samples. The results obtained show that the air-dried, 10 percent moisture-added soxhlet procedure provides significantly greater efficiency than any of the other methods tested.

In addition, a study of the recovery of Aroclor 1242 and 1254 from dosed bottom samples was carried out using three methods—mechanical dispersion, column II, and the soxhlet. Results of this test (Table 7) show the superiority of the air-dried moisture-added soxhlet extraction pro-

TABLE 7—*Recovery of PCB's from dosed bottom samples (dose 237 μg/kg total PCB's).* [a]

Method	Ref	Dry Matter, %	μg Recovered		Average Recovery, %
			1	2	
High frequency dispersion	[17]	52.5	[b]	210	88.7
Column II	[16]	89.7	208	187	83.5
Soxhlet	[14]	98.4	230	226	96.2

[a] Mixture of Aroclors 1242 and 1254, 118.5 μg/kg of each.
[b] Sample lost due to malfunction of dispersion apparatus.

cedure over the other extraction procedures as far as bottom sediments are concerned.

We acquired a sewage treatment plant sludge which was found to contain PCB's. Aroclors 1242 and 1260 were identified in the sample. Two extraction techniques were employed in the analysis—mechanical dispersion and soxhlet extraction. The results are listed in Table 8. In this case, equivalent results were obtained with the two methods.

TABLE 8—*Recovery of PCB's from environmentally contaminated treatment plant sludge.*

			Aroclor Recovered (mg/kg)	
Sample No.	Method	Dry Matter, %	1242	1260
831-A	high frequency dispersion	18.1	34.4	14.2
831-B	soxhlet	93.2	34.4	12.5

The results of these studies show the air-dried, 10 percent moisture-added soxhlet extraction procedure to be the most efficient method for recovery of PCB's from bottom samples. This is consistent with the results reported by others for recovery of certain organochlorine pesticides from soils [*22-25*]. The better efficiency of this procedure is probably due to the smaller particle size achieved when pulverizing the dry sediment and to the longer solvent contact time provided by the soxhlet method as reported by Saha et al [*26*] and Chiba and Morley [*27*]. Nash et al [*28*] evaluated soxhlet, shake, and column procedures somewhat different from those that we studied and concluded that his soxhlet and shake procedures gave near equivalent recoveries for nine pesticides from soil. In this case, the samples were not air dried or ground with a mortar and pestle prior to soxhlet extraction.

The cleanup procedures employed, the standard Florisil and the micro silica gel columns, were adequate for all industrial waste and sludge samples. However, all bottom samples required further treatment with mercury to remove sulfur. All procedures which required a water wash of the extracts, the shake, Column II, blender, and the high frequency dispersion methods, produced severe emulsions and additional manipulative steps not required by the soxhlet procedure.

The high frequency dispersion technique looks promising, at least, for sludge samples and should be evaluated further. However, some problems have been encountered in its use. Small stones, pea size and even smaller, are a hazard. They are thrown at terrific speeds into the wall of the container and will shatter glassware. Further, even small particle size sand causes excessive wear on the stator and rotor, in which case frequent parts replacement is required.

Conclusions

The separatory funnel liquid-liquid extraction procedure is the method of choice for recovery of PCB's from natural waters. The air-dried soxhlet extraction procedure is the method of choice for recovery of PCB's from bottom and sludge samples. Formaldehyde effectively preserves PCB's in dosed natural water samples, at least up to two weeks.

Acknowledgments

The authors wish to thank James O'Dell for his assistance in collection, preparation, and extraction of the samples used in this study. The GC-MS confirmatory analyses were performed by J.W. Eichelberger and L.E. Harris.

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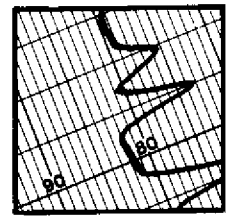
Qualitative and Quantitative Analyses of Polychlorinated Biphenyls by Gas-Liquid Chromatography

B. Bøe and E. Egaas



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QUALITATIVE AND QUANTITATIVE ANALYSES OF POLYCHLORINATED BIPHENYLS BY GAS–LIQUID CHROMATOGRAPHY

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(First received April 23rd, 1979; revised manuscript received June 26th, 1979)

SUMMARY

A model is presented of the relationship between the relative responses of flame-ionization and electron-capture detectors and the structure of polychlorinated biphenyls (PCBs). The model permits the calculation of detector responses for all PCBs and opens the possibility of detailed structural analyses.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are chemically and microbiologically resistant environmental contaminants, and are therefore incorporated into various biological life cycles. Through the food chain they accumulate in the higher members of the chain and may cause undesirable effects.

The analysis of PCBs is not easy owing to the large number of possible components, *i.e.*, 209 plus biphenyls. Quantitative analyses are routinely based on a comparison of the gas–liquid chromatographic (GLC) pattern of the sample with the PCB profiles of commercial products, and results are based on one or several prominent peaks. This method may give realistic values provided that the PCB pattern in the sample is similar to that found in a commercial PCB sample, or alternatively similar to mixtures of several commercial PCBs. Unfortunately, it is not possible in practice to verify the assumption of identical, or even similar, PCB patterns owing to the complexity of the chromatograms from biological samples; for this reason, quantitative figures for PCBs reported on the basis of this method should be regarded as only approximate.

A more satisfactory method consists in determining the amounts of all of the individual components and summing them to give the total amount of PCBs. At present glass capillary column GLC with an electron-capture detector (ECD) provides the best specificity and sensitivity, and is the method of choice. It is, however, necessary to calibrate the detector against standards for each PCB to be determined. It is known that the ECD response is dependent on the structure of the PCB and not only on the number of chlorine atoms[1,2]. Only a limited number of PCBs have been synthesized, and their ECD responses have not been systematically studied.

A model is presented here that permits the calculation of ECD calibration factors for all PCBs. With this model it becomes possible to obtain both quantitative and detailed qualitative information on the components of PCBs.

EXPERIMENTAL

Twenty-one pure PCBs and DDE (purity >99%) were obtained from Analabs, North Haven, Conn., U.S.A. The GLC equipment consisted of a Perkin-Elmer F22 two-channel gas chromatograph fitted with a flame-ionization detector (FID) and an (ECD. The FID channel was used with a 25 m × 0.25 mm I.D. OV-101 glass capillary column (Perkin-Elmer) and the ECD was connected to a 20 m × 0.25 mm I.D. OV-1 (Jaeggi, Trogen, Switzerland) column. The oven temperature was kept at 22° and injector and detector were held at 290° and 300°, respectively. Argon–methane was used as the carrier gas with the ECD channel and nitrogen with the FID channel. Peak areas were measured with a Spectra-Physics SP 4000 data system.

Mixtures of baseline-separated PCBs and DDE in *n*-hexane were prepared such that the ratio between the highest and lowest concentrations of a given PCB relative to DDE was 16. The amount of sample injected by the splitless technique was 1 μl. Concentrations of PCBs analysed with use of the ECD ranged from 20 to 100 mg/l, and were chosen so as to minimize column adsorption without saturation of the detector.

The samples analysed with use of the FID were 5–10 times more concentrated. The relative detector calibration factors, KF_{rel}, were determined using DDE as an internal standard. When the PCB in question had a relative retention time (RRT) close to unity, a second PCB was used as an intermediate internal standard. Detector calibration lines were calculated by linear regression.

RESULTS AND DISCUSSION

The approach was to measure the ECD and FID responses for a given PCB relative to DDE as an internal standard. The calibration factor, KF, is defined as the ratio between the amount of compound and the area under the corresponding peak on the chromatogram:

$$KF = \text{amount/area} = X/A \tag{1}$$

$$\text{The relative response is } KF_{rel} = \frac{X_{PCB}/A_{PCB}}{X_{DDE}/A_{DDE}}$$

These calibration factors were measured for 21 PCBs using the FID and ECD and are listed in Table I. Each KF_{rel} value was determined by linear regression based on 6–18 points. It was established that the constant term b in the expression $X_{rel} = KF_{rel} \cdot A_{rel} + b$ in all instances was less than 1% of the highest value of X. All regression lines therefore passed through the origin. A more easily accessible empirical parameter is obtained by dividing relative responses for the FID and ECD:

$$KF_{relFID}/KF_{relECD} = \frac{X_{PCB}/A_{PCB}}{X_{DDE}/A_{DDE}}\,(\text{FID}) \cdot \frac{X_{DDE}/A_{DDE}}{X_{PCB}/A_{PCB}}\,(\text{ECD})$$

and if X_{PCB}/X_{DDE} is kept constant:

$$KF_{relFID}/KF_{relECD} = \frac{A_{PCB}/A_{DDE}\,(ECD)}{A_{PCB}/A_{DDE}\,(FID)} \tag{2}$$

The ratio between the relative areas obtained with the two detectors is identical with the ratio between the relative calibration factors. Thus this ratio may be obtained for all well separated peaks without knowing the identity of the peaks, and without a knowledge of the corresponding amounts of compounds.

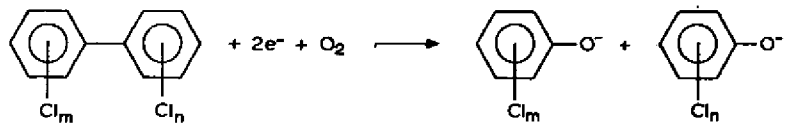
For both types of detector there is a linear relationship between area and amount of compound[3]. It was ascertained that all measurements with the ECD were made within the linear range of the detector. As the PCBs and the internal standard are contained in the same volume, we can substitute concentration for amount:

$$KF_{relFID}/KF_{relECD} = \text{Constant} \cdot \frac{[PCB]/[DDE]\,(ECD)}{[PCB]/[DDE]\,(FID)} \tag{3}$$

The expression on the right-hand side is mathematically equivalent to the expression for an equilibrium constant. In order to develop a model for relative responses it is therefore reasonable to search for linear free energy relationships involving the logarithm of KF_{relFID}/KF_{relECD}.

As can be seen from Table I, the KF_{relFID} values increase gradually with the number of chlorine atoms, from 0.910 for the dichloro isomer to 2.18 for the decachloro isomer. On the other hand, the KF_{relECD} values are more variable. Although there is a general tendency towards lower values with an increasing number of chloro substituents, there are very large differences between isomers that have the same number of chlorine atoms. For example, for the tetrachloro-PCBs, the highest KF_{relECD} value is 14.0 and the lowest value is 2.41. The ratio between KF_{relFID} and KF_{relECD} therefore reflects primarily the behaviour of KF_{relECD} values, and it becomes necessary to consider the processes taking place in the ECD.

By means of negative ion mass spectrometry it was shown that phenoxy anions are formed in the ECD during analysis of PCBs[4]. The anions may be formed by reaction with trace amounts of oxygen in the carrier gas according to the equation



(4)

The detector signal is proportional to the number of electrons being captured per second, and this depends on the stability of the phenoxy anions and on the strength of the C–C biphenyl bond.

The following Hammett-like equation was applied:

$$\log (KF_{relFID}/KF_{relECD}) = a_0 + a_1 \Sigma\sigma_{o,m,p} + a_2\Sigma\sigma_{o,m} \tag{5}$$

TABLE I

FID AND ECD RESPONSE FACTORS FOR POLYCHLORINATED BIPHENYLS, RELATIVE TO DDE

Structure	*FID, 25-m OV-101*				*ECD, 20-m OV-1 column*				$KF_{FID}/KF_{ECD} \times 10^4$	*Log* (KF_{FID}/KF_{ECD})	
(ring 1-ring 2)	*RRT*	KF_{rel}*	*Points*	*r*	*RRT*	KF_{rel}*	*Points*	*r*		*Found*	*Calculated*
26	0.453	0.910	9	0.9932	0.564	16.9	13	0.9967	538	2.73	2.84
234	0.639	0.814	13	0.9997	0.715	3.24	14	0.9998	2515	3.40	3.54
236	0.569	0.782	12	0.9991	0.652	8.16	12	0.9974	960	2.98	3.07
25-3	0.601	1.01	9	0.9897	0.680	15.8	12	0.9922	639	2.81	2.65
2345	0.826	0.975	15	0.9994	0.857	2.41	6	0.9878	4046	3.61	3.77
2356	0.715	0.948	9	0.9993	0.766	3.96	11	0.9981	2394	3.38	3.29
24-25	0.688	1.17	9	0.9985	0.750	12.9	8	0.9983	907	2.96	3.21
24-34	0.831	1.19	13	0.9998	0.859	4.34	8	0.9987	2742	3.44	3.54
25-25	0.678	0.984	9	0.9980	0.748	14.0	11	0.9993	703	2.85	2.80
23-25	0.723	0.910	12	0.9985	0.795	10.3	9	0.9974	883	2.95	2.80
245-23	0.987	1.19	18	0.9983	0.973	4.75	10	0.9976	2505	3.40	3.54
234-23	1.02	1.20	10	0.9980	1.00	1.46	6	0.9931	8235	3.92	3.54
245-25	0.904	1.11	13	0.9980	0.928	2.96	9	0.9972	3750	3.57	3.54
234-25	1.00	1.17	10	0.9992	1.00	3.25	7	0.9986	3604	3.56	3.54
236-236	1.02	1.17	18	0.9982	1.00	7.48	9	0.9986	1563	3.19	3.19
245-245	1.28	1.32	16	0.9956	1.21	1.49	9	0.9942	8859	3.95	3.84
234-245	1.42	1.31	16	0.9960	1.32	1.41	11	0.9985	9291	3.97	3.84
23456-25	1.68	1.38	13	0.9946	1.51	0.946	6	0.9891	14581	4.16	4.18
2345-2345	3.29	1.55	14	0.9984	2.71	0.792	9	0.9963	19570	4.29	4.06
23456-2345	3.97	—	11	0.9987	3.31	—	6	0.9955	20807	4.32	4.18
23456-23456	4.84	2.18	8	0.9918	3.89	1.01	10	0.9961	21584	4.33	4.45

* KF = amount/area.

where $\sigma_o = 0.68$, $\sigma_m = 0.37$ and $\sigma_p = 0.23$. The second term is the sum of substituent constants for chlorine atoms in the PCB molecule, and this term reflects the stability of phenoxy anions. The σ_o constant is taken from the ionization of phenols[5], and the other two constants are ordinary substituent constants.

The last term contains σ constants for *o*- and *m*-positions relative to the central C–C bond, and reflects the inductive substituent effects on this bond. By applying eqn. 5 to data from Table I, it was found necessary to divide the PCBs into two classes, *viz.*, those compounds containing the same number of chlorine atoms in both rings in class 1, and all of the others in class 2.

For compounds in class 1 it is reasonable that both of the rings in the PCB molecule contribute to the detector signal. The following regression equation was obtained:

$$\log (KF_{\text{relFID}}/KF_{\text{relECD}}) = 2.19 + 2.27\,\Sigma\sigma_{o,m,p} - 1.98\Sigma\sigma_{o,m} \quad (6)$$

where $R = 0.9324$ (9 points). In this equation the sum of σ values is taken for the total number of chlorine atoms in the PCB molecule.

For PCBs in class 2 the following equation was obtained:

$$\log (KF_{\text{relFID}}/KF_{\text{relECD}}) = 2.01 + 3.87\,\Sigma\sigma_{o,m,p} - 3.26\,\Sigma\sigma_{o,m} \quad (7)$$

where $R = 0.8956$ (12 points). In eqn. 7 the σ values are summed for only the most heavily substituted ring in the PCB molecule.

Calculated values for $\log (KF_{\text{relFID}}/KF_{\text{relECD}})$ are compared with the experimental values in Table I, and it can be seen that the agreement is reasonable.

Qualitative analyses of PCBs can be performed by comparing calculated with experimental values, and the number of structural possibilities is thus narrowed. There is, however, a certain degree of overlapping of theoretical $\log (KF_{\text{relFID}}/KF_{\text{relECD}})$ values for isomers containing the same number of chlorine atoms if they do not belong to the same class (1 or 2). Pairs of components containing different numbers of chlorine atoms may also overlap if they contain the same number of chlorine atoms in their most heavily substituted rings. In these instances further information on the structures of the PCBs can be obtained by comparing measured relative retention times with those calculated on the basis of additivity of retention indices (*RI*) for the two substituted rings[6]. Relative retention times in Table I measured on the OV-101 capillary column follow closely the theoretical values based on measurements on packed columns with the same stationary phase[6] (see Table II).

By means of mass spectrometry the number of chlorine atoms in a given PCB can be determined[7], and this information, together with that provided by GLC according to the present method, may allow unique structural assignments to individual PCBs. The type of information available can be illustrated by considering in detail the results for tetrachlorobiphenyls. Calculated and experimental values for retention times and relative detector responses are given in Table II. Based on a closeness criterion of $\Delta\text{RI} \geqslant 10$ (ref. 6) and $\Delta\log(KF_{\text{relFID}}/KF_{\text{relECD}}) \geqslant 0.2$, unique structures can be assigned to 29 isomers, while there are two or three possibilities for each of the remaining 13 isomers.

TABLE II

CALCULATED AND EXPERIMENTAL VALUES FOR TETRACHLOROBIPHENYL ISOMERS

No. of chlorine atoms in position, used for calculation			*Log (KF_{relFID}/KF_{relECD})*		*RI (calculated) (unique isomers in italics)*	*RRT*	
			Calculated	*Found*		*Calculated*	*Found*
o-	*m-*	*p-*					
)	4	0	2.63		*2046*		
l	3	0	2.72		*1983, 2021*		
2	2	0	2.80	2.85 2.95	1920, *1944, 1958*, 1996	0.678 0.726	0.678 0.723
l	2	0	2.88		*1931*, 1997, *2007*		
)	1	0	2.90		*1881*, 1919		
4	0	0	2.99	*1842*	*1842*		
)	3	1	3.04		*2095*		
!	1	0	3.06		*1899*, 1965, *1975*		
	2	1	3.13		1993, *2032, 2070*		
	1	1	3.21	2.96	*1930*, 1968, 1993	0.691	0.688
	2	0*	3.29	3.38	*1955*	0.722	0.715
3	0	1	3.31		*1891*		
0	2	1	3.36		2044, *2110, 2120*		
0	2	2	3.45		*2144*		
/	1	2	3.54	3.44	2042	0.831	0.831
/	1	1	3.54		1943, *1979, 2009, 2019*, 2045, *2055*		
2	0	2	3.63		1940		
2	0	1	3.72		*1849, 1915, 1925*		
/	2	1*	3.77	3.61	*2039*	0.827	0.826
2	1	1*	3.95		*1953*		

* Only one ring substituted.

Having established the identity of PCBs, quantitative analyses can be made on the basis of calculated KF_{relECD} values. For samples containing a reasonable number of individual PCBs the deviations of *KF* from theory will tend to cancel out. and reliable values for the total amount of PCB can be obtained.

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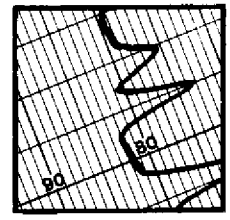
Quantitation of Polychlorinated Biphenyl Residues by Electron Capture Gas-Liquid Chromatography: Reference Material Characterization and Preliminary Study

L. D. Sawyer



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Quantitation of Polychlorinated Biphenyl Residues by Electron Capture Gas-Liquid Chromatography: Reference Material Characterization and Preliminary Study

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Weight per cent compositions of individual peaks of Aroclors 1016, 1242, 1248, 1254, and 1260 were determined under standard gas-liquid chromatographic (GLC) conditions. The GLC peak compositions were determined by using a Hall electrolytic conductivity detector for chlorine measurement and chemical ionization mass spectrometry with single ion monitoring for molecular weight characterization. The Aroclors used are available as reference materials for individual peak quantitation of polychlorinated biphenyl (PCB) residues by electron capture GLC. On the basis of a limited interlaboratory study and a collaborative study, the individual peak method shows improved interlaboratory precision and/or accuracy in PCB quantitation over existing methods.

In 1973, Webb and McCall (1) discussed the complications that exist with the quantitation of polychlorinated biphenyl (PCB) residues with the widely used electron capture gas-liquid chromatographic (ECGLC) detector. At the same time, they proposed a method of quantitation to minimize these complications. Their proposed method is based on individual ECGLC peak comparisons between the residue peaks and peaks of identical retention times from Aroclor® reference materials in which each individual Aroclor peak is a known weight percentage of the total. Weight percentages of the Aroclors were determined by using common GLC separations and halogen-specific detection for determining total chlorine content, and mass spectrometry (MS) for determining the average molecular weight for each peak.

The current AOAC methods for quantitating PCB residues, **29.018**, use a total response comparison (either area or peak height) between the reference Aroclor(s) and the residue. "Totals" are used in an attempt to obtain the best average ECGLC response per weight for both the sample residue and the reference material. The quantitation methods in sec. **29.018** were collaboratively studied in 1972, and the results were published in 1973 (2) with a caution that the methods may not be applicable in every instance. Of particular concern are those cases when the residue is highly altered compared to the reference, and a "good" average response may not be attainable. As a consequence, the analytical result may be biased by the predominance of either "high" or "low" ECGLC-responding chlorobiphenyls in the sample residue.

The applicability of the individual peak approach was investigated with the data that were submitted with the collaborative study mentioned above (2). All spiked materials and incurred residues were recalculated, using the published weight per cent values for the applicable Aroclor references (1). In all cases, the individual peak method demonstrated greater precision than the total area and total peak height methods. The average amounts of PCB determined were not significantly different, except for an incurred Aroclor 1242 residue in chicken fat. In this case, the individual peak method averaged 8.3 ppm compared to reported average values of 10.2 and 9.2 ppm by total area and total peak height methods, respectively. The chicken fat residue had a significantly altered GLC pattern compared to that of Aroclor 1242. Its total response (both area and peak height) is apparently dominated by chlorobiphenyls that cause a disproportionately "high" response by the ECGLC detector. The results of this and related investigations have been previously reported (3, 4). It was concluded that using the individual peak method should improve interlaboratory precision and/or accuracy in the ECGLC analysis of PCB, and should be considered as either a replacement or alternative approach to the existing AOAC methods. In addition, Chau and Sampson (5) have also addressed the PCB quantitation problem and concluded that the individual peak method, using ECGLC, best fulfilled the objectives of their study.

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A 10-laboratory collaborative study (6) has since been completed, in which the individual peak method was compared to the existing total response methods for quantitating the PCB content in synthetically prepared chlorobiphenyl mixtures, spiked milk samples, and chicken fat and milk samples with incurred residues. The study demonstrated that the interlaboratory precision of the individual peak method was better than or equal to the total response methods as evidenced by the coefficients of variation. The method has been adopted as first action, as an alternative approach for PCB quantitation.

This report describes the analytical procedures used and the results of a composition study of the 5 Aroclor reference materials that were supplied to the collaborators. In addition, the results of a limited method trial involving 3 laboratories are presented. In this trial, conducted prior to the collaborative study (6), useful data and suggestions were obtained for planning the more comprehensive study. The trial also indicated that the individual peak method could be readily used by chemists who were unfamiliar with the technique.

Some basic differences from those reported by Webb and McCall (1) were used in the experimental approach and include the following: (*a*) a Hall electrolytic conductivity detector (HECD) was used for chlorine detection instead of the Coulson detector; (*b*) single ion monitoring chemical ionization mass spectrometry instead of the electron impact mode was used for the molecular weight determinations; (*c*) constant temperature GLC conditions that are consistent with those used in multiresidue pesticide analysis, 29.018, and the FDA *Pesticide Analytical Manual* (7), instead of 3 different temperatures; (*d*) the chlorine response of the HECD was determined with a 3-component mixture of pure chlorobiphenyls instead of *p,p'*-DDE. The peak identification format proposed by Webb and McCall (1) in which each PCB peak is identified by a whole number relative to the retention time of *p,p'*-DDE (assigned a value of 100) was used. All peak identification numbers used throughout the text are in this format: R_{DDE} ($\times$ 100).

Food and Drug Administration (FDA) Reference Aroclors 1016, 1242, 1248, 1254, and 1260 were analyzed. These materials (except 1016) are from individual lots that have been extensively used by FDA for quantitation standards and a reserve of 3–4 lb of each is currently available. Approximately 1 lb of 1016 is available in reserve and was obtained directly from the Monsanto Co.

Experimental

Reagents

Distilled-in-Glass isooctane was used to prepare all chromatographic solutions (Burdick & Jackson Laboratories, Inc., Muskegon, MI 49443).

(a) *Aroclor solutions.*—Separate solutions of each of the following Aroclors were accurately prepared to contain ca 0.2 mg/ml: Aroclors 1242, 1248, 1254, and 1260 (obtained from FDA Industrial Chemical Repository, Division of Chemical Technology, Washington, DC 20204); Aroclor 1016 (Monsanto Co., St. Louis, MO 63166).

(b) *Chlorine response calibration solution.*—Aliquots of accurately prepared stock solutions of 2,2',5-trichlorobiphenyl, 2,5,2',5'-tetrachlorobiphenyl and 2,3,4,5,6-pentachlorobiphenyl (99+% pure, Analabs, Inc., North Haven, CT 06473) were diluted in combination to obtain an equal chlorine contribution from each homolog per volume of solvent. The final solution contained ca 0.022, 0.018, and 0.016 mg tri-, tetra-, and pentachlorobiphenyl/ml, respectively.

(c) *Retention standard solution.*—About 0.02 mg each of aldrin, *p,p'*-DDE, and *p,p'*-DDT/ml.

Apparatus for Chlorine Determinations

(a) *Gas chromatographic conditions.* — Model 7621-S GC (Packard, Downer's Grove, IL 60515), with 6' $\times$ 4 mm glass column packed with 5% OV-101 on 80–100 mesh Chromosorb W (HP). Operating conditions: column flow 60 ml nitrogen/min; column inlet and transfer line temperature, 225°C; column temperature adjusted to give *p,p'*-DDT retention time of 3.03 ($\pm$2%) relative to aldrin, ca 200°C.

(b) *Detection system.*—Model 310 HECD (Tracor, Inc., Austin, TX 78721) operated in reductive mode. Reactant gas 20–25 ml hydrogen/min; conductivity solvent isopropanol-water (1+1 or 1+4); solvent flow rate 0.5–1.0 ml/min; conductivity range and attenuator adjusted for optimum "noise-free" operation.

(c) *Furnace.*—Coulson furnace, Tracor No. 141771, interfaced between column and detector. Combustion tube, 11' $\times$ 4 mm quartz maintained at 860°C; inlet of furnace equipped with T-type toggle valve vent (8) to eliminate 90% of injection solvent, inlet maintained at 225°C.

(d) *Signal measurement.*—HECD signal monitored simultaneously with Model 3380A reporting

Table 1. Composition of Aroclor 1016 (Lot No. KB-06-256; 41.4% total Cl determined)

Peak R_{DDE} (×100)	Mean wt%	Coeff. of var., %[a]	Chlorine atoms No.[b]	Chlorine atoms % of total
11	0.2	23.9	1	100
16	3.8	8.1	1	4
			2	96
21	8.1	7.1	3	100
24	1.2	8.0	3	100
28	16.8	6.6	2	16
			3	84
32	7.6	7.7	2	4
			3	96
37	18.5	7.2	3	100
40	14.6	7.0	3	92
			4	8
47	11.6	7.3	4	100
54	7.7	6.5	4	100
58	6.4	8.3	4	100
70	3.4	10.8	4	84
			5	16

[a] Based on the average of 10 separate determinations by HECD. Areas were measured by electronic integration.
[b] Determined by chemical ionization mass spectrometry with single ion monitoring detection.

integrator (Hewlett-Packard, Avondale, PA 19311) and Model 21 multirange strip chart recorder (Westronics, Tracor, Inc.).

Apparatus for Molecular Weight Determinations

(a) *Mass spectrometer.*—Model 3300 GLC-MS system (Finnigan, Sunnyvale, CA 94086) operated in chemical ionization mode. Ion source was operated at 1.0 torr.

(b) *Gas chromatographic conditions.*—Glass column, 5′ × 4 mm, packed with 5% OV-101 on Chromosorb W (HP); carrier and reactant gas 30 ml methane/min; column inlet 250°C, column temperature adjusted to give *p,p′*-DDT retention time of 3.03 (±2%) relative to aldrin, ca 190°C.

(c) *Data system.*—Finnigan Model 6000.

Chlorine Determination

After the proper GLC conditions were established with the retention standard solution, the HECD responses to chlorine were obtained from alternate injections of the calibration mixture and the Aroclor being determined; 9 or 10 separate injections of each solution were made. The average chlorine content for each individual Aroclor peak was calculated from the average chlorine response obtained from the 3 chlorobiphenyls in the calibration solution, for a total of 27 or 30 determinations.

Areas of the individual peaks were determined either from the integrator printout or by planimetry of the strip chart chromatogram. When peaks were not resolved, a perpendicular line was drawn from the minimum point between the 2 peaks to the baseline. The integrator does this automatically when it is operating properly. When the integrator malfunctioned and planimetry was used (Aroclor 1248 and 1260 determinations), the baseline was drawn as a single line extending from the beginning of the chromatogram to the recorder baseline established after the last peak appeared.

The chlorine content of each Aroclor was determined over the course of 1 day's operation. Cell parameters and sensitivity settings of the HECD, integrator, and recorder input range were separately optimized for each Aroclor to obtain the best reproducible and noise-free signal of usable intensity.

Molecular Weight Determination

Full details on the average molecular weight determination by GLC-MS are reported elsewhere (9) and the technique will be only briefly described here. After GLC conditions that would duplicate those used in the chlorine determination were established, the molecular weight species in each Aroclor peak was characterized by chemical ionization mass spectrometry with single ion monitoring techniques. Ions with m/e values of 189, 223, 257, 293, 326.8, and 394.8, corresponding to the most intense ion within the molecular cluster

Table 2. Composition of Aroclor 1242 (FDA Lot No. 71-696; 41% total Cl determined)

Peak R_{DDE} (×100)	Mean wt%	Coeff. of var., %[a]	Chlorine atoms No.[b]	Chlorine atoms % of total
16	3.4	2.8	1	4
			2	96
21	10.3	2.6	2	100
24	1.1	2.1	3	100
28	15.8	2.3	2	16
			3	84
32	7.3	2.2	2	6
			3	94
37	17.0	2.6	3	100
40	13.0	2.3	3	81
			4	19
47	9.9	2.1	4	100
54	7.1	2.6	4	100
58	4.4	2.2	4	100
70	8.7	2.2	4	93
			5	7
78	1.9	2.8	5	100
84	ND[c]	—	5	100
98	ND	—	5	100
104	ND	—	5	100
125	ND	—	5	100

[a,b] See footnotes, Table 1.
[c] ND = not determined. HECD Cl responses were too low for accurate measurement.

of the protonated mono-, di-, tri-, tetra-, penta-, hexa-, and heptachlorobiphenyls, respectively, were monitored during the chromatographic analysis for each Aroclor. Area measurements for quantitation of each individual peak occurring in the single ion monitoring chromatograms were calculated by using the data system and calibration plots obtained from a selected representative of each molecular weight group. A basic assumption was made that responses to different isomers within a molecular weight grouping would be exactly the same as the selected representative of that group.

Results and Discussion

With the determination of the average chlorine content and the average molecular weight of each peak, the weight per cent represented by that peak can be calculated from the formula:

$$\text{Wt \%} = [(\text{ng Cl in peak/total ng Aroclor}) \times (\text{av. MW of peak/av. no. of Cl atoms in peak} \times 35.46)] \times 100$$

Table 3. Composition of Aroclor 1248 (FDA Lot No. 71-697; 48.4% total Cl determined)

Peak R_{DDE} (×100)	Mean wt%	Coeff. of var., %[a]	Chlorine atoms No.[b]	Chlorine atoms % of total
16	0.3	18.4	1	6
			2	94
21	1.1	8.3	2	100
24	0.2	8.3	3	100
28	6.0	5.5	2	7
			3	93
32	2.6	5.3	2	4
			3	96
37	8.7	5.7	3	100
40	7.4	3.9	3	62
			4	38
47	15.7	3.9	3	10
			4	90
54	9.3	4.9	3	14
			4	86
58	8.3	5.6	3	10
			4	90
70	18.2	4.2	4	90
			5	10
78	6.4	6.2	4	100
84	4.6	5.2	5	100
98	3.4	4.9	5	100
104	3.3	5.3	5	100
112	1.0[c]	12.4	ND[c]	—
125	2.3	4.9	5	100
146	1.2	9.6	5	100

[a] Based on the average of 9 separate determinations by HECD. Areas were measured by a planimeter.
[b] Determined by chemical ionization mass spectrometry with single ion monitoring detection.
[c] The number of chlorine atoms was not determined. Weight per cent was calculated by assuming that number of chlorine atoms = 5.

Table 4. Composition of Aroclor 1254 (FDA Lot No. 71-698; 52.2% total Cl determined)

Peak R_{DDE} (×100)	Mean wt%	Coeff. of var., %[a]	Chlorine atoms No.[b]	Chlorine atoms % of total
47	7.1	3.5	3	8
			4	92
54	2.7	3.0	4	100
58	1.2	7.6	4	100
70	14.7	3.2	4	57
			5	43
84	18.6	2.8	4	4
			5	96
98	8.3	3.1	4	100
104	14.1	3.8	4	6
			5	94
125	15.6	2.3	4	40
			5	60
146	9.0	3.2	5	15
			6	85
160	ND[c]	—	6	100
174	7.4	3.4	6	100
203	1.3	11.0	6	100
232	ND	—	6	100
280	ND	—	6	100
332	ND	—	6	100

[a,b] See footnotes, Table 3.
[c] ND = not determined. HECD Cl responses were too low for accurate measurement.

Using these predetermined weight per cent factors, PCB residues can be calculated by direct ECGLC response comparisons on an individual peak basis, if the sample and reference peaks exhibit identical retention times and the GLC conditions used provide the same peak resolutions as those used for determining the factors.

The compositions of the 5 Aroclors determined are summarized in Tables 1–5 with their ECGLC profiles obtained at the specified conditions illustrated in the corresponding figures, 1–5. Aroclors 1242 and 1254 (Figs 2 and 4) exhibit ECGLC peaks for which weight per cent factors are not listed in Tables 2 and 4. The HECD response to the chlorine in these later-eluting peaks, R_{DDE} (× 100) ≥84 for 1242 and ≥232 for 1254, was not adequate for accurate measurement. Residues that exhibit ECGLC responses at these retention times can be calculated from the corresponding weight per cent factors determined for Aroclors 1248 and 1260.

Figure 6 is an HECD chromatogram of the chlorine response calibration mixture used. The chlorine response tended to increase over the course of a single day's performance but, by bracketing each Aroclor injection with a calibration mixture injection, this could be tolerated.

Table 5. Composition of Aroclor 1260 (FDA Lot No. 71-699; 58.4% total Cl determined)

Peak R_{DDE} (×100)	Mean wt%	Coeff. of var., %[a]	Chlorine atoms No.[b]	Chlorine atoms % of total
70	2.4	5.9	5	100
84	3.6	10.4	5	100
98 & 104	2.8	6.7	5	47
			6	53
117	4.4	6.1	6	100
125	11.0	6.3	5	9
			6	91
146	13.3	5.2	5	6
			6	94
160	5.5	6.5	6	36
			7	64
174	10.0	5.0	6	100
203	10.9	6.5	7	100
232 & 244	11.2	6.6	7	100
280	12.5	8.1	7	100
332	4.2	11.3	7	100
360 & 372	5.4	10.0	7	100
448	0.8	17.6	7	100
528	2.0	9.6	7	100

[a,b] See footnotes, Table 1.

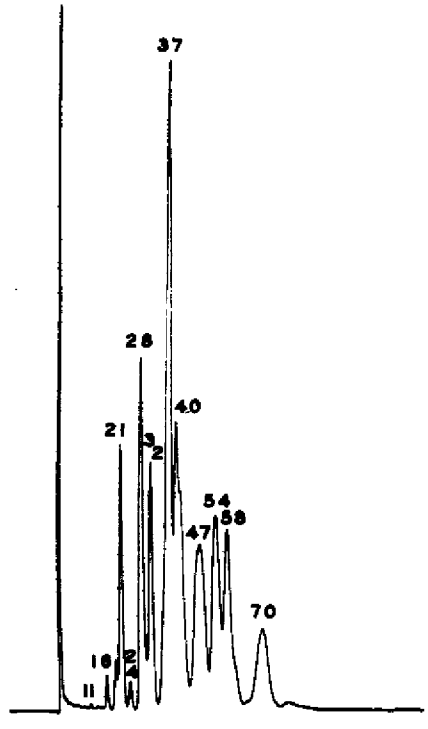

FIG. 1—Peak resolution of Aroclor 1016 at standardized GLC conditions. Peak numbers are relative to *p,p'*-DDE = 100 (ca 12 min). Chromatogram obtained with a Hewlett-Packard linearized ^{63}Ni EC detector.

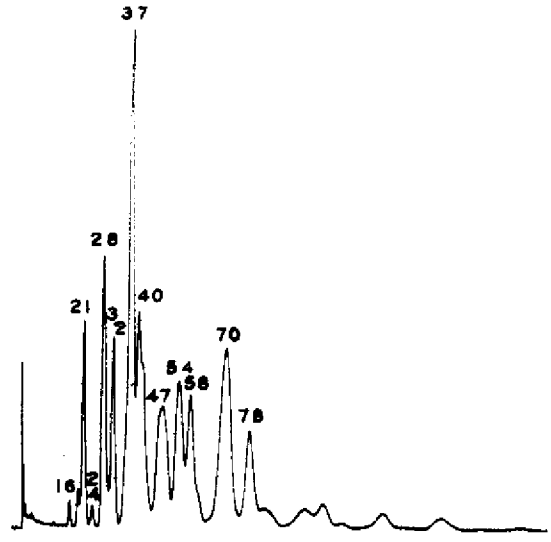

FIG. 2—Peak resolution of Aroclor 1242 at standardized GLC conditions. See Fig. 1 for details.

The average coefficients of variation for the HECD response to chlorine obtained during the individual Aroclor determinations were 5.2, 2.2, 6.0, 3.9, and 5.5% for 1016, 1242, 1248, 1254, and 1260, respectively. The average chlorine responses (area/ng Cl) for Aroclors 1016, 1254, 1260 were determined from 9 separate injections of the mixture (27 values) and those for 1242 and 1248, from 10 separate injections (30 values).

In the determination of chlorine in Aroclors 1248 and 1260, the electronic integrator consistently malfunctioned when the later-eluting peaks were being measured and the areas were determined with the recorder-planimeter combination. There were no significant differences between the 2 methods of area determination, as can be seen in Tables 6 and 7.

After the Aroclor standardization study was completed, the reference materials, standardization data, and cleaned up samples of chicken fat and milk fat containing incurred PCB residues were supplied to 2 other laboratories besides the Associate Referee's. These laboratories were asked to quantitate the residues by the existing AOAC and individual peak methods, using the same sample-reference chromatogram pair for each separate calculation.

The chicken fat sample contained an incurred 1242 residue (Fig. 7) but its profile exhibited enhanced later-eluting responses similar to 1248. Consequently, it was requested that this residue

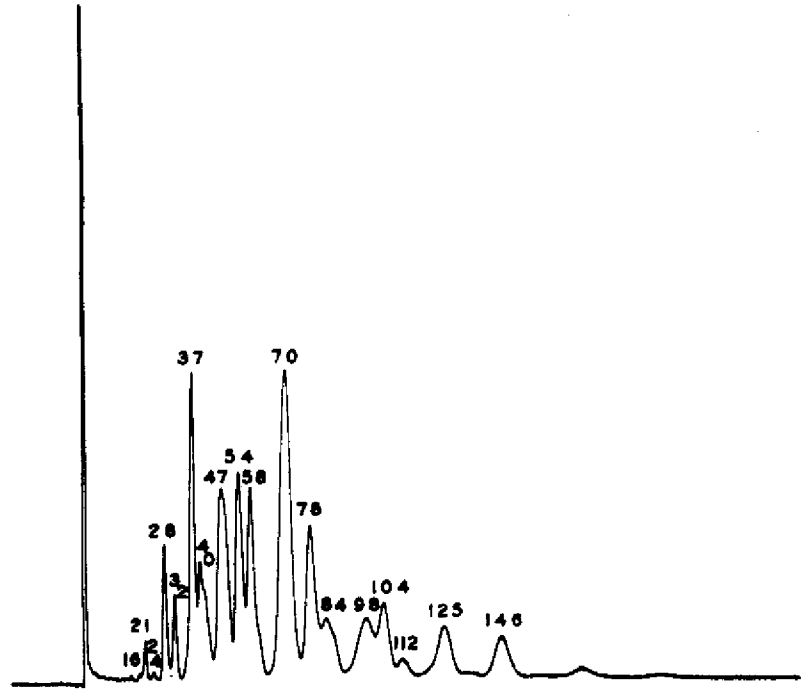

FIG. 3—Peak resolution of Aroclor 1248 at standardized GLC conditions. See Fig. 1 for details.

be calculated with both Aroclor 1242 and 1248 reference materials. The results obtained from this sample are given in Table 8, where it can be seen that agreement between laboratories was much better with the individual peak method. The AOAC results ranged from 5.21 (total area as 1248) to 9.57 ppm (total area as 1242). The individual peak values ranged from 5.78 ppm (as 1242) to 6.30 ppm (as 1248). The individual peak values obtained by using 1248 as the reference are understandably higher than the 1242 values, since weight factors for peaks in 1242 with retentions of R_{DDE} ($\times$ 100) $\geq$84 were not determined.

The results of the individual peak values in the chicken fat determined from both Aroclor materials are shown in Table 9. Because only 3 laboratories were involved, the data were not statistically analyzed; however, there are no obvious unexpected discrepancies.

The milk fat sample (Fig. 8) was analyzed similarly to the chicken fat sample, except that only Aroclor 1254 was used for quantitation. The 3-method comparison results are in Table

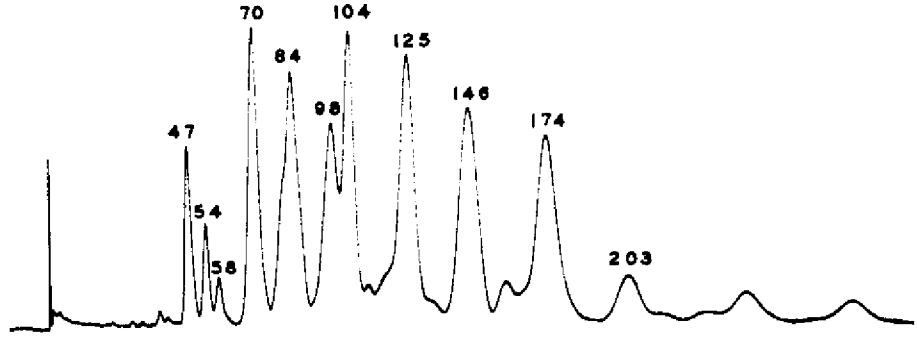

FIG. 4—Peak resolution for Aroclor 1254 at standardized GLC conditions. See Fig. 1 for details.

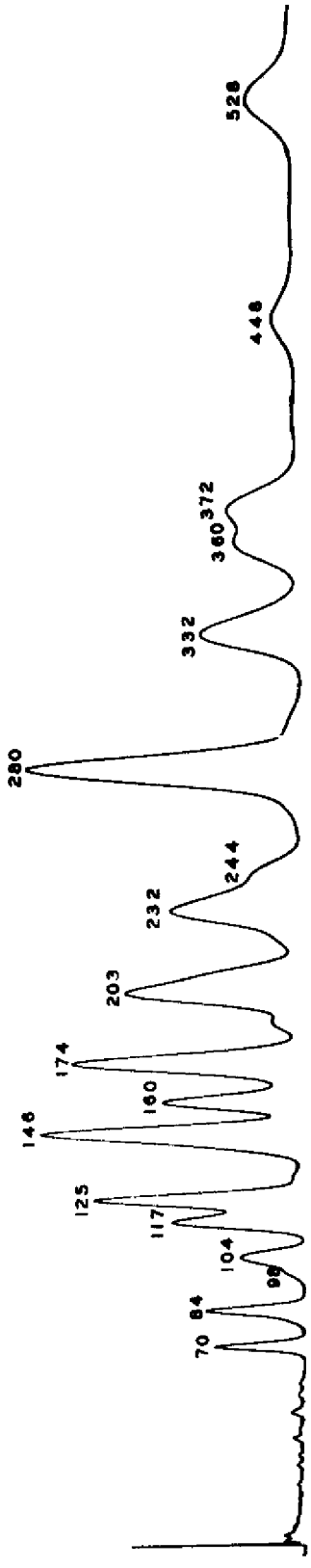

FIG. 5—Peak resolution of Aroclor 1260 at standardized GLC conditions. See Fig. 1 for details.

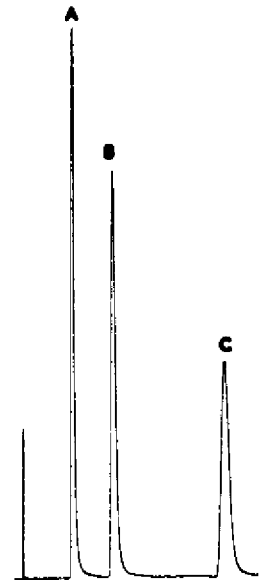

FIG. 6—HECD chromatogram of chlorine response calibration mixture. A, 130 ng 2,2',5'-trichlorobiphenyl; B, 108 ng 2,2'5,5'-tetrachlorobiphenyl; C, 100 ng 2,3,4,5,6-pentachlorobiphenyl. Retention time of C is ca 12 min.

10; the individual peak results are in Table 11. Only 4 peaks in the sample were used to calculate total PCB residues. Peaks with retention times of R_{DDE} ($\times$ 100) $<$125 are due to PCB, based on a GLC-MS determination. However, their absolute retention times do not exactly match any reference Aroclor peaks. Based on GLC retention times alone, these peaks cannot

Table 6. Comparison of integrator- and planimeter-determined chlorine content in selected peaks from Aroclor 1248 (1,239.6 ng injected)

Peak R_{DDE} ($\times$100)	ng Cl		Coeff. of var., %	
	Integrator[a]	Planimeter[b]	Integrator	Planimeter
16	1.1	1.2	19.3	18.4
21	4.1	4.3	11.0	8.3
24	0.9	1.0	22.5	8.3
28	30.1	30.8	5.6	5.5
32	13.0	13.5	7.0	5.3
37	44.4	45.8	6.3	5.7
40	39.7	40.8	4.7	3.9
47	94.6	94.9	4.1	3.9
54	55.9	55.7	4.1	4.9
58	49.4	50.5	5.1	5.6
70	113.4	113.2	4.0	4.2

[a] Average of 9 determinations except for peaks 58 and 70 where 7 values were averaged. Peaks compared and determinations averaged depend on the number of properly integrated areas reported by the integrator.
[b] Average of 9 determinations.

Table 7. Comparison of integrator- and planimeter-determined chlorine content in selected peaks from Aroclor 1260 (1,506.4 ng injected)

Peak R_{DDE} (×100)	ng Cl		Coeff. of var., %	
	Integrator[a]	Planimeter[b]	Integrator	Planimeter
70	20.3	19.0	6.2	5.9
84	27.8	27.8	6.0	10.4
98 & 104	21.2	23.3	7.0	6.7
146	116.1	113.4	6.9	5.2
160	46.3	48.9	8.4	6.5

[a] Average of 9 determinations except peaks 146 and 160 where 8 values were averaged. Peaks compared and determinations averaged depend on the number of properly integrated areas reported by the integrator.
[b] Average of 10 determinations.

Table 8. Comparison of AOAC and individual peak methods for calculating an incurred PCB residue (ppm) in chicken fat, using standardized Aroclors 1242 and 1248 reference materials, and ECGLC

Lab.	As Aroclor 1242			As Aroclor 1248		
	AOAC total area	AOAC total peak ht	Ind. peak[a]	AOAC total area	AOAC total peak ht	Ind. peak[b]
1	7.38	6.64	5.78	5.76	6.82	6.00
2	8.88	7.63	5.79	5.21	6.44	6.07
3	9.57	7.56	5.78	7.16	8.10	6.30

[a] Totals do not include residue peaks with an R_{DDE} (×100) > 78.
[b] Totals include all residue peaks.

be distinguished from commonly occurring chlorinated pesticides. Peaks with retention times of R_{DDE} (× 100) >203 are also attributable to PCB, but they would have to be calculated from an Aroclor 1260 reference, since those weight factors for 1254 were not determined, as previously discussed.

Again, as in the chicken fat sample, better interlaboratory agreement was noted in the individual peak method of quantitation with results of 1.23, 1.26, and 1.31 ppm reported. The AOAC method gave results of 1.51, 2.25, and 2.42 ppm for total area, and 1.34, 1.82, and 2.02 ppm for total peak height comparisons.

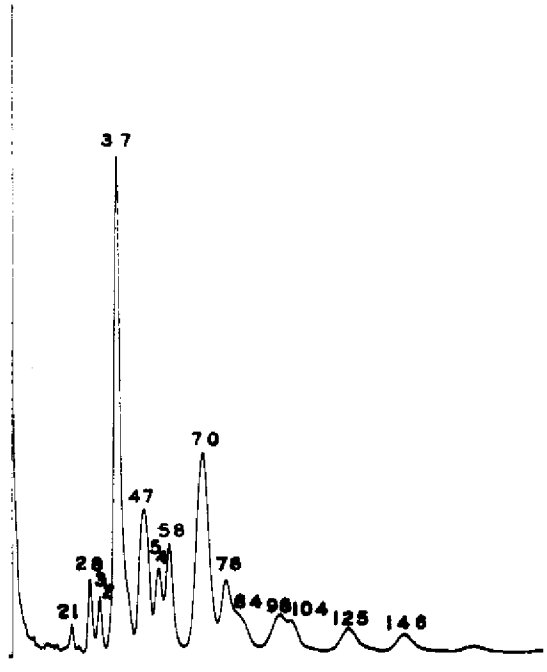

FIG. 7—Linearized ^{63}Ni (Hewlett-Packard) EC chromatogram of an incurred residue of Aroclor 1242 in chicken fat at standardized GLC conditions. Peak numbers are relative to *p,p'*-DDE = 100 (ca 12 min).

Conclusions

Based on the preliminary investigations previously reported (3–5), the limited interlaboratory data presented here, and the results of the completed collaborative study (6), the use of the individual peak method for PCB quantitation by ECGLC will tend to improve interlaboratory precision compared to the existing methods. Its major advantage is that it eliminates many of

Table 9. Comparison of individual peak results (ppm) for calculating an incurred PCB residue in chicken fat, using standardized Aroclor 1242 and 1248 reference materials and ECGLC

Peak R_{DDE} (×100)	Aroclor 1242			Aroclor 1248		
	Lab. 1	Lab. 2	Lab. 3	Lab. 1	Lab. 2	Lab. 3
21	0.16	0.10	0.07	0.09	0.07	0.10
24	0.02	ND[a]	0.10	0.01	ND	0.03
28	0.44	0.34	0.28	0.31	0.28	0.35
32	0.21	0.20	0.17	0.16	0.16	0.22
37 & 40[b]	2.15	2.30	2.25	1.72	1.88	2.36
47	1.02	1.05	1.18	0.99	0.93	0.89
54	0.38	0.33	0.27	0.34	0.29	0.26
58	0.35	0.33	0.35	0.43	0.40	0.43
70	0.91	0.98	0.96	1.07	1.08	1.12
78	0.14	0.16	0.14	0.30	0.30	0.33
84	—	—	—	0.20	0.18	0.21
98	—	—	—	0.18	0.22	ND
104	—	—	—	0.04	0.12	0.14
125	—	—	—	0.10	0.11	0.13
146	—	—	—	0.06	0.05	0.07

[a] ND = not determined.
[b] Calculated by combining the individual weight factors (Tables 2 and 3) and the individual peak height responses obtained.

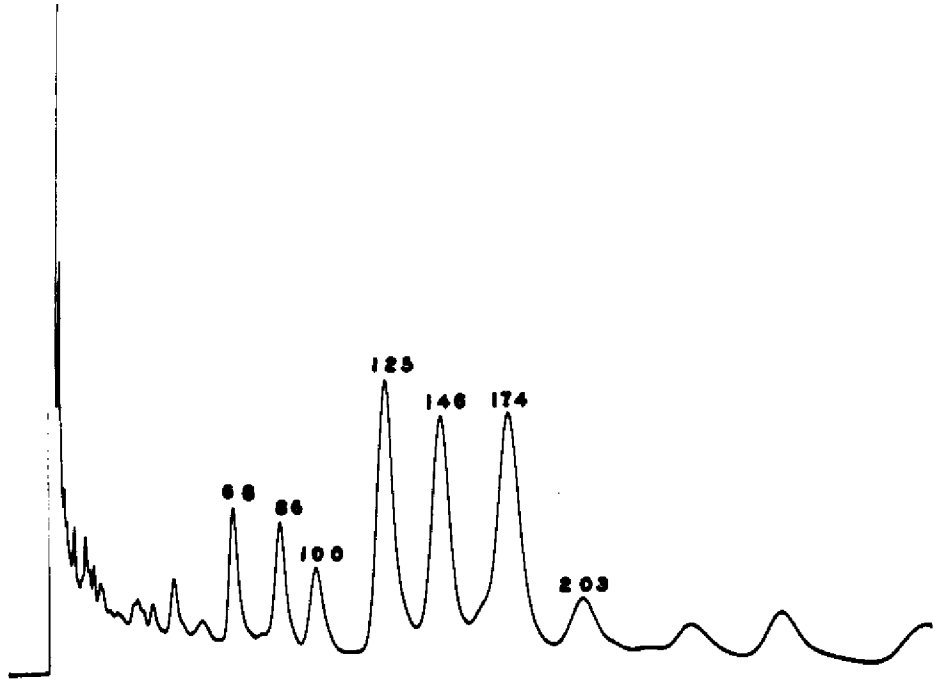

FIG. 8—Linearized ^{63}Ni (Hewlett-Packard) chromatogram of an incurred PCB residue in milk fat at standardized GLC conditions. Peak numbers are relative to p,p'-DDE = 100 (ca 12 min).

the problems associated with disproportionate detector responses to the chlorobiphenyls, which may or may not "average out" in the existing AOAC methods. However, this method is limited to one set of chromatographic conditions and one set of Aroclor reference materials. The weight factors presented in this report cannot be applied to Aroclors other than the specified FDA lot numbers. Also, positive qualitative assignment of ECGLC peaks is not possible under the conditions required for quantitation. Ancillary techniques such as alternative GLC columns and conditions, chemical treatment, or GLC-MS procedures should be utilized in conjunction with this method of quantitation to insure proper identification.

Acknowledgments

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I also thank Ronald G. Webb and Ann C. McCall of the Southeast Environmental Research Laboratory, National Research Center, Corvallis Environmental Protection Agency, Athens, GA, for graciously supplying me with solutions of their analyzed Aroclors, which were invaluable in the applicability investigations of their proposed quantitation technique.

Table 10. Comparison of AOAC and individual peak methods for calculating an incurred PCB residue (ppm) in milk fat, using standardized Aroclor 1254 as the reference material and ECGLC

Lab.	AOAC[a] total area	AOAC[a] total peak ht	Ind. peak[a]
1	1.51	1.34	1.23
2	2.25	2.02	1.26
3	2.42	1.82	1.31

[a] Based on sample peaks with R_{DDE} (×100) of 125, 146, 174, and 203 only.

Table 11. Comparison of individual peak results (ppm) for calculating an incurred PCB residue in milk fat, using standardized Aroclor 1254 reference material and ECGLC

Peak R_{DDE} (×100)	Lab. 1	Lab. 2	Lab. 3
125	0.61	0.62	0.64
146	0.25	0.28	0.29
174	0.33	0.32	0.34
203	0.04	0.04	0.04

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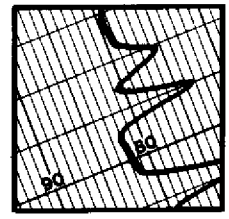
Quantitation of Polychlorinated Biphenyl Residues by Electron Capture Gas-Liquid Chromatography: Collaborative Study

L. D. Sawyer



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Quantitation of Polychlorinated Biphenyl Residues by Electron Capture Gas-Liquid Chromatography: Collaborative Study

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Ten collaborators quantitated 2 synthetically prepared polychlorinated biphenyl (PCB) mixtures, 2 PCB-fortified milk samples, and an incurred PCB residue in milk and chicken fat. Three electron capture gas-liquid chromatographic (GLC) methods were used for quantitating each unknown PCB against Aroclor reference materials. Two of the methods were existing AOAC total response comparisons and the third was a proposed individual peak comparison. In addition, existing AOAC multiresidue pesticide methodology was employed for determining PCB recovery from whole milk. The average combined recovery of Aroclor 1254 from milk fortified at 1.4 and 2.7 ppm (fat) was approximately 85% (coefficient of variation, 15%) with no significant difference by the methods of quantitation. The incurred PCB residue in milk (as 1254) was determined to be 1.3 (30%), 1.7 (31%), and 1.2 ppm (18%) by total peak height, total area, and individual peak quantitation methods, respectively. In the same order, incurred PCB in chicken fat was determined to be 6.8 (13%), 7.5 (16%), and 5.6 ppm (8%) as 1242, or 6.9 (6%), 5.9 (8%), and 6.3 ppm (8%) as 1248. Total PCB quantitated individually in a 3- and a 4-component chlorobiphenyl mixture, using 1248 as the reference standard, resulted in the following averages of actual amounts present: 3-component—109% (23%), 95% (18%), and 95% (6%); 4-component—116% (16%), 112% (29%), and 104% (7%), respectively, for total peak height, total area, and individual peak quantitation methods. Both the individual peak quantitation method and the AOAC multi-pesticide methodology have been adopted as official first action methods for PCB.

The existing AOAC methods (1) for quantitating polychlorinated biphenyl (PCB) residues as outlined in **29.018** were comparatively studied (2) with a quantitative approach proposed by Webb and McCall (3). The conclusion of this study (2) indicated that interlaboratory precision (and probably accuracy) in PCB quantitation may be improved if the proposed approach (3) is used, particularly when the composition of the PCB residue is significantly different from the reference material.

Besides the quantitative aspects of PCB, the existing methods (1) lack validation data for commodities other than chicken fat and fish. Expansion of the methods (1) to include milk would be desirable, since PCB residues are often isolated from this and related dairy commodities. The object of this study is to address both the quantitative and recovery aspects of PCB.

Instructions to Collaborators

Each collaborator was supplied 2 solutions of synthetically prepared chlorobiphenyl mixtures, 1 solution of cleaned up chicken fat, and 4 samples of frozen milk. Aroclor® reference materials and related composition data (2) needed to complete the study were also provided.

The synthetic mixtures and the chicken fat sample were to be analyzed directly by electron capture GLC with no further cleanup or dilution. The mixtures were to be quantitated from an Aroclor 1248 reference, and the chicken fat residue (incurred 1242) was to be quantitated from both Aroclor 1242 and 1248.

The milk samples (1 blank, 2 spikes, and 1 incurred residue) were to be thawed and analyzed as described in **29.012(c)**, **29.014**, and **29.015** with the following exceptions: (*a*) 100 g milk was weighed instead of measuring 100 ml; (*b*) the secondary partitioning step in **29.014** was not used; (*c*) only the 6% ethyl ether elution in **29.015** was required. The 2 spikes and the incurred residue were to be quantitated from an Aroclor 1254 reference. For more accurate recovery data, instructions were provided to subtract the responses of background pesticide and/or PCB detected in the blank from the spike sample responses.

Any ECGLC system could be used for quantitation, as long as it was equipped with a methyl silicone column that would duplicate the peak resolution and sensitivity defined by the operating instructions in **29.018** and the FDA *Pesticide Analytical Manual* (4). The exact criterion required for proper peak resolution was that *p,p'*-DDT elute at a retention time of 3.03 (±2%) relative to aldrin. Sensitivity of the detector was to be adjusted so that 1 ng heptachlor epoxide would produce a 50% recorder response.

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Each PCB mixture, residue, and spiked Aroclor material was to be quantitated by 3 different methods, using the same sample-reference chromatogram pair for each method. Two of the 3 methods were electron capture response comparisons as defined in 29.018; these methods require summation of the total area or total peak height of the reference Aroclor and comparison to the total area or total peak height of the PCB residue. Only those responses in the residue chromatogram that are conclusively attributable to PCB were to be summed for the unknown response. This meant that peaks that did not exactly match a corresponding reference Aroclor peak in retention time or suspect PCB peaks that showed interference from pesticide or product artifact materials were not to be included in the residue response summations. These methods can be summarized by the following equation:

PCB, ppm = (ng reference Aroclor/mg sample) × (total response (area or peak ht) of residue/total response (area or peak ht) of Aroclor).

The third method tested was an individual peak comparison (2, 3): Each PCB residue peak is quantitated separately against an Aroclor peak with an identical retention time. To accomplish this, the weight percentage represented by each Aroclor peak to the total Aroclor weight must be known. The total PCB found is then readily calculated by summing each individual peak value. The following equation summarizes the calculations in this method.

PCB, ppm residue peak = (ng reference Aroclor/mg sample) × (area of residue peak/area of reference peak) × wt% factor for reference peak.

The weight factors for 5 different Aroclor materials from specific Food and Drug Administration (FDA) Lot Nos. have been determined (2). These materials and the related composition data were supplied to each collaborator with instructions not to use any reference materials other than those supplied.

The collaborators were asked to use the PCB peak identification format proposed by Webb and McCall (3) for the individual peak method. This technique identifies each peak as a whole number relative to the retention time of *p,p'*-DDE, where *p,p'*-DDE is assigned a value of 100 (designated as R_{DDE} (× 100)).

The unknown PCB and the reference responses were to be matched as closely as possible and quantitation was not to be done when the major PCB peaks did not exceed a 25% recorder response (applicable only to the milk samples). When the 25% response criterion was not met, instructions were to inject more sample equivalent to meet the response criterion rather than increase the detector sensitivity.

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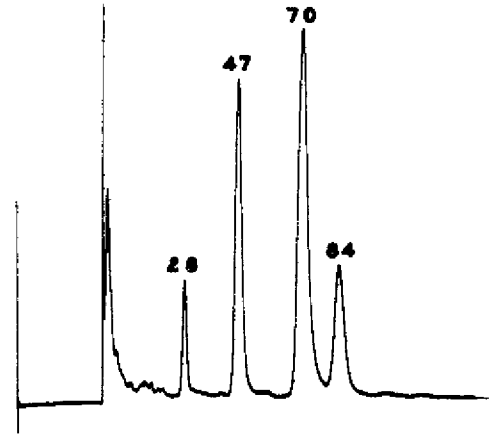

FIG. 1—Chromatogram of chlorobiphenyl synthetic mixture B. Peak numbers are relative to *p,p'*-DDE = 100 (ca 12 min). Peak 28, 2,2'5-trichlorobiphenyl (0.95 ng); 47, 2,2',5,5'-tetrachlorobiphenyl (2.65 ng); 70, 2,3',4',5-tetrachlorobiphenyl (2.75 ng); 84, 2,2',4,5,5'-pentachlorobiphenyl (0.50 ng).

Results and Discussion

The 2 synthetically prepared chlorobiphenyl mixtures supplied to each collaborator, A and B, were identical with the exception that mixture B contained 1 additional component. Both mixtures contained 0.19 ng 2,2',5-trichlorobiphenyl/μl, 0.53 ng 2,2',5,5'-tetrachlorobiphenyl/μl, 0.55 ng 2,3',4',5-tetrachlorobiphenyl/μl; mixture B contained, in addition, 0.10 ng 2,2',4,5,5'-pentachlorobiphenyl/μl. These chlorobiphenyls have been shown to exist in commercial Aroclor preparations (5, 6) and are commercially available (RFR Corp., Hope, RI 02831). Electron capture chromatograms of mixture B and the reference Aroclor 1248 used for quantitating the mixtures are shown in Figs 1 and 2, respectively.

Table 1 summarizes the individually calculated amounts of the individual components. Collaborators 3, 5, 6, 8, and 10 all reported their results from peak height response comparisons, even though area comparisons were requested. These results were recomputed on an area basis before statistical treatment of the data. The mean per cent of actual found for 2,2',5-tri-, 2,2',5,5'-tetra-, 2,3',4',5-tetra, and 2,2',4,5,5'-pentachlorobiphenyl were, respectively, 87.4 (coefficient of variation, 12.1%), 89.5 (10.9%), 104.1 (10.1%), and 232 (19.7%). Quantitation of the pentachlorobiphenyl, R_{DDE} (× 100) = 84 (Fig. 1), is heavily

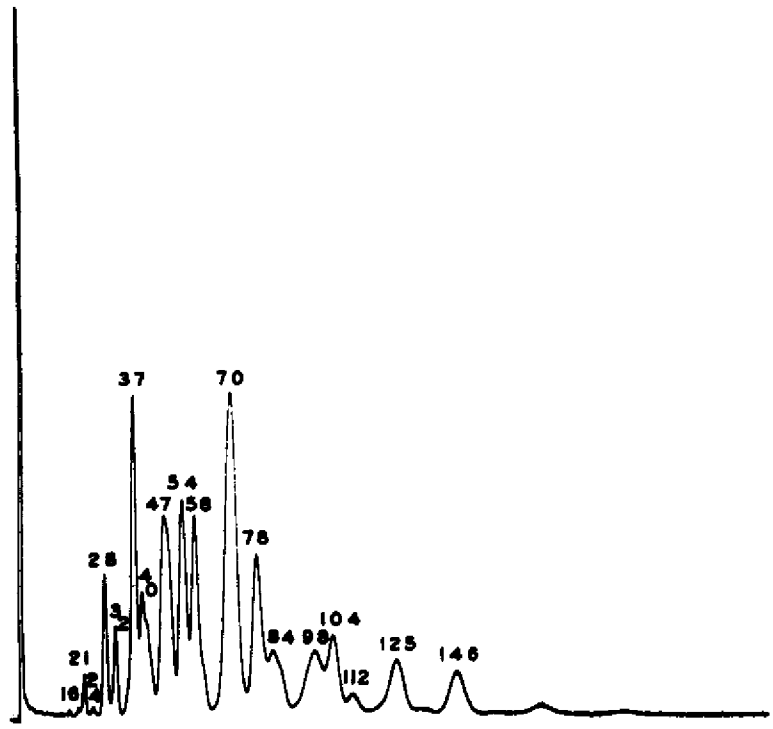

FIG. 2—Chromatogram of Aroclor 1248 (ca 20 ng). Peak numbers are relative to *p,p'*-DDE = 100 (ca 12 min).

Table 1. Collaborative results (ng/μl) for 2 chlorobiphenyl mixtures calculated from identical retention time peaks in Aroclor 1248, using individual peak weight factors

	Peaks—R_{DDE} (×100)[a]						
	28		47		70		84,
Coll.	A	B	A	B	A	B	B
1	0.17	0.17	0.47	0.48	0.55	0.55	0.24
2	0.18	0.18	0.48	0.49	0.58	0.57	0.23
3	0.17	0.17	0.53	0.46	0.54	0.50	0.20
			(0.82)[b]	(0.84)	(0.71)	(0.73)	(0.26)
4	0.16	0.16	0.45	0.45	0.54	0.53	0.21
5	0.17	0.17	0.31	0.38	0.66	0.64	0.17
			(0.88)	(0.91)	(0.97)	(0.94)	(0.30)
6	0.14	0.16	0.42	0.45	0.69	0.62	0.24
		(0.18)	(0.84)	(0.86)	(0.86)	(0.88)	(0.36)
7	0.17	0.17	0.46	0.47	0.56	0.57	0.27
8	0.21	0.20	0.55	0.54	0.53	0.52	0.17
			(0.88)	(0.86)	(0.80)	(0.80)	(0.45)
9	0.13	0.14	0.44	0.51	0.50	0.51	0.29
10	0.13	0.17	0.55	0.54	0.64	0.65	0.30
	(0.16)		(0.83)	(0.83)	(0.86)	(0.86)	
Amt added	0.19		0.53		0.55		0.10
Av. found	0.166		0.474		0.572		0.232
Std dev.	0.020		0.052		0.058		0.046
Mean % found	87.4		89.5		104.1		232.0
Coeff. of var., %	12.1		10.9		10.1		19.7
Std. error, %	2.4		2.2		2.4		14.5

[a] Peaks = R_{DDE} (×100). Peaks 28, 47, 70, and 84 correspond, respectively, to 2,2',5-trl-, 2,2',5,5'-tetra-, 2,3',4',5-tetra-, and 2,2',4,5,5'-pentachlorobiphenyls.

[b] Results in parentheses were obtained by using peak height response comparisons; area response comparisons were used for calculating actual amounts found.

Table 2. Comparison of total response and individual peak methods of quantitation for total PCB (ng/μl) found in 2 chlorobiphenyl mixtures, using Aroclor 1248 as the reference material

	Mixture A			Mixture B		
Coll.	Total peak ht	Total area	Ind. peak	Total peak ht	Total area	Ind. peak
1	1.40	1.03	1.18	1.60	1.26	1.43
2	1.33	1.04	1.24	1.50	1.34	1.47
3	1.25	1.65	1.24 (1.70)[a]	1.46	1.85	1.33 (2.00)
4	0.92 (2.92)[b]	1.28 (4.03)[b]	1.16	1.11 (3.09)[b]	1.47 (4.17)[b]	1.35
5	1.50	1.18	1.20 (2.02)	1.88	1.36	1.36 (2.32)
6	2.16	1.57	1.25 (1.84)	2.10	2.72	1.47 (2.28)
7	1.33	1.05	1.18	1.55	1.31	1.48
8	1.45	1.12	1.29 (1.89)	1.61	1.33	1.43 (2.29)
9	1.16	1.05	1.07	1.50	1.33	1.45
10	1.39	1.16	1.35 (1.86)	1.67	1.48	1.66 (2.17)
Amt added		1.28			1.38	
Av. found	1.39	1.21	1.22	1.60	1.54	1.44
Std dev.	0.318	0.224	0.077	0.262	0.445	0.093
Mean % found	108.6	94.5	95.3	115.9	111.6	104.4
Coeff. of var., %	22.9	18.5	6.3	16.4	28.9	6.5
Std error, %	7.8	5.5	1.9	6.0	10.2	2.1

[a] Except for footnote *b*, results in parentheses were reported by using peak height response comparisons; area response comparisons were used for calculating actual amounts found.

[b] Results in parentheses, Collaborator 4, were calculated by using only responses from the corresponding peak in the reference material; total reference responses were used for calculating actual amounts found.

influenced by the co-eluting peak of R_{DDE} ($\times$ 100) = 78 (Fig. 2) in the reference material, making an accurate reference area measurement almost impossible.

The "total" PCB found in the 2 mixtures calculated by the existing total response methods and the individual peak method is compared in Table 2. Again, the GLC electron capture individual peak values were recalculated by using areas when peak height values had been reported. Mixture A containing 1.28 ng total PCB/μl gave mean values of actual found of 108.6% (22.9%), 94.5% (18.5%), and 95.3% (6.3%), by the total peak height, total area, and individual peak height methods, respectively. Mixture B contained 1.38 ng/μl total PCB, and the respective mean values of 115.9% (16.4%), 111.6% (28.9%), and 104.4% (6.5%) were obtained.

Collaborator 4 reported values for the total response methods based on the summation of only those reference peaks that matched those in the mixture and, thus, the total weight of Aroclor 1248 injected was taken as giving only 3 or 4 responses, causing the quantitative answer to be inordinately "high." These values were recalculated by using the total responses as directed.

Table 3 summarizes the 3-method comparative data obtained for the incurred Aroclor 1242 residue in the chicken fat. This residue is chromatographically illustrated in Fig. 3 and individually quantitated from Aroclors 1242 (Fig. 4) and 1248 (Fig. 2). Although this residue was obtained from a feeding study using Aroclor 1242, the detector profile exhibits many similarities to 1248 in that it exhibits lower responses in the earlier part of the chromatogram and enhanced responses in the latter part compared to 1242. This sample had been previously used in a collaborative study (7) in which 4 of 9 collaborators indicated they thought it more closely resembled 1248.

The average 1242 results (in ppm) obtained were 6.78 (12.6%), 7.54 (16.0%), and 5.62 (8.1%) by total peak height, total area, and individual peak area methods, respectively. The 1248 results, in the same order, were 6.94 (5.5%), 5.89 (7.7%), and 6.31 (7.8%). Closer agreement of results and precision with the 1248

Table 3. Collaborative results (ppm) for chicken fat containing an incurred Aroclor 1242 residue, quantitated by total response and individual peak methods, using Aroclor 1242 and 1248 reference materials

	Total peak ht		Total area		Ind. peak	
Coll.	As 1242	As 1248	As 1242	As 1248	As 1242	As 1248
1	6.40	6.71	7.51	5.98	5.74	6.17
2	6.36	6.75	7.74	5.92	5.75	6.08
3	5.35	6.69	6.07	5.43	4.53	5.42
4	6.72	7.44	6.96	5.60	5.55	6.15
5	7.78	6.69	8.90	5.85	5.92[a]	6.56
6	7.98	6.79	9.23	5.69	5.81[b]	7.02
7	5.83	6.62	5.84	6.14	5.69	6.15
8	7.54	7.73	8.54	6.86	6.25	7.00
9	7.25	7.21	6.34	5.24	5.65	5.94
10	6.63	6.81	8.32	6.18	5.28	6.58
Av.	6.78	6.94	7.54	5.89	5.62	6.31
Std dev.	0.852	0.380	1.21	0.454	0.456	0.491
Coeff. of var., %	12.6	5.5	16.0	7.7	8.1	7.8
Std error, ppm	0.27	0.12	0.04	0.14	0.14	0.16

[a] Does not include a reported 0.34 ppm for peak R_{DDE} (×100) = 16. Calculation was based on a peak response of <1% recorder deflection.

[b] Does not include a reported 1.03 ppm for peak R_{DDE} (×100) = 16. Calculation based on a peak response of <1% recorder deflection.

data indicates that it should be the reference of choice. The higher 1248 value from the individual peak area technique compared to the 1242 result by the same technique, 6.31 vs. 5.62 ppm, arises from the fact that no weight factors for 1242 with peaks of R_{DDE} (×100) >78 were determined (2). The average individual peak values obtained for the 2 reference materials show good agreement (Table 4). Residue peaks with an R_{DDE} (×100) range of 21 to 78 total 5.56 ppm from the 1242 reference and 5.63 ppm from the 1248 reference.

Although this chicken fat sample was analyzed in a previous collaborative study (7), significantly lower results were obtained here compared to those previously reported using the total response values for comparison. The previous values (ppm) reported, using a 1242 refer-

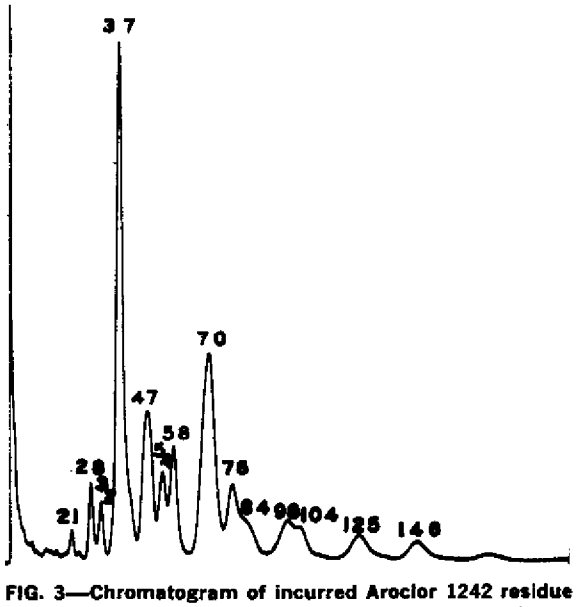

FIG. 3—Chromatogram of incurred Aroclor 1242 residue in chicken fat (ca 2 mg fat). Peak numbers are relative to *p,p'*-DDE = 100 (ca 12 min).

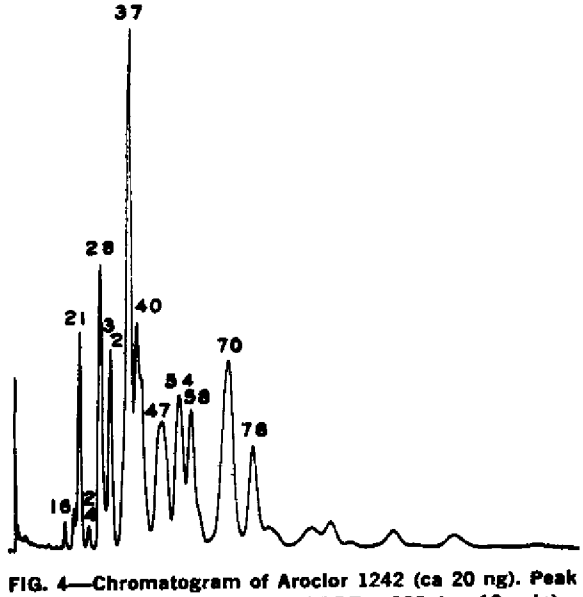

FIG. 4—Chromatogram of Aroclor 1242 (ca 20 ng). Peak numbers are relative to *p,p'*-DDE = 100 (ca 12 min).

Table 4. Comparison of average individual peak values (ppm) in an incurred Aroclor 1242 residue quantitated from both Aroclor 1242 and 1248 reference materials

Peak R_{DDE} (×100)	Av. of 10 detns		Coeff. of var., %	
	1242	1248	1242	1248
21	0.16	0.08	32.9	39.7
28	0.39	0.32	19.2	12.8
32	0.20	0.17	21.0	15.1
37 & 40	2.05	1.93	9.9	18.2
47	1.01	0.99	7.9	7.2
54	0.34	0.32	15.6	10.8
58	0.33	0.41	12.4	11.0
70	0.96	1.12	10.5	7.4
78	0.12	0.29	19.3	12.1
Total	5.56	5.63	—	—
Av. coeff. of var.	—	—	16.5	14.9

ence material, were 9.24 (8.0%) and 10.2 (17.2%) by total peak height and peak area, respectively. By the same methods of quantitation, the results obtained here were 6.63 (12.6%) and 8.32 (16.0%). The previously reported values (7) were obtained after fat cleanup by appropriate AOAC multiresidue methodology (1). The values reported here were obtained after cleanup by an automated gel permeation chromatography (GPC) system (GPC Autoprep, Model 1001, Analytical Biochemistry Laboratories, Inc., Columbia, MO 65201). The GPC eluates from 23 individual cleanups were combined, mixed, and concentrated to an appropriate strength before being distributed to the collaborators. No direct recovery comparison between the 2 cleanup techniques was attempted and this may account for the lower values reported in this study.

Recoveries of Aroclor 1254 from 2 fortified milk samples analyzed by **29.001–29.018** and quantitated by the 3 ECGLC methods are presented in Table 5. The individual samples were fortified on the whole-product basis by adding 1.0 ml of an ethanolic spike solution to about 2.2 kg milk and mixing for 1 hr. The blank was prepared similarly, with the addition of 1.0 ml ethanol.

After preparation, the blank and both spiked samples were each analyzed in duplicate to determine the average per cent fat. The average of the 6 determinations was 3.22 (2.8%). All recovery data are based on this fat content.

Spiking levels were chosen to approximate the temporary tolerances for this product, both the proposed 1.5 ppm level (8) and the existing 2.5 ppm level (9). Mean per cent recoveries obtained for the "low" 1.38 ppm spike level were 82.6 (16.8%), 85.5 (11.3%), and 84.1 (15.2%) by the peak height, total area, and individual peak methods, respectively. In the same order, mean per cent recoveries for the "high" 2.72 ppm spike level were 85.3 (18.8%), 87.1 (14.3%) and 86.8 (18.2%).

Table 5. Recovery of Aroclor 1254 (ppm, fat basis) from fortified milk samples, analyzed by AOAC multiresidue method and quantitated by total response and individual peak methods

Coll.[a]	Sample 1			Sample 2		
	Total peak ht	Total area	Ind. peak	Total peak ht	Total area	Ind. peak
1	1.07	1.20	1.15	1.97	2.20	2.15
2	1.34	1.24	1.27	2.89	2.87	2.90
3	1.40	1.37	1.38	2.89	2.66	2.86
4	1.06	1.15	1.12	1.91	2.03	1.96
5	1.37	1.29	1.39	2.64	2.59	2.69
6[b]	0.89	0.92	0.91	2.52	2.68	2.62
7	1.13	1.13	1.12	2.38	2.36	2.34
8	0.89	1.05	0.90	1.76	1.98	1.77
10	1.14	1.23	1.18	1.96	1.99	1.91
Amt added		1.38			2.72	
Av.	1.14	1.18	1.16	2.32	2.37	2.36
Std dev.	0.193	0.133	0.176	0.437	0.339	0.429
Mean % recd	82.6	85.5	84.1	85.3	87.1	86.8
Coeff. of var., %	16.8	11.3	15.2	18.8	14.3	18.2
Std error, ppm	0.06	0.04	0.06	0.14	0.11	0.14

[a] The data reported by Collaborator 9 are not included, since he modified the method.
[b] Sample 1 results do not include peaks with R_{DDE} (×100) values of 70, 98, and 104 because of background interference.

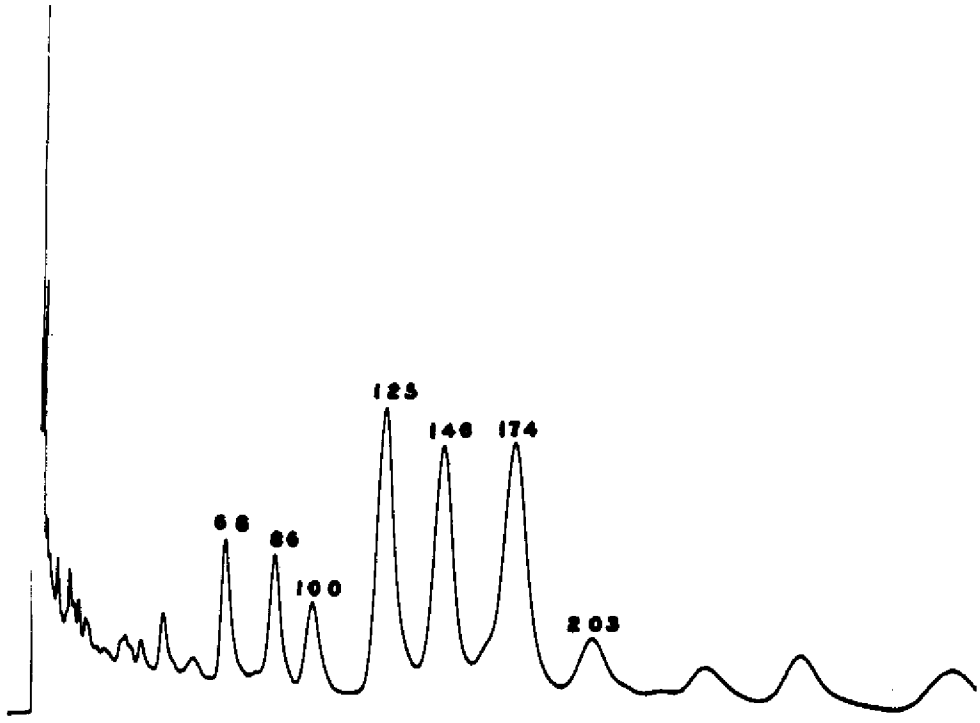

FIG. 5—Chromatogram of PCB residue in milk fat (ca 6 mg). Peak numbers are relative to p,p'-DDE = 100 (ca 12 min).

Collaborators 6 and 9 both reported blanks containing a high PCB level. No evidence of contamination in the spiked samples was noted for Collaborator 6, but some residual "apparent" pesticide peaks interfered with the low level peaks with R_{DDE} (× 100) of 70, 98, and 104. These peaks were not included in any of this collaborator's calculations for the low level, causing lower than actual recoveries to be reported. At the high level, the background responses were considered insignificant and were included in the calculations, causing somewhat higher than actual recoveries to be reported. Besides obtaining a high blank, Collaborator 9 used an additional silica gel cleanup prior to quantitation and recovered approximately 50% of the general range of the other collaborators. Consequently, the milk recovery data from this collaborator were not considered valid. Regardless of the method used for quantitation, the recovery through the AOAC methodology at both levels studied approximated 85% (15%).

Figure 5 is a chromatogram of the incurred PCB residue isolated from a raw whole milk sample, and Fig. 6 is the chromatogram of Aroclor 1254 used for the reference. Table 6 lists

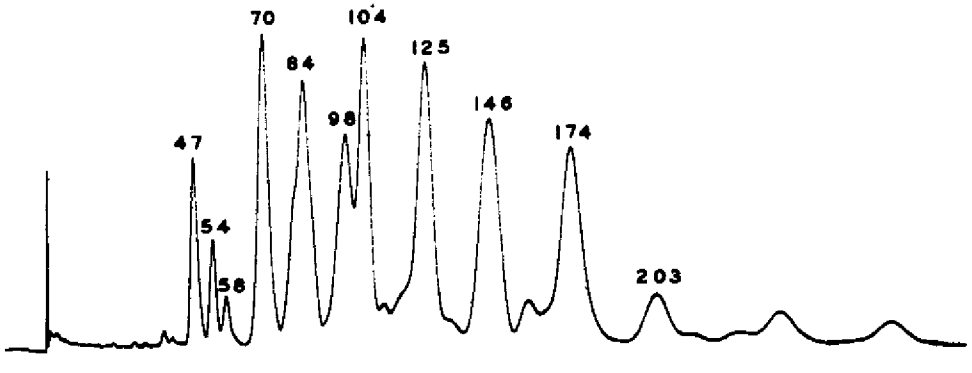

FIG. 6—Chromatogram of Aroclor 1254 (ca 20 mg). Peak numbers are relative to p,p'-DDE = 100 (ca 12 min).

Table 6. Individual peak values (ppm fat basis) reported for an incurred PCB residue in milk analyzed by the AOAC multiresidue method and quantitated by using Aroclor 1254 reference material

Coll.[a]	Peaks—R_{DDE} (×100)										
	47	54	58	70	84	98	104	125	146	174	203
1	—	—	—	—	—	—	—	0.50	0.25	0.34	0.05
2	0.01	—	—	—	—	—	—	0.57	0.35	0.51	0.05
3	—	—	—	0.15	0.25	0.09	—	0.51	0.26	0.30	0.05
4	0.01	—	—	—	—	—	—	0.42	0.22	0.23	0.04
5	0.13	0.06	—	—	—	—	—	0.67	0.37	0.34	0.05
6	—	—	—	—	—	—	—	0.58	0.30	0.32	0.06
7	—	—	—	—	0.25	—	—	0.41	0.25	0.30	0.06
8	0.02	0.02	0.01	0.16	0.22	0.09	—	0.37	0.20	0.23	0.05
10	—	0.02	0.03	0.44	0.18	—	0.08	0.57	0.31	0.27	0.04
Av.								0.511	0.279	0.316	0.050
Std dev.								0.097	0.058	0.084	0.007
Coeff. of var., %								19.0	20.6	26.6	14.1
Std error, ppm								0.032	0.019	0.028	0.002

[a] The data reported by Collaborator 9 are not included because he modified the method.

each collaborator's individual peak interpretation of the residue. Only peaks with R_{DDE} (× 100) values of 125, 146, 174, and 203 were uniformly assigned as PCB and only these data are statistically summarized. In all chromatograms submitted with this study, peaks eluting earlier than peak 125, particularly those designated as 68, 86, and 100 in Fig. 5, did not chromatograph at the absolute retention times as peaks in Aroclor 1254. Those eluting later than peak 125 did correspond exactly by absolute retention. The 68, 86, and 100 peaks have been confirmed to be chlorobiphenyls by GLC-mass spectrometry (GLC-MS). However, at the GLC conditions required for this study, these peaks could be all or in part qualitatively assigned to organochlorinated pesticide chemicals. The individual peaks with R_{DDE} (× 100) values of 125, 146, 174, and 203 were determined at average ppm values of 0.511 (19.0%), 0.279 (20.6%), 0.316 (26.6%), and 0.050 (14.1%) by the individual peak techniques.

Table 7 lists the comparative data for the incurred residue in the milk sample quantitated by the 3 ECGLC methods. Again, only the 4-peak data are statistically compared. The average values (ppm) are 1.34 (30.4%), 1.66 (31.4%), and 1.15 (18.4%) for the peak height, total area, and individual peak methods, respectively.

The larger coefficients of variation obtained in the milk sample quantitations are understandably higher than those obtained in either the synthetic mixture or chicken fat quantitations, since these samples underwent laboratory analytical procedures other than ECGLC quantitation. Of particular interest is the comparative change noted in both the average found and the coefficients of variation in the milk spike calculations compared to the incurred residue. The

Table 7. Collaborative results (ppm fat basis) for an incurred PCB residue in milk analyzed by the AOAC multiresidue method and quantitated by total response and individual peak methods, using Aroclor 1254 reference material

Coll.[a]	Total peak ht	Total area	Ind. peak
1	1.15	1.63	1.14
2	1.50	2.10	1.48
			(1.49)[b]
3	0.95	1.17	1.12
	(1.43)	(1.48)	(1.60)
4	1.09	1.36	0.91
5	2.05	2.49	1.43
	(2.29)	(2.52)	(1.62)
6	1.78	2.15	1.26
7	1.15	1.06	1.02
	(1.39)	(1.24)	(1.27)
8	0.83	1.10	0.85
	(1.57)	(1.90)	(1.36)
10	1.55	1.90	1.19
	(2.37)	(2.54)	(1.94)
Av.	1.34	1.66	1.15
Std dev.	0.407	0.523	0.212
Coeff. of var., %	30.4	31.4	18.4
Std error, ppm	0.136	0.174	0.071

[a] The data reported by Collaborator 9 are not included because he modified the method.

[b] Results in parentheses represent reported values which include all peaks calculated by the collaborators as PCB. Statistical analysis is based on only the values obtained for peaks with R_{DDE} (×100) values of 125, 146, 174, and 203.

average spike recoveries and precision between laboratories were nearly constant, regardless of the method of quantitation. On the basis of coefficients of variation, the individual peak method for the incurred residue showed a significantly lower value at about twice the precision as the peak height and total area methods.

Observations and Comments

No problems were reported or observed in establishing the proper GLC separations required. Collaborators 8 and 9 experienced linearity problems at the sensitivity setting based on 1 ng heptachlor epoxide, giving a 50% recorder response. They and Collaborator 7 operated at "higher" sensitivities (>50% full scale deflection) to stay within the linear range of their detectors.

Collaborators 1, 2, 3, 4, 7, and 8 used some type of linearized ^{63}Ni detector, Collaborators 5, 6, and 10 used a concentric design ^{3}H detector, and Collaborator 9 used a nonlinearized ^{63}Ni detector. Collaborators 1, 2, 4, 7, 9, and 10 used a data system or microprocessing integration for area measurements; Collaborators 3 and 6, manual planimetry; and Collaborators 5 and 8, disk integration. The collaborators who used electronic integration systems reported fewer hours in completing the study, but also experienced more calculation errors due to faulty integration. No difference in accuracy for area measurement by any of the techniques used was readily apparent, except for the few faulty electronic integrations that were overlooked by the collaborators.

Four of the 10 collaborators commented on the applicability of the individual peak method of quantitation. All that did felt it would be inherently more accurate than the existing techniques.

Conclusions and Recommendations

An individual peak method of PCB quantitation, using ECGLC as outlined previously (2,3) and collaboratively studied here, has been shown to be at least equivalent to and, generally, an improvement on interlaboratory precision compared to existing **29.018** methods. This is demonstrated by the ECGLC analysis of synthetically prepared chlorobiphenyl mixtures, incurred residues in chicken fat and milk, and spiked milk samples. In the case of the synthetic mixtures, the individual peak method also demonstrated better accuracy, but only when individual peak area responses are compared. It is therefore recommended that the individual peak method of quantitating PCB residues be adopted as an official first action method as an alternative approach to the existing **29.018** methods. It should be the method of choice when residues are encountered that do not exhibit an ECGLC profile similar to the selected reference Aroclor(s). The method can only be applied accurately when proper GLC conditions are established and area measurements of residue and reference Aroclors are compared. Also, the method as outlined can be used only with the specified lots (2) of Aroclor materials.

Even though the individual method demonstrated better overall precision in this study than the existing AOAC methods, the previously recommended guidelines (7) for PCB quantitation are just as applicable in this as well as other quantitative methods. Briefly, these guidelines are: (*a*) Use alternative columns and halogen-specific detection to substantiate original ECGLC findings; (*b*) use only those peak areas that are conclusively due to PCB, and these corresponding areas must be demonstrated (by retention time) to be present in the reference Aroclor(s); exclude all areas attributable to pesticide and/or product artifact materials; (*c*) attempt to duplicate the residue GLC pattern with the reference(s) as closely as possible in both peak number and intensity; and (*d*) routinely analyze reagent blanks to check for intralaboratory PCB contamination.

The applicable AOAC multiresidue methods in Chapter 29 were found adequate for the recovery of Aroclor 1254 at levels of 1.4 and 2.7 ppm in the fat of whole milk. The combined recoveries of the 2 levels were 85% (15%), using ECGLC as the determinative step, which is consistent with the previously reported PCB recov-

The recommendations of the Associate Referee were approved by the General Referee and by Subcommittee E and were adopted by the Association. See (1978) *J. Assoc. Off. Anal. Chem.* **61**, this issue.

This paper was presented as part of the report of the Associate Referee at the 91st Annual Meeting of the AOAC, Oct. 17-20, 1977, at Washington, DC.

ery and related discussion (7) in fatty food types. It is therefore recommended that the applicable sections of Chapter 29 be adopted as official first action for the determination of PCB residues in milk and other fatty dairy products.

Acknowledgments

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Interlaboratory Study of the Determination of Polychlorinated Biphenyls in a Paper Mill Effluent

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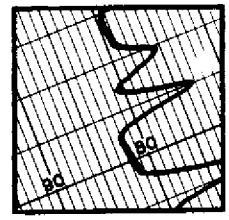

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Interlaboratory Study of the Determination of Polychlorinated Biphenyls in a Paper Mill Effluent

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Six laboratories collaboratively studied a method for determining polychlorinated biphenyls (PCBs) in paper mill effluent. In preliminary studies, the recovery and relative standard deviation (RSD) for the PCB Aroclor 1242 added to and extracted from distilled water were 95.6% and 14.7%, respectively. Because the RSD of data from direct injection of Aroclor 1242 solutions into the gas chromatograph was of similar magnitude, 15.6%, gas chromatographic analysis appeared to provide the principal source of variation in the overall determination. Participating laboratories achieved an average 93.7% recovery of Aroclor 1242 added to a paper mill effluent; their data had a RSD of 16.0%. The results indicate that the method is satisfactory for use with paper mill effluents having PCB concentrations above 2 μg/L and it compares favorably with findings from studies in other environmental matrices. Greater variation might be expected from effluents containing significant interferences.

Polychlorinated biphenyls (PCBs) were formerly used in carbonless copy papers, but this practice was terminated in 1971 (*1*, *2*). Small amounts of PCBs, particularly the Aroclor 1242 mixture, are still entering paper mills which recycle used paper fibers as part of their manufacturing process. It has not been logistically nor economically feasible to completely separate carbonless copy paper from the other waste papers that are being recycled (*1*). Thus, until all of the PCB-containing papers still in circulation cease to appear in recycled fiber, small quantities of PCBs will continue to be discharged in the mills' aqueous effluents. The amounts of PCBs discharged can be lowered by reducing the suspended solids in the effluent from the mill (*3*).

A procedure for determining PCBs in industrial effluents has been issued by the Environmental Protection Agency (EPA) (*4*). In our collaborative investigation, we modified the EPA method to apply specifically to paper mill effluents and

aimed to document the precision of the modified method when used in several laboratories. However, the modified method described here has not been submitted to the EPA for approval—an action that would be necessary if the data were to be included in an EPA-required monitoring program. Participants in the study included industry, universities, independent laboratories, and government agencies.

The promulgated EPA method for determining PCBs in industrial effluents involves liquid–liquid extraction, Florisil cleanup (Florisil is a registered Trade Mark of the Floridin Company, Pittsburgh, Pa. 15235), and electron capture gas chromatography. Previous work has revealed deficiencies when this procedure is used on in-mill process streams containing large amounts of cellulose fibers (*2*). Complete removal of the PCBs from cellulose fiber suspensions required alcoholic KOH reflux of the isolated fibers subsequent to liquid–liquid extraction. Samples of paper mill effluent for PCB monitoring are typically taken following waste treatment. Because a large percentage of the suspended fibers is removed in the treatment system, these samples should not require PCB isolation procedures beyond those specified in the EPA method (*4*). Therefore, the procedure used in this investigation retained many features of the EPA method (*4*) and was judged suitable by the collaborators for the purposes of this study. As described below, some modifications were incorporated to make the method easier to use on effluents in which organochlorine pesticides were not expected or generally observed in previous analyses.

EXPERIMENTAL

The interlaboratory study was performed in two parts. Phase 1 was designed to determine the comparability of PCB methodologies in use in each laboratory and to assess the ability of the participating analysts to perform the basic operations employed in PCB determinations. Phase 2 consisted of application of the modified method to determination of Aroclor 1242 in a paper mill effluent.

Phase 1. Each participating analyst was provided with septa-sealed vials containing acetone solutions of Aroclor mixtures. Each laboratory was asked to analyze the PCB mixture by (a) direct injection into a gas chromatograph (GC) employing an electron capture detector, and (b) addition of 1 mL of the unknown to 1000 mL of distilled water followed by solvent extraction, concentration, and then injection into the GC. Procedures for these operations were left to the discretion of the analysts.

Phase 2. *Validation of Sample Preparation Procedure.* Prior to collection of paper mill effluent samples to be used for the PCB determinations, a separate study was performed to evaluate the study coordinator's ability to provide equivalent effluent samples to each participant. Because PCBs tend to sorb onto suspended solids, samples for collaborative study must contain equivalent suspended solids contents. A large volume of paper mill effluent was placed in a metal container and was mechanically stirred. Aliquots (250 mL) were removed and sequentially added to each of ten separate 2.5-L glass containers. (Bottles were rinsed with hexane several times to remove possible contaminants before being used for paper mill effluent samples. The hexane was drained and the bottles air dried prior to use. Aluminum foil was used to line the bottle caps.) The process was repeated ten times until each 2.5-L container was filled. Suspended solids were determined on the contents of each container.

Instructions to Analysts. Each analyst received two 2.5-L paper mill effluent samples and three sealed glass ampules containing Aroclor 1242. Two of the three ampules contained Aroclor 1242 in isooctane; one ampule was designated a "known" and contained 13.6 μg/10 mL; the second ampule was an "unknown" and contained 35.2 μg/10 mL. Participants were asked to analyze each solution by direct injection into the GC. The third ampule contained an unknown concentration of Aroclor 1242 in methanol and was to be added directly to one of the two paper mill effluent sample bottles. The ampule was designed to deliver 6.8 μg of Aroclor 1242 directly into the paper mill effluent sample bottle.

The study plan called for each participant to divide the contents of one of the paper mill effluent samples into two equal portions. Then, each portion was to be extracted and the Aroclor 1242 concentration of each portion determined by GC. Each analyst was also instructed to add the ampule containing the 6.8 μg of Aroclor 1242 to the second 2.5-L sample container, break the ampule inside the container, mix well, and let this "spiked" sample stand for 24 h before beginning extraction and analysis by GC. As before, this "spiked" effluent sample was also to be analyzed in duplicate by dividing the 2.5-L sample into two equal portions with each one being analyzed separately.

Determination of PCBs in Paper Mill Effluent. In the promulgated method for PCBs in industrial effluents (*4*), PCBs and organochlorine pesticides are coextracted from the sample by liquid–liquid extraction. A silica gel microcolumn procedure and standard Florisil column cleanup are prescribed for separating PCBs from pesticides and for dividing the pesticides into subgroups. Because pesticides are unlikely constituents of paper mill effluents and were not of concern here, the EPA method (*4*) was modified for use in this study by removing from the procedure those steps necessary for extraction, separation, and determination of pesticides. The features of the EPA method (*4*) which were modified for application to paper mill effluent in this investigation are:

(1) Hexane and petroleum ether (30–60 °C) were independently shown by the collaborators to be suitable alternates to 15% methylene chloride in hexane for separatory funnel extraction of effluent. Hexane extraction has been shown to recover PCBs almost quantitatively from effluents with low fiber contents (*2*). Solvents of higher polarity, such as methylene chloride in hexane, extract excessive amounts of non-PCB materials without improving PCB recovery.

(2) To assure consistent performance of the electron capture detector and to minimize down time for detector cleaning, all extracts were subjected to Florisil column cleanup prior to gas chromatographic analysis.

(3) In addition to the specifications for Florisil columns (*4*), other column sizes and amounts of Florisil and eluting solvent were acceptable for PCB determination provided that (a) all PCBs were completely eluted, and (b) chromatogram quality signified that samples had been adequately cleaned up. Elution of PCBs from the Florisil column with hexane or petroleum ether as well as with 6% ethyl ether in petroleum ether was permitted. Use of petroleum ether alone for elution is standard practice in determining PCBs in paper and paperboard (*5*).

(4) The silica gel microcolumn procedure for separating PCBs from pesticides was deleted from the method.

(5) Gas chromatographic column liquid phases specified in the EPA method (*4*) include SE-30 or OV-1, and OV-17/QF-1. Other silicone liquid phases used successfully for determining PCBs in Phase 1 of this study included OV-17, OV-210, DC-200, OV-101, OV-225, and equivalent SP phases. An earlier collaborative study has indicated that several column materials are useful for PCB determinations (*6*). Therefore, the phases listed above were considered acceptable for this study. Also accepted were stainless steel as well as glass columns.

(6) Unknown Aroclors were identified by matching retention times and relative peak heights with peaks in reference Aroclors. To ensure valid quantitation, amounts were injected such that the size of the peaks from the sample and the standard were within ±25%. When quantitation was based upon peak heights, at least four peaks were used.

RESULTS AND DISCUSSION

Phase 1. The results of the GC analyses of Aroclor 1242 in acetone are presented in Table I. The average PCB concentration (as Aroclor 1242) based on direct injection into the GC was 1.47 ng/μL, representing an average recovery of 98% of the 1.5 ng/μL present in the acetone solution. Therefore, on the average, the results indicated that the eight participants in Phase 1 had good GC technique and could quantify PCBs.

While the results of the direct injection experiment were good, some variation among the analysts was evident as shown by the standard deviation (0.23 ng/μL), relative standard deviation (RSD) (15.6%) and range (1.05–1.76 ng/μL). While

Table I. Determination of PCBs as Aroclor 1242 in Acetone Solution by Direct Injection and Extraction from Distilled Water (Phase 1)

analyst	PCB by direct injection, ng/μL[a]	PCB extraction from distilled water, %
1	1.52	90.0
2	1.41	99.3
3	1.46	89.6
4	1.70	>100[c]
5	1.59	114
6	1.26	106
7	1.76	70.1
8	1.05	100
average	1.47 ng/μL[b]	95.6%[d]
range	1.05-1.76 ng/μL	70.1-114%
standard deviation	0.23 ng/μL	14.1%
rel. std. deviation	15.6%	14.7%

[a] Concentration of Aroclor 1242 = 1.50 ng/μL. [b] Represents average finding by direction injection = 98%. [c] Treated as outlier as analyst was unable to quantitate recovery except as reported. [d] Data from analyst no. 4 excluded in statistical calculations.

Table II. Test for Determining Suspended Solids Concentrations in Representative Samples

run	suspended solids, mg/L
1	73.2
2	80.8
3	74.8
4	74.8
5	75.6
6	73.6
7	77.6
8	77.6
9	74.4
10	72.4
average	75.5 mg/L
range	72.4-80.8 mg/L
std. deviation	2.53 mg/L
rel. std. dev.	3.3%

this indicated more variation than might be desired in an interlaboratory study, it is shown later that a RSD of 15.6% is typical for PCB determinations involving environmental matrices.

The extraction of Aroclor 1242 added to distilled water resulted in an average recovery of 95.6%. This was satisfactory, although the variation was again relatively high as indicated by the RSD (14.7%) and the range (70–114%). Because this sample was free from interferences, the precision represents that which is attainable under unusually favorable analytical conditions.

The RSDs obtained in the direct injection experiment and in determination of Aroclor 1242 added to distilled water were of similar magnitude. This suggests that GC analysis provided the major sources of between-laboratory variation in the overall analytical scheme. Likely contributors to this variation included: (a) use of different Aroclor 1242 standards with slightly different PCB isomeride composition, and (b) use of different quantitation methods, including measurement of peak heights, peak areas, or weight percentages of individual peaks (*7–9*). The contribution of different GC columns and conditions is difficult to assess, although it was possibly advantageous for the analysts to use their own columns which produced familiar Aroclor chromatograms.

Phase 2. This phase of the study again involved determination of Aroclor 1242 by direct injection of solvent solutions into the GC and, of greater importance, also included determination of Aroclor 1242 in a paper mill effluent. The effluent was studied as collected and after addition of a known amount of an Aroclor 1242 standard.

The ability of the proposed sample collection procedure to supply equivalent mill effluent samples for collaborators was tested by determining if the procedure could provide samples of equivalent suspended solids content. Results are given in Table II. One analyst removed aliquots from each of ten different 2.5-L sample bottles, filled as described earlier, and performed the standard suspended solids measurement (*10*). The data indicate that representative suspended solids distribution could be achieved by the sampling technique, since the RSD experienced was 3.3%. The published RSD for suspended solids determinations ranges from 0.76 to 33% depending on the actual suspended solids concentration present in the sample (*10*).

Known and unknown Aroclor 1242 concentrations were determined by direct GC injection from glass ampules. The results are shown in Table III. The known solution allowed participating analysts to check their in-house standards with one prepared by the coordinating laboratory and also provided a reference standard to be used for the spiking, extraction, and recovery experiment involving the paper mill effluent sample.

The determination of the known and unknown PCB concentrations by direct injection into the GC yielded essentially the same average calculated recovery data, i.e., 98% and 97%, respectively (Table III). However, the variation among seven reporting analysts was somewhat greater for the unknown standard solution (RSD = 12.6%) than for the known standard solution (RSD = 7.5%). There is no im-

Table III. Determination of PCBs as Aroclor 1242 in Isooctane Solution by Direct Injection (Phase 2)

	PCB concentration as Aroclor 1242			
	known (13.6 μg/10 mL)		unknown (35.2 μg/10 mL)	
analyst	concn reported, μg/10 mL	calcd recovery, %	concn reported, μg/10 mL	calcd recovery, %
1	14.2	104	37.0	105
2	13.6	100	34.0	97
3	12.5	92	36.0	102
5	12.5	92	27.3	78
6	12.8	94	31.0	88
7	15.0	110	40.7	116
8	12.5	92	33.4	95
average	13.3 μg/10 mL	98%	34.2 μg/10 mL	97%
range	12.5-15 μg/10 mL	92-110%	27.3-40.7 μg/10 mL	78-116%
std. dev.	1.0 μg/10 mL	7.2%	4.3 μg/10 mL	12.2%
rel. std. dev.	7.5%		12.6%	

Table IV. Determination of PCBs as Aroclor 1242 in a Paper Mill Effluent (Phase 2)

analyst	replicate, μg/L no. 1	replicate, μg/L no. 2	average PCB concn reported by each analyst, μg/L
1	2.70	2.09	2.40
2	3.48	3.88	3.36
3	2.45	2.89	2.67
6	2.26	2.32	2.36[a]
7	2.33	2.73	2.53[a]
8	2.98	2.84	2.95[a]
average (n = 12)		2.74 μg/L	2.71 μg/L[b]
range		2.09–3.88 μg/L	2.36–3.36 μg/L
std. dev.		0.52 μg/L	0.39 μg/L
rel. std. dev.		19.0%	14.4%

[a] Includes small additional amount of PCB obtained by rinsing sample bottle with solvent after removal of sample. [b] Statistics developed by considering only the average PCB concentration reported by each analyst.

mediate explanation for this except that the unknown solution was ca. 2.5 times more concentrated than the known solution. This resulted in an additional dilution step to keep the unknown Aroclor on scale. This could have introduced additional error and the slightly higher RSD.

The PCB concentration in the paper mill effluent was determined in duplicate by six analysts according to the modified analytical procedure. The results are given in Table IV. The paper mill effluent was also analyzed in duplicate following addition of a methanol-based Aroclor 1242 standard. These data appear in Table V. Relative standard deviations of the PCB determinations performed on the effluent as collected and following addition of Aroclor 1242 were 19.0% and 16.0%, respectively.

The variations in the results for the determination of Aroclor 1242 in the paper mill effluent among the six analysts who completed Phase 2 were not very different from the variations noted for the direct GC injection of Aroclor 1242 solutions (Tables I and III). This suggests, as did the Phase 1 findings, that only small additional errors were introduced by the sample extraction and Florisil cleanup steps.

As indicated in Table V, the average recovery of the added Aroclor 1242 was 93.7%. This average is slightly misleading since three results clustered near 100% and the remainder ranged from 84 to 88%.

Following Florisil cleanup of the paper mill effluent used in this study, all collaborators obtained characteristic Aroclor 1242 chromatograms. Some other paper mill effluents contain interfering materials that cannot be removed on Florisil and which produce badly distorted chromatograms (*11*). Between-laboratory variation in PCB determinations conducted on effluents containing intractable interferences would undoubtedly be greater than that experienced in the current investigation.

Table VI. Some Examples of Interlaboratory Variation Based on Collaborative Studies Involving PCBs

sample matrix	rel. std. dev., %	no. of analysts	ref.
paperboard	15-22	11	(*6*)
milk	18-31[a]	10	(*9*)
chicken fat	6-16[a]	10	(*9*)
marine wildlife	21	14	(*12*)
shark liver homogenate	27	6	(*13*)
marine sediments	22	10	(*14*)
fish	27-37	7-13	(*15*)
paper mill effluent	15-19	6	this study

[a] RSD varied with method used for GC quantitation.

Other observations reported by the collaborators in Phase 2 included (a) formation of emulsions during solvent extraction of the effluent, and (b) small differences in peak ratios between the individual laboratory's Aroclor 1242 standards and the standard provided by the coordinating laboratory. Emulsions were broken by centrifugation or addition of Na_2SO_4. The problem of variations in PCB standards could be obviated by providing Aroclor standards from a common source to all laboratories conducting PCB determinations.

Considering the nature of the paper mill effluent matrix, the results of this interlabortory study were good. This can be substantiated by comparison of the RSDs reported in this study with those reported for PCB collaborative studies involving other complex environmental matrices (Table VI). It is clear, however, that analysts desiring to compare their results for the determination of PCBs in environmental samples must anticipate variations within the range of 15–20% expressed as the RSD.

CONCLUSIONS

Based on this interlaboratory study, the method described herein for PCBs in paper mill effluents appeared satisfactory. However, the statistics developed in this work were derived from determination of Aroclor 1242 mixtures in the concentration range of 2–6 μg/L and on an effluent from which interferences were readily removed. Different precision and accuracy findings could occur when the method is applied to paper mill effluents having different contents of PCBs and of materials which interfere in the determination. Gas chromatographic standards and techniques appear to have been the principal sources of variation in this study. It is

Tavle V. Determination of PCBs as Aroclor 1242 in a Paper Mill Effluent to Which a Standard Solution of Aroclor 1242 Was Added

analyst	replicate, μg/L no. 1	replicate, μg/L no. 2	average PCB concn reported by each analyst, μg/L	calcd recovery of std. PCB,[b] %
1	4.30	4.90	4.60	88
2	6.26	5.95	6.10	100
3	6.56	4.50	5.53	103
6	4.32	4.15	4.32[a]	84[a]
7	5.80	4.96	5.42[a]	101[a]
8	4.64	5.17	4.96[a]	86[a]
average (n = 12)		5.13 μg/L	5.16 μg/L[a]	93.7%[c]
range		4.15–6.56 μg/L	4.32–6.10 μg/L	84–103%
std. dev.		0.82 μg/L	0.63 μg/L	8.53%
rel. std. dev.		16.0%	12.2%	9.1%

[a] Includes small additional amount of PCB obtained by rinsing sample bottle with solvent after removal of sample. [b] Based on average PCB concentration reported by analysts in Table IV. [c] Statistics developed by considering only the average PCB concentration reported by each analyst.

important that regulatory officials seeking to establish effluent standards for PCBs in discharge media such as paper mill effluent take the findings of this and other related studies into consideration, so that the standards may be enforced rationally in light of analytical variability.

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High-Volume Sampling of Airborne Polychlorobiphenyls with Amberlite XAD-2 Resin

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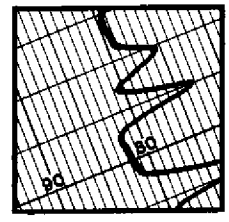

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HIGH-VOLUME SAMPLING OF AIRBORNE POLYCHLOROBIPHENYLS WITH AMBERLITE XAD-2 RESIN

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SUMMARY

Polyurethane foam, polyurethane foam coated with DC-200, Florisil, and Amberlite XAD-2 resin have been evaluated in a small-scale comparative study of their ability to sample airborne polychlorobiphenyls. XAD-2 resin has an excellent collection efficiency for tetrachlorobiphenyl at 1 l min^{-1} flow rates and is also suitable for high-volume air sampling. A high-volume air sampler was modified to sample both particulate and vapor-phase polychlorobiphenyls by incorporating the XAD-2 resin behind a glass fiber filter. When the sampling system was operated at a flow rate of 0.7 m^3 min^{-1} for 24 h, the collection efficiencies for tetrachlorobiphenyl and Aroclor 1221 were 96.5% and 83.0%, respectively.

Polychlorobiphenyls (PCBs) have been discovered in the North Atlantic atmosphere [1—3] and in Antarctic snow samples [4]. These findings suggest that the atmosphere is a major transport route. Murphy and Rzeszutko [5] found that precipitation is at present the major source of PCBs to Lake Michigan. These discoveries indicate the necessity to collect and quantify airborne PCBs. However, at present no collection method is uniformly accepted.

Polyurethane foam, which has been used extensively for high-volume sampling of airborne PCBs [2, 3], has been submitted to rigorous collection efficiency tests [6, 7]; collection efficiencies range from ca. 75 to 100% for different PCB isomers and mixtures. Murphy and Rzeszutko [5] reported a collection efficiency of 88% for polyurethane foam coated with DC-200 silicone oil. Giam et al. [8] used Florisil to sample laboratory air and reported a collection efficiency of 100%.

The number of methods available and the range of collection efficiencies reported make it necessary for a quantitative sampling methodology, which could easily be applied and which would simplify the intercomparison of data, to be found. This paper presents results from a comparison and evaluation of different collection methods for airborne PCBs.

A small-scale comparative study was initiated to evaluate the retention and collection efficiencies of polyurethane foam, polyurethane foam coated with DC-200, Florisil, and XAD-2 resin, a porous styrene—divinylbenzene copolymer, which has not been used to sample PCBs in air but has been

applied to collect chlorinated hydrocarbons and PCBs from water [9, 10].

The criteria which a collection method for atmospheric PCBs must meet are: the sampler must have a high collection efficiency for all PCB isomers, even the most volatile species; as the concentrations in air are usually in the low ng m^{-3} range, a substantial flow rate through the collection medium is essential to avoid extremely long sampling periods; it must be relatively easy to recover PCBs from the collection medium as only a small quantity is collected and lengthy procedures would allow more opportunity for loss of sample; interfering substances from the blank medium must be few to minimize difficulties in the quantification and interpretation of gas chromatograms.

Rather than try to adapt a high-volume air sampler to accommodate each of the adsorbent materials, the collection and retention efficiencies were evaluated under similar experimental conditions on a small scale at flow rates orders of magnitude lower than those experienced during high-volume air sampling; the results indicated trends which were used to determine the methods best suited for high-volume air sampling. A high-volume air sampler adapted to accommodate the medium was subsequently tested to determine its PCB collection efficiency.

EXPERIMENTAL

In the small-scale experiments the PCB adsorbent materials were held within glass tubing (0.95 cm i.d.) by a glass wool plug. The columns also contained 1 g of sodium sulfate. The Florisil column contained 0.3 g of 100—200 mesh Florisil (3% H_2O, w/w). The Amberlite XAD-2 resin column consisted of 0.4 g of dry XAD-2 resin (20—50 mesh). The polyurethane column contained a plug (1.3 cm diam.) cut from 3.8-cm thick polyurethane foam. Polyurethane foam coated with a solution of 1% DC-200 in hexane was also used.

Glass wool, Na_2SO_4, XAD-2 resin, and Florisil were cleaned by Soxhlet extraction (24 h) with petroleum ether. The polyurethane foam was rinsed with distilled water and Soxhlet-extracted for 12 h with petroleum ether and 12 h with acetone, then air-dried. Sodium sulfate and Florisil were activated at 320°C for 24 h. XAD-2 resin was dried at 60°C for 24 h. All solvents were of pesticide quality or double-distilled in glass.

A small-scale sampling apparatus was developed to test each adsorbent. The prefilter consisted of a Millipore holder containing a glass fiber filter for particulate matter and two XAD-2 resin columns for organic vapors. Two (three in certain experiments) air sampling columns were connected to both a vacuum pump and the prefilter. Flow rates were determined by a tri-flat variable-area flowmeter (Fischer and Porter Co.). All experiments were performed in the laboratory.

In the comparative study, ^{14}C-labelled 2,5,2′,5′-tetrachlorobiphenyl (1 μCi/0.03 mg) was used as the spiking material. Three different small-scale

experiments were performed: (1) elution efficiencies were determined by directly spiking the adsorbent, letting the spike air-dry, then eluting with petroleum ether; (2) retention efficiencies were determined by directly spiking the adsorbent, letting the spike air-dry, then drawing air through the column; (3) collection efficiencies were determined by sampling PCB vapor then drawing air through the columns for various lengths of time.

The collection efficiency experiments consisted of vaporizing PCBs in the injection port of a gas chromatograph. The spike was injected into a glass "T" joint joined to both the prefilter and sampling columns. The injection port was heated to 200—210°C before injection of the spike.

^{14}C-labelled 2,5,2′,5′-tetrachlorobiphenyl (TCB) was eluted from the adsorbent materials by allowing petroleum ether to equilibrate with the column for 30 min after which time the eluate was collected in a graduated receiving vessel. The column was then eluted with additional petroleum ether. The eluate was made up to 5 ml and transferred to a scintillation vial containing 10 ml of scintillation cocktail which contained toluene as the solvent and PPO and dimethyl-POPOP as fluors. The final concentration of fluors in the 15-ml counting solution was 0.5% PPO and 0.03% dimethyl-POPOP. ^{14}C was determined by the method of quench correction [11].

After completing the comparative study, a high-volume air sampler (GCA Precision Scientific, equipped with a Sierra model 310 flow controller) was modified to incorporate XAD-2 resin behind the filter holder assembly (Fig. 1). Particulates were sampled with a glass fiber filter (General Metal Works) and organic vapors with XAD-2 resin located in a screen-enclosed metal chamber (21-cm long). The chamber fits within a 30-cm long metal cylinder (9 cm i.d), threaded to accommodate both the filter holder assembly and the motor. The XAD-2 resin capsule fits tightly within the cylinder and is flanged at the top to prevent air flow around the chamber. The chamber parts are removable. The top and bottom are enclosed with 60-mesh screens to prevent the XAD-2 resin from entering the motor.

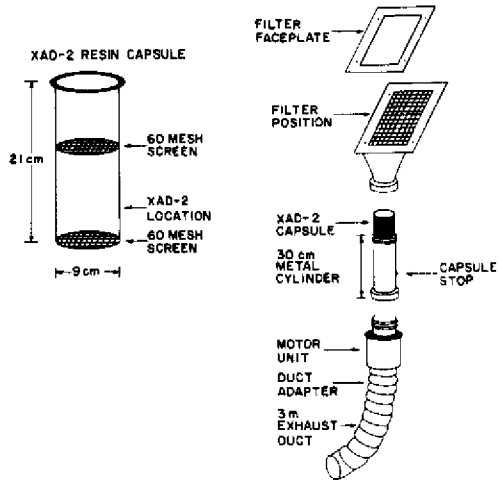

Fig. 1. Exploded view of high-volume PCB sampler and XAD-2 resin capsule.

The collection efficiency of the modified sampler was tested with a PCB vapor sampling technique. A g.c. injection port, into which a curved glass tube was inserted, was used to generate PCB vapor. The experimental procedure consisted of positioning the injection port above the filter, heating it to 200—210°C, and then injecting the spike into a 5 ml min^{-1} nitrogen carrier gas stream which subsequently flowed into the air stream of the sampler. After the sampling period had been completed, the tube, filter, and XAD-2 resin were analyzed for the PCB spike.

Retention and collection efficiency experiments were performed with TCB as the spiking material. Retention efficiency experiments consisted of spiking two 70-g portions of XAD-2 resin with TCB; one portion was placed in the sampler and the other was used as the analytical control. After the sampling period had been completed, the resin was Soxhlet-extracted for 24 h with 500 ml of petroleum ether. The extract was then concentrated (Kuderna—Danish concentrator) and prepared for scintillation counting. The collection efficiency experiments incorporated the vapor-generating device. Both retention and collection efficiency experiments were performed without a prefilter.

A second set of experiments tested the ability of the sampler to retain the most volatile PCB isomers. Aroclor 1221, which is composed predominantly of monochloro- and dichloro-biphenyl, was used as the spiking material. Two samplers were interconnected, one being used as a prefilter. A 70-g quantity of XAD-2 resin, spiked with Aroclor 1221, was placed in the second sampler. Each experiment included an analytical recovery control. In the analytical scheme, a 4-g alumina (6% H_2O, w/w) column (1 cm i.d.) was employed to remove interfering substances.

An experiment was also performed to obtain the collection efficiency of XAD-2 resin for Aroclor 1221 vapor. The vapor was generated in the g.c. injection port and subsequently collected by the XAD-2 resin. Air was sampled for a shortened period of 30 min to avoid collection of an excessive amount of interfering organic vapors. An air blank was subtracted from the actual sample.

Cleaning XAD-2 resin to obtain low blanks has been reported to be a problem [12]. For air sampling purposes, the XAD-2 resin is dried at 60°C for 24 h, Soxhlet-extracted with petroleum ether for 72 h (changing the solvent every 24 h), and dried overnight at 60°C. Quantities of clean resin (70 g) are stored in glass jars with foil-lined caps. Figure 2 presents chromatograms of an XAD-2 resin extract containing Aroclor 1242 before and after alumina cleanup which removes many of the early eluting resin contaminants from the extract.

After extraction of a collected air sample, the resin can be dried, stored, and reused. Some problems have been experienced with yellowing of the resin and permanent contamination upon collection of samples in heavily polluted areas. A more extensive cleanup of the resin, possibly with a polar solvent, may be necessary but has not been investigated.

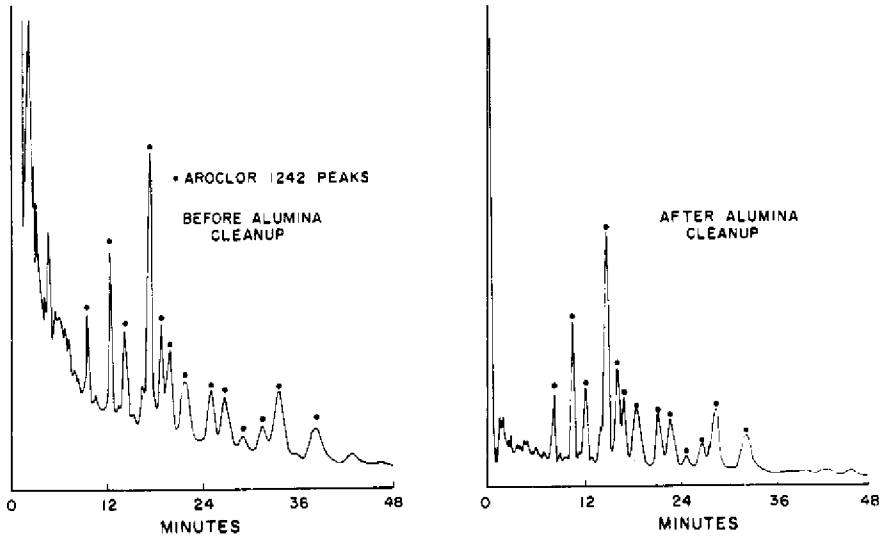

Fig. 2. XAD-2 resin extract containing Aroclor 1242 before and after alumina cleanup.

Analyses for carbon-14 were performed on a Packard model 3320 scintillation counter. Gas chromatographic analyses were performed on a Varian Aerograph Series 1700 chromatograph equipped with two scandium (^3H) foils. A 3.3-m column (2 mm i.d.) packed with 1.5% OV-17/1.95% QF-1 on 80—100 mesh GasChrom W(AW), and a 3.3-m column (2 mm i.d.) packed with 4% SE-30/6% OV-210 on 80—100 mesh GasChrom W(AW) were used for quantification. Nitrogen was used as the carrier gas at 12 ml min^{-1}. Aroclor 1221 was chromatographed isothermally at 165°C.

RESULTS AND DISCUSSION

The small-scale experiments were designed to compare the four adsorbent materials under similar experimental conditions. The retention and collection efficiencies were calculated by dividing the amount of TCB recovered from the first column by the total amount of TCB recovered. This method of calculation was considered to be necessary because of minor difficulties experienced in accurately reproducing the exact amount of PCB spike. XAD-2 resin, Florisil, polyurethane foam, and coated polyurethane foam had average retention efficiencies of 100%, 97%, 80% and 58%, respectively (Table 1). The collection efficiencies of XAD-2 resin, Florisil, and polyurethane foam were 99%, 100%, and 50%, respectively (Table 2); the collection efficiency of coated polyurethane foam was not determined because of its poor performance in the retention efficiency experiments.

Florisil and XAD-2 were efficient retainers and adsorbers of PCB, even in very small amounts. Difficulties were experienced with the mesh size of Florisil. The fine mesh adsorbent creates a large pressure drop across the column; this subsequently produces extremely low flow rates which make it difficult to include Florisil in a high-volume air sampling system in its present form.

TABLE 1

TCB retention efficiencies

Adsorbent	Flow rate (cm^3 min^{-1})	Volume of air sampled (m^3)	Recovery (%)[a]	1st column (ng)	2nd column (ng)	Retention efficiency (%)
XAD-2	7000	10.1	100	301	0	100
	6200	8.6	77	223	0	100
	6400	8.8	72	215	0	100
			Av. 83 (92)[b]			Av. 100
Florisil	6000	8.0	91	272	0	100
	5600	7.4	94	282	0	100
	4200	11.3	94	272	0	97
	3800	18.5	104	300	12	96
	3400	16.5	90	255	15	94
			Av. 95 (99)			Av. 97
Polyurethane	7200	8.2	81	202	41	83
	7300	9.2	77	161	71	69
	7000	8.8	80	213	26	89
			Av. 79 (79)			Av. 80
Coated polyurethane	7300	9.2	84	182	70	72
	6500	8.9	71	96	116	45
	6800	9.3	81	140	103	58
			Av. 79 (79)			Av. 58

[a]TCB spike = 300 ng = 2.22×10^4 dpm.
[b]Average elution efficiencies from separate recovery experiment run in triplicate.

TABLE 2

TCB vapor collection efficiencies

Adsorbent	Flow rate (cm^3 min^{-1})	Volume of air sampled (m^3)	Recovery[a] (%)	1st column (ng)	2nd column (ng)	3rd column (ng)	Collection efficiency (%)
XAD-2	8500	9.6	95	141	1	0	99
	5700	5.5	113	168	1	0	99
	9400	10.6	71	106	0	0	100
	8500	17.1	113	163	4	2	97
			Av. 98 (92)[b]				Av. 99
Florisil	4900	16.5	100 (99)	149	0	0	100
Polyurethane	15800	15.9	75 (79)	57	45	11	50

[a]TCB spike = 150 ng= 1.11×10^4 dpm.
[b]Average elution efficiencies from separate recovery experiment run in triplicate.

Based on the same criteria for all experiments, the retention efficiency of polyurethane foam was much lower than that of either XAD-2 resin or Florisil. As similar flow rates were used, differences in retention efficiencies from differences in flow rates were not a factor. The flow rate for the

polyurethane collection efficiency experiment was increased to ca. double that in the direct spiking experiment. The collection efficiency was reduced significantly. A rather small plug was used; the first plug in the sampling train may therefore have become saturated with PCBs. The actual depth of adsorbent (3.8 cm) was similar to the 5-cm plugs employed by Bidelman and Olney [2]. However, the volume of polyurethane used in this study (5.0 cm^3) was much less than the 393 cm^3 used by Bidelman and Olney [2] or the 181-cm^3 employed by Lewis et al. [7]. Using the first two plugs (total depth 7.6 cm) in the sampling train as a basis for the calculation resulted in a collection efficiency of 90%, which is in the same range as the collection efficiencies reported for polyurethane foam [2, 7].

The small-scale TCB experiments were used only as a preliminary test. Flow rates were in the l min^{-1} range and the amount of air sampled ranged from 7 to 19 m^3. The results cannot be used to indicate the performance characteristics of the adsorbent media under the m^3 min^{-1} flow rate conditions of high-volume air sampling. However, certain trends were evident. The TCB breakthrough for polyurethane was greater than that of XAD-2 resin or Florisil. This would seem to indicate that XAD-2 resin and Florisil are more efficient adsorbers. However, the use of a larger amount of polyurethane foam might produce greater collection efficiencies. The results indicated that XAD-2 resin had an excellent retention and collection efficiency for TCB. Its mesh size was also coarse enough, unlike Florisil, for incorporation into a high-volume air sampling system. Also unlike polyurethane, which must be cut into plugs from sheets of the material, XAD-2 resin can be used without further alteration.

The XAD-2 resin incorporated into the high-volume sampling system described in the experimental section was tested as discussed. The amount of XAD-2 resin used was determined from the small-scale experiments which indicated that a total depth of 2.8 cm of XAD-2 resin produced a sufficient collection efficiency. The thickness of the XAD-2 resin was the most important parameter. A depth of 2.8 cm in the capsule required 70 g of XAD-2 resin. The results from the TCB retention and collection efficiency experiments are presented in Table 3. In all experiments the tube and filter contained undetectable levels of the spike. Average recoveries of the sampling experiments (96.5%) were greater than those of analytical controls (93.4%) in all cases. Correcting the recoveries from the collection or retention efficiency experiments for analytical recovery would result in an efficiency of over 100%, and the retention or collection efficiencies were therefore reported as the analytical recoveries of the sampling experiments. Combining the results from the retention and collection efficiency experiments, the average efficiency for TCB was 96.5%. The resin retained both large (2283 ng) and small (218 ng) amounts of TCB; only a negligible amount was lost even after collection for 3 days.

In the Aroclor 1221 experiments (Table 4) the average recovery was 64.9% and recoveries for direct spikes on the resin with TCB and Aroclor

TABLE 3

TCB retention and collection efficiencies for PCB sampler

Sample[a]	ng/spike	Flow rate (m^3 min^{-1})	Volume of air sampled (m^3)	Analytical recovery (%)	Retention or collection efficiency (%)[b]
TCB DS1	257	0.7	1153	91.5	92.3
TCB DS2	257	0.7	1019		98.9
TCB DS3	242	0.7	3127		Av. 92.2
TCB VS6	218	0.7	719	94.2	101.2
TCB VS7	2283	0.7	1037	94.6	98.0
				Av. 93.4	Av. 96.5

[a]DS, direct spike applied to resin before putting it in air sampler. VS, spike was vaporized in g.c. injection port before collection.
[b]Recovery from collection efficiency experiment (not corrected for analytical recovery).

TABLE 4

Aroclor 1221 retention and collection efficiencies for PCB sampler

Sample[a]	ng/spike	Flow rate (m^3 min^{-1})	Volume of air sampled (m^3)	Analytical recovery (%)	Retention or collection efficiency (%)[b]
1221 DS1	4848	0.5	1344	60.3	75.6
1221 DS2	4848	0.5	1430	62.1	82.8
1221 DS3	4848	0.5	1492	72.3	90.6
				Av. 64.9	Av. 83.0
1221 VS1	4040	0.6	18	64.9	91.0

[a]DS, direct spike applied to resin before putting it in air sampler. VS, spike was vaporized in g.c. injection port before collection.
[b]Corrected for analytical recovery.

1242, with subsequent elution of the extract through alumina, were 85.5% and 80.1%, respectively. The low recoveries for Aroclor 1221 are probably a result of the high volatility of the mixture. For comparison with the TCB experiments, the retention efficiences were corrected for analytical recovery. The retention efficiency varied, the more volatile components being retained less efficiently. The monochloro- and dichloro-biphenyl components were collected with average efficiences of 72% and 86%, respectively. When three major peaks from Aroclor 1221 were employed for quantification, the average retention efficiency for the mixture was 83.0%.

In the Aroclor 1221 collection efficiency experiment (Table 4) the collection efficiencies for the mixture and and its monochloro- and dichloro-biphenyl components were 91%, 91% and 90%, respectively. Comparison

with the results of the direct spike experiments indicates that the resin initially collected Aroclor 1221 with a high efficiency. The components were stripped from the resin to a small extent during a 24-h sampling period, the amount retained being directly proportional to the volatility of the component. All of the present sampling techniques appear to underestimate the amount of mono- and dichloro-biphenyl in the atmosphere.

Conclusions

A modified PCB sampling technique with XAD-2 resin meets the criteria which a collection method for atmospheric PCBs must possess. The sampler has a high collection efficiency for TCB (96.5%) and a lower efficiency for Aroclor 1221 (83.0%). The low retention efficiencies for monochloro- (72%) and dichloro-biphenyl (86%) show that the system is not equally efficient for all PCB isomers. The maximum flow rate of the modified sampler (0.7 m^3 min^{-1}) makes extremely long sampling periods unnecessary. Recovery of PCBs from XAD-2 resin is relatively easy by Soxhlet extraction with subsequent elution of the concentrated petroleum ether extract through alumina. Analytical recoveries for TCB, Aroclor 1242, and Aroclor 1221 are 85.5%, 80.1% and 64.9%, respectively. For XAD-2 resin, blanks which are free of PCB-interfering substances can easily be obtained.

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An Expanded Approach to the Study and Measurement of PCBs and Selected Planar Halogenated Aromatic Environmental Pollutants

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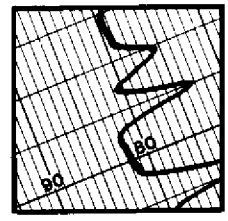

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AN EXPANDED APPROACH TO THE STUDY AND MEASUREMENT OF PCBs AND SELECTED PLANAR HALOGENATED AROMATIC ENVIRONMENTAL POLLUTANTS*

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Analysis of environmental polychlorinated biphenyl (PCB) residues is complicated by the presence of a large number of congeners in the various commercial mixtures. Currently, environmental levels of PCBs are often reported in terms of total PCB concentration or Aroclor® content. Marked differences exist in the toxicity of individuals chlorobiphenyls and impurities in technical PCB mixtures.[1,2] To predict adequately the environmental hazards associated with PCB pollution, detailed information is required on the composition of technical PCB mixtures and environmental PCB residues. Assessment of the biologic significance of environmental PCB residues necessitates the establishment of definitive relationships between PCB structure and toxicity. Recent studies by Goldstein *et al*[1] and Poland *et al.*[2] indicate that PCB components lacking *o,o'*-Chlorine (Cl) substitution and having four or more Cl atoms, may account for a significant portion of the toxicologic activity resulting from acute exposures to PCB mixtures.

To date, use of high resolution (HR) wall-coated open tubular (WCOT) glass capillary gas chromatographic (GC) columns has provided the most complete separation of PCB congeners in technical mixtures.[3,4] Earlier, we found that 28M WCOT glass capillary C_{87},[5] Apiezon-L and SE-30 columns failed to resolve PCBs lacking *o,o'*-Cl substitution from several other components present in Aroclors 1248 and 1254.[4] However, application of PX-21 carbon or PX-21 carbon/foam column chromatography[6] provided separations adequate for the quantitation of non-*o,o'*Cl-substituted PCBs and trace planar impurities in technical mixtures.[4] The retention volumes of PCB components on carbon columns generally increased with decreasing *o,o'*-Cl substitution and with *meta* and *para* Cl substitution. Large volumes of toluene were required to elute planar non-*o,o'*-substituted PCBs, chlorinated dibenzofurans, and dioxins from granular (>325 mesh) PX-21 carbon columns.[4] Use of small amounts of PX-21 carbon on foam as an adsorbent for the isolation of these compounds permitted a significant reduction in the solvent required.

We report the carbon separation of Aroclor 1248 into four fractions to determine the toxicity of structurally related PCB components to fish. PCB components in each fraction were characterized using HRGC 28M WCOT glass capillary columns of Apiezon-L, and C_{87} hydrocarbon. Rainbow trout (*Salmo gairdneri*) were exposed to Aroclor 1248 fractions containing 1–4 *o,o'*-Cl for 28 days with no visible effects at

*Reference to trade names does not imply government endorsement of commercial products.

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concentrations equal to or less than 8 μg/1. However, all fish exposed to 0.1–1.3 μg/1 of non *o,o'*-Cl substituted 3,4,3',4'-tetrachlorobiphenyl (TCB) exhibited poor growth and impaired equilibrium after 34 days exposure.

EXPERIMENTAL

Reagents

Solvents were nanograde (distilled in glass) from Burdick and Jackson, Muskegon, MI. AMOCO carbon grade PX-21 was obtained from AMOCO Research Corporation, 200 East Randolph Drive, P.O. Box 5910-A, Chicago, IL. Polyurethane plugs (Gaymar Identi Plugs #L800) were obtained from Arthur H. Thomas Company, Philadelphia, PA. Silica gel was CC-7 Silicar 70-230 mesh from Mallinckrodt, P.O. Box 5439, St. Louis, MO. Chlorobiphenyl standards were obtained from RFR Inc., 1 Main Street, Hope, RI; Analabs Inc., 80 Republic Drive, North Haven, CT.; and as a gift of Göran Sundström, National Swedish Environment Protection Board, Wallenberg Laboratory, Stockholm, Sweden.

Apparatus

Apiezon-L and C_{87} WCOT glass capillary columns (0.22 mm × 28 m) were purchased from the Quadrex Corporation, P.O. Box 3881 Amity Station, New Haven, CT. Aroclor 1248 and carbon fractions were characterized using a Varian model 3700 gas chromatograph equipped with small volume pulsed ^{63}Ni electron capture detectors and a capillary injection system. A 2 mm id × 4.6 m glass GC column with 2.5% purified Apiezon-L[7] on Chromasorb W HP (w/w) was employed for the analysis of most fish extracts. The gel permeation chromatograph (GPC) utilized for sample cleanup was an Autoprep 1001, Analytical Biochemistry, P.O. Box 1097, Columbia, MO. A flow-through proportional diluter system as described by McAllister *et al.*[8] was used for rainbow trout exposures.

Method

PX-21 carbon was sieved into batches >325 mesh and <325 mesh. Particles <325 mesh were used in the preparation of carbon/foam adsorbent as described by Huckins *et al.*[6] and carbon particles >325 mesh were used without modification. Chromatographic columns were 1 cm id glass, filled to 10 cm bed depth with carbon or carbon/foam. A stepwise gradient of toluene in cyclohexane (Figures 1–3) was utilized for the fractionation of PCBs and PCB impurities on columns of carbon and carbon/foam. Column flow rate was maintained at 3 ml/min and the recovery of non-*o,o'*-Cl PCBs from Aroclor 1248 was determined using carbon purified Aroclor 1248 spiked with known quantities of non-*o,o'*-Cl PCBs. The sample capacity of a 1.75 g (>325 mesh) PX-21 carbon column for 1-4 *o,o'*-Cl substituted PCB mixtures was 200 mg. Similar sized carbon columns removed >95% of non-*o,o'*-Cl-substituted PCBs from 1 g quantities of Aroclor 1248. Columns of PX-21 carbon (>325 mesh) were used for the fractionation of 1–4 *o,o'*-Cl Aroclor 1248 components for toxicity studies. Structural assignments of PCB congeners present in 0–4 *o,o'*-Cl carbon fractions of Aroclor 1248 (TABLE 1) were based on HRGC comparison with PCB

standards and previously published data by Jensen and Sundström[7] and Sisson and Welti.[9]

Rainbow trout fingerlings (0.3–1.0g) reared at the Columbia National Fisheries Research Laboratory were exposed to analytic grade (>95 pure) 3,4,3′,4′-TCB and 1–4 *o,o′*-Cl carbon fractions of Aroclor 1248 in a flow-through diluter system.[8] Exposure times were 28 or 50 days and selected fish were maintained in fresh water for a period of 28 days after cessation of exposure (TABLES 2 & 3). Analytic grade 3,4,3′,4′-TCB was used for the non-*o,o′*-Cl study because isolation of sufficient

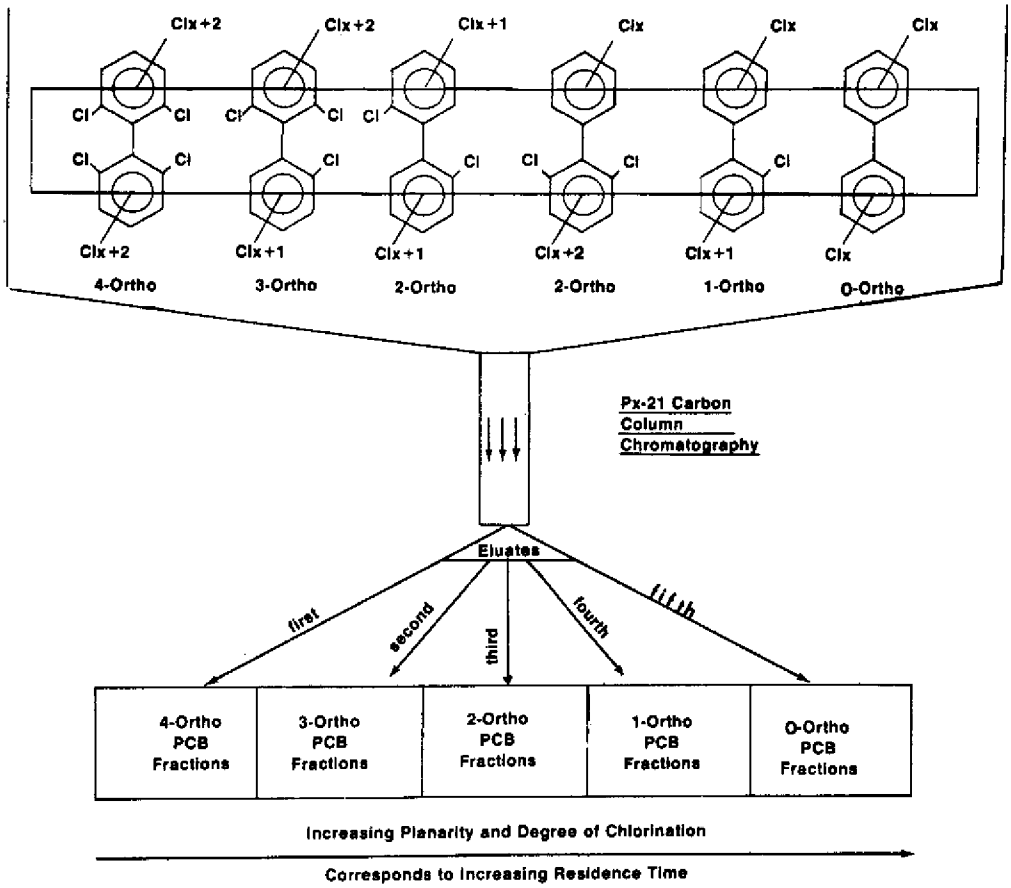


FIGURE 1. Elution order of the six possible *o,o′*-Cl substitution patterns of PCBs from PX-21 carbon or carbon/foam columns.[14] See FIGURE 2 for solvent system.

quantities of 3,4,3′4′-TCB for a flow-through exposure would have required the carbon fractionation of 25 g of Aroclor 1248.

Whole body fish samples (<5 g) were ground with four times their weight of anhydrous sodium sulfate and placed in 1 cm id × 10 cm glass columns fitted with Teflon® stopcocks.[10] PCB residues (neutrals) were extracted with 150 ml of 5% diethyl ether in petroleum ether using a column flow rate of 2–3 ml/min. Sample cleanup was accomplished using automated GPC[11] and Florisil chromatography. The GPC column (2.5 cm id × 30 cm.) was packed with 35 g of Bio Beads SX-3 and eluted with 25%

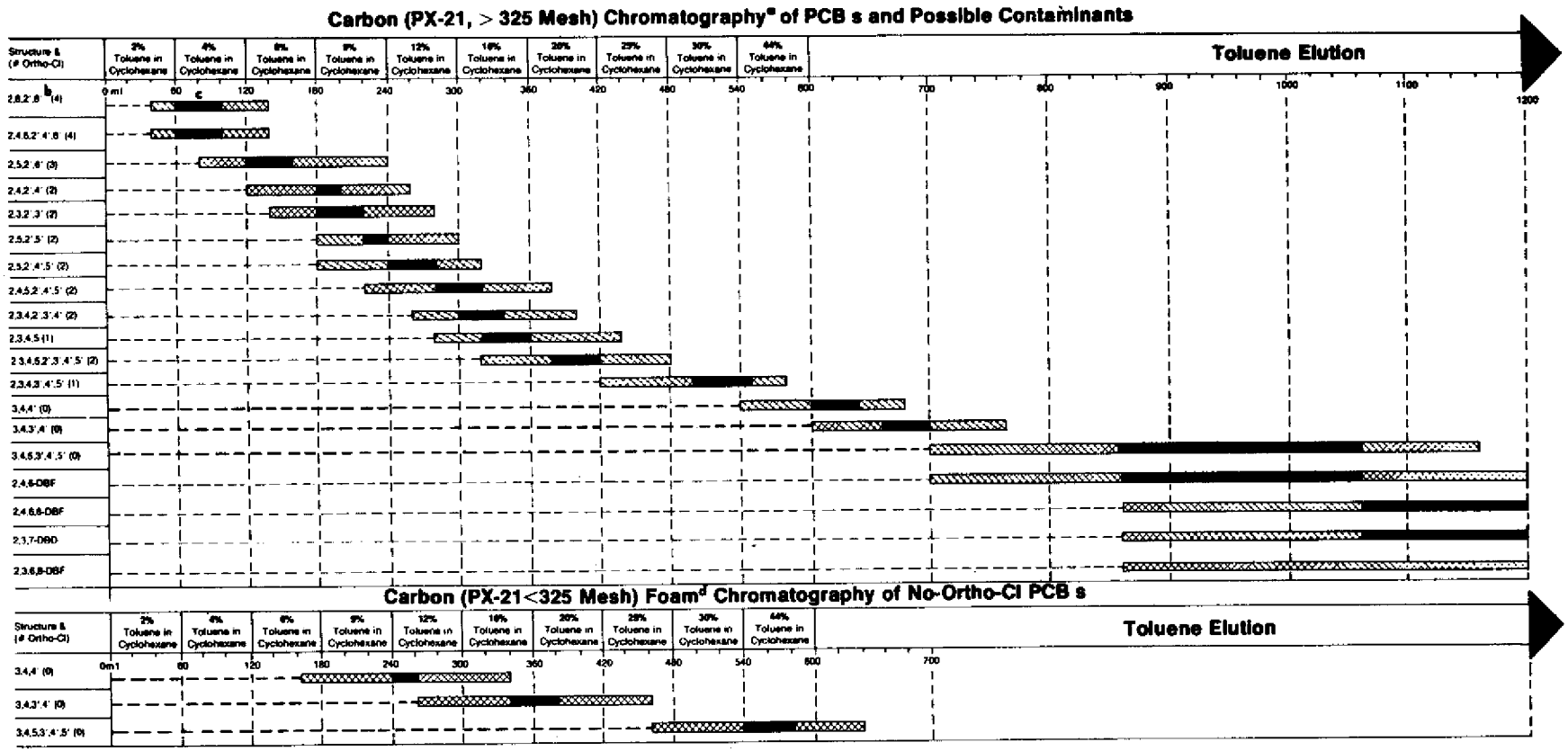


FIGURE 2. PX-21 carbon and carbon/foam chromatography of selected PCBs, chlorinated dibenzofurans (DBF), and a dibenzo-*p*-dioxin (DBD). *a*, Stepwise elution of two 1 cm id × 10 cm carbon (1.75g) columns; *b*, carbon columns were spiked with 20 μg of each PCB and 5 μg of DBF and DBD, *c*, dark portion of bar represents maximum concentration; *d*, stepwise elution of a 1 cm id × 10 cm carbon/foam (1.1g, 15% carbon w/w) column.

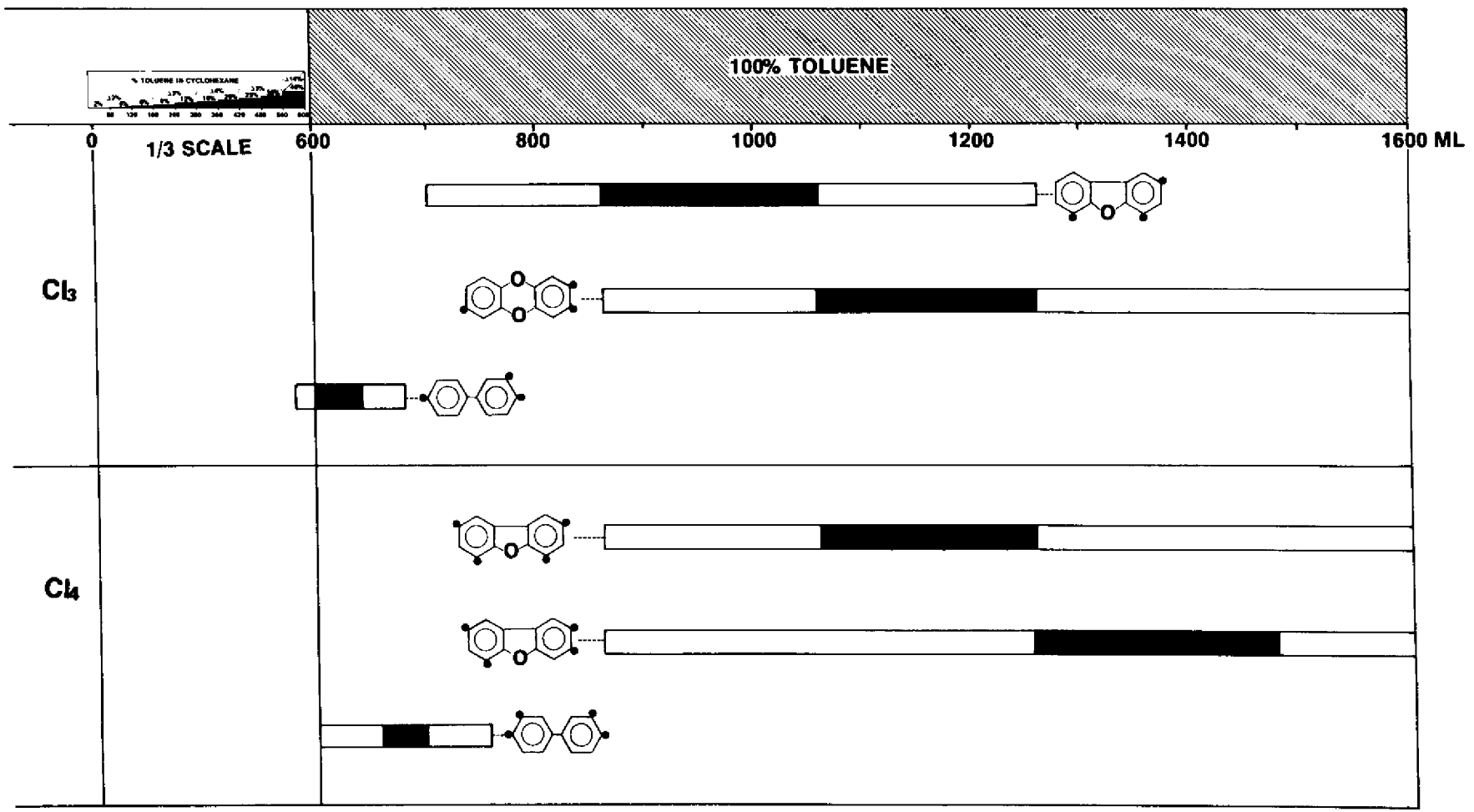


FIGURE 3. Elution profiles of planar PCBs, chlorinated DBFs, and a DBD from PX-21 carbon (>325 mesh) columns (1 cm id × 10 cm).

TABLE 1

MAJOR AROCLOR® 1248 COMPONENTS USED IN FOUR PCB EXPOSURES TO RAINBOW TROUT

40–180 ml* 2–4 *o,o'*-Cl (GC Peak #)	140–320 ml 1&2 *o,o'*-Cl (GC Peak #)	340–520 ml 1 *o,o'*-Cl (GC Peak #)	540–780 ml 0 *o,o'*-Cl (GC Peak #)
2,4' (8)†	2,3',5' (15)	3,4,2',5' (8)	3,4,3',4'
2,2',5' (9)	4,2'5' (16)	2,3',4',5'	
2,2',4' (10)	3,2',4' (17)	3,2',3',4' ‡	
2,2',3' (11)	2,3',4' (20)	2,4,3',4'	
2,6,2',6'§	2,3,4' (20)	2,3,3',4'	
	2,5,2', 5' (1)		
2,2',4',6' (14)	2,4,2',5' (2)	3,4,2',4',5' (25)	
2,5,2',5' (1)	2,3,2',5' (3)	3,4,2',3',4' (28)	
2,4,2',5' (2)	2,2',3',4' (4)		
2,3,2',5' (3)	2,6,3',4'‡		
2,4,2',4'	4,2',3',6' ¶		
2,2',3',4' (4)	2,3,2',4' ¶		
2,5,2',3',6' (6)	3,4,2',5' (8)		
2,3,2',3',6' (7)	2,5,2',4',5' (12)		
	2,4,2',4',5' (13)		
	2,3,2',4',5' (14)		
	2,5,2',3',4' (15)		
	3,4,2',3',6' (17)		
	2,3,2',3',4' (16)		

*Carbon fraction containing maximum concentration of the 1248 components listed.
†A 1 *o,o'*-Cl PCB; numbers with dots represent different GC peaks, see FIGURE 4.
‡Identities are tentative.
§A 4 *o,o'*-Cl PCB; coelutes with GC peak 11.
¶Two possible structures for a single GC peak.

TABLE 2

WHOLE BODY RESIDUES (μg/g) IN RAINBOW TROUT* EXPOSED TO 3,4,3',4'-TCB AND 1 *o,o'*-Cl 1248 COMPONENTS IN A FLOW-THROUGH DILUTER SYSTEM

Toxicant (# of *o,o'*-Cl)	Mean† H_2O Concentration (μg/l)	Days Exposure 16	29	50	Days Elimination 7	14	28
3,4,3',4' (0 *o,o'*-Cl)	0.1			0.8	0.4	0.2	ND‡
	0.4			1.9	—	—	—
	0.7			5.1	4.7	3.8	1.9
	1.4			12.0	—	—	—
(1 *o,o'*-Cl)§	3.8		82.2		62.2	34.6	26.9
	7.8	60.2¶	—		—	—	—

*Control fish contained <0.1 μg/g total PCB residue and each sample was a composite of 2 to 4 fish.
†Water samples were analyzed weekly.
‡None detected; minimum detection limit of 3,4,3',4' was 5 ng/g.
§See TABLE 1 for identity of 1248 components in the 1 *o,o'*-Cl carbon fraction.
¶Complete mortality due to a diluter malfunction was observed at 16 days in the 7.8 μg/l concentration.

toluene in ethyl acetate. The flow rate of the column was maintained at 4–5 ml/min. The first 105 ml of eluate from the column was discarded and the 105–200 ml fraction containing the PCBs was collected. Subsequent purification with Florisil was necessary for samples with less than 5 μg/g of PCBs. GPC fractions containing PCBs were concentrated to 5 ml volume in isooctane and chromatographed using a 1 cm id column containing 4 g of activated Florisil. PCBs were eluted with 40 ml of 10% diethyl ether in petroleum ether at a flow rate of 3–5 ml/min. A second column wash of 40 ml of 20% diethyl ether in petroleum ether was also collected to monitor PCB recovery from Florisil columns.

Residues of PCBs accumulated by fish were measured by GC using a electron capture detector. GC conditions are given in apparatus section. The peak heights of the major components in each fraction were summed and the totals of these peak heights were used to prepare calibration curves. These curves were used to quantitate the PCBs accumulated by fish during the exposure periods.

TABLE 3

WHOLE BODY RESIDUES (μg/g) IN RAINBOW TROUT* EXPOSED TO 1 AND 2 *o,o'*-Cl AND 2–4 *o,o'*-Cl 1248 COMPONENTS IN A FLOW-THROUGH DILUTER SYSTEM

Toxicant (# of *o,o'*-Cl)	Mean† H_2O Concentration (μg/l)	Days of Exposure		Days Elimination		
		10	29	7	14	28
(1&2 *o,o'*-Cl)‡	4.5		36.5	30.0	14.9	—
	19.4		72.1§	27.5	13.2	7.1¶
(2–4 *o,o'*-Cl)‡	3.9		46.5	19.8	17.4	8.2
	16.0		144.7	74.0	42.9	27.0

*Control fish contained <0.1 μg/g total PCB residue and each sample analyzed was a composite of 2 to 4 fish.

†Water samples were analyzed weekly.

‡See TABLE 1 for identity of 1248 components.

§Represents mortalities.

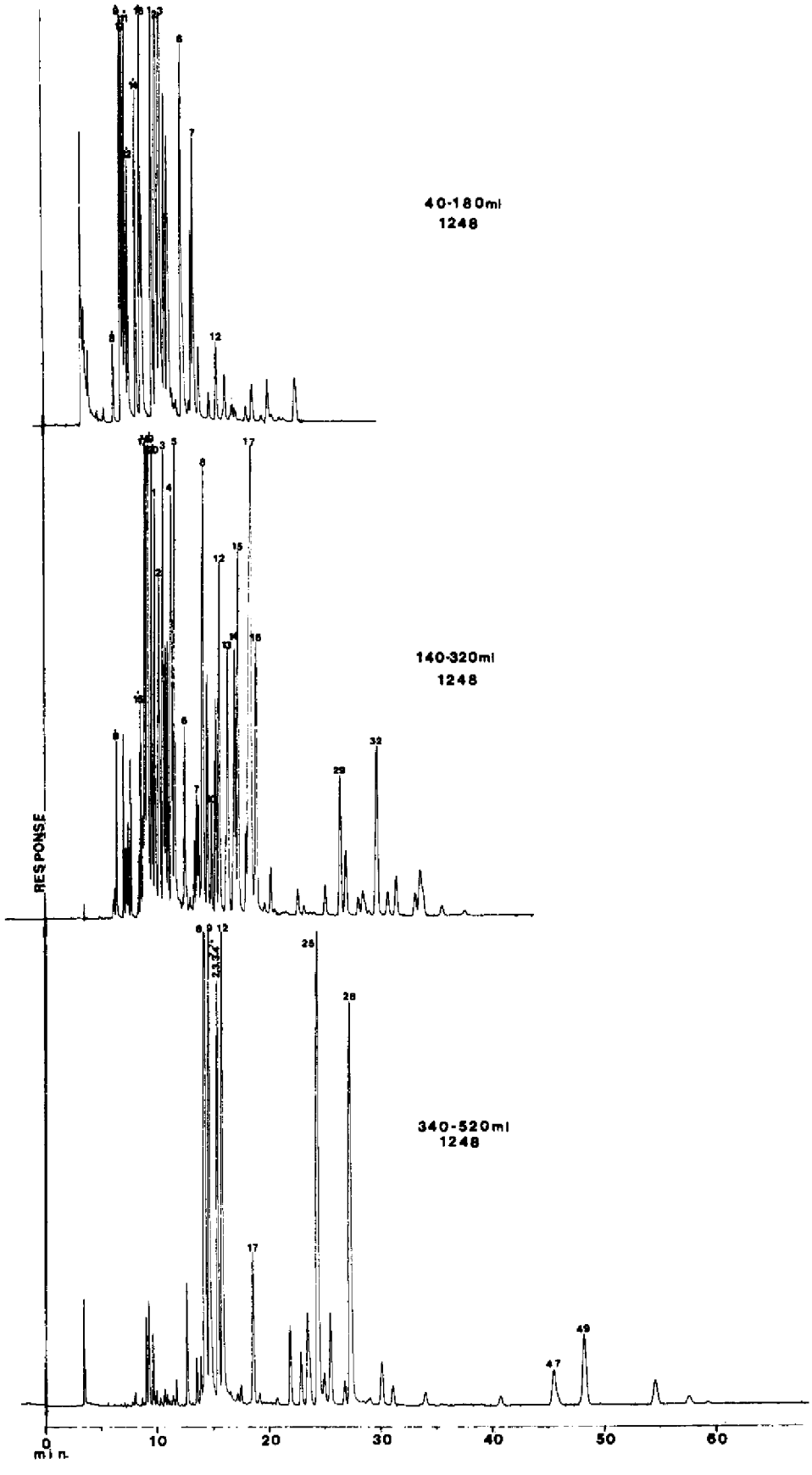
¶One fish sampled.

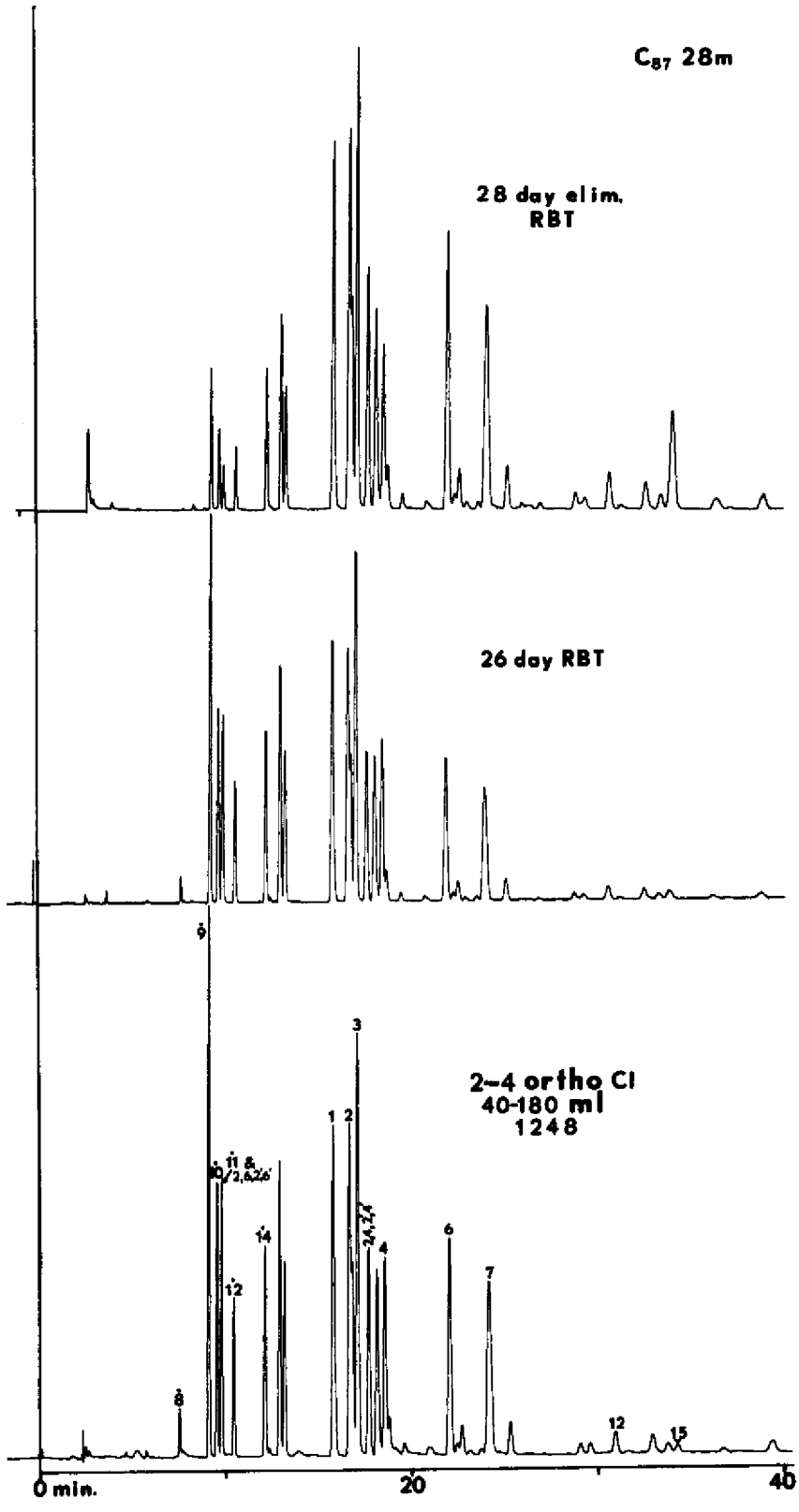
Results and Discussion

Goldstein[1] and Poland[2] have indicated that adverse physiologic activity associated with technical PCB mixtures may be due to the presence of small quantities of non-*o,o'*-Cl-substituted PCBs. The hazards posed to aquatic organisms by the presence of trace levels of toxic chlorinated dibenzofurans in PCBs[12,13] are still unclear.

The major objective of this study was to determine if differences in PCB toxicity relate to *o,o'*-Cl substitution. Assignment of Aroclor toxicity to the various PCB classes of *o,o'*-Cl substitution necessitated that structurally related subgroups be available for testing. In addition, an effective means was required to minimize any influence of toxic PCB impurities such as chlorinated dibenzofurans. Preparative carbon column chromatography as described in the method section was used to

FIGURE 4. HRGC of 2–4 *o,o'*-Cl, 1 and 2 *o,o'*-Cl, and 1 *o,o'*-Cl carbon fractions. Capillary column was 28m Apiezon-L and column temperature was set at 240°C. See TABLE 1 for identities of numbered peaks.


40-180ml
1248
140-320ml
1248
340-520ml
1248
RESPONSE
0
min.
10
20
30
40
50
60


C_{87} 28m
28 day elim.
RBT
26 day RBT
2-4 ortho Cl
40-180 ml
1248
0 min.
20
40

provide 1–4 *o,o'*-Cl fractions for toxicity testing. The 1–4 *o,o'*-Cl PCB fractions were assumed to be free of chlorinated dibenzofurans because of the large volumes of toluene required for the elution of these planar compounds from carbon columns (Figure 2).

We found that PCB components are more strongly retained by PX-21 carbon as: (1) substitution of the *o,o'* position decreases, and (2) total Cl substitution increases.[4] Elution volume of non-*o,o'*-Cl-substituted PCB components examined (3,4,4'-trichlorobiphenyl, 3,4,3',4'-TCB, and 3,4,5,3',4',5'-hexachlorobiphenyl) increased with their degree of chlorination (Figure 2). The presence of a 1 *o,o'*-Cl substituted PCB (2,4'dichlorobiphenyl) in the fraction containing the 2–4 *o,o'*-Cl substituted PCBs illustrates the decreased interaction of carbon with less chlorinated components (TABLE 1).

HR Apiezon-L and C_{87} glass capillary GC was used to characterize 1–4 *o,o'*-Cl fractions (TABLE 1 & FIGURES 4 & 5). PCB components without *o,o'*-Cl substitution and those with 4 *o,o'*-Cl substitution represented $<1.0\%$ (w/w) of the PCBs present in Aroclor 1248. Earlier, we quantitated the non-*o,o'*-Cl substituted PCBs present in several Aroclor mixtures.[4] We found that Aroclor 1248 contained the largest amount of non-*o,o'*-Cl-substituted PCBs with 4 or more Cl atoms. The major non-*o,o'*-Cl isomer (3,4,3',4'-TCB) represented 0.22% (w/w) of the PCBs in Aroclor 1248.

We exposed rainbow trout to several concentrations of each Aroclor 1248 fraction (TABLE 1), and analytic grade non-*o,o'*-Cl-substituted 3,4,3'4'-TCB. Residue data from whole body fish samples are summarized in TABLES 2 and 3. Unfortunately, a malfunction of the diluter system used for the 1 *o,o'*-Cl exposure (TABLE 2) resulted in a temporary rise in the water concentration of toxicant and may have significantly increased residues in fish sampled during the experiment. The half-life of total PCB residues in fish exposed to the various concentrations of the four PCB fractions did not differ greatly, and ranged from 7 to 14 days. However, comparison of the PCBs accumulated in fish to the components in the original fractions revealed major differences in the elimination rates of individual PCBs in the 1 and 2 *o,o'*-Cl, and 2-4 *o,o'*-Cl fractions (FIGURE 5). Elimination rates of chlorobiphenyls appeared to relate directly to the degree of chlorination and number and ring position of vicinal unsubstituted carbon atoms.

No major difference in toxicity was observed in rainbow trout exposed to similar concentrations of 1–4 *o,o'*-Cl fractions of Aroclor 1248. Fish exposed to less than 8 $\mu g/l$ were not visibly affected (TABLE 4). However, exposure to all concentrations (0.1–1.4 $\mu g/l$) of the non-*o,o'*-Cl-substituted 3,4,3',4'-TCB resulted in poor growth, reduced food consumption, and disorientation in test fish. Mortalities were not observed until 28 days following cessation of the 1.4 $\mu g/l$ 3,4,3',4'-TCB exposure. Rainbow trout used for the 3,4,3',4'-TCB study had an average weight of 0.6 g at the start of the dilutor exposure. After 50 days fish maintained in untreated water weighed an average of 3.1 g. However, fish exposed to 0.1 and 1.4 $\mu g/l$ of 3,4,3',4'-TCB for 50 days weighted an average of 2.2 and 1.2 g respectively. Fish sampled during the 3,4,3',4'-TCB study were pooled for residue analysis and each of the above average weights represented 5 to 14 individuals.

The recent detection of 3,4,3',4'-TCB in a composite fish sample (33% largemouth bass and 67% goldfish, w/w) from the Hudson River[4] underscores the need for

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FIGURE 5. Bottom chromatogram, C_{87} HRGC of 2–4 *o,o'*-Cl PCB components from carbon fractionation of Aroclor 1248. *Middle chromatogram*, extract from 26-day exposure of rainbow trout (RBT) to same PCB components. *Top chromatogram*, extract of rainbow trout exposed to same PCBs for 26 days and then maintained in fresh water for 28 days. HRGC conditions similar in all chromatograms; see text.

TABLE 4

INCIDENCE OF ADVERSE EFFECTS (AE) FROM FLOW-THROUGH EXPOSURES OF RAINBOW TROUT* TO AROCLOR® 1248 COMPONENTS

Toxicant (# of *o,o'*-Cl)	Mean H_2O Concentration ($\mu g/l$)	Days of Exposure		Days Elimination
		29	50	28
3,4,3',4' (0 *o,o'*-Cl)	0.1		AE†	AE
	0.4		AE	AE
	1.4		AE	AE (25%m)‡
(1 *o,o'*-Cl)§	0.75	NE¶		NE
	3.8	NE		NE
	7.8	NE		NE
(1&2 *o,o'*-Cl)§	2.0	NE		NE
	4.5	NE		NE
	11.4	AE (16%m)		NE
(2–4 *o,o'*-Cl)§	2.4	NE		NE
	3.9	NE		NE
	16.0	AE (25%m)		NE

*Control fish appeared to be healthy during the studies and no mortalities were observed.

†Adverse effects consisted of poor growth, reduced food consumption and disorientation; these effects were first observed at day 16 in the 0.4 and 1.4 $\mu g/l$ 3,4,3',4' concentrations.

‡Percent mortalities of beginning population.

§See TABLE 1 for identify of 1248 components in these fractions.

¶No effect observed.

additional research on non-*o,o'*-Cl-substituted PCBs. Routine detection of non-*o,o'*-Cl-substituted PCBs, chlorinated dibenzofurans, and dioxins in environmental extracts containing technical PCB mixtures will require delineation of their behavior through current multiresidue analytic procedures and the use of carbon or carbon/foam chromatography. Carbon chromatography using PX-21 will permit fractionation of chlorinated aromatic mixtures into structurally related subgroups for future structure/toxicity studies. Correlation of environmental residues of non-*o,o'*-Cl-substituted PCBs and planar contaminants, such as chlorinated dibenzofurans and dioxins, to similar concentrations in laboratory studies should provide a better perspective on their environmental impact.

ACKNOWLEDGMENTS

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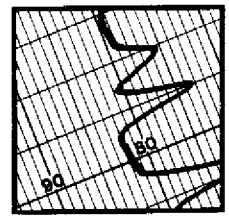
Column Chromatographic Method for Cleaning up Extracts from Biological Material and Simultaneous Separation of PCBs and DDE

J. J. Kveseth and E. M. Brevik



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Column Chromatographic Method for Cleaning Up Extracts from Biological Material and Simultaneous Separation of PCBs and DDE

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Gas chromatographic determination of organochlorine insecticides in biological material is often complicated because of the simultaneous presence of polychlorinated biphenyls (PCBs). A separation is therefore advantageous. Several column chromatographic procedures are described in the literature (REYNOLD 1969, HOLDEN and MARSDEN 1969, ARMOUR and BURKE 1970, SNYDER and REINERT 1971, BERG et al.1972, ERNEY 1974).

In order to establish such a column chromatographic separation procedure at our department, we used as a base those methods which had been reported to be successful in separation of PCBs and DDE. Our duplication efforts did not prove to be very satisfactory. For one reason the fine grained silica gel types used gave slow elution and therefore the large quantities of eluting agent necessary led to a prolonged eluting time. We therefore chose a coarser grained silica gel. In addition to a separation procedure, we also wanted a simultaneous clean-up of extracts of biological material. Such a combination column is described in this report.

MATERIALS AND METHODS

Reagents

a) Adsorbents: Silica gel, Woelm 0.063-0.2 mm or 0.05-0.2 mm for column chromatography (M.Woelm,Eschwege, W.Germany). Kiesel gel 60 for column chromatography, grain size 0.063-0.125 mm (Art.9386,Merck), Silicic acid, 100 mesh, AR (Mallinckrodt No. 2847).

These reagents were Soxhlet extracted with n-hexane for 4 hr and activated at 130°C for at least 24 hr. Thereafter they were transferred to a flask with a tightly fitting glass stopper and placed in a desiccator. After cooling, known quantities of water were added in some cases and the flask, shaken thoroughly for an hour, was then stored in a desiccator.

Aluminum oxide 90 active neutral, for column chromatography (Art.1077 Merck). It was Soxhlet extracted with n-hexane 4 hr and activated at 800°C for at least 8 hr. After cooling, the aluminum oxide was deactivated with 5% distilled water and thereafter

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treated in the same way as the silica gel.

b) Solvents: n-hexane Art.9688 Merck, petroleum ether boiling point 40-60°C, Art.909 Merck, diethyl ether supplied by A/S Den norske Eterfabrikk. The solvents were redistilled in glass and tested before use by concentrating 50 fold before injection into the gas chromatograph.

c) Standards: p,p'-DDE, p,p'-DDD, p,p'-DDT and lindane, purchased from Analytical Standards, Sweden. PCBs, Clophen A50, from Farbenfabriken Bayer Aktiengesellschaft Leverkusen.

Apparatus

a) Gas chromatograph: Varian 1700 with electroncapture detector(^{3}H). Columns: 150 cm x 2 mm ID glass columns, one packed with 10% QF-1 and one with 4% SF-96 on Chromosorb W, 100-120 mesh. Operating conditions: Nitrogen 30 ml/min, injector column and detector temperatures were 190°, 180°, and 200°C, respectively.

b) Clean-up/separation column: Glass column with inner diameter of 0.8 cm, with teflon stopcock and a 50 ml reservoir at the top.

Experimental procedure

A hexane-washed cotton wool plug was first placed into the glass column, which was then filled with hexane and packed with 2 to 6 g of the described silica gel types. Up to 1 ml of a standard solution of Clophen A 50 (4000 ng/ml), lindane (50 ng/ml), DDE (200 ng/ml), DDD (500 ng/ml), and DDT (800 ng/ml) were introduced into the column. Elution was carried out either with hexane or petroleum ether, and separation was obtained by after a predetermined volume adding 10% diethyl ether. Elution rate was regulated to about 1.5 ml/min. The course of elution as regards the individual components was determined by fractionating the eluate and analysing each fraction by gas chromatography. Thereafter a combination column was made which in addition to the most suitable silica gel type was packed with 2 g of aluminum oxide at the top. An elution trial was carried out with a sample from the same standard mixture. The same components were thereafter added to uncontaminated cod liver oil and trial solutions with 20-60 mg of this cod liver oil were introduced into the column in order to simultaneously test separation and clean-up capacity. Finally, samples of extracts of biological material were introduced into the column. Evaporation of small volumes of solvent was carried out over a water bath at 40°C, and in a stream of dry air, whilst large volumes were evaporated using a rotary evaporator and a round flask to which was welded a 5 cm long glass tube with a diameter of 0.5 cm and marked with divisions at 0.5 and 1 ml.

RESULTS AND DISCUSSION

Separation trials were carried out using the three described silica gel types with the standard mixture. With 3-10 g kiesel gel 60 or silicic acid slow elution and unsatisfactory separation resulted, especially of PCBs and DDE which were the most difficult to separate. By using 4 g silica gel with a grain size of 0.063-0.2 mm or 0.05-0.2 mm, it was possible to separate PCBs and DDE. The separation efficiency of this column was dependent to a marked extent on the degree of activation of the silica gel. Deactivation with 0-0.5% water gave satisfactory separation, but already with 1.5% water added to the silica gel, PCBs and DDE were eluted partially together as shown in Fig.1. Hexane and petroleum ether were found to be equally effective as eluting agents. To simplify matters, only hexane is mentioned in the text, henceforward.

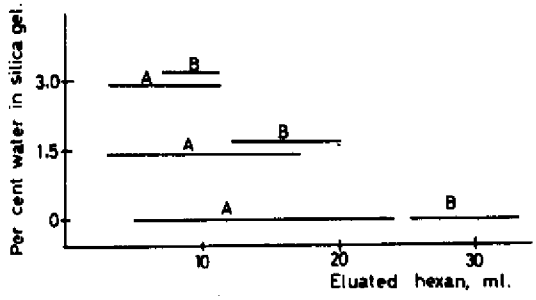

Fig.1. Effect of silica gel water content on elution and separation of Clophen A 50 (A) and p,p'-DDE (B). 1-5 ml fractions of hexane eluate analyzed.

In order to achieve a simultaneous separation and clean-up of extracts from biological material, the column was packed with silica gel and aluminum oxcide as mentioned previously. This did not change the elution process as far as the mentioned components were concerned. PCBs (Clophen A 50) was eluted with around 24 ml hexane, whilst DDE as the first of the remaining components began to come around 25 ml eluted hexane (Fig.2). Every new batch should be checked for separation ability, as small changes in eluating volumes have been observed between batches.

The aluminum oxide/silica gel column was tested with samples of biological material to investigate repeatability and recovery. Fourteen samples of 20 mg of old uncontaminated cod liver oil were fortified with the standard solution and introduced into the column. The elution volume for PCBs (Clophen A 50) did not exceed 24 ml hexane, called fraction A, whilst DDT, DDD, DDE and lindane were thereafter eluted with 20 ml hexane containing 10% diethyl ether, fraction B. The

results of the recovery experiments are shown in Table I.

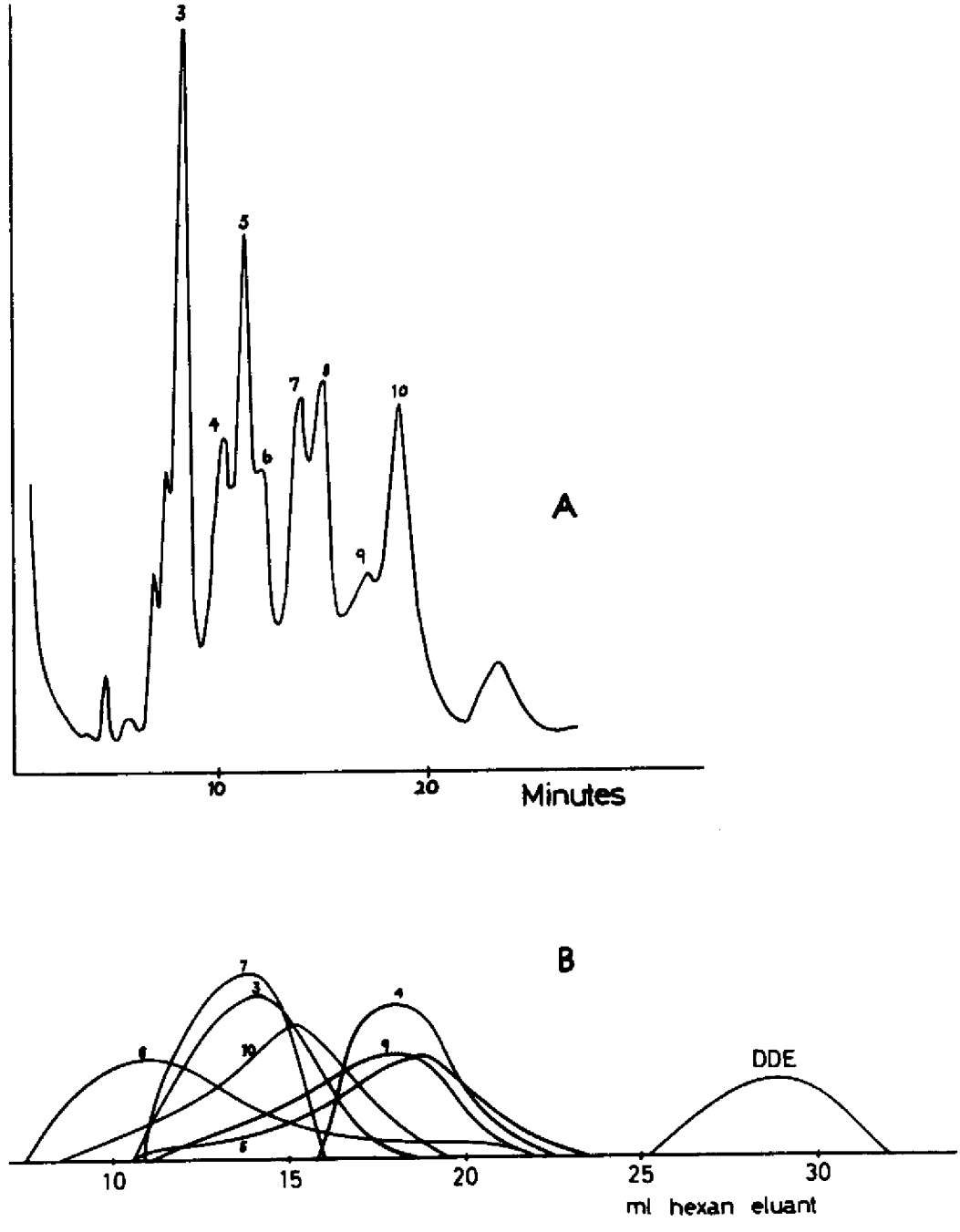

Fig.2. Gas chromatogram of Clophen A 50 (A), and hexane elution pattern of Clophen A 50 and p,p'-DDE (B) from a silica gel-aluminum oxide column.

The separation efficiency of the column proved to be dependent on the concentration of the organochlorines in the sample. For example, good separation was achieved with 1 µg/ml PCBs and 0.5 µg/ml DDE, whilst a ten-fold increase in concentration led to 4-5% of the total amount of PCBs being eluted together with 5-6% of the total DDE. In cases where biological material has residues of organochlorines exceeding these concentration levels, regard should be given to this by, for example, diluting the lipid extract of the sample in order to achieve optimal conditions for separation.

TABLE I

Recovery experiments of PCBs, lindane, DDT and metabolites in fortified cod liver oil samples, by use of deactivated aluminum oxide and silica gel column chromatography.

Fraction	Test substance	Concentration of test substance, µg/ml	Percentage recovery of 14 parallels Mean	Range
A hexane	PCB	0.8 - 8	99	(89 - 112)
B 20 ml hexane and 10% diethyl ether	Lindane	0.01 - 0.1	93	(87 - 112)
	DDE	0.04 - 0.4	93	(80 - 104)
	DDD	0.1 - 1.0	97	(87 - 114)
	DDT	1.6 - 16	97	(83 - 116)

The amount of fat introduced into the column was varied from 20 to 60 mg without this having any demonstrable effect on the separation of PCBs and DDE.

The method was used in connection with the analysis of contaminated seabird egg material and cod liver oil. 20 mg of extracted fat dissolved in hexane was introduced into the column. A better separation of PCBs and DDE was found with this material than in the described experiments in which pure standards were used. Fig.3 shows a gas chromatogram of a seabird egg with 88 ppm PCBs and 33 ppm DDE calculated on fat basis before and after separation. This seems to indicate that the degree of chlorination of PCBs influences the column's separation characteristics. This supposition is supported by MASUMOTO (1975) who, using a column packed with silicic acid, found that of the analysed PCB compounds, only the most chlorinated, Arochlor 1260, was satisfactorily separated from DDE.

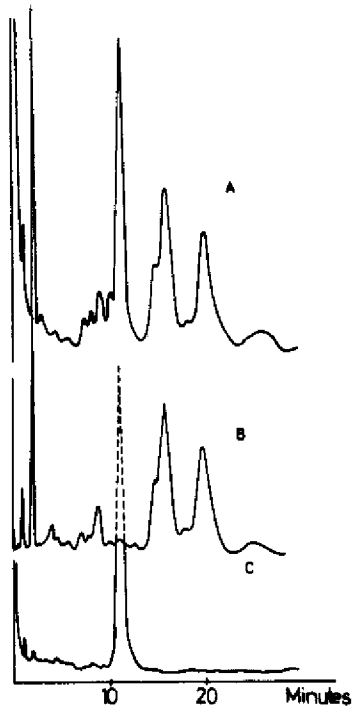

Fig.3. Chromatograms of a seabird egg extract containing residues of PCBs and p,p'-DDE before and after separation on silica gel-aluminum oxide column. A: the extract treated with sulfuric acid, B: hexane eluate and C: hexane with 10% diethyl ether eluate from the column.

The method described for cleaning up extracts of biological material and simultaneous separations of PCBs and DDE is relatively rapid and requires comparatively small amounts of chemicals. The silica gel seemed to be stable during prolonged storage in a desiccator, in that it was used up to one month after activation without any differences in its characteristics being observed.

If the method's premises regarding the amount of fat and concentration of organochlorines are fulfilled, the method should be very suitable for use in connection with gas chromatographic analysis of biological material with regard to organochlorine insecticides and PCBs.

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Preparative Method for Gas Chromatographic/Mass Spectral Analysis of Trace Quantities of Pesticides in Fish Tissues

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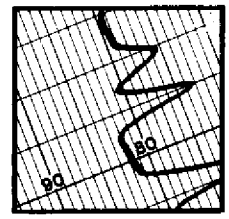

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Preparative Method for Gas Chromatographic/Mass Spectral Analysis of Trace Quantities of Pesticides in Fish Tissue

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A large capacity, low efficiency pesticide cleanup method is combined with a small capacity, high efficiency method for gas chromatographic/mass spectral analysis of fish tissue. Trace chemicals are extracted from the tissue, removed from the bulk of the co-extracted lipids through selective desorption from Micro Cel-E, and isolated from the remaining lipids by gel permeation chromatography. The procedure is capable of cleaning up the extract from several hundred grams of fish, minimizes sample contamination, and permits identification of xenobiotic chemicals present at ng/g concentrations.

Although the development of computerized gas chromatographic/mass spectral (GC/MS) techniques has been a major instrumental advancement in the identification of trace xenobiotic chemicals in biological tissues, the methodology for preparing samples for multiresidue analysis by GC/MS has been largely restricted to established analytical-scale techniques. The unequivocal identification of potentially hazardous chemicals at concentrations below 0.05 μg/g tissue dictates that the sensitivity of instrumentation must be further increased, and more efficient methods must be developed to provide greater enrichment of the trace chemicals.

Efforts to increase the sensitivity of GC/MS analyses have centered around limited mass measurements or mass fragmentography (1, 2) where the intensity of selected ions is monitored continuously rather than acquiring entire spectra. Although mass fragmentography greatly increases the sensitivity, the technique also increases the uncertainty of identification, particularly in samples containing much co-extracted material.

For determinations of chemicals such as chlorodioxins, which are likely to be present at concentrations <1 ng/g, Baughman and Meselson (3) have shown that the specificity of limited-mass mass spectral analyses can be retained by working at greater than 10,000 resolution. This eliminates the possible interference by DDE, DDD, DDT, and polychlorinated biphenyls (PCBs) in the dioxin analyses. However, signal-averaging techniques and the use of direct probe MS rather than GC/MS were needed to achieve the necessary sensitivity.

Where the sensitivity of existing GC/MS facilities are optimal, efforts to improve the detection limits have generally centered around scaling up analytical cleanup techniques. For example, Rogers (4) found that Micro Cel-E had 490 weight per cent capacity for oils because of the 200 sq m/g surface area, and he developed a batch technique to extract pesticides from 30 g lipids impregnated on Micro Cel-E. Florisil was used similarly by Porter and Burke (5), although substantially less lipid per g Florisil could be sampled. Moreover, the isolated pesticides from these techniques require further cleanup of the extract on Florisil, and the final concentrated extract is acceptable only for electron capture gas-liquid chromatographic (GLC) analyses where the detector is specific for chlorinated chemicals. These techniques are not efficient enough for GC/MS analyses because of the lipid interferences.

Stalling *et al.* (6) developed a gel permeation chromatographic technique for the efficient removal of pesticides from lipids based on differences in molecular volume. Although the pesticides can be isolated within 1 hr, the capacity of the gel permeation column is generally <500

mg lipids, and the cleanup of large quantities of lipids by repeated injections becomes time consuming. Moreover, each 500 mg lipid sample is diluted by 100 ml solvent which must be removed before analysis, and the solvent impurities may increase the GC/MS background and interfere with the analysis (7). Therefore, rapid semipreparative cleanup techniques are needed which are capable of isolating trace chemicals from large quantities of tissue for GC/MS analyses with a minimum introduction of impurities. This communication presents an evaluation of one such method which combines the advantages of the techniques reported by Rogers (4) and Stalling *et al.* (6).

METHOD

Reagents and Apparatus

All solvents are pesticide quality and should be redistilled before use.

(a) *Sodium sulfate.*—Anhydrous. Extract 36 hr with hexane-acetone (59+41), oven-dry to remove moisture and possible trace organic impurities, and store in Teflon-capped glass bottles.

(b) *Micro Cel-E.*—(Johns-Manville Products Corp., Celite Division, Lampoc, CA). Prepare similarly to Na_2SO_4.

(c) *Bio-Beads SX-2.*—200–400 mesh (Bio-Rad Laboratories, Richmond, CA).

(d) *Gel permeation chromatographic unit.*—Manual or semiautomatic (Analytical Biochemistry Laboratories, Inc., Columbia, MO).

(e) *Ultraviolet (UV) liquid chromatographic detector.*—Luv Monitor (Laboratory Data Control, Riviera Beach, FL).

(f) *Gas chromatograph.*—2 m × 2 mm id column packed with OV-17–SP 2401 (1.9 + 1.5%) on 80–100 mesh acid-washed Supelco operated at 180°C with ^{63}Ni electron capture detector.

(g) *Gas chromatograph-mass spectrometer.*—Varian MAT CH5 mass spectrometer interfaced with Varian 1700 gas chromatograph and Varian Spectro System 100 data system.

Procedure

Homogenize whole fish while frozen and dry 100–400 g aliquots by mixing with 3 g Na_2SO_4/g tissue. Extract mixture overnight with hexane-acetone (59+41) in large Soxhlet extractor. Remove solvent from extract in Kuderna-Danish apparatus with 3-ball Synder column.

Thoroughly mix 30 g extracted lipids with 15 g prepurified Micro Cel-E. Separate pesticides and related materials from bulk of lipid on Micro Cel-E by procedure of Rogers (4), except omit Celite and Whatman No. 589 filter paper to reduce possible contamination of extract as discussed by Lamont and Cromartie (7). Dry the resulting 100 ml petroleum ether extract containing trace organic chemicals over anhydrous Na_2SO_4, and repeat procedure until necessary mass of trace materials is isolated.

Concentrate combined extract from Micro Cel-E enrichment step to 10 ml and dilute with redistilled cyclohexane to 100 mg lipid/ml. Place 5 ml aliquots of extract sequentially on 25 × 230 mm column of SX-2 Bio-Beads and elute with redistilled cyclohexane at 3.5 ml/min. Collect pesticides and related chemicals from column by monitoring eluant with either UV detector or automatic collection device which has been calibrated to isolate lower molecular weight components on column (8). In general, composite Micro Cell-E extract requires only 2 or 3 "batch" separations on gel permeation column, eliminating need for more expensive automatic gel permeation units and excessive solvents. Concentrate to ca 100 µl in Concentratubes (Laboratory Research Co., Los Angeles, CA) for GC/MS analysis.

Results

Figure 1a is a gel permeation chromatogram of a typical fish extract as monitored with a UV detector. The large peak eluting with the first 100 ml contains the lipids; the small shoulder eluting in 100–200 ml solvent contains the pesticides. After preliminary cleanup on Micro Cel-E, the pesticides are substantially enriched with respect to the lipids (Fig. 1b). When the pesticide fraction is collected as indicated in Fig. 1b and re-injected onto the gel column, the chromatogram in Fig. 1c results. Finally, combining several fractions from the gel column yields the chromatogram in Fig. 1d in which the trace components are greatly enriched.

Rogers (4) has shown that the acetone-acetonitrile extraction of lipids which are impregnated onto Micro Cel-E removes more than 85% of all chlorinated pesticides studied. Stalling *et al.* (6) and Tindle and Stalling (8) have demonstrated that lindane, heptachlor, *cis-* and *trans-*chlordane, dieldrin, endrin, DDT, and DDE are eluted with greater than 95% recovery from the gel permeation cleanup. Although the use of these 2 techniques in a semipreparative manner as outlined above provides optimal isolation

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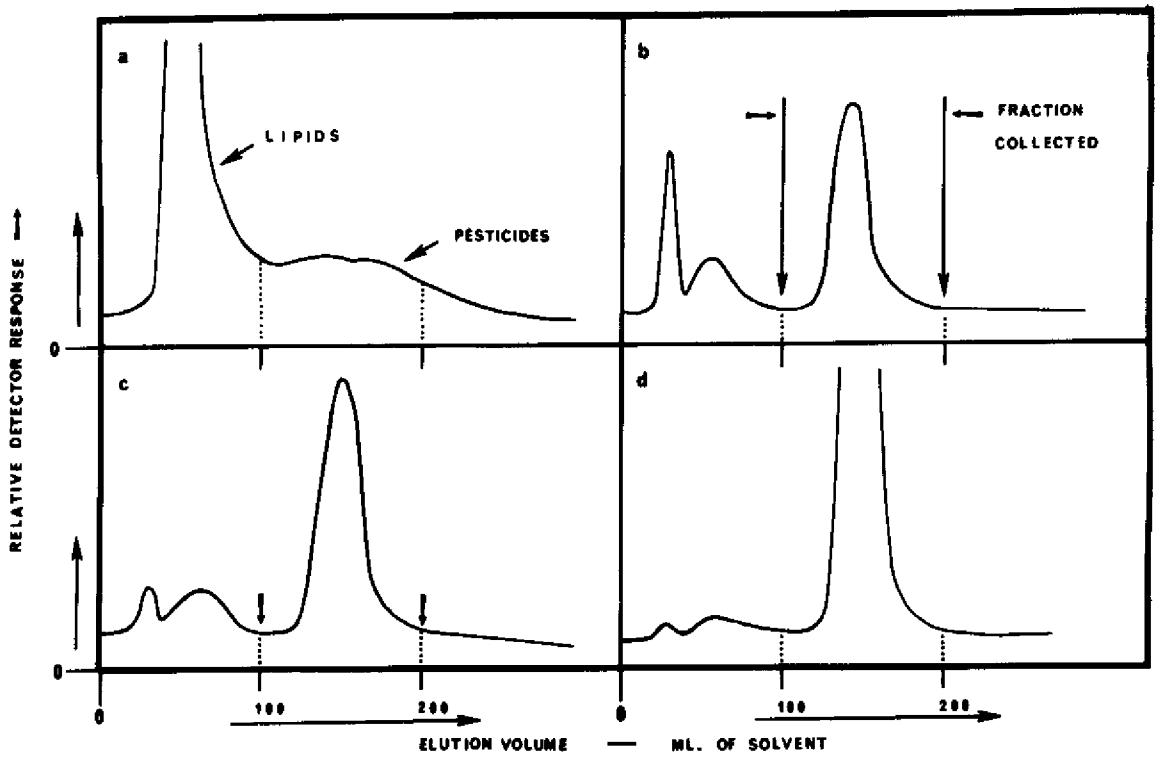

FIG. 1—Gel permeation chromatograms of pesticides in fish extracts after a, extraction; b, Micro Cel-E cleanup; c, gel permeation cleanup; d, final gel permeation cleanup.

of ultra-trace chemicals for qualitative rather than quantitative analyses, all chlorinated pesticides, chlorinated industrial chemicals, and carbamates such as carbaryl which have been tested are isolated with approximately 70% recovery, depending on the volume of solvent collected from the gel permeation column. Moreover, mixtures of chemicals such as PCBs, toxaphene, technical chlordane, and chlorinated paraffins elute in the pesticide fraction (Fig. 1b).

Even though the collection of fractions from the gel column largely determines the recovery, the ability to examine 300–500 g tissue greatly expands the capability of exploration studies of ultra-trace chemicals. In a study of coho salmon from Lake Michigan, chemicals such as hexachlorobenzene, chlordane, and dieldrin which are present at less than 0.05-0.10 μg/g in the tissue are sufficient to produce spectra in subsequent GC/MS analyses in which the intensity of the parent or predominant ion clusters is greater than 50% of the working range of the computer. For example, Fig. 2a is a spectrum of dieldrin obtained from Lake Michigan fish in which the predominant ions in the regions of m/e 260–280, 340–350, and 375–385 are clearly evident and, together with GLC retention time, give an explicit confirmation. Figure 2b is a spectrum of a dieldrin reference to illustrate the similarities between the 2 spectra.

The usefulness of isolating larger quantities of trace chemicals is also demonstrated in the examination of the component in Lake Michigan fish which is eluted by 15% ether from Florisil, is resistant to saponification, and is eluted from a methyl silicone GLC column with a retention time of approximately 1.25 relative to aldrin. This component has been assumed to be heptachlor epoxide (9–11), but explicit confirmations have not been reported because of the inability to isolate sufficient quantities of the component. A mass spectrum (without background subtraction) of the component (Fig. 3) shows that the component is not heptachlor epoxide, and no evidence was found for the predominant heptachlor epoxide fragments at m/e 351–359. Rather, Fig. 3 has been identified as the spectrum of dichlorobenzophenone with the M^+ at m/e = 250

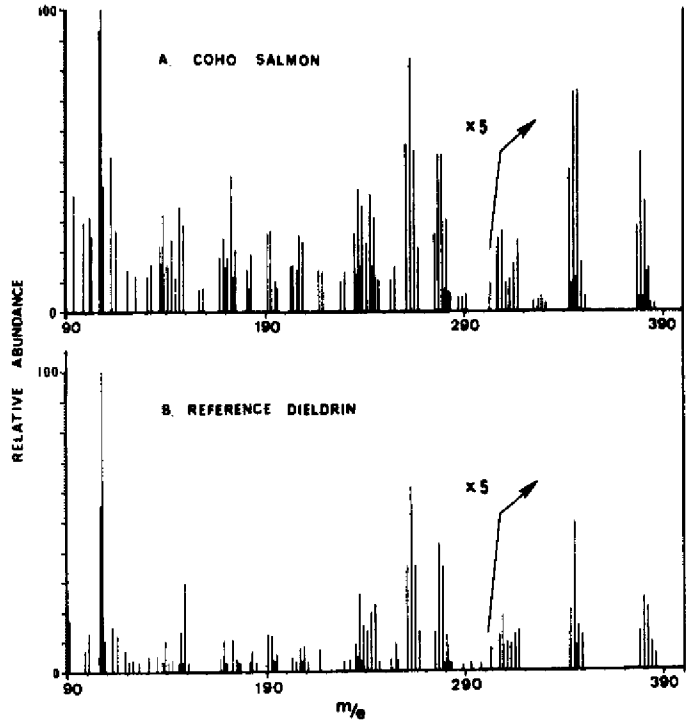

FIG. 2—Mass spectrum of A, dieldrin obtained from Lake Michigan fish containing 0.1 μg dieldrin/g via GLC, and B, dieldrin reference.

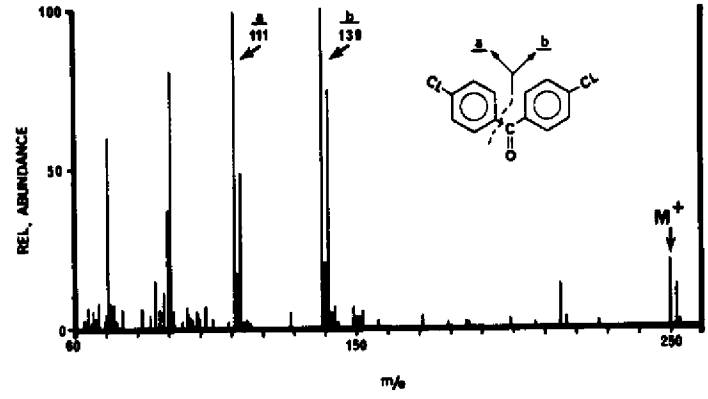

FIG. 3—Mass spectrum of the "apparent heptachlor epoxide" component in Lake Michigan fish—confirmed as dichlorobenzophenone.

and the major chlorinated fragments at m/e = 111 and 139. The dichlorobenzophenone may arise from the metabolism of DDT (12) or DDA (13) and, in part, from the decomposition of the DDT metabolite bis(*p*-chlorophenyl)-2,2,2-trichloroethanol in the GC/MS injector (14, 15).

Summary

An evaluation of cleanup methodology has shown that present methods are not adequate for GC/MS analyses of residues at ng/g concentrations. Methods with a large sample capacity do not remove the interferences from lipids, and those which efficiently isolate the

trace chemicals have such small sample capacity that the sample size is restricted. The use of Micro Cel-E in conjunction with gel permeation chromatography permits readily interpretable mass spectra to be obtained from chemicals present at ng/g concentrations. The procedure eliminates the time-consuming repetitive operations and minimizes the use of solvents. Approximately 300 g tissue can be cleaned up by this procedure in a normal work day.

Acknowledgment

We wish to thank the Johns-Manville Products Corp., Celite Division, which provided the Micro Cel-E.

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Residues of Organochlorine Insecticides and Polychlorinated Biphenyls in Fish from Lakes Huron and Superior, Canada, 1968-76

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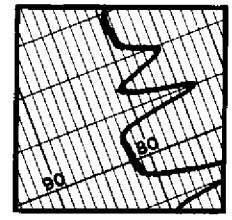

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Residues of Organochlorine Insecticides and Polychlorinated Biphenyls in Fish from Lakes Huron and Superior, Canada—1968–76 [1]

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Douglas P. Dodge,[3] and George E. Sprangler[4]

ABSTRACT

Five species of fish from Lake Superior and 12 species from Lake Huron were analyzed for organochlorine pesticides and polychlorinated biphenyls (PCBs) between 1968 and 1975. Mean residues of ΣDDT peaked at 1.72 ppm and 7.60 ppm in lake trout (Salvelinus namaycush) *from Lakes Superior and Huron, respectively. By 1975, the mean level of ΣDDT had decreased in lake trout and was highest in bloaters* (Coregonus hoyi) *from both lakes: 1.06 ppm and 1.87 ppm, respectively. Dieldrin levels in fish from Lake Superior changed little over the same period. However, in 1969–70, dieldrin levels in fish from Lake Huron exceeded the 0.3 ppm tolerance level set by Health and Welfare Canada or the Food and Drug Administration, U.S. Department of Health, Education, and Welfare in 5 percent of lake whitefish* (Coregonus clupeaformis) *and 10 percent of bloaters. By 1975, 50 percent of bloaters caught in Georgian Bay and North Channel had dieldrin levels above 0.3 ppm. PCB residues declined in lake trout and lake whitefish caught in Lake Superior between 1971 and 1975, but increased slightly in bloaters and white sucker* (Catostomus commersoni). *Mean PCB residues in bloaters caught in Lake Huron in 1969–71 and 1975–76, and splake* (Salvelinus fontinalis *and* S. namaycush) *and cisco* (Coregonus artedii) *caught in 1975 exceeded the 2 ppm tolerance level.*

Introduction

The Great Lakes are surrounded by land that is highly developed for urban, industrial, agricultural, and recreational activities. Since outflow of the Great Lakes is limited, chemical discharges into the lakes are very persistent. For the past decade organochlorines have been identified as a serious contaminant in fish, resulting in long-range detrimental effects to private and commercial fishing.

Organochlorine insecticides and polychlorinated biphenyls (PCBs) have been identified in fish caught in Lakes Huron and Superior. Reinhert reported residues of 0.2–7.4 ppm ΣDDT and 0.01–0.05 ppm dieldrin in several species of fish caught in Lake Superior in 1967–68 *(7)*. Reinke et al. reported that two fish species caught in 1970 from the same lake had mean residues of 0.2 ppm and 1.3 ppm ΣDDT and 0.06 ppm dieldrin *(9)*. Four species, also caught in Lake Superior in 1974–75, cited by the Upper Great Lakes Reference Group, contained mean residues of 0.2–4.4 ppm ΣDDT and 0.01–0.15 ppm dieldrin *(11)*. Residues of chlordane, lindane, and PCBs were also reported in these four species

Reinhert found mean residues of 0.8–6.9 ppm ΣDDT and 0.02–0.11 ppm dieldrin in nine species of fish from Lake Huron in 1967–68 *(7)*. Reinke et al. reported mean residues of 0.5–16.4 ppm ΣDDT and 0.01–0.31 ppm dieldrin in the same major fish species in Lake Huron in 1970 *(9)*. The Upper Great Lakes Reference Group cited considerably lower residues of ΣDDT in three fish species caught in 1974–75 *(11)*, but levels of dieldrin, lindane, chlordane, and PCBs were similar to those found in other studies.

Studies on the distribution of organochlorines in water, sediment, and seston in Lakes Superior and Huron reveal that these compounds are widespread in the Great Lakes ecosystem *(3)*. Miles and Harris reported that the Muskoka River discharged large amounts of ΣDDT to Georgian Bay *(6)*. Peak discharges of 5.4 kg/week occurred in May 1971, but the quantity declined rapidly from May to October, averaging 0.9 kg ΣDDT/week. Frank et al. found that fish in the Muskoka Lake–Muskoka River system contained some of the highest residue levels found in fish from inland lakes of Ontario *(2)*. Fourteen species had mean residues of 0.22–22.4 ppm ΣDDT; sediments in this lake–river system contained ΣDDT residues as high as 2.9 ppm.

The present study, begun in 1968, was originally intended to identify and measure organochlorine residue.

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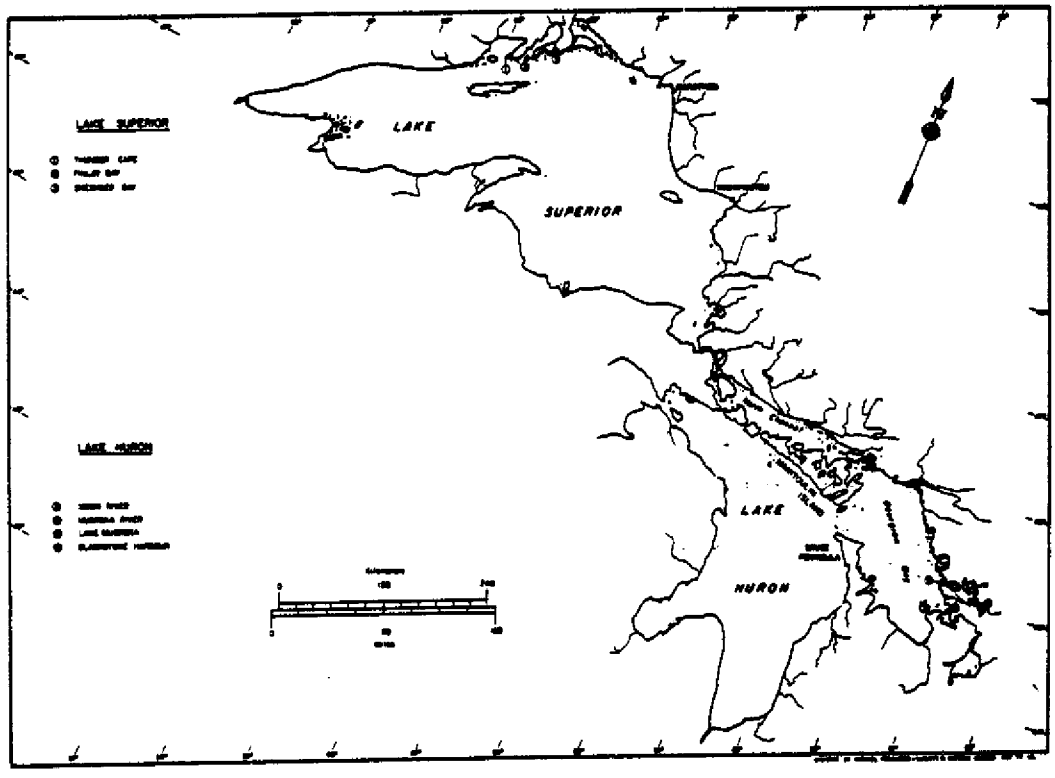

FIGURE 1. *Map of Lakes Huron and Superior showing fish collection areas*

in fish from the Great Lakes. However, it was broadened following restrictions on the use of aldrin, dieldrin, and heptachlor in Canada in 1969, DDT in 1970, and the voluntary restrictions on the use of PCBs in 1971 within the Province of Ontario. Authors wished to determine whether these use restrictions were significantly reflected in organochlorine residues in fish from Lakes Huron and Superior.

Methods and Materials

FIELD COLLECTION

Fifteen species (843 fish) were caught by net, line, or trap between 1968 and 1976 from Lakes Huron and Superior; many of the larger fish were obtained from commercial catches. Five species (115 fish) were caught in the Canadian waters of eastern Lake Superior between Michipicoten and the entrance to the North Channel (Figure 1). Between 1968 and 1976, 14 species (728 fish) were caught in Lake Huron. Of these, 481 fish of 12 species were from the Canadian waters of Lake Huron, 142 fish of five species were from Georgian Bay, and 105 fish of five species were from the North Channel. Bloaters *(Coregonus hoyi)*, coho salmon *(Oncorhynchus kisutch)*, and walleye *(Stizostedion vitreum vitreum)* were caught in southern Lake Huron, walleye caught in Georgian Bay at the mouth of the Moon River, and rainbow trout *(Salmo gairdneri)* and lake trout *(Salvelinus namaycush)* came from the south shore of Georgian Bay. Other species were caught between the Bruce Peninsula and Manitoulin Island.

Fish species were identified and named according to the nomenclature of the American Fisheries Society *(1)*.

SAMPLE PREPARATION

Fish were measured, weighed, and where possible, the sex was determined. Heads and viscera were removed and the remainder of the fish was macerated in a Hobart meat grinder. A 150–200-g subsample was stored in a sealed glass jar at −20°C; storage time varied from a few days to four months. Individual fish were analyzed when the sample size was not limiting. Alewife, shiners, smelt, and other small fish were prepared as composites of similar sized fish. They were weighed and measured individually before being ground.

ANALYTICAL PROCEDURE

Ten grams of tissue homogenate was ground with 100 g anhydrous sodium sulfate and 25 g Ottawa sand. The mixture was extracted with 300 ml hexane for 7 hours in a Soxhlet extractor. Solvent was evaporated by rotary vacuum and the percentage fat was determined gravimetrically.

A one-step Florisil column cleanup method described by Langlois et al. *(5)* was used to isolate organochlorine insecticides and PCBs. A maximum of 1 g fat was mixed with conditioned Florisil and placed above another layer of Florisil. The column was eluted with a 300-ml 1:4 mixture of dichloromethane–hexane. Solvent was evaporated by rotary vacuum.

PCBs, Hexachlorobenzene (HCB), and organochlorine insecticides were separated on a charcoal column according to the method described by Holdrinet *(4)*. Analyses were performed with a Tracor Model 550 gas-liquid chromatograph (GLC). Instrument parameters and operating conditions follow.

Detector:	^{63}Ni
Column:	15 cm × 0.64 cm OD glass, packed with a mixture of 4 percent SE-30 and 6 percent QF-1 on 80–100-mesh Chromosorb W
Temperature:	180°C
Carrier gas:	nitrogen flowing at 60 ml/minute
Injection volume:	5 µl was equivalent to 1 ng fat sample

Two-dimensional thin-layer chromatography was used on random samples for confirmation. Samples were removed, redissolved, and re-injected into the GLC column.

Recoveries were checked periodically by fortification of tissue homogenates prior to extraction. Average recoveries were:

Residue	%	Residue	%
o,p'–DDT	91	Dieldrin	89
p,p'–DDT	89	*cis*-Chlordane	98
p,p'–TDE	94	*trans*-Chlordane	90
p,p'–DDE	96	PCBs	85–90

The data were not corrected for recoveries. Detection limits were 0.005 ppm for organochlorines and 0.05 ppm for PCBs. PCBs were identified by comparing them with mixtures of Aroclors 1254 and 1260 and checking for a resemblance to peaks VII, VIII, and X on sample chromatograms according to Reynolds *(10)*.

Analysis was begun in 1968 when the known main contaminants in fish were *p,p'*-DDT and its analogs plus dieldrin and heptachlor epoxide; PCB values prior to 1970 were estimated. With the introduction of a column fractionation technique in 1970 for the separation of PCBs from organochlorine insecticides, the measurement of PCB residues became more precise. Analysis for HCB was included in 1973 but was discontinued because of the low level and incidence of HCB found in the samples. The analysis and confirmation for *cis*- and *trans*-chlordane was refined in 1975; analyses for mirex and oxychlordane were introduced in 1976.

Results

LAKE SUPERIOR

ΣDDT—None of the five fish species caught in Lake Superior contained annual mean residues in excess of the 5 ppm tolerance level established by Health and Welfare Canada or the Food and Drug Administration, U.S. Department of Health, Education, and Welfare. The highest mean residue of 2.7 ppm was found in lake trout caught in 1968. However, of 18 lake trout analyzed, three contained residues of ΣDDT that exceeded 5 ppm (Table 1): a 1544-g fish caught in Shesheep Bay contained 14.1 ppm; a 2906-g fish caught off Thunder Cape contained 7.9 ppm; and a 3314-g fish caught in Finlay Bay contained 5.2 ppm (Figure 1). Lake trout caught in 1971 and bloaters caught in 1971 and 1975 contained the second highest mean ΣDDT residues of 1.16 ppm and 1.06 ppm, respectively, but no individuals exceeded the tolerance level.

Residues of ΣDDT declined in both lake trout and lake whitefish *(Coregonus clupeaformis)* between 1971 and 1975, but no trend was apparent in either bloater or white sucker *(Coregonus commersoni)*. The ratio of DDE plus TDE to ΣDDT increased in lake trout and lake whitefish from 1971 to 1975, indicating a metabolic breakdown of *o,p'*- and *p,p'*-DDT; this was not so apparent in bloaters and white sucker (Table 2). The decline is more evident in lake trout when similar weight classes are compared (Table 3). In spite of higher fat content in fish caught in 1975, ΣDDT is only a fraction of the residue found in 1968–70.

Dieldrin—No fish species contained mean residues that exceeded 0.08 ppm dieldrin, and no individual fish contained residues which exceeded the 0.3 ppm guideline set by FDA. The highest level of dieldrin found in an individual fish was 0.26 ppm in a lake trout caught in 1968. In general, levels of dieldrin were low, but the rate of disappearance of dieldrin since 1971 also has been slow. On the basis of a ΣDDT/dieldrin ratio, ΣDDT declined more rapidly than dieldrin between 1971 and 1975 (Table 2). Lake trout exhibited a decline in the ratio between 1968 and 1975 of 91 to 5. The ratio of PCBs to dieldrin changed little between 1971 and 1975. This was borne out when similar weight classes of lake trout were compared (Table 3).

PCBs—None of the five fish species caught in Lake Superior contained mean residues of PCBs greater than the 2 ppm tolerance level set by Health and Welfare Canada (Table 1). However, two individual trout caught

TABLE 1. *Organochlorine residues in five fish species caught in the Canadian waters of eastern Lake Superior, 1969–75*

Species	Year	No. of Analyses	Mean and Range Weight, g	Mean and Range Fat, %	Mean Content and Range of Contaminants in Fish Puree, ppm[1,2] DDE	TDE	DDT	ΣDDT	Dieldrin	PCBs
Catostomidae										
White sucker	1971	5	1102	2.2	0.08	0.01	0.04	0.13	0.01	0.2
			988–1202	0.9–5.0	<0.01–0.15	<0.01–0.02	<0.01–0.07	0.01–0.24		<0.1–0.5
	1975	8	946	3.1	0.14	0.01	0.05	0.20	0.02	0.3
			696–1154	0.7–7.1	0.03–0.46	<0.01–0.03	<0.01–0.15	0.08–0.59	<0.01–0.06	0.1–0.7
Esocidae										
Northern pike	1971	5	2044	1.2	0.23	0.03	0.14	0.40	<0.01	0.3
			1474–2752	0.8–1.8	0.08–0.48	0.01–0.07	0.02–0.41	0.11–0.96		0.1–0.6
Salmonidae										
Bloater	1971	4(19)[3]	149	9.7	0.68	0.07	0.41	1.16	0.02	0.6
			145–175	9.4–10.0	0.56–0.75	0.06–0.08	0.34–0.45	0.96–1.36	0.01–0.06	0.5–0.7
	1975	10	169	10.2	0.52	0.07	0.47	1.06	0.04	1.0
			112–268	3.1–18.7	0.07–1.76	0.02–0.16	0.12–1.39	0.22–3.23	0.01–0.09	0.3–3.7
Lake trout	1968	18	2016	8.0	1.44	0.24	1.04	2.72	0.08	0.7
			455–5506	1.3–14.7	0.16–7.11	0.01–1.32	0.02–5.68	0.27–14.1	0.01–0.26	<0.1–2.0
	1969	20	734	6.4	0.43	0.12	0.43	0.98	0.03	0.3
			409–1700	1.7–14.4	0.20–0.75	0.04–0.20	0.19–0.77	0.43–1.69	<0.01–0.05	0.1–0.6
	1971	5	1901	17.4	0.98	0.09	0.65	1.72	0.03	1.8
			1572–2728	15.7–22.1	0.59–1.25	0.06–0.11	0.38–0.82	1.03–2.18	0.02–0.05	1.1–2.3
	1975	10	1121	20.7	0.11	0.01	0.05	0.17	0.04	0.4
			555–1432	14.7–29.4	0.09–0.16	<0.01–0.03	0.02–0.09	0.10–0.24	0.03–0.05	0.3–0.6
Lake whitefish	1971	5	959	12.0	0.35	0.04	0.35	0.74	0.04	0.8
			895–1060	8.5–14.2	0.29–0.45	0.03–0.05	0.30–0.43	0.63–0.93	0.03–0.05	<0.1–1.0
	1975	10	1135	10.8	0.16	0.02	0.06	0.24	0.07	0.3
			766–1400	6.2–12.2	0.09–0.29	0.01–0.03	<0.01–0.16	0.12–0.48	0.04–0.11	0.1–0.7

[1] In 1975 traces (0.004 ppm) of *cis*- and *trans*-chlordane were detected in some bloater, white sucker, lake trout, and lake whitefish.
[2] <0.01 ppm represents a trace of contaminant above the level of detection (0.001 ppm) but below 0.010 ppm.
[3] Composite of 19 fish.

off Grass Cap Point in 1971 had residues of 2.2 ppm and 2.3 ppm PCBs and two bloaters caught commercially in 1975 had residues of 2.1 ppm and 3.7 ppm. Mean residues for lake trout in 1971 and bloaters in 1975 were 1.8 ppm and 1.0 ppm, respectively.

TABLE 2. *Ratios of organochlorine contaminants in four species of fish caught in Lake Superior, Lake Huron, and Georgian Bay, 1968–76*

Species	Year	DDE+TDE / ΣDDT	ΣDDT / PCBs	ΣDDT / Dieldrin	PCBs / Dieldrin
Lake Superior					
Bloater	1971	0.65	2.0	50	30
	1975	0.56	1.1	30	25
White sucker	1971	0.69	0.5	21	20
	1975	0.75	0.8	10	15
Lake trout	1968	0.62	3.9	91	9
	1969	0.56	3.3	33	10
	1971	0.62	0.9	50	60
	1975	0.70	0.4	5	10
Lake whitefish	1971	0.53	0.9	19	20
	1975	0.75	0.8	3	4
Lake Huron (Main Lake)					
Bloater	1969	0.74	3.5	69	20
	1970	0.52	1.8	29	16
	1971	0.64	2.1	94	44
Cisco	1969	0.66	6.1	61	10
	1976	0.95	1.0	6	7
Coho salmon	1968	0.54	0.5	26	50
	1969	0.68	2.5	51	20
	1970	0.60	1.6	25	15
	1971	0.61	1.2	19	17
	1975	0.91	0.4	7	16
Lake whitefish	1969	0.36	3.6	9	2
	1972	0.60	1.4	9	7
	1973	0.60	1.2	8	7
	1976	0.80	0.6	2	3
Georgian Bay					
Bloater	1971	0.66	1.0	24	24
	1975	0.61	0.7	5	7
Cisco	1969	0.38	3.2	159	50
	1976	0.62	0.7	8	12

Mean PCB residues declined in lake trout and lake whitefish between 1971 and 1975 but increased in bloaters over the same period. Comparison of lake trout by weight class revealed no significant decline in PCB residues (Table 3). The ΣDDT/PCB ratio in all species declined, suggesting the disappearance of ΣDDT. The PCB/dieldrin ratio indicates that dieldrin is more persistent in fish tissues than are PCBs.

Other organochlorines—Trace quantities (<0.01 ppm) of *cis*- and *trans*-chlordane were detected in some bloaters, white sucker, lake trout, and lake whitefish caught in 1975, but no oxychlordane, endrin, or heptachlor epoxide was detected in fish caught in 1968–75.

LAKE HURON

ΣDDT—Three fish species caught in Lake Huron and Georgian Bay contained mean residues that exceeded 5 ppm. These included walleye (5.05 ppm) caught in southern Lake Huron in 1970, lake trout (7.60 ppm) caught in Georgian Bay in 1969, and bloaters (5.18 ppm) caught in 1971 in Georgian Bay (Table 4). Individual fish of five species contained ΣDDT residues in excess of 5 ppm including: bloaters (1970 and 1971), coho salmon (1970), and walleye (1970), caught in the southern half of Lake Huron: and bloaters (1971), rainbow trout (1968), lake trout (1969), and walleye (1969 and 1970) caught in Georgian Bay (Table 4, Figure 1).

ΣDDT residues declined noticeably between 1968–71 and 1975–76 in six species including alewife (*Alosa pseudoharengus*), smallmouth bass (*Micropterus dolomieui*), cisco (*Coregonus artedii*), coho salmon, rainbow

TABLE 3. *Comparison of organochlorine residues in two weight classes of splake, lake trout, and lake whitefish caught in Lake Huron and Lake Superior, 1969–76*

Species and Location	Year	0.5–1.0 kg Class						1.0–1.5 kg Class					
		No. of Fish	Weight, g	Fat, %	ΣDDT, ppm	Dieldrin, ppm	PCBs, ppm	No. of Fish	Weight, g	Fat, %	ΣDDT, ppm	Dieldrin, ppm	PCBs, ppm
Splake													
Lake Huron	1969	3	821	6.8	1.61	0.06	0.2	5	1351	6.9	0.86	0.03	0.3
	1970	[illegible]	784	13.2	1.16	0.06	1.6	8	1220	17.6	1.35	0.07	1.5
	1972	3	787	10.8	0.87	0.05	0.7						
	1973	10	690	6.6	0.77	0.03	0.6	4	1108	12.2	0.75	0.06	0.9
	1974	1	526	3.3	0.11	<0.01	0.1	4	1271	4.4	0.15	0.02	0.3
Georgian Bay	1975	6	747	11.9	0.78	0.14	1.4	6	1185	14.1	0.87	0.16	1.[illegible]
Whitefish													
Lake Superior	1971	3	910	12.5	0.71	0.04	0.8	2	1032	11.3	0.78	0.04	0.8
	1975	1	766	8.6	0.28	0.04	0.2	9	1176	11.0	0.22	0.07	0.[illegible]
Lake Huron	1969	22	730	5.4	0.40	0.05	0.1	1	1180	3.7	0.25	0.03	<0.1
	1972	12	813	8.2	0.55	0.07	0.3	7	1142	12.3	0.87	0.09	0.6
	1973							7	1172	17.1	0.64	0.08	0.4
	1976	2	850	3.7	0.08	0.03	0.1	10	1237	6.3	0.12	0.07	0.2
North Channel	1969	6	936	3.2	0.14	0.01	<0.1	8	1187	6.1	0.89	0.10	0.1
	1970	1	980	8.6	0.80	0.05	0.4	2	1285	10.6	0.71	0.07	0.4
Georgian Bay	1969	4	939	4.5	0.44	0.01	0.1	6	1131	3.7	0.54	0.01	0.2
Lake Trout													
Lake Superior	1968	8	698	4.8	0.818	0.046	0.21	4	1566	10.6	4.94	0.128	1.19
	1969	10	619	3.8	0.731	0.024	0.25	10	1308	8.8	1.25	0.040	0.34
	1970							4	1694	17.9	1.75	0.033	1.83
	1975	3	768	18.6	0.192	0.037	0.33	7	1272	21.6	0.17	0.037	0.49

smelt *(Osmerus mordax)*, and walleye from the main waters of Lake Huron, and bloaters from Georgian Bay. ΣDDT mean residues were erratic or unchanged in cisco, splake *(Salvelinus fontinalis* and *S. namaycush)*, and walleye caught in Georgian Bay and in splake and lake whitefish caught in the main lake.

To determine whether ΣDDT residues in splake and lake whitefish had declined, similar weight classes were compared (Table 3). ΣDDT levels in splake with an average weight of 1250 g declined between 1971 and 1974 from 1.35 ppm to 0.15 ppm. A similar decline in ΣDDT residues in lake whitefish was noted between 1972 and 1976. Cisco, coho salmon, and lake whitefish all showed a marked increase in the DDE+TDE/ΣDDT ratio during the present study (Table 2), suggesting a lower intake of the parent compound and/or degradation to metabolites; this decline was not evident in bloaters.

Dieldrin—Mean residues for all species investigated did not exceed the 0.3 ppm tolerance level set by FDA. However, individual fish of three species exceeded the level. One of 20 lake whitefish caught in the North Channel in 1969 contained 0.58 ppm dieldrin; one of 10 bloaters caught in Lake Huron in 1970 had a residue of 0.44 ppm dieldrin; five of 10 bloaters caught in Georgian Bay in 1975 contained dieldrin levels of 0.34–0.50 ppm; 10 of 20 bloaters caught in the North Channel in 1975 contained residues of 0.3–0.6 ppm dieldrin; and two large splake caught in Lake Huron contained residues of 0.43 ppm and 0.53 ppm dieldrin. The 10 bloaters caught in the North Channel during 1975, which had residues above the tolerance level, weighed an average of 333 g and contained an average of 0.40 ppm dieldrin. The remaining 10 bloaters, which averaged 236 g, contained a mean residue of 0.19 ppm dieldrin. In this instance, and in the case of the splake, higher dieldrin residues were associated with larger fish, but this relationship was not apparent in the 10 bloaters caught in Georgian Bay in 1975 (Table 4).

Dieldrin levels increased in alewife, bloaters, cisco, yellow perch *(Perca flavescens)*, coho salmon, and splake during 1968–71 and 1975–76; levels in other species showed little change. Assessment of dieldrin levels on the basis of similar weight classes of lake whitefish and splake indicate that residues declined in lake whitefish and increased in splake (Table 3). A marked decline was noted in the ΣDDT/dieldrin ratio in four species: in cisco, for example, the ratio declined from 61 to 6 between 1969 and 1976. The PCB/dieldrin ratio also declined in the same four species suggesting declining PCB residues and static or increasing dieldrin residues (Table 2).

PCBs—Three fish species contained mean PCB residues which exceeded the 2 ppm tolerance level set by Health and Welfare Canada. Bloaters from the main lake (1970 and 1971), from Georgian Bay (1971 and 1975), and from the North Channel (1975) contained mean residues of 2.2–5.2 ppm. Individual bloaters had residues as high as 5.0 ppm and 6.4 ppm (Table 4). Cisco netted in Georgian Bay during 1975 contained a mean PCB residue of 2.2 ppm and a high level of 4.6 ppm in individual fish. Two large splake taken from the main waters of Lake Huron in 1975 contained levels of 5.5 ppm and 6.4 ppm PCBs.

TABLE 4. *Organochlorine residues in 14 fish species caught in the North Channel, Georgian Bay, and Canadian waters of Lake Huron, 1968–76*

Species	Year	Location	No. of analyses[1]	Mean and Range Weight, g	Mean and Range Fat, %	Mean Content and Range of Contaminants in Fish Puree, ppm[2] DDE	TDE	DDT	Σ DDT	Dieldrin	PCBs
Catostomidae											
White sucker	1972	Huron	5	723	2.5	0.08	0.01	0.02	0.11	<0.01	0.1
				550–909	1.8–3.3	0.05–0.13	<0.01–0.03	<0.01–0.06	0.06–0.22		<0.1–0.2
	1973	Georgian Bay	4	131	0.7	<0.01	<0.01	<0.01	0.01	<0.01	0.1
				66–212	0.2–1.0	<0.01–0.02			<0.01–0.03		<0.1–0.2
	1976	Huron	10	977	0.6	0.06	<0.01	0.02	0.09	<0.01	0.1
				738–1837	0.1–1.1	<0.01–0.20		<0.01–0.14	<0.01–0.37		<0.1–0.2
Centrarchidae											
Smallmouth bass	1968	Huron	3	499	3.1	0.68	0.76	0.53	1.97	<0.01	0.9
				429–630	2.0–4.9	0.12–1.69	0.15–2.02	0.12–1.23	0.30–4.94		0.2–2.0
	1972	Huron	5	353	3.7	0.12	0.01	0.03	0.16	0.01	0.4
				298–437	2.6–4.5	0.11–0.13		0.02–0.04	0.15–0.18	<0.01–0.03	0.3–0.5
	1972	Georgian Bay	6	281	2.8	0.05	0.01	<0.01	0.07	<0.01	0.01
				270–300	1.6–4.0	0.04–0.07	0.01–0.02		0.06–0.10		0.1–0.1
	1975	Georgian Bay	9	364	3.2	0.17	0.01	0.03	0.21	0.03	0.6
				275–562	1.7–4.5	0.09–0.28	<0.01–0.04	<0.01–0.08	0.12–0.36	<0.01–0.09	0.4–0.9
Clupeidae											
Alewife	1970	Huron	8(21)	33	7.5	0.76	0.23	0.64	1.63	0.08	1.1
				26–40	1.5–13.2	0.16–1.40	0.04–0.52	0.22–1.48	0.27–3.40	0.01–0.22	0.5–2.0
	1976	Huron	5(23)	23	10.7	0.44	0.10	0.26	0.80	0.14	0.3
				3–49	5.8–16.9	0.04–1.08	0.01–0.12	0.01–0.54	0.06–1.74	<0.01–0.25	0.1–0.6
Osmeridae											
Rainbow smelt	1970	Huron	8(21)	22	6.5	0.36	0.12	0.32	0.80	0.04	0.7
				12–67	4.0–8.4	0.06–0.97	0.01–0.25	0.02–0.80	0.11–1.86	<0.01–0.15	0.2–1.0
	1970	N. Channel	5(24)	26	3.6	0.12	0.04	0.15	0.31	0.02	0.1
				18–44	2.8–4.4	0.05–0.20	0.03–0.05	0.08–0.20	0.15–0.45	<0.01–0.03	
	1976	Huron	7(32)	14	2.7	0.11	0.02	0.02	0.15	0.01	0.01
				7–30	1.2–3.9	0.05–0.19	0.01–0.02	<0.01–0.03	0.08–0.23	<0.01–0.02	<0.1–0.2
Percidae											
Yellow perch	1968	Huron	5	335	0.8	0.20	0.12	0.20	0.52	<0.01	0.2
				118–426	0.5–1.0	0.06–0.61	0.02–0.47	0.08–0.51	0.16–1.59		<0.1–0.5
	1969	N. Channel	20	201	1.4	0.03	0.01	0.03	0.07	<0.01	<0.1
				167–341	0.5–2.4	<0.01–0.08	<0.01–0.03	<0.01–0.05	<0.01–0.18		
	1972	Huron	5	67	4.4	0.07	0.01	0.03	0.11	0.01	0.1
				64–74	3.8–5.3	0.06–0.08	0.01	0.02–0.03	0.09–0.12	<0.01–0.02	
	1975	N. Channel	10	175	6.1	0.36	0.03	0.09	0.48	0.05	0.9
				150–197	3.5–8.6	0.13–0.57	<0.01–0.05	<0.01–0.17	0.13–0.72	0.02–0.09	0.4–1.4
	1976	Huron	17	236	2.5	0.21	0.03	0.08	0.32	0.02	0.2
				66–481	0.7–5.4	0.06–0.68	0.01–0.08	0.01–0.55	0.07–1.31	<0.01–0.05	<0.1–0.4
Walleye	1968	Huron	3	409	0.8	0.12	0.04	0.13	0.29	<0.01	0.1
				390–426	0.6–0.9	0.06–0.22	0.02–0.07	0.08–0.21	0.16–0.50		<0.1–0.1
	1969	Georgian Bay	15	2073	2.6	1.05	0.24	1.08	2.37	0.02	1.5
				792–4190	0.6–6.0	0.23–3.53	0.06–0.81	0.23–4.03	0.54–8.36	<0.01–0.07	0.5–2.1
	1970	Huron	2	2083	10.1	2.37	0.64	2.04	5.05	0.08	1.3
				1910–2255	9.7–10.4	1.80–2.91	0.43–0.84	1.65–2.47	3.88–6.22	0.06–0.08	0.7–1.9
	1970	Georgian Bay	21	3236	4.7	0.94	0.23	0.98	2.15	0.04	1.4
				1721–4760	1.2–10.6	0.23–3.70	0.04–0.85	0.18–3.88	0.45–8.33	<0.01–0.16	0.5–2.3
	1970	N. Channel	3	605	2.1	0.19	0.04	0.16	0.39	<0.01	0.1
				526–715	1.3–3.5	0.16–0.23	0.04–0.05	0.13–0.21	0.34–0.49		<0.1–0.1
	1971	Georgian Bay	10	2859	5.8	1.74	0.21	1.11	3.06	0.03	1.8
				1132–4756	2.9–11.6	0.08–4.08	0.04–0.37	0.04–2.32	0.16–6.93	<0.01–0.07	0.1–3.9
	1975	Huron	10	2539	4.7	0.38	0.05	0.27	0.70	0.03	0.5
				722–5218	1.5–13.3	0.16–1.01	<0.01–0.15	0.03–1.01	0.27–2.17	0.01–0.13	0.3–2.5
Salmonidae											
Bloater	1969	Huron	15	97	3.0	0.39	0.12	0.18	0.69	0.01	0.2
				55–143	1.4–7.7	0.06–1.54	0.02–0.50	0.04–0.66	0.20–2.71	<0.01–0.02	<0.1–0.7
	1970	Huron	10	260	16.0	1.95	0.49	2.24	4.68	0.16	2.6
				148–307	8.1–26.7	1.00–3.69	0.20–1.03	1.01–5.17	2.48–9.88	0.04–0.44	1.5–5.0
	1971	Huron	6	71	15.0	2.73	0.29	1.69	4.71	0.05	2.2
				61–101	7.2–21.1	2.02–4.34	0.05–0.78	1.14–2.40	3.44–7.52	0.03–0.07	1.1–3.2
	1971	Georgian Bay	4(12)	259	20.1	2.90	0.53	1.75	5.18	0.22	5.2
				140–433	18.0–23.0	2.34–3.83	0.29–0.75	1.63–1.85	4.26–6.43	0.18–0.28	4.3–6.4
	1975	Georgian Bay	10	219	16.3	0.71	0.16	0.56	1.43	0.30	2.2
				179–250	8.1–20.1	0.33–1.15	0.01–0.30	0.15–1.11	0.49–2.56	0.10–0.50	0.8–4.4
	1975	N. Channel	20	285	22.9	1.16	0.17	0.54	1.87	0.29	2.6
				134–560	15.5–29.8	0.56–2.34	<0.01–0.41	0.01–1.48	0.74–4.18	<0.01–0.60	0.6–5.2
Cisco	1969	Huron	2	180	5.4	0.35	0.05	0.21	0.61	0.01	0.1
				100–260	2.0–8.8	0.18–0.52	0.02–0.07	0.07–0.35	0.27–0.94	<0.01–0.02	<0.1–0.2
	1969	Georgian Bay	10	820	7.2	0.41	0.19	0.99	1.59	0.01	0.5
				337–937	4.8–9.4	0.22–1.01	0.08–0.40	0.54–2.58	0.83–3.99	<0.01–0.02	0.2–1.1
	1975	Georgian Bay	6	543	18.0	0.78	0.15	0.58	1.51	0.19	2.2
				352–710	11.3–24.7	0.47–1.14	0.09–0.29	0.26–1.22	0.83–2.65	0.12–0.30	1.3–4.6
	1976	Huron	9(11)	138	5.0	0.14	0.05	0.01	0.20	0.03	0.2
				47–242	3.0–7.5	0.11–0.19	0.03–0.09	<0.01–0.02	0.14–0.29	0.01–0.08	0.1–0.4

(*continued next page*)

TABLE 4. (cont.d.) *Organochlorine residues in 14 fish species caught in the North Channel, Georgian Bay, and Canadian waters of Lake Huron, 1968–76*

Species	Year	Location	No. of Analyses[1]	Mean and Range Weight, g	Fat, %	Mean Content and Range of Contaminants in Fish Puree, ppm[2] DDE	TDE	DDT	ΣDDT	Dieldrin	PCBs
Coho salmon	1968	Huron	8	81	3.9	0.11	0.03	0.12	0.26	<0.01	0.5
				39–163	1.2–5.4	0.04–0.42	0.01–0.12	0.04–0.43	0.09–0.97	0.01–0.03	0.2–1.0
	1969	Huron	5	1885	5.8	0.88	0.16	0.48	1.52	0.03	0.6
				1138–3335	5.1–6.5	0.31–2.01	0.13–0.21	0.35–0.62	0.87–2.84	0.01–0.05	0.3–1.2
	1970	Huron	41	1031	4.9	0.48	0.11	0.39	0.98	0.04	0.6
				754–1595	0.8–11.1	0.9–5.2	0.03–0.66	0.08–1.89	0.22–7.75	0.01–0.24	0.1–7.0
	1971	Huron	10	936	8.0	0.50	0.20	0.45	1.15	0.06	1.0
				475–1395	4.9–13.0	0.23–1.15	0.07–0.62	0.16–0.80	0.48–2.09	0.02–0.13	0.2–2.1
	1975	Huron	11	2284	5.8	0.43	0.05	0.05	0.53	0.08	1.3
				280–4356	3.9–8.4	0.04–0.94	<0.01–0.14	<0.01–0.19	0.05–1.22	0.01–0.16	0.1–3.3
Kokanee salmon	1968	Huron	2	95	3.5	0.44	0.12	0.59	1.15	0.04	0.3
				94–96	2.8–4.2	0.08–0.80	0.02–0.22	0.10–1.08	0.20–2.00	<0.01–0.07	0.1–0.4
	1969	Huron	11	98	3.3	0.10	0.03	0.10	0.23	0.01	<0.1
				58–375	1.4–5.3	0.02–0.80	<0.01–0.06	0.03–0.18	0.06–0.57	<0.01–0.03	
	1970	Huron	15	512	4.1	0.38	0.12	0.45	0.95	0.04	0.6
				204–1098	0.9–7.8	0.17–0.67	0.05–0.24	0.16–1.03	0.78–1.76	<0.01–0.12	0.2–1.0
Splake	1969	Huron	20	1113	6.3	0.43	0.14	0.46	1.03	0.04	0.2
				25–2354	1.8–10.1	0.03–0.85	0.02–0.33	0.03–0.85	0.08–2.11	<0.01–0.09	<0.1–0.5
	1970	Huron	23	810	10.2	0.37	0.13	0.50	1.00	0.04	1.0
				208–1420	3.2–15.7	0.12–0.82	0.04–0.20	0.01–1.03	0.19–1.81	<0.01–0.12	0.1–1.5
	1972	Huron	5	544	8.5	0.30	0.04	0.28	0.62	0.03	0.5
				138–877	5.1–11.7	0.14–0.44	0.01–0.04	0.08–0.56	0.23–1.10	0.01–0.05	0.2–0.9
	1973	Huron	26	556	8.8	0.38	0.03	0.06	0.47	0.03	0.4
				96–450	3.6–16.1	0.03–1.20	<0.01–0.13	<0.01–0.16	0.04–1.31	<0.01–0.10	<0.1–1.5
	1974	Huron	7	1238	4.1	0.11	0.01	0.03	0.15	0.02	0.2
				526–1540	2.7–5.9	0.06–0.19	<0.01–0.02	<0.01–0.05	0.08–0.25	<0.01–0.04	<0.1–0.7
	1975	Huron	2	2127	17.2	1.80	0.42	0.46	2.68	0.48	6.0
				1997–2256	13.6–20.8	1.58–2.02	0.40–0.44	0.37–0.55	2.39–2.97	0.43–0.53	5.5–6.4
	1975	Georgian Bay	17	1048	13.3	0.52	0.08	0.18	0.78	0.15	1.6
				458–1798	8.5–17.4	0.18–0.88	0.03–0.11	0.10–0.37	0.28–1.12	0.08–0.18	0.6–2.3
Rainbow trout	1968	Georgian Bay	12	857	5.5	0.74	0.17	0.84	1.75	0.04	0.3
				284–1850	3.2–7.8	0.12–6.17	0.02–1.27	0.13–5.70	0.27–13.1	<0.01–0.19	<0.1–1.5
Lake trout	1969	Georgian Bay	4	6328	13.4	4.04	0.50	3.06	7.60	0.07	0.7
				4200–8740	12.9–13.8	3.14–5.51	0.45–0.63	2.66–3.71	6.28–9.85	0.06–0.09	0.4–0.9
Lake whitefish	1969	N. Channel	20	1430	4.8	0.13	0.07	0.36	0.56	0.06	0.1
				854–2785	0.8–8.9	0.03–1.58	0.01–0.31	0.66–2.66	0.10–4.75	<0.01–0.58	<0.1–0.7
	1969	Huron	26	711	5.0	0.13	0.05	0.18	0.36	0.04	0.1
				386–1080	2.9–9.9	0.07–0.24	0.02–0.10	0.04–0.36	0.16–0.73	<0.01–0.08	<0.1–0.3
	1969	Georgian Bay	10	1054	4.0	0.20	0.09	0.22	0.51	0.01	0.2
				881–1241	3.2–6.4	0.10–0.31	0.03–0.13	0.09–0.33	0.22–0.78	<0.01–0.02	<0.1–0.3
	1970	N. Channel	3	1183	9.9	0.27	0.07	0.40	0.74	0.06	0.4
				980–1361	8.6–11.7	0.23–0.29	0.07	0.33–0.45	0.63–0.80	0.05–0.09	0.2–0.5
	1972	Huron	25	761	8.2	0.28	0.65	0.22	0.55	0.06	0.04
				80–1393	1.8–21.9	0.07–1.02	0.01–0.16	0.03–0.42	0.11–1.47	0.01–0.11	0.1–0.8
	1973	Huron	19	804	14.2	0.23	0.05	0.19	0.47	0.06	0.04
				108–1747	6.9–26.5	0.03–1.48	0.01–0.21	0.02–0.92	0.07–2.61	<0.01–0.25	<0.1–0.6
	1976	Huron	15	1323	5.8	0.08	0.02	0.03	0.13	0.06	0.2
				802–2495	2.5–11.6	0.04–0.15	0.01–0.03	0.02–0.05	0.07–0.23	0.03–0.14	0.1–0.5

[1] Most analyses were performed on single fish; where composites were analyzed, the number of fish is given in parentheses. Composities were of similar weight.
[2] <0.01 ppm represents a trace of contaminant above the level of detection (0.001 ppm) but below 0.010 ppm.

Alewife, rainbow smelt, and walleye caught in the main waters of Lake Huron were the only species in which PCB levels declined between 1968–71 and 1975–76. Residues in cisco from Georgian Bay, yellow perch from the North Channel, and coho salmon and splake from the main lake increased during these periods. In the above species caught in other locations and in other species, no trend was evident; PCB levels were static. Analysis of splake and lake whitefish on the basis of similar weight classes indicates that PCB levels peaked in splake in 1970 and then declined between 1970 and 1974. PCB levels in lake whitefish declined between 1972 and 1976 (Table 3).

In general, the ΣDDT/PCB and PCB/dieldrin ratios declined between 1968–71 and 1975–76 in four species: bloaters, cisco, coho salmon, and lake whitefish; this supported the finding that ΣDDT residues were declining, dieldrin residues were increasing, and PCB residues were static or declining slowly (Table 2).

Chlordane—Residues of chlordane were detected in smallmouth bass and walleye caught in Georgian Bay in 1975 at mean levels of 0.01 ppm and 0.05 ppm, respectively (sum of *cis*- and *trans*-isomers). Trace levels (<0.01 ppm) were suspected in bloaters, cisco, coho salmon, and splake in 1975. Oxychlordane analysis was included in 1976. Total chlordane levels found in 1976 ranged from traces in yellow perch to 0.039 ppm in alewife; both species were caught in the open part of Lake Huron (Table 5).

TABLE 5. *Residues of chlordane and heptachlor epoxide in fish from the main waters of Lake Huron and Georgian Bay, 1975–76*

Location and Species	No. of Fish[1]	Average Weight, g	Average Fat, %	Residue, ppm	
				Chlordane[2]	Heptachlor Epoxide
1975 Georgian Bay					
Smallmouth bass	9	364	3.2	0.01 <0.01–0.04	ND
Walleye	10	2539	4.7	0.05 <0.01–0.19	ND
1976 Lake Huron					
Alewife	23(5)[3]	23	10.7	0.039 0.004–0.060	0.026 ND–0.100
Cisco	11(9)	138	5.0	0.025 0.015–0.040	0.013 ND–0.044
Yellow perch	17	236	2.5	<0.001 ND–0.013	0.002 ND–0.009
Rainbow smelt	32(7)	14	2.7	0.002 ND–0.013	0.004 ND–0.007
White sucker	10	977	0.6	0.001 ND–0.013	0.002 ND–0.006
Lake whitefish	15	1323	5.8	0.047 0.025–0.087	0.026 0.013–0.065

NOTE: ND=not detected to less than 0.005 ppm.
[1] Number in brackets represents number of analysis.
[2] 1975 analyses included *cis*- and *trans*-isomers; 1976 analyses included *cis*- and *trans*-chlordane and oxychlordane.

Heptachlor epoxide—No heptachlor epoxide was identified in fish caught prior to 1976. Mean residues in alewife caught in 1976 ranged from 0.002 ppm in yellow perch to 0.026 ppm in alewife caught in the main lake (Table 5).

Hexachlorobenzene (HCB)—Analyses for HCB in fish tissues were not routinely carried out during the study period. An indication of the extent of HCB in fish was obtained from samples caught in 1972 and 1973 from Lake Huron. One of five splake caught in the open lake contained 0.001 ppb HCB, and smelt caught off Black Stone Harbour in Georgian Bay contained 0.03 ppm. HCB was not detected in a limited number of smallmouth bass, yellow perch, or lake whitefish from either Georgian Bay or the main lake.

Discussion

Lake Superior water analyzed by Glooschenko et al. *(3)* was free of DDT, dieldrin, and PCBs down to the detection limit. However, residues of these contaminants were found in sediment and seston. Sediment samples taken from various sites in the Canadian waters of Lake Superior had measurable amounts (0.005 ppm) of dieldrin and ΣDDT in 14 percent and 5 percent, respectively. PCB residues were present in all sediments at all sites; highest level reported was 1.3 ppm in samples collected near Marathon. Seston contained only traces of ΣDDT and dieldrin, but the mean level of PCBs was 1.3 ppm, identical to that in the sediments.

Levels of ΣDDT and dieldrin in lake trout caught in 1970 in Lake Superior correspond closely with those reported by Reinhert *(7)* in 1966–67. Residues in lake trout reported in the present study did not agree with those cited in the Upper Great Lakes Reference Group report *(11)*. However, bloaters contained similar residues in two studies.

Measurable levels of ΣDDT were reported by Glooschenko et al. in 29 percent of sediments taken from various sites in the Canadian waters of Lake Huron and in 14 percent of sediments taken from Georgian Bay *(3)*; maximum levels in both Lake Huron and Georgian Bay were 0.02 ppm. Dieldrin was present at trace levels, and PCBs ranged up to 0.02 ppm. ΣDDT and dieldrin in sediments from the North Channel were below detection levels, but traces of PCBs were found. Organochlorines were highest in seston from the open lake, ranging from 0.8 to 8.1 ppm compared to 0.7 to 6.7 ppm in Georgian Bay and a high level of 1.0 ppm in the North Channel.

Residue levels of ΣDDT and dieldrin in fish from Lake Huron and Georgian Bay reported in this paper correspond closely to the levels reported previously by Reinhert *(7)* and Reinke et al. *(9)* for alewife, bloaters, kokanee *(Oncorhynchus nerka)*, rainbow smelt, and walleye, but discrepancies are evident in yellow perch, lake whitefish, and rainbow trout. ΣDDT mean residues of 2.44 ppm are reported by Reinhert for alewife caught in 1966–67 *(7)*; the present study reveals a decline to 1.63 ppm ΣDDT mean residues in 1970 and a further decline to 0.80 ppm ΣDDT by 1976; conversely, dieldrin levels were slightly higher in 1976 (0.14 ppm) than in 1966–67 (0.05 ppm). Levels of ΣDDT in rainbow trout show little change between the 1966–67 study and those detected in 1970 in the present study, 0.75 and 0.8 ppm, respectively. However, a marked decline to a mean residue of 0.15 ppm by 1976 occurred in rainbow trout caught in Lake Huron. A mean level of 4.7 ppm ΣDDT in bloaters caught in 1970–71 in the present study is similar to levels of 3.6 ppm and 3.08 ppm reported respectively by Reinhert *(7)* in 1966 and Reinke et al. *(9)* in 1970. Mean levels of ΣDDT, dieldrin, and PCBs in bloaters in the present study closely parallel those reported by the Upper Great Lakes Reference Group *(11)* for 1975–76.

Reinke et al. found 6.02 ppm ΣDDT in walleye caught in 1970 in the main waters of Lake Huron *(9)*; this is close to the mean level of 5.05 ppm reported here. Reinke et al. reported 0.47 ppm ΣDDT in walleye caught in 1970 in Georgian Bay *(9)*, but the present study reports mean levels of 2.2 ppm and 3.1 ppm, respectively, for 1970 and 1971. This discrepancy may be partly explained by the fact that the walleye in the present study were obtained at the mouth of the Moon River, an area where high DDT residues were reported *(2, 6)*.

Residues in kokanee from Lake Huron reported here are similar to those reported by Reinke et al. *(9)* but ΣDDT

residues of 0.52 ppm in yellow perch reported in the present study are considerably lower than the mean values of 1.59 ppm in 1966–67 and 1.46 ppm in 1970 reported by Reinhert *(7)* and Reinke et al. *(9)*, respectively. Mean ΣDDT residues in lake whitefish in the present study are also markedly lower than those reported previously *(7, 9)*.

Although there are differences in the data for ΣDDT levels in coho salmon between the present study and earlier reports, there is more similarity among coho salmon from the same location. Reinke et al. reported a mean of 1.26 ppm ΣDDT and 0.08 ppm dieldrin for fish caught in northern Lake Huron *(9)*; the present study shows mean levels of 0.98 ppm ΣDDT and 0.04 ppm dieldrin for 41 coho salmon caught in the same area. ΣDDT levels in rainbow trout caught in southern Georgian Bay vary considerably from those reported previously. Reinke et al. reported a mean of 8.7 ppm ΣDDT in rainbow trout caught in 1970 *(9)*, but only 1.75 ppm ΣDDT was found in the same species caught in the same location in 1968 for the present survey. This discrepancy may be due to local differences in ΣDDT use.

Despite the number of variables which are associated with a sampling study of this kind, it is remarkable that such close agreement is found between different studies in different time frames for such large bodies of water as Lakes Superior and Huron. Other factors that cause fluctuations in contaminant concentrations in fish tissues are spawning times and changes in fat content.

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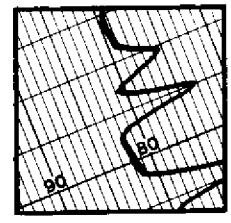

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ORGANOCHLORINE INSECTICIDES AND PCB IN THE SEDIMENTS OF LAKE HURON (1969) AND GEORGIAN BAY AND NORTH CHANNEL (1973)

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ABSTRACT

Surficial and core sediments were collected from the main body of Lake Huron in 1969 and Georgian Bay and North Channel in 1973. These were analysed for organochlorine insecticides and PCB. Residues of organochlorines were higher in the twelve depositional basins in Lake Huron and Georgian Bay than in sediment in the non-depositional zones. PCB was present at similar concentrations to ΣDDT; with mean levels of 13 and 10 ng/g for PCB and ΣDDT in the main body of Lake Huron and 11 and 5 ng/g for PCB and ΣDDT in Georgian Bay. Residues of PCB varied from 9—33 ng/g in the 12 basins. With respect to ΣDDT, both parent DDT and its two metabolites were present in sediment at a mean residue of 7.8 ng/g for the whole lake. The main lake had residues of 10.2 ng/g while Georgian Bay had 5.8 ng/g and North Channel 4.1 ng/g in keeping with use patterns since 1943. HEOD was present in only 5.7% of sediment samples from main Lake Huron, 30% from Georgian Bay and 15% from North Channel. The highest residues 1.7 ng/g occurred in the North Channel.

No chlordane was detected, however, heptachlor epoxide was identified in 8.5% of sediments collected in Lake Huron, 23% from Georgian Bay and 14% in North Channel. Endosulfan appeared in 4% of samples from both Georgian Bay and North Channel.

INTRODUCTION

Persistent organochlorine insecticides and industrial chemicals have been found as contaminants of water, fish and sediment in the Great Lakes Basin. Organochlorine insecticides and PCB have been identified and measured in the sediments of Southern Lake Michigan by Leland et al. (1973), and in sediments of Lakes St. Clair, Erie and Ontario by Frank et al. (1977, 1978) and Holdrinet (1978). Studies on the distribution of pesticides and PCB in water, sediment and seston in the Upper Great Lakes (Superior and Huron) have been carried out by Glooschenko et al. (1976). All demonstrate the widespread presence of these compounds in the Great Lakes system. Their collective results indicate DDT and its metabolities, PCB, HEOD and mirex constitute reservoirs of stable organochlorine residues in lake sediments.

Organochlorine insecticides and polychlorinated biphenyls (PCB) have been identif d in fish caught in Lakes Huron and Superior by Frank et al. (1978), Reinert (1970), Reinke et al. (1972), and by the IJC (1976).

Over the last decade, organochlorines have been identified as a serious contamination problem which is affecting both the commercial and sports fishing industries.

The present study was intended to map the concentrations of organochlorine insecticides and PCB in the upper few centimeters of the bottom sediments in Lake Huron, including Georgian Bay and North Channel. The sampling of sediments in Lake Huron was carried out as part of an ongoing study on the regional sedimentology and geochemistry of the Great Lakes being conducted at the Canada Centre for Inland Waters. A description of the sediments of Lake Huron has been presented by Thomas et al. (1973) and Geogian Bay by the IJC (1977). For the purpose of this paper, the term Lake Huron is used to describe the main body of the lake exclusive of Georgian Bay and North Channel which are identified separately.

METHODS AND MATERIALS

Samples were collected using a Shipek bottom sampler on alternate samples of a 10 Km Universal Transverse Mercater (U.T.M.) grid in Lake Huron in 1969 (Fig. 1). In 1973, similar samples were taken on a 10 Km U.T.M. grid in Georgian Bay and on alternate samples of a 5 Km U.T.M. grid in North Channel (Fig. 1). In all samples, the surficial 3 cm of sediment was carefully sub-sampled aboard the Canadian survey ship 'LIMNOS' and freeze-dried for subsequent geochemical analysis. The freeze-dried samples were later sieved at 20 mesh to remove pebble size material and ground to pass 100 mesh to ensure complete homogeneity of the sample.

In addition, two Benthos cores were taken from Lake Huron (1971), three from Georgian Bay (1974) and a further core in South Bay (1970). Locations and sub-sampling procedures for these cores have been given by Kemp and Harper (1977) and Kemp and Thomas (1976) and locations are shown in Fig. 1.

ANALYTICAL PROCEDURE

Ten grams of dried sediment* were extracted with a mixture of hexane and acetone in a procedure described by Chiba and Morley (1968). The extract was cleaned up and fractionated on a Florisil column as described by Mills et al. (1972). Further fractionation of PCB and organochlorine insecticides was carried out on a second column consisting of charcoal following the method described by Berg et al. (1972) and Holdrinet (1974).

*Analyses carried out in 1977 on freeze-dried material held in inventory after sampling in 1969 and 1973.

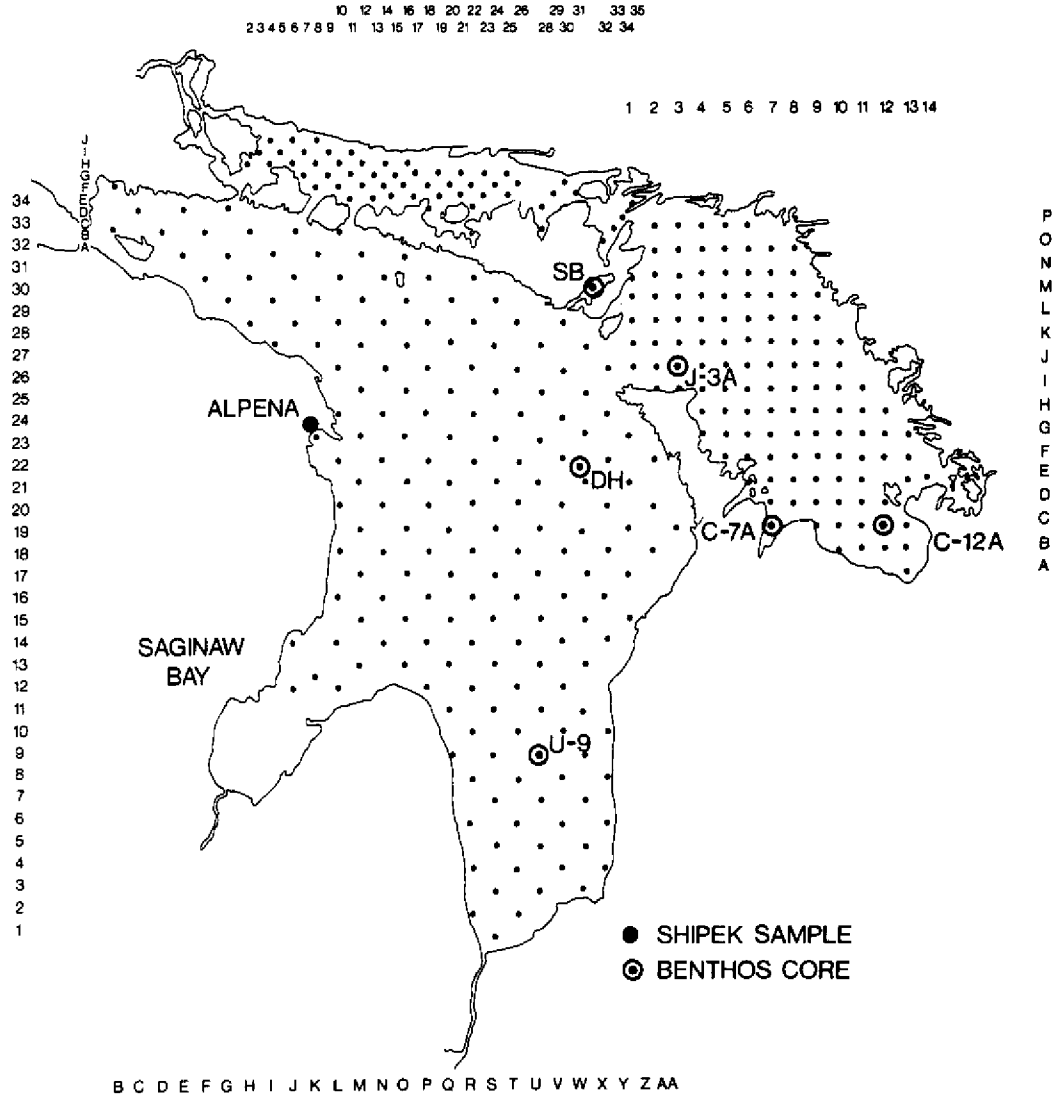

Fig. 1. Sample grid for shipek samples from Lake Huron, 1969, Georgian Bay and North Channel 1973 and Benthos core samples 1970, 1971 and 1974 (Lake Huron, South Bay and Georgian Bay respectively).

Micro-Tek Model MT 220 and 550 gas chromatographs equipped with Ni^{63} electron capture detectors were used. Columns were packed with 4% SE-30 and 6% QF-1 on a 80—100 mesh Chromosorb W which had been acid washed and treated with dimethylchlorosilone. Columns were pre-conditioned for 72 h at 225 °C and 30 ml nitrogen per minute. Operating parameters were: Nitrogen carrier gas at 60 ml/min; Injector temperature, 220 °C; Column temperature, 180 °C; Detector temperature, 300 °C.

Recovery studies were undertaken using natural and spiked samples. The following recovery percentages were obtained: DDE, 92; TDE, 91; DDT, 90; PCB, 89; and HEOD, 88. The procedure described by Reynolds (1971) was employed to estimate the level of PCB in samples.

Total quartz determinations were carried out gravimetrically using a potassium pyrosulphate fusion after the technique of Trostell and Wynne (1940). The major elements, Al_2O_3, K_2O, MgO and Fe_2O_3 were analysed by X-ray fluorescence, organic carbon by (Leco) induction furnace carbon analyser and mercury by flameless atomic absorption.

RESULTS AND DISCUSSION

Data between Lake Huron and Georgian Bay—North Channel are not strictly comparable because samples from the former were collected in 1969 and from the latter in 1973. However, sedimentation rates in these lakes are so low per annum (Kemp and Harper 1977) as to make such comparisons valid.

The surficial sediment distribution in Lake Huron was described by Thomas et al. (1973) and a similar description for Georgian Bay was provided in the Upper Lakes Reference Report (IJC, 1977). These publications further defined the Lake in terms of non-depositional zones and basins of active sediment accumulation (Fig. 2).

Fig. 2. Distribution of non-depositional and depositional basins in Lake Huron and Georgian Bay.

Surficial sediment

Lake Huron — ΣDDT. All four major components associated with ΣDDT were identified in Lake Huron sediments. The parent components *o,p'*- and *p,p'*- DDT were identified in 50 and 92.5% of samples respectively. The metabolities, *p,p'*-DDE and *p,p'*-TDE were present in 93 and 92% of sediments respectively (Tables 1 and 2). The mean ΣDDT residue from the lake was 10.2 ng/g. Contamination of sediments in the basins (18.4 ng/g) was greater than in the non-depositional zone (3.8 ng/g). Among the six basins identified by Thomas *et al.* (1973) the Goderich Basin contained the highest mean resides (31.1 ng/g). Four basins had ΣDDT residues that ranged from 10.6 to 17.9 ng/g whilst the Mackinac Basin had the least contaminated sediment with a mean of 5.7 ng/g ΣDDT. This was only fractionally higher than the 3.8 ng/g mean residue in the non-depositional zone (Table 2).

DDE averaged 32 and 29% of the ΣDDT residue respectively in the

TABLE 1

CONTENT OF ΣDDT AND ITS COMPONENTS IN DRIED LAKE SEDIMENTS FROM LAKE HURON (1969), GEORGIAN BAY AND NORTH CHANNEL (1973)

	Content in dried sediment (ng/g)			ND 0.2	0.2 2.0	2.1 20.0	2.1 20.0
Contaminant	Mean	Min.[a]	Max.	(%)	(%)	(%)	(%)
Lake Huron (main lake — 174 samples)							
p,p'-DDE	3.0	ND	21.0	6.9	50.0	42.5	0.6
p,p'-TDE	1.7	ND	44.0	8.0	76.4	15.6	0.6
o,p'-DDT	0.5	ND	12.0	50.0	46.7	3.3	0.0
p,p'-DDT	5.0	ND	150.0	7.5	43.7	46.0	2.8
ΣDDT	10.2	ND	220.0	2.3	27.0	56.3	14.4[a]
Georgian Bay (115 samples)							
p,p'-DDE	2.5	ND	16.0	12.2	53.0	34.8	0.0
p,p'-TDE	1.6	ND	8.6	13.0	65.2	21.8	0.0
o,p'-DDT	0.1	ND	1.0	83.5	16.5	0.0	0.0
p,p'-DDT	1.6	ND	12.0	39.1	33.9	27.0	0.0
ΣDDT	5.8	ND	35.5	7.0	26.1	61.7	5.2
North Channel (55 samples)							
p,p'-DDE	1.9	ND	10.0	16.4	49.1	34.5	0.0
p,p'-TDE	0.9	ND	4.7	12.7	80.0	7.3	0.0
o,p'-DDT	0.1	ND	0.9	76.4	23.6	0.0	0.0
p,p'-DDT	1.2	ND	9.2	12.7	70.9	16.4	0.0
ΣDDT	4.1	ND	20.9	7.3	45.4	45.4	1.9
Whole lake (344 samples)							
ΣDDT	7.8	ND	220.0	4.7	29.7	56.3	9.3

[a]ND = <0.2 ppb.

TABLE 2

RESIDUES OF DDT, DDT METABOLITES, PCB AND HEOD IN LAKE HURON SEDIMENTS AND THE CONCENTRATIONS IN NON-DEPOSITIONAL AND BASIN DEPOSITIONAL ZONES

	Content in freeze-dried sediments (0—3 cm) in ng/g								
Contaminant	Total lake	Non-depositional zones	Basin zones	Mackinac Basin	Manitoulin Basin	Port Huron Basin	Goderich Basin	Alpena Basin	Saginaw Basin
No. of samples	174	97	77	8	42	5	16	2	4
DDE Mean	3.0	1.2	5.4	2.1	5.8	2.8	7.0	5.0	4.6
SD	4.0	1.5	4.9	2.1	5.1	2.3	5.8	—	2.6
Min.	<0.2	<0.2	<0.2	0.3	<0.2	0.3	0.8	—	2.0
Max.	21.0	9.0	21.0	5.0	21.0	6.1	19.0	—	7.1
TDE Mean	1.7	0.6	3.1	1.0	2.5	0.9	7.2	1.4	1.8
SD	4.1	0.5	5.8	0.5	3.1	0.5	11.0	—	1.5
Min.	<0.2	<0.2	<0.2	0.5	<0.2	0.3	0.3	—	1.0
Max.	44.0	2.6	44.0	2.1	12.0	1.3	44.0	—	4.0
DDT Mean	5.5	2.0	9.9	2.6	9.6	6.9	16.9	4.8	5.9
SD	13.5	2.4	19.3	2.8	9.2	10.3	39.0	—	3.3
Min.	<0.2	<0.2	<0.2	0.8	<0.2	0.4	1.0	—	2.1
Max.	162.0	15.2	162.0	9.2	38.4	24.4	162.0	—	10.1
ΣDDT Mean	10.3	3.8	18.4	5.7	17.9	10.6	31.1	11.1	12.2
SD	19.7	3.8	27.2	4.8	15.8	12.6	52.1	—	5.8
Min.	<0.6	<0.6	<0.6	2.0	<0.6	1.2	2.3	—	5.1
Max.	220.0	23.2	220.0	16.0	64.2	31.6	220.0	—	18.3
PCB Mean	13	11	15	18	12	17	19	9	33
SD	10	7	13	15	6	5	14	—	38
Min.	3	3	3	5	3	10	7	—	8
Max.	90	43	90	50	30	23	65	—	90
HEOD Mean	<0.2	<0.2	<0.2	0.2	<0.2	<0.2	<0.2	<0.2	<0.2
SD	—	—	—	—	—	—	—	—	—
Min.	<0.2	<0.2	<0.2	<0.2	—	—	<0.2	—	—
Max.	1.3	1.3	0.5	0.5	—	—	1.2	—	—

TABLE 3

RESIDUES OF DDT AND DDT METABOLITES, PCB AND HEOD IN GEORGIAN BAY AND NORTH CHANNEL SEDIMENTS AND THE CONCENTRATIONS IN NON-DEPOSITIONAL AND BASIN DEPOSITIONAL ZONES

	Content in freeze-dried sediments (0.3 cm) in ng/g									
Contaminant	Total lake	Non-depositional zones	Basin zones	Nottawasaga Basin	Owen Sound Trough	Lions Trough	Cabot Basin	French River Basin	Flowerpot Basin	North Channel
No. of samples	168	75	93	14	6	2	4	2	7	54
DDE Mean	2.8	1.4	3.0	5.3	3.8	8.4	6.4	2.4	3.1	1.9
SD	2.8	1.7	3.3	4.4	4.5	—	3.9	—	1.3	2.2
Min.	<0.2	<0.2	<0.2	1.0	0.9	—	1.6	—	1.8	<0.2
Max.	16.0	7.6	16.0	16.0	12.0	—	11.0	—	4.9	10.0
TDE Mean	1.3	1.0	1.6	4.3	1.9	1.7	2.4	0.8	1.8	0.9
SD	1.5	0.8	1.8	2.6	1.6	—	1.0	—	1.3	1.0
Min.	<0.2	<0.2	<0.2	1.0	0.3	—	1.5	—	0.8	0.2
Max.	8.5	5.4	8.6	8.6	3.7	—	3.7	—	4.5	4.7
DDT Mean	1.7	1.0	2.2	4.0	2.6	5.9	4.6	4.2	2.3	1.4
SD	2.3	1.6	2.7	4.1	3.8	—	3.1	—	0.6	1.6
Min.	<0.2	<0.2	<0.2	0.2	0.2	—	0.4	—	1.4	<0.2
Max.	12.7	8.1	12.7	12.7	8.6	—	7.7	—	3.2	10.1
ΣDDT Mean	5.3	3.4	6.8	13.7	8.4	15.9	13.3	7.3	7.2	4.1
SD	5.9	3.5	7.0	9.7	9.5	—	7.7	—	2.9	4.1
Min.	<0.6	<0.6	<0.6	2.2	1.4	—	3.7	—	4.0	<0.6
Max.	35.5	17.3	35.5	35.5	24.3	—	22.4	—	11.9	20.9
PCB Mean	11	11	11	20	9	18	16	24	9	8
SD	11	13	8	12	6	—	4	—	3	4
Min.	3	3	3	5	4	—	12	—	6	3
Max.	110	110	50	50	20	—	20	—	13	20
HEOD Mean	0.2	<0.2	0.3	0.4	0.3	1.4	0.4	0.4	<0.2	<0.2
SD	0.3	0.2	0.4	0.4	0.3	—	0.4	—	—	—
Min.	<0.2	<0.2	<0.2	<0.2	<0.2	—	<0.2	—	<0.2	<0.2
Max.	1.7	1.0	1.7	1.0	0.8	—	1.0	—	0.5	1.7

non-depositional and basin zones. For TDE, the percentages were 16 and 17% respectively and for parent DDT it was 52 and 54%.

Georgian Bay—North Channel — ΣDDT. All four components of ΣDDT were present in both the Georgian Bay and North Channel sediments (Tables 1 and 3). DDE was present in 88 and 84% of samples respectively; TDE was present in 87% of samples in both and DDT in 61 and 87% of samples respectively. The mean ΣDDT residues were 5.8 ng/g in Georgian Bay and 4.1 ng/g at North Channel. In the North Channel, the sediment environment was not mapped in terms of non-depositional and basin zones due to grid dimension and the occurrence of shallows. The statistical breakdown for this body of water is for all samples only and shown in Table 3. In Georgian Bay, mean residues for ΣDDT were 6.8 ng/g in basins and 3.4 ng/g in non-depositional zones. In the 4 basins and 2 troughs of Georgian Bay, three had mean residues ranging from 13.3 to 15.9 ng/g and the remainder gave mean residues ranging from 7.2 to 8.4 ng/g. The higher levels were in the Cabot Basin opposite the Muskoka Lakes, in the Nottawasaga Basin opposite the Nottawasaga River and in the Lions Trough (Fig. 2).

The component composition of ΣDDT between the basins and non-depositional zone showed little difference, DDE represented 44 and 41%, TDE represented 24 and 29% and DDT 32 and 30% respectively.

Distribution of ΣDDT. The distribution of ΣDDT is given in Fig. 3A. This shows a distinct increase in ΣDDT in the sediments of the depositional basins as compared to the non-depositional zone. In Georgian Bay, the higher concentrations can be observed in the southern and south eastern part.

Quartz corrected ΣDDT distributions are shown in Fig. 3B. The utility of the quartz correction has been demonstrated and discussed by Frank et al. (1977) and basically serves to eliminate variations in concentration due to textural variation. Such a distribution is thus more sensitive in elucidating source and dispersion of contaminated sediment. From Fig. 3B, a wide dispersion of ΣDDT can be seen which presumably relates to fairly widespread diffuse loadings at low levels. However, three clear sources of ΣDDT can be inferred from the distribution, one in Saginaw Bay in western Lake Huron, Wasaga Beach in southern Georgian Bay (into the Nottawasaga Basin) and in the central part of eastern Georgian Bay. This latter source is uncertain but may well be due to northward coastal movement of DDT contaminated sediment derived from DDT use in the Muskoka Highland (Frank et al., 1974; Miles and Harris, 1973).

Lake Huron — HEOD. HEOD was identified in only 5.7% of the sediment samples with residues at or below 1.3 ng/g. Samples containing HEOD were located in the Goderich and Mackinac Basins and in the non-depositional zone near Saginaw Bay (Tables 2 and 4). The mean residue for the lake was below the <0.2 ng/g detection limit, based on the assumption that samples where no HEOD was detected had a level of 0.1 ng/g.

Georgian Bay—North Channel — HEOD. HEOD was identified in 30% of the sediments from Georgian Bay and 15% of those from the North Channel. The highest residues were 1.7 ng/g and occurred in the North Channel

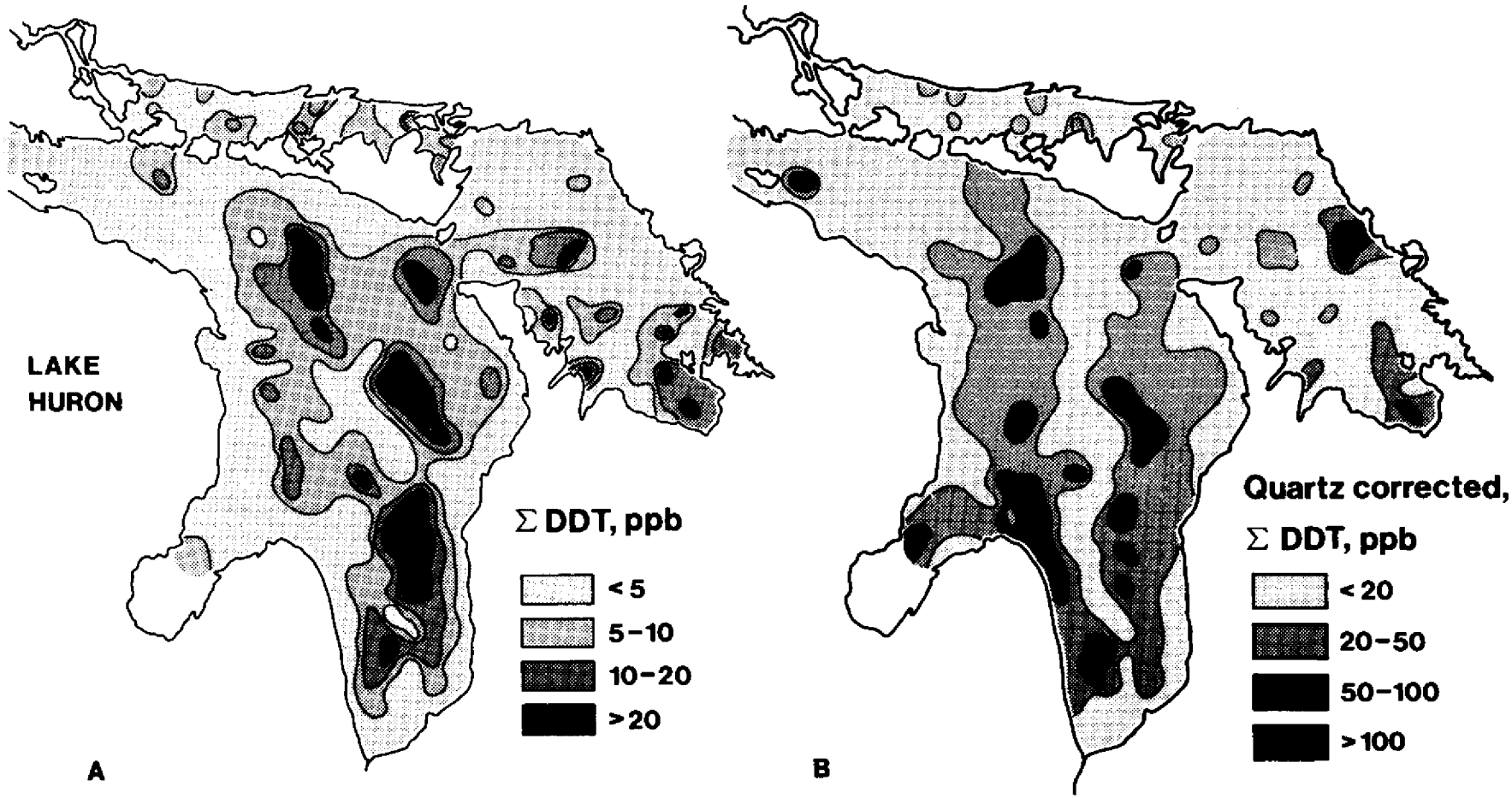

Fig. 3. Distribution of ΣDDT in freeze-dried sediments from Lake Huron, Georgian Bay and North Channel. A: observed values; B: quartz corrected values (0—3 cm).

(Table 3). Residues of HEOD were only fractionally higher in the basins (0.3 ng/g) than the non-depositional zones (<0.2 ng/g). The highest single readings appeared in the North Channel, Lions Trough and the Nottawasaga Basin (Table 3). Mean residues were lower in the North Channel (0.08 ng/g) than in Georgian Bay (0.31 ng/g) (Table 4).

Lake Huron—Heptachlor Epoxide (HE). HE was identified in 8.5% of samples with a maximum residue of 1.0 ng/g. Samples with HE occur primarily on the U.S. side around Saginaw Bay and the upper peninsula of Michigan. Occasional samples in which HE could be identified were also found in the Mackinac Basin (Table 4).

Georgian Bay—North Channel — Heptachlor Epoxide (HE). HE was present in 23% of sediments from Georgian Bay and 14% from North Channel. Mean residues for these two bodies of water were 0.14 ng/g and 0.15 ng/g respectively (Table 4). HE was identified in both the inlet and outlet areas to the North Channel.

TABLE 4

CONTENT OF CYCLODIENES AND PCB IN DRIED LAKE SEDIMENTS FROM THE LAKE HURON, (1969), GEORGIAN BAY AND NORTH CHANNEL (1973)

	Content in dried sediment (ng/g)			ND <0.2	0.2 2.0	2.1 20.0	21 200
Contaminants	Mean	Min.[a]	Max.	(%)	(%)	(%)	(%)
Lake Huron (Main Lake — 174 samples)							
HEOD	0.09	ND	1.3	94.3	5.7	0.0	0.0
Heptachlor epoxide	0.07	ND	1.0	91.4	8.6	0.0	0.0
Endosulfan	0.00	ND	ND	100.0	0.0	0.0	0.0
PCB	13.00	3	90.00		0.0	87.9	12.1
Georgian Bay (115 samples)							
HEOD	0.21	ND	1.7	70.4	29.6	0.0	0.0
Heptachlor epoxide	0.14	ND	1.1	77.4	22.6	0.0	0.0
Endosulfan	0.15	ND	7.3	95.7	0.0	4.3	0.0
PCB	13.00	3	110.0		0.0	88.7	11.3
North Channel (55 samples)							
HEOD	0.08	ND	1.7	85.4	14.6	0.0	0.0
Heptachlor epoxide	0.15	ND	1.4	83.6	16.4	0.0	0.0
Endosulfan	0.03	ND	1.2	96.4	3.6	0.0	0.0
PCB	8.00	3	20.0		1.8	98.0	0.0
Whole lake (344 samples)							
HEOD	0.13	ND	1.7	91.6	8.4	0.0	0.0
Heptachlor epoxide	0.11	ND	1.4	82.9	17.1	0.0	0.0
Endosulfan	0.05	ND	7.3	98.0	0.6	1.4	0.0
PCB	12.00	3	110.0		0.3	89.8	9.9

[a]ND { <0.2 (HEOD) (heptachlor epoxide)
<0.5 (endosulfan)

Its presence in Georgian Bay was primarily off Manitoulin Island near the outlet to North Channel and in upper Georgian Bay. Residues were also found in the lower central reaches of Georgian Bay between the Muskoka River and the Bruce Peninsula.

Georgian Bay—North Channel — Endosulfan. Endosulfan was present in 4% of samples from both Georgian Bay and North Channel, but no residues were identified in sediments from Lake Huron (Table 4). This percentage consists of five sediments in Georgian Bay and two in North Channel. The highest concentration in a single sediment occurred in the Owen Sound Trough. Other values occurred in single samples at widely separated points and remained unexplained. Residues contained 0.1 to 2.3 β-endosulfan and 0.3 and 5.5 endosulfan sulfate. No α-isomer was identified. The mean of these 7 readings was 1.2 ng/g β-endosulfan and 1.8 ng/g endosulfan sulfate.

Lake Huron — PCB. All sediment samples contained PCB residues with a range in values of 3 to 90 ng/g, and a mean residue of 13 ng/g (Table 2). Unlike DDT, PCB residues in the non-depositional zone (11 ng/g) were similar to the levels observed in the basins (15 ng/g). Among the basins, Saginaw Basin (33 ng/g) contained the highest mean residue and Alpena Basin the lowest (9 ng/g).

Georgian—Bay North Channel — PCB. All sediments from both Georgian Bay and North Channel contained detectable residues of PCB. The mean concentrations were respectively 13 and 8 ng/g for Georgian Bay and North Channel (Table 3). Similar residues were found in both the non-depositional zones (11 ng/g) and the basin zones (11 ng/g). Among the basins in Georgian Bay, the Owen Sound Trough and Flowerpot Basin were lowest (9 ng/g) while the remainder ranged from 16 to 24 ng/g.

Distribution of PCB. The distribution of PCB and quartz corrected PCB are given in Figs. 4A and 4B. The distribution of PCB in Fig. 4A shows only a general relationship to the depositional basins, with higher values in the deeper water depositional regions. This relationship is masked in part by nearshore areas of high concentration which appear to be related to direct inputs (Fig. 4A). These sources can be better observed in the quartz corrected values given in Fig. 4B. In Lake Huron, two sources are significant, one from Alpena and the other from Saginaw Bay. The input from Alpena has a small areal impact whereas the Saginaw Bay input has had a more extensive impact on the lake. From Fig. 4B high values from Saginaw Bay can be observed dispersed to the north with a second zone extending eastwards around the "thumb" of Michigan and continuing southwards to the St. Clair River. This pattern is similar to that observed by Thomas (1973) for sediment-bound mercury and agrees with the known surface water movements of southern Lake Huron. Transportation of the PCB associated with this sediment presumably forms the major source of PCB to Lake St. Clair (Frank et al., 1977) In Georgian Bay, substantial inputs of PCBs appear to be occurring from the northeast shoreline (Fig. 4B), yet the direct source is unknown.

Other organochlorines. No residues of aldrin, chlordane, endrin, heptachlor, methoxychlor or mirex were detected in sediments from all three waters. In addition, no endosulfan was detected in the main lake.

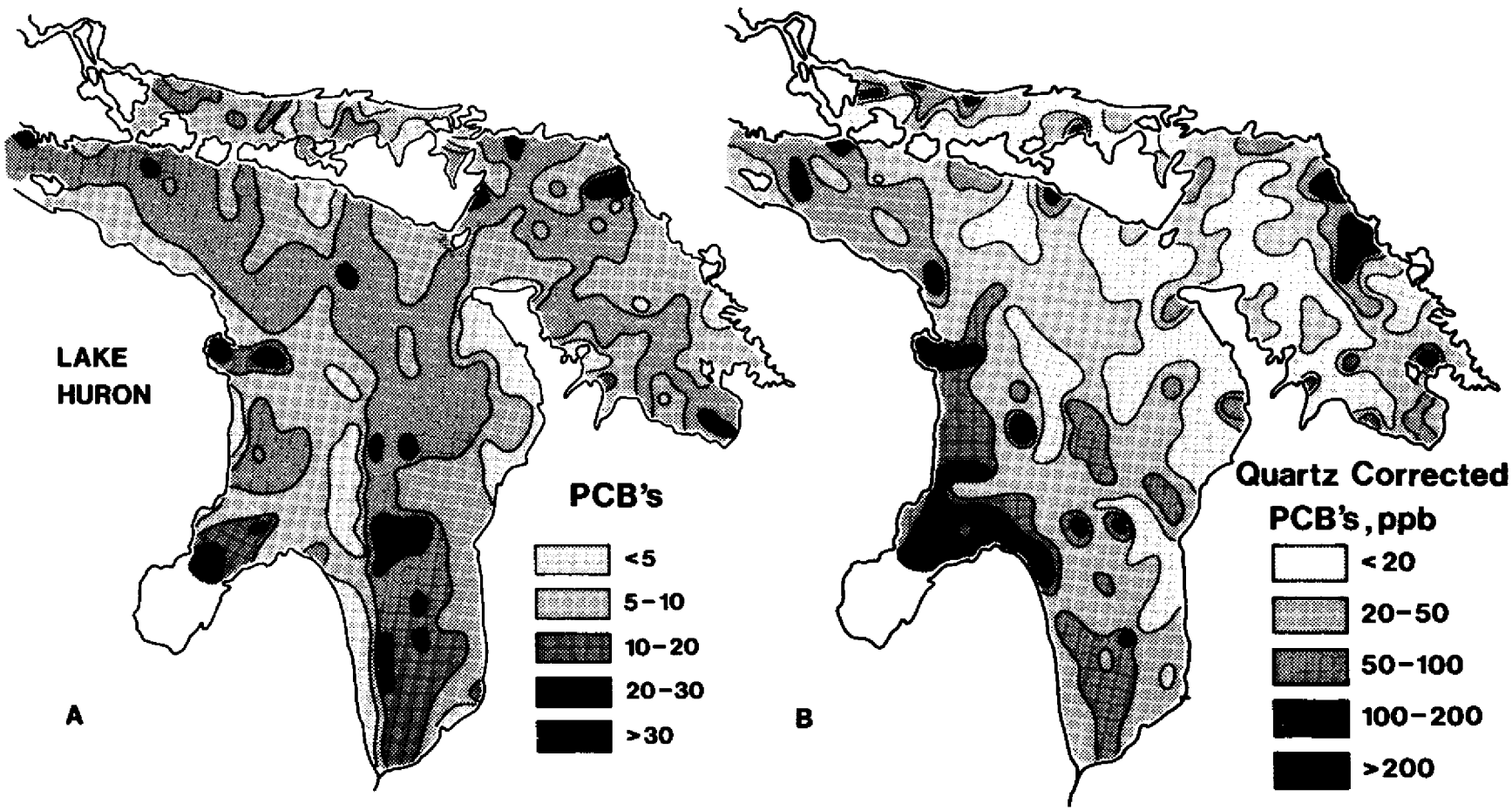

Fig. 4. Distribution of PCB in freeze-dried sediments from Lake Huron, Georian Bay and North Channel. A: observed values; B: quartz corrected values (0—3 cm).

Core sediments

Sedimentation rates in the cores analysed from Lake Huron and Georgian Bay are extremely low (Kemp and Harper, 1977). Values range from 0.2 mm/yr in cores C-7A and C-12A to 1.7 mm/yr in core U-9. One centimeter of sediment accumulation thus represents a range of 6 to 50 years of accumulation and any core sub-sampled at 2.0 cm increments cannot give a precise measurement of the date of appearance of a compound. This is very different to the situation observed in Lake Erie where high sedimentation rates enabled the establishment of relatively precise chronology of the appearance of DDT and PCB (Frank et al., 1977).

ΣDDT. In the two cores in Lake Huron (DH and U-9), DDE, TDE and DDT were all detected in the 0—2 cm depth at both locations and only DDE at 2—4 cm in U-9 (Table 5). At location U-9 a total of 8 ng/g ΣDDT was detected in the surface 0—2 cm representing accumulation from 1960—1971. This was based on the sedimentation rate reported by Kemp and Harper (1977). The residue in the 2—4 cm layer was 4 ng/g and represented accumulation from 1948—1959, indicating that DDT first occurs in the early to mid 1950's.

In the second core taken from the deep hole (DH) in the Manitoulin Basin

TABLE 5

RESIDUES IN TWO CORES TAKEN IN LAKE HURON, GEORGIAN BAY

Site years		Depth (cm)	Contents in dried sediment (ng/g)				
			DDE	TDE	DDT	ΣDDT	PCB
Lake Huron							
D.H.	1943—1971	0—2	8	5	9	22	100
(1971)	1915—1942	2—4	ND	ND	ND	ND	ND
(0.7 mm/yr)	1829—1914	4—10	ND	ND	ND	ND	ND
U9	1960—1971	0—2	3	2	3	8	30
(1971)	1948—1959	2—4	4	ND	ND	4	10
(1.7 mm/yr)	1936—1947	4—6	ND	ND	ND	ND	10
	1894—1935	6—10	ND	ND	ND	ND	ND
Georgian bay							
C7A	1924—1974	0—1	14	6	8	28	20
(1974)	1424—1924	1—10	ND	ND	ND	ND	ND
(0.2 mm/yr)							
C12A	1924—74	0.1	8	2	7	17	9
(1974)	1824—1923	1—3	ND	ND	ND	ND	ND
(0.2 mm/yr)							
J3A	1950—1974	0—1	14	5	10	29	5
(1974)	1925—1949	1—2	5	5	2	12	5
(0.4 mm/yr)	1725—1925	2—10	ND	ND	ND	ND	ND
South bay							
SB	1920—1970	0—3	18	9	9	36	20
(1970)	1808—1920	3—10	ND	ND	ND	ND	ND
(0.6 mm/yr)							

the surficial 2 cm contained 22 ng/g ΣDDT which represents accumulation from 1943 to 1971. This, again, is compatible with a first occurrence of DDT in the 1950's.

In the three cores in Georgian Bay, DDE, TDE and DDT were detected in the 0—1 cm depth (Table 5). At site C-7A a mean of 28 ng/g was detected in the surficial one centimeter representing accumulation from 1924—1974. At site C-12A the residue was 17 ng/g and again represented accumulation from 1924—74 based on sedimentation rates by Kemp and Harper (1977). At site J-3A DDT was present in both the 0—1 and 1—2 cm layers. The 1—2 cm represents accumulation between 1925 and 1949 and the occurrence of DDT here is not compatible with a first appearance of the compound in the 1950's as postulated from the other cores. This is as yet unexplained but may well represent a 2-cm mixing zone resulting from bioturbation.

In the core from South Bay, DDE, TDE and DDT were detected in the 0—3 cm depth at 36 ng/g representing accumulation from 1920—1970.

PCB. At site U-9 in Lake Huron PCB residues were found to a depth of 6 cm covering the period 1936—1971. In sediments from the deep hole (DH), PCB residues were present to a depth of 2 cm and represented sedimentation between 1943 and 1971. PCBs were first introduced in North America around 1938. However, in earlier work on Lake Erie, Frank et al. (1977) noted that PCB first occurred in 1954. The evidence in all the cores exclusive of U-9 and J-3A are quite compatible with this timing. U-9 probably presents the first evidence for the occurrence of PCBs in the Great Lakes prior to the 1950's with introduction in the period 1936 to 1947. As noted earlier for DDT, core J-3A may have been subjected to bioturbation accounting for the occurrence of PCB in the time period 1925 to 1947. It is quite evident that fine resolution of the historical evolution of organochlorine contamination cannot be worked out on the basis of a 1.0 or 2.0 centimeter sediment increment with the low sedimentation rate occurring in this lake. Finer sub-sampling will have to be undertaken in future studies.

Annual loadings of ΣDDT and PCB

The annual loadings of ΣDDT and PCB (Table 6) were calculated from the sedimentation rates given by Kemp and Harper (1977). The long-term annual sedimentation rates in the depositional regions of Lake Huron only were multiplied by the mean concentration of the parameter of interest for the same depositional sediment. Kemp and Harper (1977) give a range of accumulation of between 0.3 and 3.1 mm/yr for Lake Huron. The highest loading occurs in the Goderich Basin, in the lower part of the lake for both ΣDDT and PCB. The total annual accumulation of 55.2 and 48.7 kg/annum of ΣDDT and PCB respectively is low when compared with the estimated loading in Lake Ontario of 246 and 352 kg/annum and for Lake Erie 1000 and 3400 kg/annum of ΣDDT and PCB respectively (Frank et al., 1978; 1977).

The values given above are for the main body of the lake since no values for annual sediment accretion are available for Georgian Bay and North Channel.

TABLE 6

ESTIMATED ANNUAL ACCUMULATION OF SEDIMENT AND ASSOCIATED ΣDDT AND PCB IN LAKE HURON BASIN SEDIMENTS

Basin	Sediment accumulation (10^3 metric tons/yr)	Concentration		Accumulation	
		ΣDDT (ng/g)	PCB (ng/g)	ΣDDT (Kg/annum)	PCB (Kg/annum)
Mackinac	224	5.7	18	1.3	4.0
Manitoulin	907	17.9	12	16.2	10.9
Alpena	188	11.1	9	2.1	1.7
Saginaw	240	12.2	33	2.9	7.9
Port Huron	408	10.6	17	4.3	6.9
Goderich	913	31.1	19	28.4	17.3
Total as mean	2,880	18.4	15	55.2	48.7

Inter-relationship between parameters

Linear correlation matrices for Lakes Huron and Georgian Bay are given in Tables 7 and 8 respectively. In Lake Huron (Table 7) highly significant relationships can be observed between Al_2O_3, K_2O and Org C reflecting the illitic nature of Lake Huron sediment and associated organic matter (Sly and Thomas, 1974). Additionally, significant relationships can be observed between Org C and DDT and its metabolities suggesting that these compounds are sorbed or chelated by the organic matter. A further high degree of correlation can be observed between ΣDDT, HEOD and PCB indicating a general association of these compounds, even though a direct relationship of PCB and HEOD to organic matter cannot be inferred from these data. This is likely due to slight variations in the use patterns in the basin and, in the case of PCBs the greater significance of more localized discharge regions in Saginaw Bay and Alpena.

TABLE 7

CORRELATION MATRIX SHOWING DEGREE OF LINEAR RELATIONSHIP BETWEEN VARIABLES FOR 110 SAMPLES FROM LAKE HURON

Confidence limit 99.0%, $r = 0.320$; 95.0%, $r = 0.195$.

	Al_2O_3	K_2O	Org C	DDE	TDE	DDT	ΣDDT	HEOD	PCB
Al_2O_3	1.0								
K_2O	0.942	1.0							
Org C	0.561	0.607	1.0						
DDE	0.414	0.423	0.620	1.0					
TDE	0.187	0.190	0.422	0.590	1.0				
DDT	0.196	0.195	0.349	0.567	0.897	1.0			
ΣDDT	0.245	0.246	0.436	0.687	0.936	0.983	1.0		
HEOD	0.025	0.058	0.129	0.194	0.569	0.761	0.712	1.0	
PCB	0.204	0.244	0.245	0.319	0.319	0.573	0.574	0.528	1.0

TABLE 8

CORRELATION MATRIX SHOWING DEGREE OF LINEAR RELATIONSHIP BETWEEN VARIABLES FOR 129 SAMPLES FROM GEORGIAN BAY AND NORTH CHANNEL

Confidence limit 99.0%, $r = 0.320$; 95.0%, $r = 0.195$.

	Al_2O_3	K_2O	Org C	DDE	TDE	DDT	ΣDDT	HEOD	PCB
Al_2O_3	1.0								
K_2O	0.826	1.0							
Org C	0.278	0.396	1.0						
DDE	0.289	0.345	0.589	1.0					
TDE	0.226	0.254	0.491	0.620	1.0				
DDT	0.207	0.214	0.412	0.875	0.423	1.0			
ΣDDT	0.278	0.314	0.566	0.973	0.716	0.912	1.0		
HEOD	0.164	0.237	0.374	0.353	0.237	0.193	0.303	1.0	
PCB	0.058	0.019	0.178	0.375	0.197	0.411	0.388	0.251	1.0

The relationships observed in the sediments of Georgian Bay and North Channel (Table 8) parallel those observed in Lake Huron though values for the correlation coefficients are of a lower level of significance. The relationships, however, may be explained in a similar manner as for Lake Huron discussed above.

CONCLUSION

Residues of both ΣDDT and PCB are widely distributed in Lake Huron, Georgian Bay and North Channel sediments. These residues are considerably lower than those found in Lakes Erie and Ontario (Frank et al., 1977 and 1978). This is also reflected by the lower estimated annual loadings of these two compounds to the lake sediments. From the distribution of both ΣDDT and PCB, some "regional point sources" can be identified the most significant one of which is Sagenaw Bay. It is uncertain whether the point sources can account for the total observed distribution which may in part be related to atmospheric flux.

The resolution of PCB and ΣDDT profiles in cores prove to be insufficient to provide a good estimate of the onset of loadings to the lake. However, a general inference of early 1950's as noted in previous studies on Lake Erie can be made.

PCB, HEOD and DDT showed a high degree of statistical relationship. DDT in turn showed affinity to organic carbon. It appears that the compounds are bound by organic matter in the lake sediments with variations attributable to variations in use pattern and localized sources.

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Confirmation of Mirex and *cis*- and *trans*-Chlordane in the Presence of Other Organochlorine Insecticides and Polychlorinated Biphenyls

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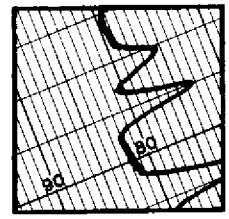

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Confirmation of Mirex and *cis*- and *trans*-Chlordane in the Presence of other Organochlorine Insecticides and Polychlorinated Biphenyls

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The ability of certain pesticides to withstand strong acid treatment was utilized in developing a confirmation procedure for mirex and *cis*- and *trans*-chlordane. SCHECHTER *et al.* (1945) applied intensive nitration to DDT residues from plant extracts using a 1:1 mixture of concentrated sulfuric and fuming nitric acid and subsequently determined the nitrated DDT's colorimetrically. ERRO *et al.* (1964) modified this procedure to determine toxaphene in the presence of DDT.

Mirex analysis is often complicated by the presence of PCB's and other organochlorine insecticides. On most GLC columns mirex co-elutes with a heptachlorobiphenyl compound, necessitating some form of separation before quantitation. REYNOLDS (1969), ARMOUR and BURKE (1970), and HOLDRINET (1972) modified existing adsorption chromatography systems to allow PCB and organochlorine separation from mirex before GLC determination. These methods require careful control of the adsorbant activity and are subject to non-reproducibility. Additional confirmation, especially for regulatory purposes, is required. HALLET *et al.* (1976) used perchlorination, NORSTROM (1976) described the use of the Hall detector, and KAISER (1974) and HALLET *et al.* (1976) described a GLC-MS system for mirex confirmation. Mass spectrometric determination of mirex presents some difficulties; the major fragment $C_5Cl_6^+$ can arise from a variety of compounds, particularly from PCB components, and care in the interpretation of the mass spectrometric data must be exercised.

Plant and animal co-extractives often interfere with the electron capture determination of *cis*- and *trans*-chlordane. CHAU and COCHRANE (1969) and COCHRANE (1969) developed a confirmation procedure based on dehydrochlorination. This procedure is lengthy and co-extractives present may produce a confusing chromatogram.

METHODS AND MATERIALS

After extraction and preliminary column clean-up (HOLDRINET 1974), evaporate the extract to dryness in a 125-ml boiling flask using rotary vacuum evaporation. Add 5 ml of a freshly prepared mixture of concentrated sulfuric:fuming nitric acid 1:1 (v/v). Swirl the flask to insure acid contact with the sides, stopper and place in a waterbath at 70°C for ½ hr. Transfer the acid mixture quantitatively with 3 X 10 ml of dichloromethane to a 500-ml separatory funnel containing 200 ml of tap water. Add a further

30 ml of dichloromethane, shake the funnel 1 min, allow the phases to separate and drain the dichloromethane layer into a second 500-ml separatory funnel containing 100 ml of tap water. Re-extract the original 200 ml of tap water with an additional 60 ml of dichloromethane, and add the organic layer to the second separatory funnel and discard the water. Shake the second separatory funnel 1 min, allow the phases to separate and drain the dichloromethane back into the first 500-ml separatory funnel. Add 60 ml of 5% $NaHCO_3$, shake 1 min, allow the phases to separate and filter the organic layer over Na_2SO_4 into a 250-ml boiling flask. Rinse the Na_2SO_4 with 3 X 10 ml of dichloromethane and collect the rinsings into the flask. Evaporate to dryness on a rotary evaporator, make up in hexane (10 ml) and transfer an aliquot to a Florisil clean-up column (MILLS *et al.* 1972). Collect the first fraction, evaporate on a rotary evaporator, make up with a suitable volume of hexane and proceed to GLC determination.

Gas chromatographic instrumentation and conditions used to carry out the analyses: Tracor MicroTek 550 equipped with Ni^{63} EC detector; Pyrex column, 2 m x 4 mm i.d. packed with 2.5% OV-17 plus 2.5% OV-210 on Gas Chrom Q, 100-120 mesh, at 180°C; detector temperature 310°C; nitrogen carrier flow 60 ml/min; all injections were 5 µl.

RESULTS AND DISCUSSIONS

Figure 1 illustrates a mixture of organochlorine insecticides before nitration (A) and after nitration (B). Amounts of insecticides injected were: HCB and lindane, 0.05 ng each; oxychlordane, heptachlor-epoxide, *trans*-chlordane, *cis*-chlordane, p,p'-DDE and dieldrin, 0.1 ng each; o,p'-DDT, p,p'-TDE and mirex, 0.25 ng each. After nitration and clean-up only lindane, *trans*-chlordane, *cis*-chlordane and mirex remain (same amount injected). Figures 2 and 3 show chromatograms of unnitrated (A) and nitrated (B) Aroclor 1254 and Aroclor 1260 respectively; 1 ng injected. Figure 4 shows chromatograms of a fish sample, fortified with *trans*- and *cis*-chlordane at 0.02 ppm and mirex at 0.1 ppm before nitration (A) and after nitration (B). Mirex recoveries ranged from 90% to 100% (30 determinations) and *cis*- and *trans*-chlordane recoveries ranged from 80% to 95% (10 determinations).

To insure complete nitration of PCB's a reaction time of ½ hr at 70°C is required. The column clean-up procedure is necessary to separate the nitrated products from mirex and *cis*- and *trans*-chlordane. Because of the small acid mixture volume, boiling flasks larger than 125 ml should be avoided. Care in transferring the acid to the water should be exercised.

The nitration procedure has been used successfully in this laboratory to confirm mirex, and *cis*- and *trans*-chlordane in such substrates as water, sediment, sludge, shearwaters, fish, seals, milk and human fat. Interferences were not encountered except in one sample of industrial sludge.

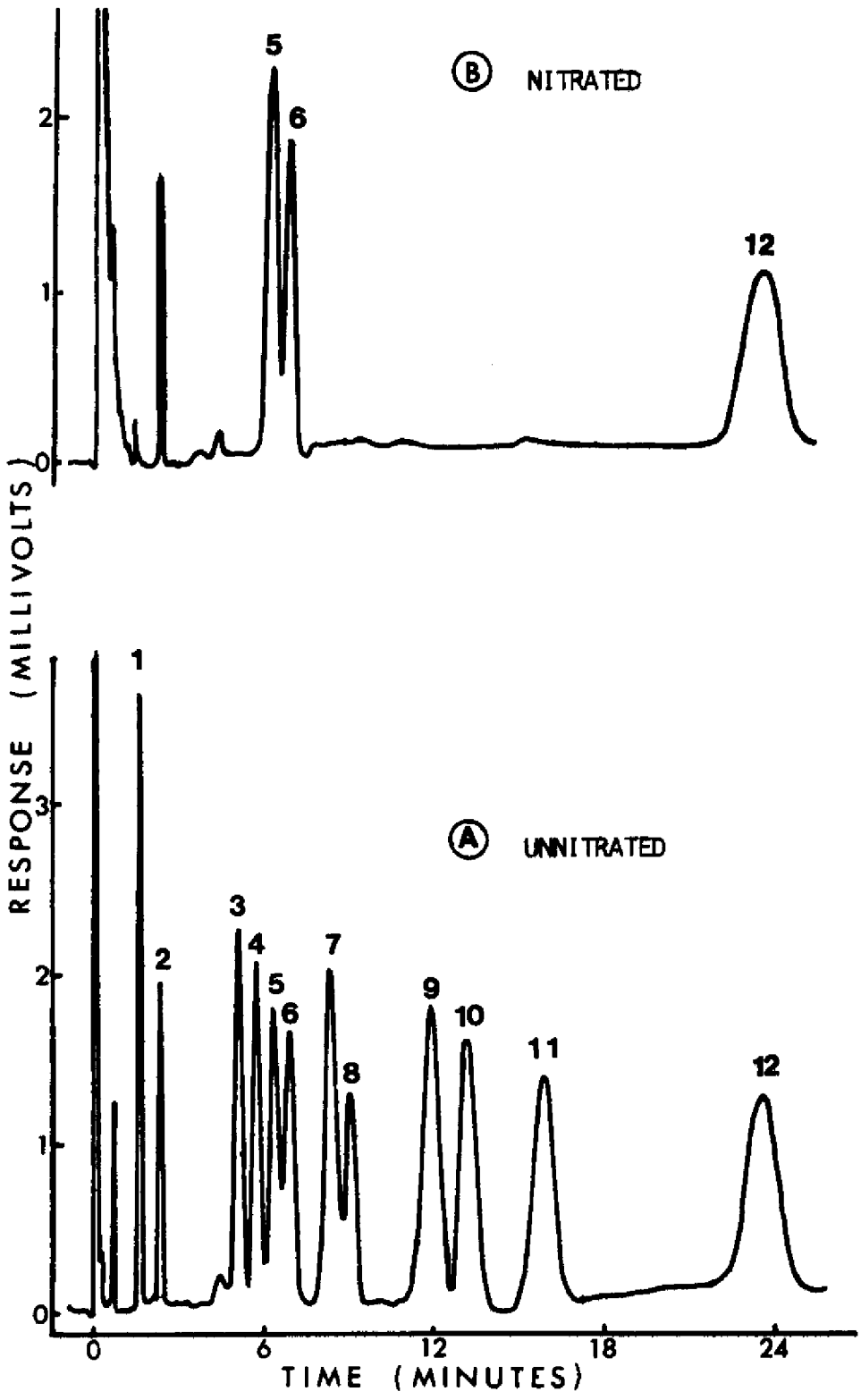

Figure 1. Gas chromatogram of an organochlorine mixture before (A) and after (B) nitration, 1-HCB, 2-lindane, 3-oxychlordane, 4-heptachlorepoxide, 5-*trans*-chlordane, 6-*cis*-chlordane, 7-p,p'-DDE, 8-dieldrin, 9-o,p'-DDT, 10-p,p'-TDE, 11-p,p'-DDT, 12-mirex.

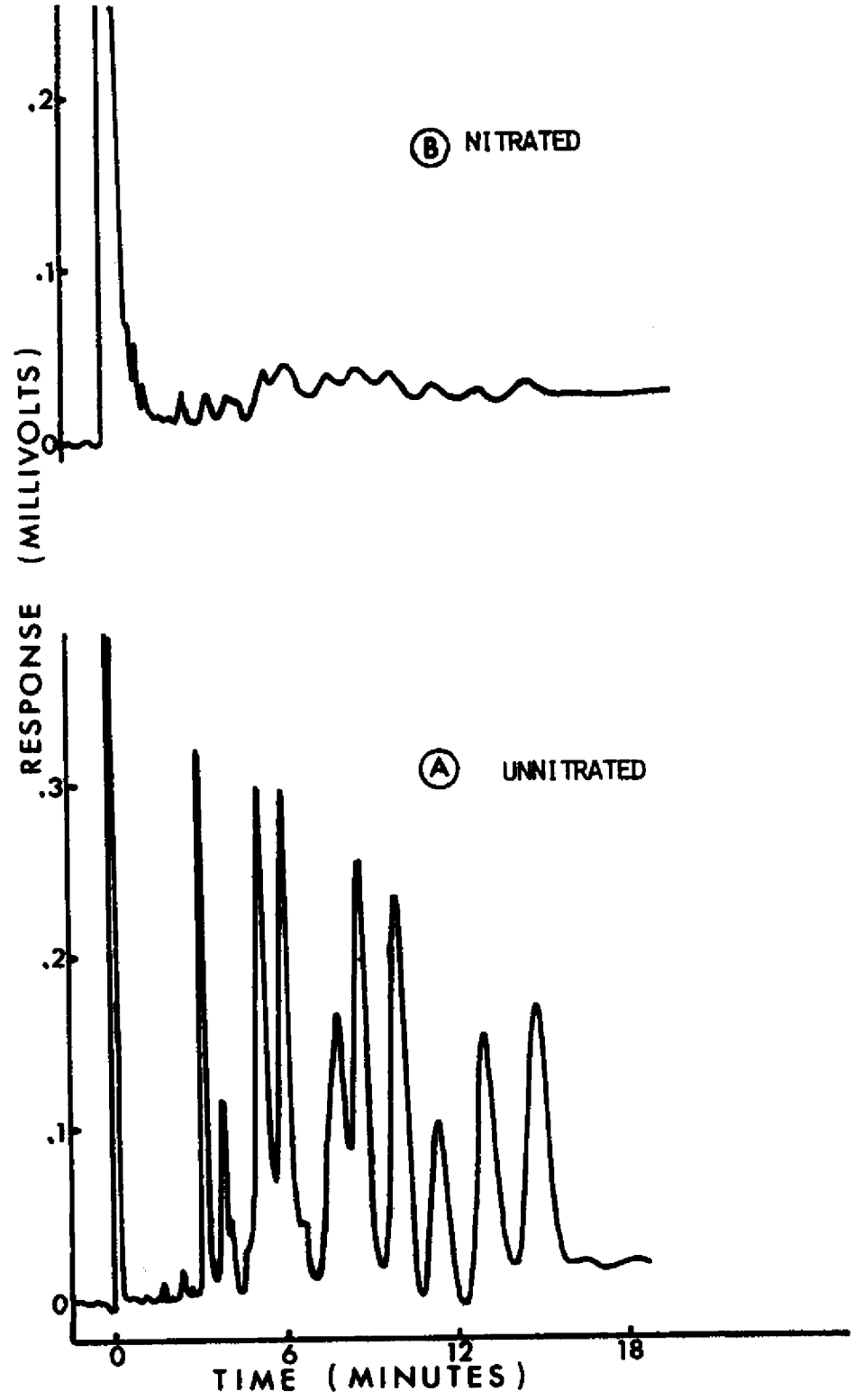

Figure 2. Gas chromatogram of Aroclor 1254 before (A) and after (B) nitration.

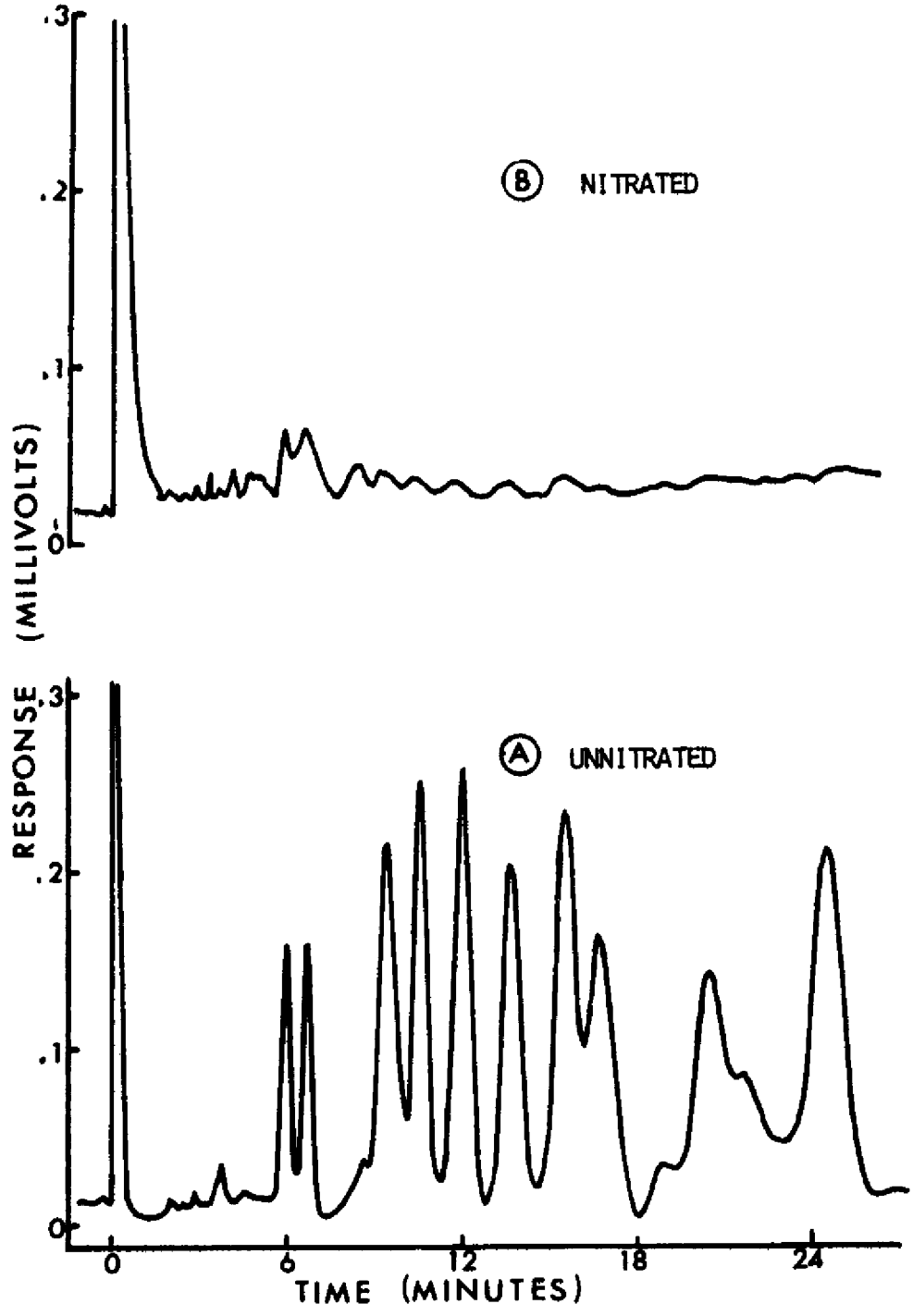

Figure 3. Gas chromatogram of Aroclor 1260 before (A) and after (B) nitration.

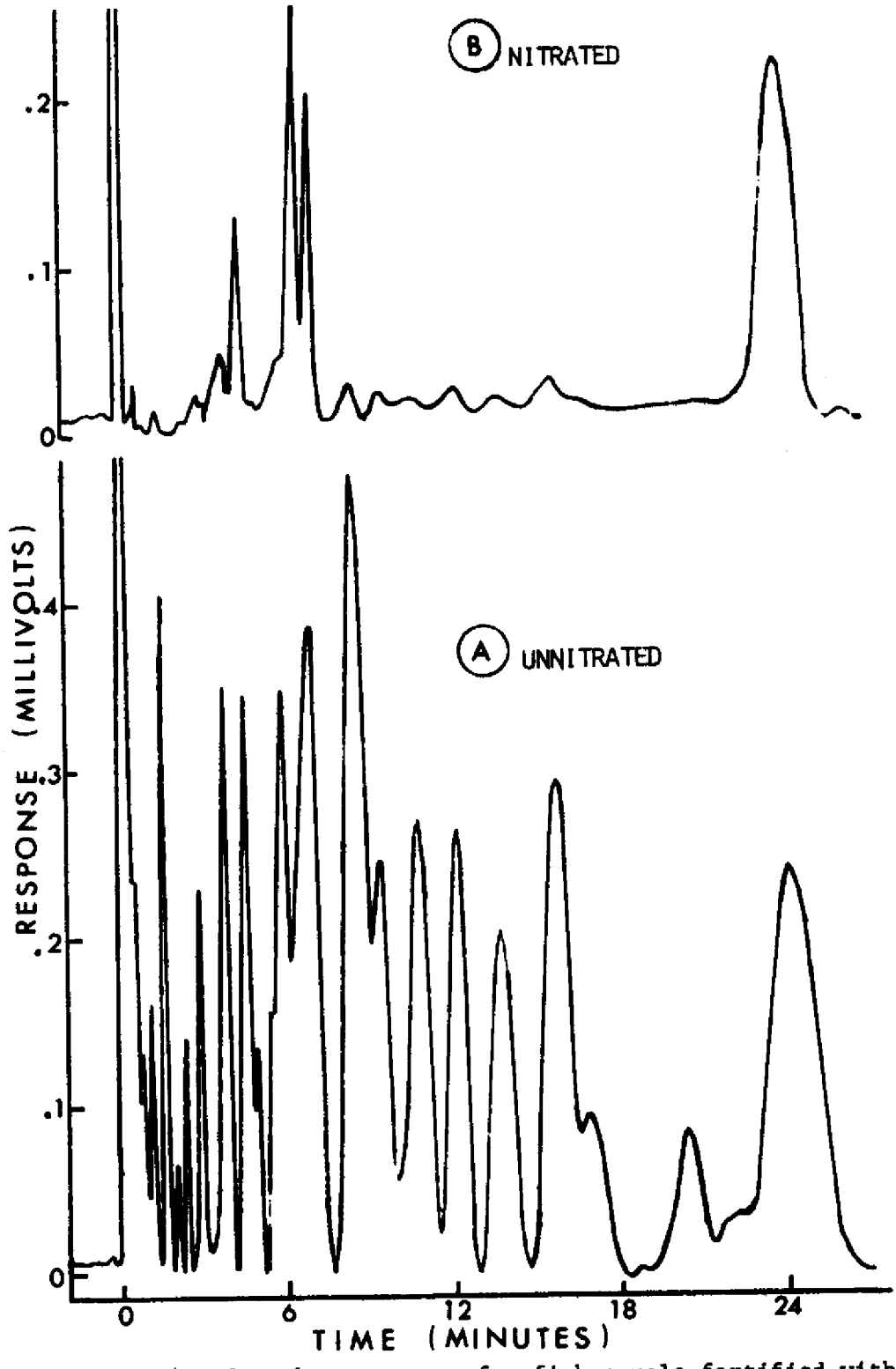

Figure 4. Gas chromatogram of a fish sample fortified with *trans*- and *cis*-chlordane and mirex (1 g fish tissue/ml) before (A) and after (B) nitration.

The method is simple, reasonably rapid and the nitrated extract can be analysed by electron capture, Hall or Coulson GLC detection systems, or by GLC-mass spectrometry without problems associated with interferences.

Lindane remains unnitrated (Fig. 1) but recoveries are erratic. Before lindane is incorporated in this procedure, further investigation is required.

This procedure may be used as a simplified analytical method when only mirex and/or *cis*- and *trans*-chlordane are to be determined.

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Determination and Confirmation of Hexachlorobenzene in Fatty Samples in the Presence of Other Residual Halogenated Hydrocarbon Pesticides and Polychlorinated Biphenyls

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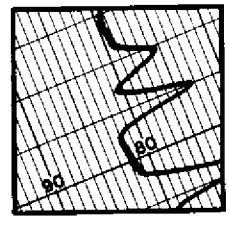

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Determination and Confirmation of Hexachlorobenzene in Fatty Samples in the Presence of Other Residual Halogenated Hydrocarbon Pesticides and Polychlorinated Biphenyls

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Hexachlorobenzene (HCB) determination and confirmation at low residual levels (parts per billion range) is hampered by the presence of electron-capturing co-extractives, other organochlorine pesticides, and polychlorinated biphenyls (PCBs). A method is described to routinely examine lipid material for residual HCB, using a column chromatographic separation system and subsequent derivatization. Following initial extraction and cleanup, organochlorines, PCBs, and HCB are successively eluted from a charcoal column. After gas chromatographic analysis of each of the fractions, the HCB fraction is subjected to caustic alkali at high temperatures, and the hydrolyzed product is methylated to yield the pentachlorophenol methyl ether derivative.

Hexachlorobenzene (HCB) is produced by catalytic chlorination of benzene (1) for use as a selective fungicide in cereal seeds, and inadvertently as a byproduct from such organic syntheses as the production of pentachloroethylene and vinyl chloride (2). Misuse of HCB as a fungicide and disposal of HCB by the plastic industry in waste waters can contaminate the entire food chain: dairy products, cereal products, domestic animals, fish, shellfish, aquatic and terrestial birds, and man. Although HCB has a low acute toxicity (500 mg/kg is not lethal to rats) (3), subacute chronic exposure is hazardous, suggesting high cumulative toxicity. Rats and quail fed subacute amounts of HCB daily for different lengths of time all exhibited adverse effects varying in intensity with the total amount of compound ingested (4–6; unpublished data, The Dow Chemical Co.). Misuse of HCB was traced as the cause of an outbreak of porphyria in Turkey (4). HCB residues have been reported in human fat in Australia (7), in birds of prey and owls in The Netherlands (8), and in tern eggs in Hamilton Harbour, Canada (9). Because HCB is not altered in biological systems, and undergoes biomagnification (10), an analytical procedure that permits confident qualitation as well as quantitation of residues in the parts per billion range is necessary. Most methods available rely on a general, electron capture gas-liquid chromatographic (GLC) screening procedure for organochlorines, with confirmation on GLC columns of different polarity (7–9, 11). Thin layer chromatography (TLC) is used only in isolated high residue cases (>0.1 ppm in extracted fat). HCB as well as electron-capturing co-extractives elute early on all GLC columns and relying solely on GLC methods for confirmation can lead to erroneous interpretation. A method is described herein that permits separation of HCB from other electron-capturing contaminants and its subsequent confirmation through derivatization.

METHOD

Apparatus and Reagents

(a) *Soxhlet extractors.*—50 mm id × 250 mm long.

(b) *Rotary vacuum evaporator.*

(c) *Chromatographic columns.*—(*1*) *Cleanup columns.*—25 × 300 mm Pyrex, fitted with Teflon stopcock and 350 ml solvent reservoir. (*2*) *Charcoal separation columns.*—9 × 250 mm Pyrex, fitted with Teflon stopcock and 200 ml solvent reservoir.

(d) *Ottawa sand.*—Fisher Scientific Co., 20–30 mesh.

(e) *Sodium sulfate.*—Anhydrous, granular.

(f) *Florisil.*—60–100 mesh (Floridin Co.), activated commercially at 1200°F and reheated 24 hr at 135°C. Partially deactivate after cooling with 5 ml water/100 g Florisil, store in air-tight container, and let equilibrate 24 hr while tumbling.

(g) *Charcoal.*—50–200 mesh (Fisher No. 5-690, no substitutes). Remove charcoal from container without pretumbling after discarding top 1" (bottom 2" also not used). Wash charcoal twice with excess acetone, let air-dry, and store in open container 72 hr at 135°C before use. Mechanically tumble container 3 min before filling column with prepared charcoal.

(h) *Solvents.*—Reagent grade ethylene glycol; distilled-in-glass methylene chloride, hexane, acetone, ethyl ether (2% ethanol added), benzene, and toluene.

(i) *Diazomethane.*—Prepare from Diazald, using diazomethane generator kit and following instruc-

tions supplied with kit to obtain diazomethane in ether solution. Exercise caution in preparation and use of this compound as it is extremely toxic and explosive. (Both Diazald and diazomethane generator kit are available from Aldrich Chemical Co., Inc.)

(j) *Detergent reagent.*—Prepare by dissolving 200 g sodium tetraphosphate in ≃2 L distilled water, add 100 ml Triton X-100, mix, and dilute mixture to 4 L with distilled water.

(k) *Gas chromatographs.—(1) Organochlorines, polychlorinated biphenyls (PCBs), and HCB.*—Micro-Tek Model MT 220 gas chromatograph equipped with ^{63}Ni electron capture detector; 6′ × ⅛″ id U-shaped glass column packed with 4% SE-30 + 6% QF-1 (applied mixed as a solution) on 80–100 mesh Chromosorb W (acid-washed, dimethylchlorosilane-treated), preconditioned 72 hr at 215°C and 30 ml nitrogen/min. Operating parameters: nitrogen carrier gas 60 ml/min, injector 220°C, column 180°C, detector 265°C, all injections 5 μl (2.5 pg HCB at 8×10^{-2} attenuation gave 0.2 mv deflection with 1 mv recorder span).

(*2*) *PCB methyl ether.*—Varian Aerograph Model 1400 gas chromatograph equipped with tritium electron capture detector; 6′ × ⅛″ id coiled Pyrex column packed with 6% Carbowax 20M on 80–100 mesh Aeropak 30, preconditioned 72 hr at 220°C and 50 ml nitrogen/min. Operating parameters: nitrogen carrier gas 60 ml/min, inlet 230°C, column 150°C, detector block 240°C to give foil temperature of 200°C, all injections 5 μl.

Extraction

(a) *Animal tissue.*—Thoroughly mix 10 g homogenized sample with 100 g Na_2SO_4 and 25 g Ottawa sand until homogeneous mixture is obtained. Transfer to Soxhlet extraction apparatus and extract 6 hr with 250 ml hexane. Remove solvent with rotary vacuum apparatus at 50°C and determine per cent fat gravimetrically.

(b) *Human fat and animal fat.*—Mix 2 g sample with 100 g Na_2SO_4 and 25 g sand and continue as in (a).

(c) *Eggs and cheese.*—Mix 5 g egg puree or cheese with 100 g Na_2SO_4 and 25 g Ottawa sand and continue as in (a).

(d) *Milk.*—To 250 ml volumetric flask, add 100 ml milk and dilute to volume with detergent reagent. Set in boiling water bath ca 1.5 hr until butterfat separates. Remove butterfat with disposable dropper.

Cleanup

Plug chromatographic column (c)(*1*) with glass wool and fill with 25 g deactivated Florisil. Prewash column with methylene chloride-hexane (1+1). In beaker add 1 g fat extract to 25 g deactivated Florisil and mix until mixture flows evenly. Top prewashed Florisil with this mixture and eluate with 300 ml methylene chloride-hexane (1+4). Remove solvent with rotary vacuum evaporation and dissolve residue in 5 ml acetone (200 mg fat/ml).

Column Chromatography

Plug column (c)(*2*) with small amount of glass wool and sandwich 3″ cooled charcoal between ½″ Ottawa sand. Prewash column with 30 ml acetone-ethyl ether (1+3). Introduce sample aliquot (in acetone from *Cleanup*), using ≤10 ml acetone to complete transfer; discard washings. Elute successively with 180 ml acetone-ethyl ether (1+3), 80 ml benzene, and 100 ml toluene, changing receivers each time. Remove solvent by rotary vacuum evaporation and dissolve residue from each fraction in 5 ml hexane for analysis by GLC, column *1*.

Derivatization

Evaporate the toluene fraction in test tube under gentle stream of nitrogen (no heat). Add 1 ml 0.2*N* KOH in ethylene glycol, seal the tube with masking tape, and heat 1 hr at 150°C in oil bath. Cool, add 10 ml water saturated with Na_2SO_4, and acidify by adding 3 drops 10*N* H_2SO_4. Transfer to separatory funnel and extract 3 times with 10 ml benzene. Combine benzene extracts and filter through small amount of Na_2SO_4; evaporate benzene with rotary vacuum apparatus. Add 4 ml diazomethane solution and let sit at room temperature 30 min. Remove solvent with gentle stream of nitrogen just to dryness, dissolve residue in 5 ml hexane, and analyze by GLC, column *2*.

Results and Discussion

HCB recoveries for pure standard (0.01–1 μg) for the detergent method (12), Soxhlet extraction method, and Langlois cleanup (13) averaged 98.2, 99.1, and 101.9%, respectively. The charcoal column (14) was able to accommodate up to 1 μg each of the organochlorines (including HCB) and 10 μg PCBs to give 85–100% separation. Overloading resulted in cross-elution. All commonly occurring organochlorine insecticides eluted in the acetone-ethyl ether, PCBs in the benzene, and HCB in the toluene eluate (*see* Table 1). To achieve optimum separation some HCB was allowed to elute in the benzene fraction, which correspondingly lowered the HCB recoveries in the toluene fraction (recoveries from 78 to 93.5%). Figures 1A, 2A, and 3A demonstrate the effectiveness of the charcoal separation method; they represent chromatograms obtained from a human fat sample which was Soxhlet extracted, cleaned up on the Langlois

Table 1. Separation on charcoal column of cleaned-up, fortified[a] fat extracts of fish, egg, beef fat, duck pectoral

Compound	Fortification, μg, on fat basis	Recovery range, %		
		Acetone-ether	Benzene[b]	Toluene
Lindane	0.5	75.2–95.2	15.0–ND[c]	ND
Heptachlor epoxide	0.5	89.8–99.2	10.5–ND	ND
p,p'-DDE	1	76.8–99.1	22.5–ND	ND
Dieldrin	1	82.2–98.2	13.6–ND	ND
o,p'-DDT	1	80.1–97.2	18.2–ND	ND
p,p'-DDD	1	82.2–98.2	14.0–ND	ND
p,p'-DDT	1	85.2–100	10.5–ND	ND
γ-Chlordane	0.5	90.5–99.5	10.0–ND	ND
α-Chlordane	0.5	94.2–99.5	8.5–ND	ND
Methoxychlor	1	80.2–95.0	10.5–ND	ND
Aroclor® 1254	10	ND–12.5	85.2–95.0	ND
Aroclor® 1260	10	ND–15.2	80.6–96.2	ND
HCB	1	ND	18.6–ND	77.8–95.5

[a] Fortification after Langlois cleanup on samples previously analyzed for residues.
[b] All results corrected, if necessary, for residue already present.
[c] ND = none detected.

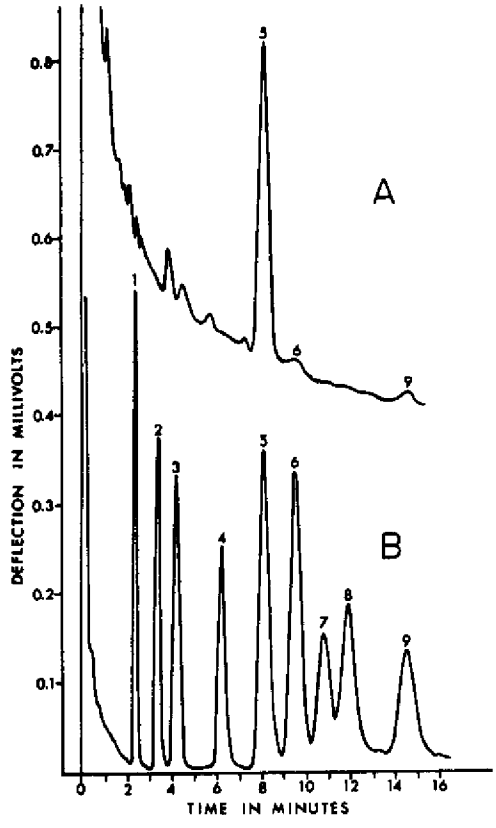

FIG. 1—Gas chromatograms with A, human fat sample, acetone-ethyl ether eluate, fortified with 0.5 ppm HCB; B, organochlorine standards: 1, lindane (0.05 ng), 2, heptachlor (0.05 ng), 3, aldrin (0.05 ng), 4, heptachlor epoxide (0.05 ng), 5, *p,p'*-DDE (0.1 ng), 6, dieldrin (0.1 ng), 7, *o,p'*-DDT (0.1 ng), 8, *p,p'*-DDD (0.1 ng), 9, *p,p'*-DDT (0.1 ng).

column, and fractionated on the charcoal column. Figure 1A represents the chromatogram obtained with the organochlorine insecticides (acetone-ethyl ether) fraction, 50 μg of sample injected; Fig. 2A the PCB fraction (benzene), 500 μg sample injected; and Fig. 3A the HCB fraction (toluene), 100 μg sample injected. Figures 1B, 2B, and 3B are chromatograms of corresponding standards.

Vacuum oven treatment prior to Soxhlet extraction, which is desirable for those samples with a high moisture content, resulted in total loss of pure HCB standard; however, in the case of substrate fortified with HCB, the loss due to volatilization was much lower and recoveries in most cases were still acceptable (Table 2).

To avoid overloading of the charcoal column, a preliminary GLC determination of the cleaned up extract was necessary for samples suspected of high DDE and/or PCB contamination.

Recoveries for the derivatization method were 90% or better (0.005–1 ppm HCB added on extracted fat basis). One hr heating at 150°C in the oil bath was necessary to insure complete conversion of HCB to the pentachlorophenol (PCP) and acidification of the aqueous phase prior to the benzene extraction was essential for the total recovery of PCP. The diazomethane-ether solution used for esterification should be deep yellow before it is used for the reaction; a lighter color implies loss of diazomethane and will result in incomplete esterification. The derivatization can be performed successfully on

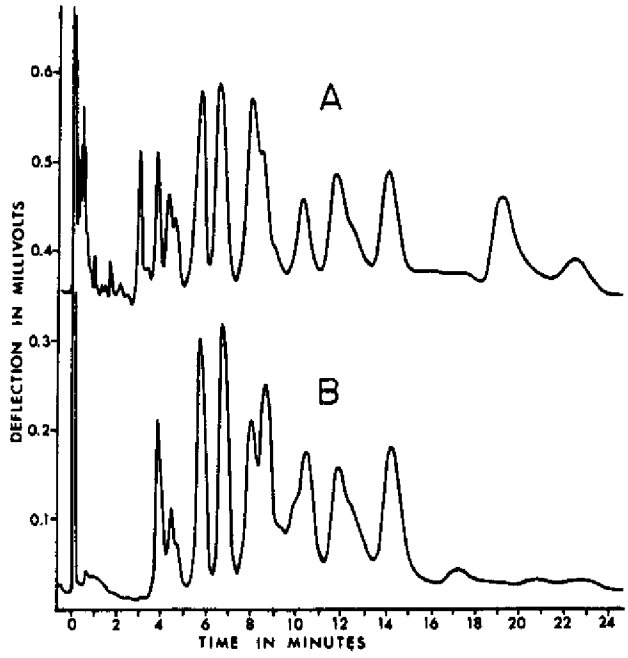

FIG. 2—Gas chromatograms of A, benzene eluate from human fat sample described in Fig. 1A; B, PCB standard (Aroclor 1254) (1 ng).

HCB residues of 0.005 ppm and more in the extracted fat. GLC sensitivity to the PCP methyl ether derivative under the conditions described is one-half that to HCB. Figure 4A represents a chromatogram obtained with the hydrolyzed and esterified toluene fraction depicted in Figure 3A, 200 μg sample injected. Figure 4B is a chromatogram of PCP methyl ether standard.

High HCB residues ($\geq$0.05 ppm) can be quickly and conveniently confirmed in a test tube by extracting the acidified aqueous phase with 5 ml benzene, removing an aliquot by pipet, and performing the methylation procedure. Recoveries for this procedure ranged between 60 and 85%.

HCB recovery data for a variety of substrates initially free of HCB, fortified with different amounts of standard, and analyzed according to the described procedure are given in Table 3. Vacuum oven treatment was not used. The detection limit for HCB quantitation in the toluene fraction was 0.002 ppm on an extracted fat basis. The system of analysis is in routine operation in this laboratory and has been successfully applied to a variety of samples.

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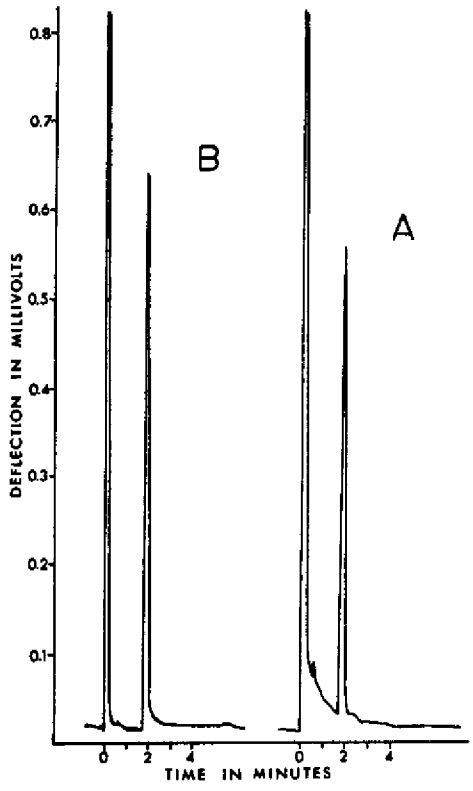

FIG. 3—Gas chromatograms of A, toluene eluate from human fat sample described in Fig. 1A; B, HCB standard (0.05 ng).

Table 2. HCB recovery data for Soxhlet vs. vacuum oven and Soxhlet

Substrate	Added, ppm, on tissue basis	Recovered, %	
		Soxhlet	Vacuum oven and Soxhlet
Fish[a]	0.05	91.3	73.5
Cheese[a]	0.3	84.3	50.3
Beef fat[a]	0.05	81.5	65.5
Egg[a]	0.05	83.8	67.3
Fish[b]	2	100	85.7
Bird liver[b]	2	104.5	83.7
Bird muscle[b]	2	98.2	90.9

[a] Recovery after Soxhlet extraction, Langlois column cleanup, and charcoal column separation.
[b] Recovery after Soxhlet extraction and Langlois column cleanup.

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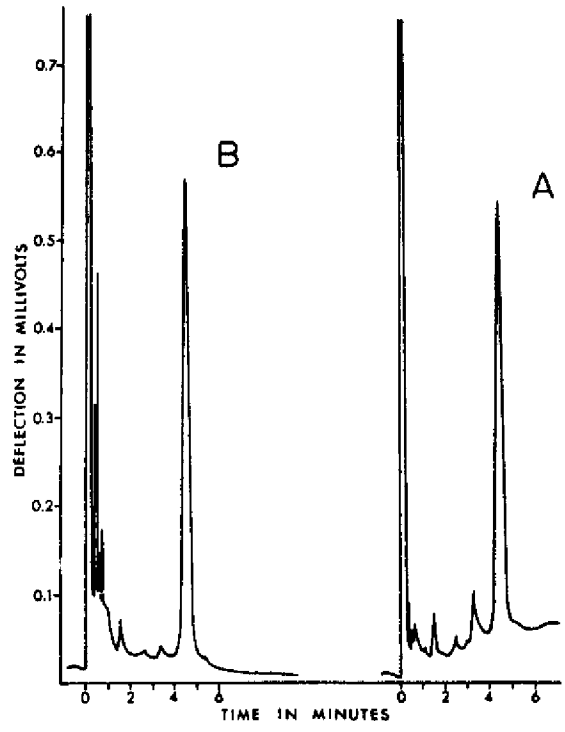

FIG. 4—Gas chromatograms of A, hydrolyzed and esterified toluene eluate from human fat sample described in Fig. 1A; B, pentachlorophenol, methyl ether standard (0.1 ng).

Table 3. Per cent HCB recovered from fortified substrates

Substrate	Added, ppm, on tissue basis			
	0.05	0.1	0.5	2.0
Hawk egg	82.5	—	79.8	—
Heron egg	91.2	—	90.1	—
Chicken egg	83.8	—	84.3	—
Owl pectoral	86.2	—	84.2	—
Loon pectoral	—	87.3	—	93.7
Duck pectoral	—	84.6	—	88.2
Beef fat	81.5	—	83.2	—
Avian fat	92.8	—	89.3	—
Human fat	—	—	82.9	83.3
Milk	—	79.5	81.2	—
Fish	91.3	—	90.5	89.6
Cheese	83.8	—	84.3	—

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Hexachlorobenzene (HCB) Levels in Lake Ontario Salmonids

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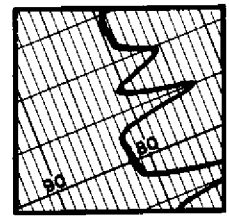

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Hexachlorobenzene (HCB) Levels in Lake Ontario Salmonids

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Hexachlorobenzene, C_6Cl_6, has been used as an agricultural fungicide until the early 1970's. HCB is also used as a peptizing agent for rubber products, a wood preservative, and a plasticizer for polyvinyl chloride (QUINLIVAN et al. 1975, PLIMMER & KLINGEBIEL 1976). HCB is also formed as a by-product during the manufacture of industrial chemicals, especially those products containing chlorine and chlorinated hydrocarbons, and the incineration of domestic and industrial waste (EPA 1973).

Once in the environment, HCB is persistent. Photodecomposition is extremely slow, and no decomposition products have been identified (PLIMMER & KLINGEBIEL 1976). No degradation of HCB have been reported by microbes (VERSCHUEREN 1977). Losses of surficial soil-bound HCB appears to be attributable to volatilization while losses of subsurface HCB appears to be negligible (BEALL 1976, ISENSEE et al. 1976). In the aquatic environment, HCB may be susceptible to partial degradation. Highly polar conjugates have been suggested as degradation products of HCB by invertebrates and fish (METCALF et al. 1973). Pentachlorophenol has also been identified as a product of HCB (SANBORN et al. 1977).

HCB has a solubility of 6 ng/g in water (GUNTHER et al. 1968). An octanol/water partition coefficient of 6.18 would suggest HCB has the propensity to bioaccumulate at high levels (NEELY et al. 1974). Laboratory model ecosystem studies have indicated HCB can be systematically accumulated through the food chain (LU & METCALF 1975, ISENSEE et al. 1976).

In view of the prevalence and persistence of HCB, measurements of environmental levels from a highly industrialized area such as the Great Lakes basin would provide an excellent reference on its occurrence. This study examined the levels of HCB in lake trout, rainbow trout, and coho salmon from Lake Ontario to confirm the bioaccumulative property of this compound in a natural water body, and to evaluate its potential impact as an important environmental contaminant.

MATERIALS AND METHODS

Lake trout (Salvelinus namaycush) were captured during the summer in the eastern basin of Lake Ontario. The 14 fish averaged

1.02 kg in weight and ranged from 0.21-2.11 kg. Fifteen rainbow trout (Salmo gairdneri), with an average weight of 2.32 kg (0.64-3.76 kg) were taken during their spring spawning migration in the Ganaraska River, at Port Hope, Ontario. Twenty coho salmon (Oncorhynchus kisutch), whose weight averaged 3.56 kg (0.72-5.34 kg), were captured during the fall spawning migration in the Credit River, near Toronto, Ontario. All fish were wrapped in acetone-washed aluminum foil after capture and frozen. After thawing and ancillary measurements were taken, individual whole fish were ground to a homogeneous composition using a Hobart grinder. An aliquot was then frozen in a glass container for analyses.

HCB Determination

The homogenates were weighed, mixed with $MgSO_4$, and allowed to dry. Each sample was Soxhlet extracted for 6 h with hexane. After extraction, the volume of the hexane was made up to 100 mL, and a 10 mL aliquot was evaporated to near dryness. This fraction was brought up to 1 mL with hexane, placed on a Florisil column, and eluted with 50 mL hexane. HCB levels were determined using a gas liquid chromatograph with an electron capture detector. A 183 cm x 2 mm ID glass column was packed with 11% OV-17, 4% QF-1 on Gas Chrom Q. Nitrogen was used as the carrier gas at 30 mL/min. Injection port, column, and detector temperatures were 235, 210, and 300° C, respectively.

RESULTS

HCB levels in whole fish homogenates averaged 80 ng/g for lake trout, 62 ng/g for rainbow trout, and 36 ng/g for coho salmon (Table 1). The levels of HCB increased significantly with body weight for all species (Table 2). For fish of the same weight, HCB levels were highest in lake trout, followed by rainbow trout, and coho salmon (Fig. 1).

TABLE 1

Hexachlorobenzene and Fat Levels in Lake Trout, Rainbow Trout, and Coho Salmon from Lake Ontario.

Species	No.	HCB in tissue, ng/g				% Fat in tissue			
		Min.	Mean	Max.	SD	Min.	Mean	Max.	SD
Lake trout	14	40	80	120	23	8.9	16.2	22.5	4.2
Rainbow trout	15	30	62	125	24	5.3	8.9	14.7	2.3
Coho salmon	20	16	36	50	9	3.7	7.5	9.7	1.6

The average level of body fat in lake trout was higher than that observed in rainbow trout and coho salmon (Table 1). Percent body fat increased significantly with weight in lake trout although

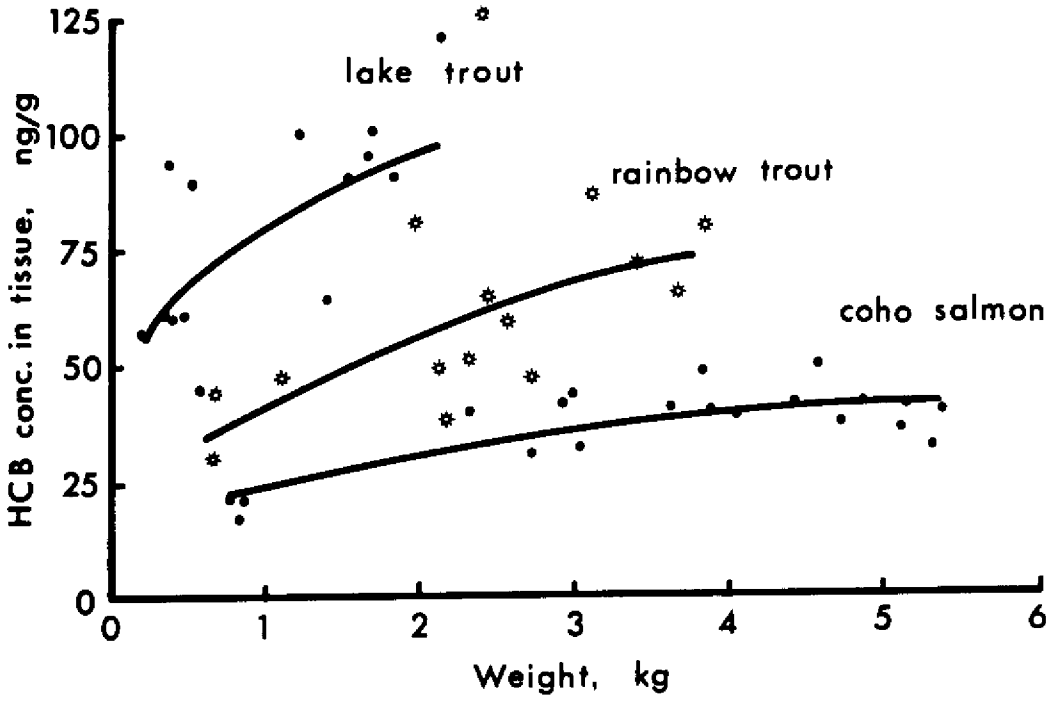

Fig. 1. Relationship between body weight and hexachlorobenzene levels in lake trout, rainbow trout, and coho salmon collected from Lake Ontario.

mean body weight and size range examined was less than the other species (Table 2). Percent body fat was not dependent on body weight for rainbow trout or coho salmon. A high preponderance of immature lake trout, female rainbow trout, and male coho salmon in the respective samples precluded any comparisons to determine differences in HCB levels and fat content that may have been attributable to sex.

TABLE 2

Regression Analyses of Lake Ontario Salmonids Expressed as $Y=aW^b$ where Y Represents HCB Levels in ng/g or % Body Fat, and W the Weight in grams. F Values with Asterisk Notes Statistical Significance at the 0.05 Level or Better.

Regression	Lake trout	Rainbow trout	Coho salmon
Weight vs HCB level in tissue	$Y=14.971W^{0.244}$ $F=6.70^*$	$Y=2.509W^{0.411}$ $F=7.61^*$	$Y=2.133W^{0.349}$ $F=26.30^*$
Weight vs % fat in tissue	$Y=2.186W^{0.295}$ $F=25.96^*$	$Y=2.618W^{0.156}$ $F=1.63$	$Y=5.938W^{0.026}$ $F=0.09$

DISCUSSION

The HCB levels reported for the three species of salmonids in this study are comparable to the average levels of 24 ng/g HCB in alewives (Alosa pseudoharengus) and smelt (Osmerus mordax), and 97 ng/g in coho salmon muscle collected from Lake Ontario (NORSTROM et al. 1978). Overall, HCB levels in fish from Lake Ontario are generally higher than those reported for a number of species surveyed throughout the United States (JOHNSON et al. 1974). Actual collection sites in that study were not sufficiently identified to establish a correlation between HCB levels and the quality of the waters that were sampled. A relationship between HCB levels in soil, water, and biota have been demonstrated for an industrialized region along the lower Mississippi River where HCB levels in the mosquito fish (Gambusia affinis) ranged from 72-380 ng/g (LASKA et al. 1976). Observations on the other Great Lakes indicate trace levels of HCB in fish tested from Lake Huron, and 1-24 ng/g HCB in fish from Lake St. Clair (FRANK et al. 1978a, FRANK et al. 1978b).

Figure 1 indicate distinct differences in the uptake kinetics of HCB among the three species examined. These differences may be partially explained when the age of the fish tested is considered. The lake trout examined included 2-5 year old fish, the rainbow trout perhaps 3-7 year old fish, and the coho salmon 3-4 year old fish. The rainbow trout appears to have originated from a self-sustaining population while the lake trout and coho salmon were fish planted as fingerlings. Differences in feeding habits may also influence HCB levels. Coho salmon feed heavily on smelt and alewives, while rainbow trout utilize other species such as perch as well. Small lake trout may feed on benthic organisms such as sculpins. The food habits of the forage species have not been sufficiently identified and monitored to establish the basis for a trophodynamic analysis of HCB in the Lake Ontario environment.

The octanol/water partition coefficient of 6.18 would suggest nearly all of the HCB in the fish homogenate would be associated with the fat fraction. This lipophilic behaviour of HCB has been well established in birds and mammals (AVRAHAMI & STEELE 1972). Based on this premise, HCB levels of the species examined were calculated from the percentage of fat in the homogenate. Mean HCB levels in fat were estimated to be 0.5 µg/g for lake trout, 0.7 µg/g for rainbow trout, and 0.5 µg/g for coho salmon (Table 3). These levels are lower than the mean values of HCB in fat of 1.0 µg/g for alewives and smelt, and 1.2 µg/g for coho salmon muscle calculated from the data reported by NORSTROM et al. (1978). To put the significance of these levels into perspective, the United States Environmental Protection Agency has recommended an interim action guideline of 0.5 µg/g in the fat of domestic animals, and the Food and Drug Administration has adopted a guideline of 0.3 µg/g in the fat of milk and dairy products. Based on these observations, further investigations on the levels of HCB in terrestrial and aquatic samples, and an assessment of the impact of HCB in highly populated areas such as the Great Lakes basin and the Mississippi River drainage would be highly desirable.

TABLE 3

Estimated Hexachlorobenzene Levels in the Fat of Lake Ontario Salmonids.

Species	No.	HCB in fat, µg/g		
		Min.	Mean	Max.
Lake trout	14	0.2	0.5	0.7
Rainbow trout	15	0.3	0.7	1.7
Coho salmon	20	0.2	0.5	1.1

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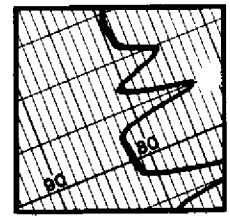
Approaches to Comprehensive Analyses of Persistent Halogenated Environmental Contaminants

D. L. Stallings, L. M. Smith and J. D. Petty



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Approaches to Comprehensive Analyses of Persistent Halogenated Environmental Contaminants[2]

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ABSTRACT: A series of chromatographic processes has been integrated into an automated sequential procedure that has been specifically developed for uninterrupted purification and fractionation of multiclass organic chemical residues from environmental samples. The effectiveness and general applicability of this sequential cleanup and fractionation procedure were demonstrated by the recovery of 39 chemicals representing a broad range of residue classes from a fortified fish sample. A chromatographic controller under construction is designed to carry out the numerous sample and solvent manipulations in an automated and continuous procedure. Alkali metal hydroxide treated silica gel effectively separated phenols and acids from neutral compounds.

KEY WORDS: multiclass organic environmental contiminants, phenols, pesticides, polychlorinated biphenyls, chlorinated dibenzo-*p*-dioxins and dibenzofurans, comprehensive cleanup, analysis, gel permeation chromatography, carbon chromatography, alkali metal hydroxide treated silica gel, chromatographic controller, microprocessor

The mission of the Columbia National Fisheries Research Laboratory (CNFRL) includes precrisis assessment of environmental contaminants. In part, this effort involves identifying and anticipating the effects of chemical pollutants on the nation's fishery resources. Many complex and uncharacterized chemical pollutants now elude measurement in environmental samples. The improvement of the probability of detecting and characterizing contaminants previously not recognized or considered important requires a comprehensive analytical approach that is applicable to diverse classes of chemical pollutants. We at this laboratory are particularly concerned with development of a scheme that is well

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[2]References to trade names do not imply government endorsement of commercial products.

suited for analyzing pollutants in the three major compartments of the aquatic environment—water, sediment, and biota.

Development of this comprehensive approach to multiclass residue analysis requires three steps: (1) establishment of a systematic chemical classification scheme to simplify the selection of the most appropriate analytical procedures; (2) identification of specific procedures for processing various types of sample matrices; and (3) a generalized procedural scheme for the collection and preparation of samples, and the enrichment, fractionation, identification, and measurement of contaminants. Such a general approach could improve the opportunity to identify a wide variety of pollutants, while minimizing interference among contaminants. Also, it could facilitate multiclass analysis by specialized procedures presently employed.

In general, most modern analytical schemes for identifying and measuring residual contaminants from the environment focus on multistep chromatographic procedures for selected chemical compounds. These procedures are not directed toward systematic identification and measurement of classes of compounds, but rather toward the determination of specific hazardous pollutants (for example, surveillance of pesticide residues in a specific matrix [*1,2*]).[3] The development of most methods for environmental samples has been pragmatic, with emphasis on overcoming specific problems of a sample matrix. Thus, most methods follow the path of least resistance toward generating acceptable contaminant data.

Recently, the Southeast Water Quality Control Laboratory of the Environmental Protection Agency (EPA) took a significant step toward a comprehensive integrated residue methodology. This goal was initiated by development of a master analytical scheme for measuring organic pollutants in water. A particularly important aspect of this analytical effort is the establishment of a classification procedure encompassing the more than 1300 organic contaminants detected in 29 types of water [*3*].

Halogenated organics are perhaps the most widely recognized class of persistent global pollutants. Selected halogenated compounds in this class are pesticides, biphenyls, naphthalenes, phenols, anisoles, and aliphatics [*4–12*]. Due to their extreme toxicity, a few isomers of the halogenated dibenzo-*p*-dioxins and dibenzofurans cause particular concern [*13,14*]. Trace level impurities of the latter compounds are often found in large-volume commercial preparations of such compounds as pentachlorophenol, polychlorinated biphenyls, and 2,4,5-trichlorophenoxyacetic acid [*15–18*]. Chlorinated dibenzofurans and dibenzo-*p*-dioxins have also been reported as by-products from combustion of materials containing chlorinated phenolic compounds [*19,20*]. These contaminants can be classified either as neutral or polar chemicals. Traditionally, adsorption chromatography is applied to the separation of neutral contaminants from coextracted biogenic compounds. Problems remain in the isolation of trace levels of polar pollutants using the common practice of liquid–liquid partitioning.

[3]The italic numbers in brackets refer to the list of references appended to this paper.

Further, difficulties exist in the direct analysis of polar compounds such as phenols or acids. Because these compounds have poor gas chromatographic properties, conversion to nonpolar derivatives such as pentafluorobenzyl ethers is required [*21*].

Chromatographic cleanup of environmental samples with Florisil [*22*] and/or silica gel [*23*] can fractionate the extracted contaminants into less complex subgroups. When combined with gas chromatographic (GC) separation and a sensitive and/or specific detector [*24,25*], this fractionation is typical of most current contaminant analysis procedures. Much of the initial progress in analyzing halogenated contaminants in the environment resulted from GC analysis using the electron capture detector (EC) [*26–28*].

The wall coated open tubular (WCOT) capillary column has increased the resolving power of gas chromatography [*29,30*]. Nevertheless, we have yet to resolve the approximately 200 polychlorinated biphenyl (PCB) components using WCOT–GC. Limitations imposed by incomplete GC resolution can be partially offset by mass spectrometric detection [*31–33*].

Even though mass spectrometry in combination with chromatographic techniques has greatly expanded the scope of contaminant survey and measurement [*34,35*], sample cleanup and contaminant fractionation remain relatively unimproved. Consequently, enrichment and fractionation of organic contaminants extracted from various sample matrices remain as prerequisites for further development of comprehensive analytical residue methodology.

Current analytical methods research at the Columbia National Fisheries Research Laboratory emphasizes development of a comprehensive approach for the analysis of environmentally persistent pollutants (primarily halogenated organic compounds). Candidates for such methodology are selected according to their (1) selectivity for structural features, functional groups, and chemical classes and (2) potential for mechanizing the procedure to facilitate the analysis of large numbers of samples.

Contaminant Enrichment Studies

Before 1970, laboratory research required an improved contaminant cleanup to eliminate coextracted biogenic material [*36*]. Our first efforts to improve fractionation of sample extracts led to a mechanized gel permeation chromatographic (GPC) system for recovering intermediate molecular weight contaminants [*37*]. Subsequently, small carbon columns were employed to isolate several groups of contaminants, including chlorinated dibenzofurans and dibenzo-*p*-dioxins from the eluate of a GPC column [*38*]. The combination of techniques has proven very useful in improving the separation of complex residues, but the development of extensively mechanized or partially automated cleanup systems for organic contaminants has proceeded very slowly [*39–41*].

In 1976, Stalling suggested that a microprocessor-controlled cleanup system, using the combined techniques of gel permeation chromatography (GPC) and

adsorption chromatography (carbon columns) sequential to the GPC column, could have considerable utility [*42*]. Herein, we report progress toward a unified analytical approach for analyzing environmentally persistent halogenated compounds and other classes of pollutants. First, modular chromatographic techniques must be designed that can be sequentially coupled and operated as a single system by means of an automated chromatography controller. Details of these steps follow.

Gel Permeation Chromatography (GPC)

The three compartments of the aquatic environment—water, sediment, and biota—each impose specific requirements for sample cleanup and analysis. A common problem with each matrix is the separation of organic contaminants from coextracted biogenic compounds.

GPC is effective in separating emulsion-forming fatty acids and other lipids in fish oil from contaminant residues. Generally, GPC is superior to liquid–liquid partitioning procedures used widely (Fig. 1). GPC is a rapid chromatographic technique with normal elution times of 30 to 60 min at a flow of 5 ml/min. It can be automated and mechanized readily. GPC gels are compatible with a wide range of organic solvents and do not require regeneration. They exhibit a relatively high capacity for lipids and allow the residues to be recovered nearly quantitatively. We observed class separations of chemical residues during GPC, but this feature still requires considerable development for our purposes. GPC separated aliphatic hydrocarbons from aromatic compounds [*43*], but the former coelute with triglycerides and other lipid moieties. GPC can recover phenolic residues along with chlorinated hydrocarbon pesticides.

As an example of the utility of GPC, the automated GPC system currently in use at CNFRL can be loaded with 23 individual fish extracts. The unit provides a dump cycle to remove coextracted biochemicals, a collect cycle to isolate contaminant residues, and a wash cycle to flush the GPC column prior to additional sample processing. Thus, lipids in 23 samples can be removed within a day without significant intervention by laboratory personnel.

Carbon-Foam Chromatography (CFC)

We have investigated a second chromatographic procedure, carbon-foam chromatography, extensively at CNFRL and feel that it integrates readily into a comprehensive scheme. Carbon has widely varying affinities for different classes of chemical compounds. Unfortunately, carbon in general is characterized by a broad spectrum of adsorptive sites, often giving solute elution profiles that are broad and tailing.

Coplanarity of closely situated aromatic systems in the molecules appears most important for adsorption on carbon. Specifically, the forces are increased by electronegative substituents (chlorine, bromine, nitro) on the aromatic systems

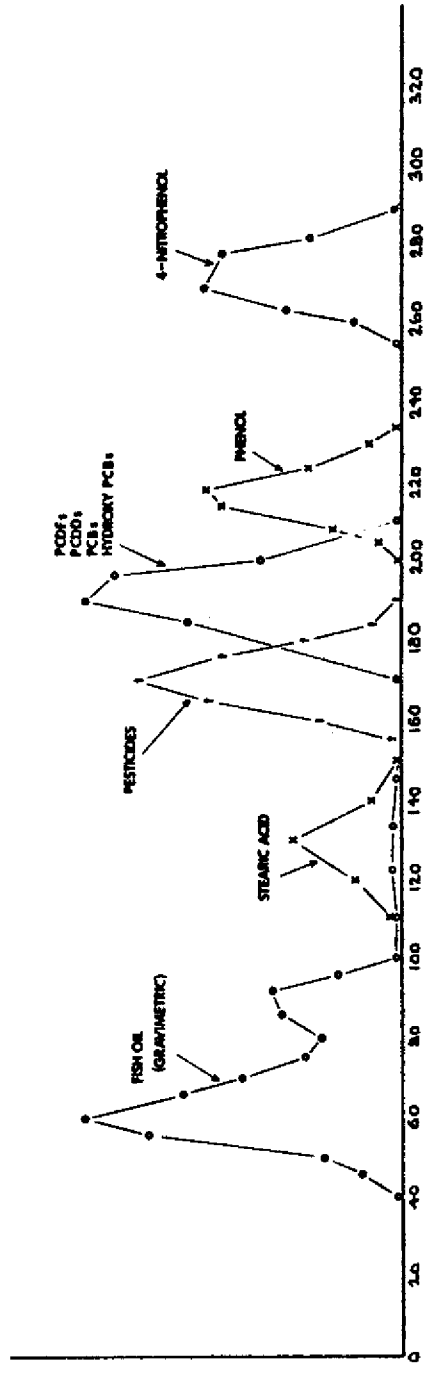


FIG. 1—*GPC elution profiles of selected biogenic materials and environmental contaminants; 60 g S-X3 Bio-Beads, 2.5 by 45 cm column, cyclohexane–methylene chloride 1:1, 5 ml/min.*

[*44*]. These forces also depend on the coplanarity and proximity of intramolecular aromatic centers. The selectivity of carbon among aromatic compounds according to coplanarity is demonstrated by the fractionation of a PCB mixture, Aroclor 1254 (Fig. 2). By choosing the eluting solvents properly, these complex mixtures of chlorinated biphenyls can be separated into fractions containing four, three, two, one, and no ortho chlorine substituents. This selectivity is consistent with the enhanced adsorption of compounds with planar aromatic moieties. For example, the non-ortho-substituted PCB components that can most readily assume a coplanar configuration are the most strongly adsorbed PCB congeners [*45*].

We found that polychlorinated dibenzofurans, dibenzo-*p*-dioxins, and naphthalenes (PCDFs, PCDDs, and PCNs, respectively) are even more strongly retained on carbon [*46,38*]. Recovery of these compound types from granular carbon columns is difficult [*38*]. Experimentally, we found the use of columns containing small amounts (50 mg) of finely divided carbon to be impractical due to limited solvent flow, even though recovery of these strongly adsorbed organics was satisfactory. Our discovery of a method for dispersing finely divided

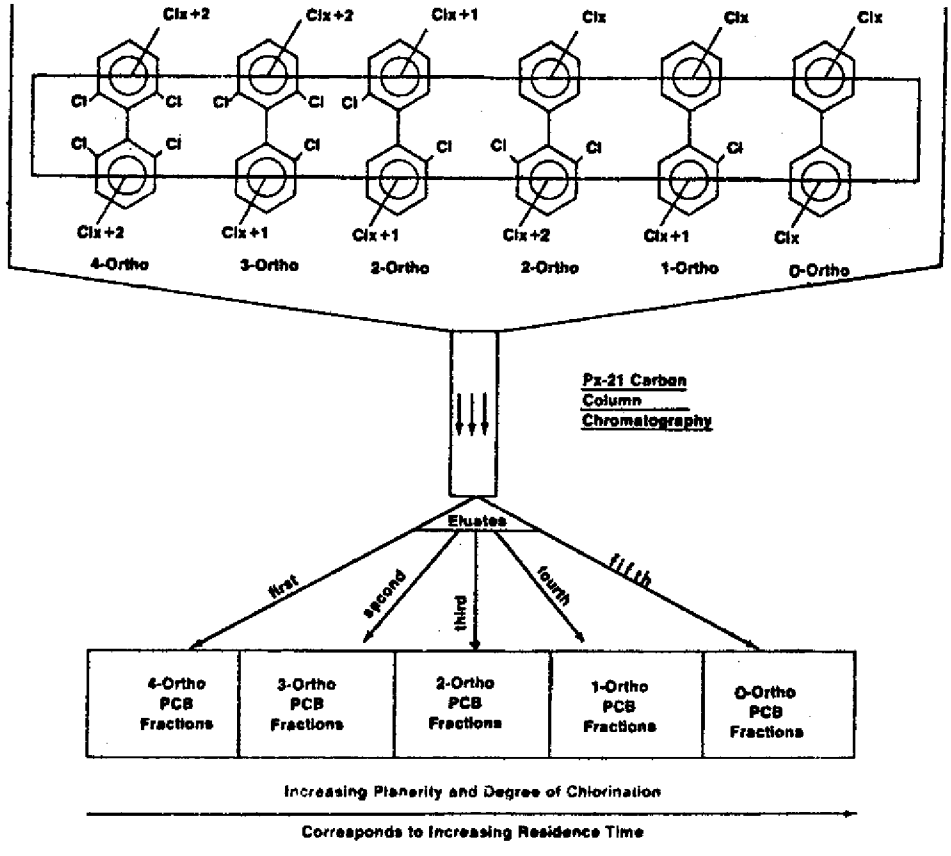


FIG. 2—*Fractionation of PCB components according to degree of ortho substitution using carbon chromatography.*

carbon on the surface of shredded polyurethane foam [*47*] overcame this problem. The carbon particles adhere to the foam strongly and are not dislodged during chromatography. The much smaller amounts of carbon employed with this procedure give improved recovery and separation of strongly adsorbed planar aromatics. We found Amoco PX-21, an experimental carbon [*48*], to be superior to coconut charcoal, which we previously used for adsorption of halogenated planar aromatic compounds [*38*]. We have developed several CFC systems using solvent mixtures to fractionate aromatic chemical residues. This procedure can be readily coupled with GPC for improved sample cleanup. Our CFC procedure for fractionating aromatic compounds (Fig. 3) employs 1 g of 5 percent (by weight) carbon dispersed on shredded polyurethane foam eluted with 1:1 cyclohexane–methylene chloride, ethyl acetate, 4 percent benzene, 20 percent benzene, and 50 percent benzene in ethyl acetate. The flow of the column was reversed, and elution was continued with toluene, the last eluting solvent. Most PCB congeners (those having at least one ortho substituent) are eluted from the CFC column with 30 ml of 1:1 methylene chloride–cyclohexane. The non-ortho-substituted PCBs elute with 50 percent benzene in ethyl acetate. Thus, 3,4,4′-trichloro- and 3,4,3′,4′-tetrachlorobiphenyl elute in 30 ml of 50 percent benzene. At this point, 2,7-dichlorodibenzo-*p*-dioxin, the first of the PCDFs and PCDDs, begins to elute. Continued elution with the strongest solvent, toluene, gives broad elution profiles for most of the PCDFs and PCDDs. Recovery of the more highly chlorinated isomers with continued toluene elution is impractical. However, we found that all PCDFs and PCDDs can be recovered from the carbon-foam column if the flow of toluene is reversed and elution is continued.

This chromatographic scheme also fractionates several other classes of aromatic compounds. Essentially all nonionic chlorinated pesticides are quantitatively recovered in the first 35 ml of the solvent elution sequence. Additionally, the thiophosphate pesticides, such as malathion and parathion, are reovered in this fraction, whereas, Guthion, due to its extended aromatic system, is retained until elution with 20 percent benzene. Hexachlorobenzene (HCB) and chlorinated naphthalenes are also retained.

To demonstrate the utility of CFC to enrich planar trace level environmental contaminants, we fortified 10.0 mg of Aroclor 1254 with a series of PCDFs at 1 μg/g and subsequently fractionated using the CFC procedure. Due to column overloading with PCBs, the 35-ml benzene eluate, enriched in non-ortho-substituted PCBs, contained 20 ppm of the Aroclor 1254 as background. In this fraction, trace level planar components of Aroclor 1254 were concentrated approximately 10 000-fold. The concentration of the major non-ortho-substituted PCB in Aroclor 1254, 3,4,3′,4′-tetrachlorobiphenyl, was estimated to be 40 ppm. The toluene fraction expected to contain PCDFs and PCDDs also contained an Aroclor 1254 background, which precluded GC–EC analysis. This problem was overcome, after toluene evaporation, by rechromatographing this fraction with CFC. The second toluene eluate was essentially free of 1254 background, and the PCDFs could be readily detected at the 1 ppm level by GC–EC (Fig. 4). In

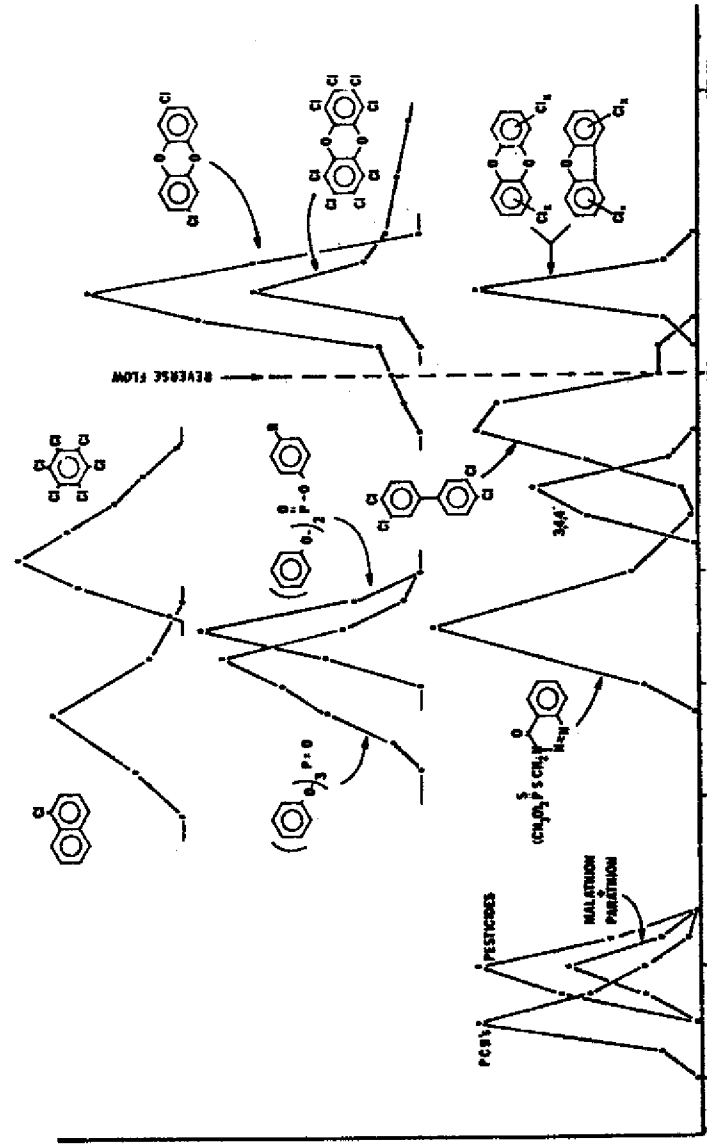


FIG. 3—*Carbon-foam chromatography of selected environmental contaminants; 1.0 g 5 percent Amoco PX-21 carbon (≤40 μm) on shredded polyurethane foam, 1.0 by 8.5 cm column.*

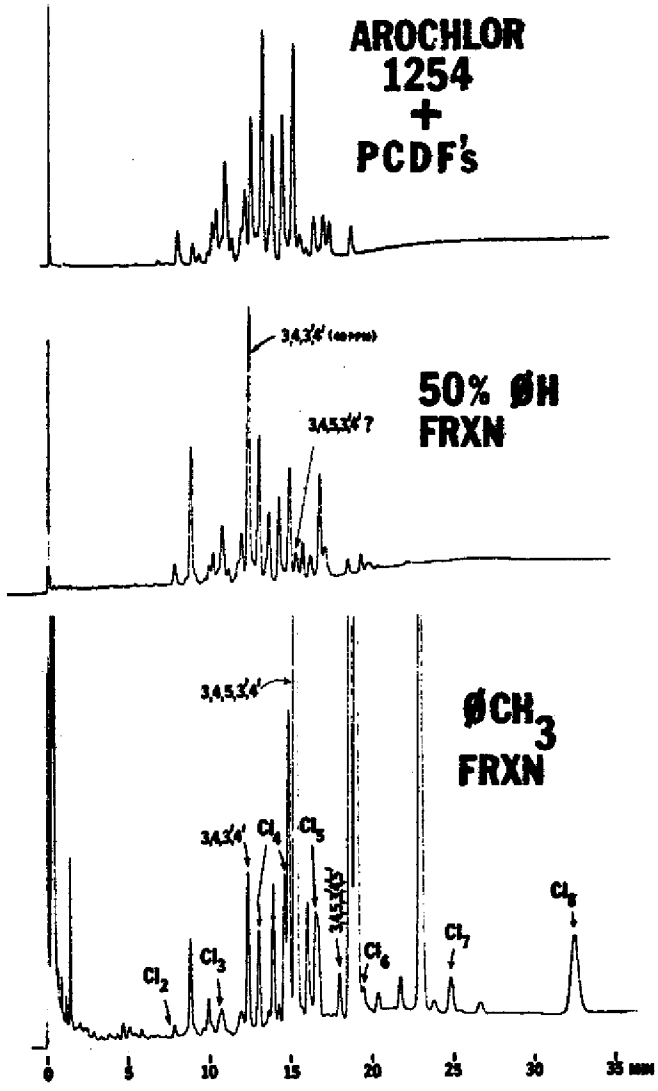


FIG. 4—*CFC of Aroclor 1254 spiked at 100 ppb with chlorinated dibenzofurans; SP 2250/2401 column.*

addition, this fraction contained a number of other chlorinated components presumably enriched from the 1254 sample. Further characterization of these components indicated the presence of chlorinated naphthalenes and methyl naphthalenes [*46*].

The CFC columns are easily regenerated, can be reused many times, and present no significant flow restriction at 5 ml/min. Because the CFC columns have little flow resistance, they are ideally suited for extending low-pressure chromatographic systems. We have found that CFC in sequence with GPC offers considerable utility for selective enrichment of planar contaminants in complex sample extracts.

Determination of Phenols

Cleanup systems that can separate phenolics and carboxylic acids generally involve ion exchange resins or solvent–base partitioning. Commonly used adsorbents are not well suited for recovery of phenols and carboxylic acids from sample extracts because of their poor chromatographic behavior. The regeneration of ion exchange resins, which is generally required after each use, impedes mechanization of the procedure. Extraction of phenols and acids with an aqueous base is often ineffective, because emulsions are formed and the partitioning of several types of phenols is incomplete.

As part of an interagency agreement between the EPA and the U.S. Fish and Wildlife Service, we undertook the analysis of the eleven EPA consent decree phenols (Table 1) in fish tissue. Our initial cleanup and analysis scheme involved GPC removal of lipids and free fatty acids, followed by acid–base extraction,

TABLE 1—*EPA Consent Decree phenols.*

Phenol
2,4-Dimethylphenol
2-Chlorophenol
p-Chloro-*m*-cresol
2,4-Dichlorophenol
2,4,6-Trichlorophenol
2-Nitrophenol
4-Nitrophenol
Pentachlorophenol
2,4-Dinitrophenol
4,6-Dinitro-2-methylphenol

derivatization of the phenols with pentafluorobenzyl bromide using a crown ether catalyst [*49*], silica gel chromatography for cleanup of the phenol derivatives [*50*], and finally, we employed temperature-programmed GC–EC analysis using at least two columns (Apezion-L and SP 2250/2401). The pentafluorobenzyl (PFB) derivatives of the phenols exhibit good GC properties and excellent EC response [*21*]. However, we did not effect reaction of the two dinitrophenols with PFBB or acylating reagents used to form EC active derivatives. After the phenols were reacted with PFBB, the dinitrophenols were extracted with a base, acidified, reextracted with methylene chloride, and methylated with diazomethane for GC–EC analysis as dinitroanisoles. Because the methoxy group does not significantly alter or add to the EC response of compounds, this derivative may be preferred for analysis of halogenated phenols. The direct GC–EC analysis of phenols can be made using a specially deactivated packing, thus permitting preliminary analyses of the chloro- and nitrophenols before derivatization.

The recoveries of the phenols as derivatives are generally good (Table 2) but may vary with sample matrices. Cleanup with silica gel following derivatization

TABLE 2—*Thirty-nine environmental contaminants used to test the multiclass residue purification scheme in the presence of whole fish tissue and resultant residue recovery from a spiked fish sample.*

Contaminant[a]	Residue Recovery, %	Contaminant[a]	Residue Recovery, %
HCB	63	*p*-Chloro-*m*-cresol	42
α-BHC	99	2,4-Dichlorophenol	78
Lindane	97	2,4,6-Trichlorophenol	86
Heptachlor	87	2-Nitrophenol	54
Aldrin	92	4-Nitrophenol	21
Heptachlor epoxide	104	Pentachlorophenol	63
trans-Chlordane	108	4,5-Dinitro-*o*-cresol	39
cis-Chlordane	108	2,4-Dinitrophenol	17
DDE	98	4-Iodophenol[b]	50
Dieldrin	98	2,3,7,8-TCDD	112
Endrin	84	OCDD	78
DDD	116	2,8-DCDF	98
DDT	103	3,6-DCDF	98
Aroclor 1260	101	2,4,5-TrCDF	98
Malathion	71	2,4,5,8-TCDF	114
Parathion	71	2,3,7,8-TCDF	112
Guthion	89	1,2,4,7,8-PnCDF	114
Phenol	18	1,2,4,5,7,9-HCDF	105
2,4-Dimethylphenol	35	1,2,3,4,6,8,9-HpCDF	91
2-Chlorophenol	47	OCDF	75

[a]In the abbreviations for the polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), descriptors for number of chlorines are: D = di, Tr = tri, T = tetra, Pn = penta, H = hexa, Hp = hepta, O = octa.
[b]Internal standard.

is necessary, to reduce interferences arising from PFB derivatives of endogenous compounds.

The PFB derivative cleanup similar to that employed by Johnson [*50*], can be summarized as follows: the phenols were applied to 1.4 g Silica Gel-60, 70–230 mesh (E. Merck, Darmstadt, Germany), in a 0.27 by 11 cm column, followed by application of 6.0 ml hexane, 8.0 ml 15 percent benzene–hexane, 8.0 ml 40 percent benzene–hexane, and 4.5 ml 75 percent benzene–hexane, in that order. Collection of the 7.0- to 15.0-ml fraction recovered all the phenol derivatives except those of the nitrophenols, which were recovered in the 23.5- to 26.5-ml fraction.

Significant improvements in the selective recovery of phenols may be obtained by using alkali metal silicate chromatography discussed later. Chromatography using this modified silica gel integrates well with GPC.

Contaminant Enrichment

Having developed the three procedural modules discussed above, we proceeded to integrate them into a more comprehensive scheme to fractionate multiple

classes of chemical residues and thus expedite later identification and quantitation of individual compounds (Fig. 5). Furthermore, several time-consuming steps in the scheme now appear amenable to mechanization that could lead to completely automated procedures.

The utility of this integrated analytical scheme was tested by processing a whole fish sample spiked at 100 ppb with 39 representative chemical contaminants (Table 2). Ground fish tissue fortified with a methylene chloride solution of the contaminants was thoroughly mixed and treated with anhydrous sodium sulfate. This tissue–sodium sulfate mixture was packed in a column, and extracted with methylene chloride. After evaporation of the solvent, the extract was fractionated by GPC (1:1, by volume, methylene chloride–cyclohexane). After solvent evaporation, the chemical residues were partitioned between 1 percent

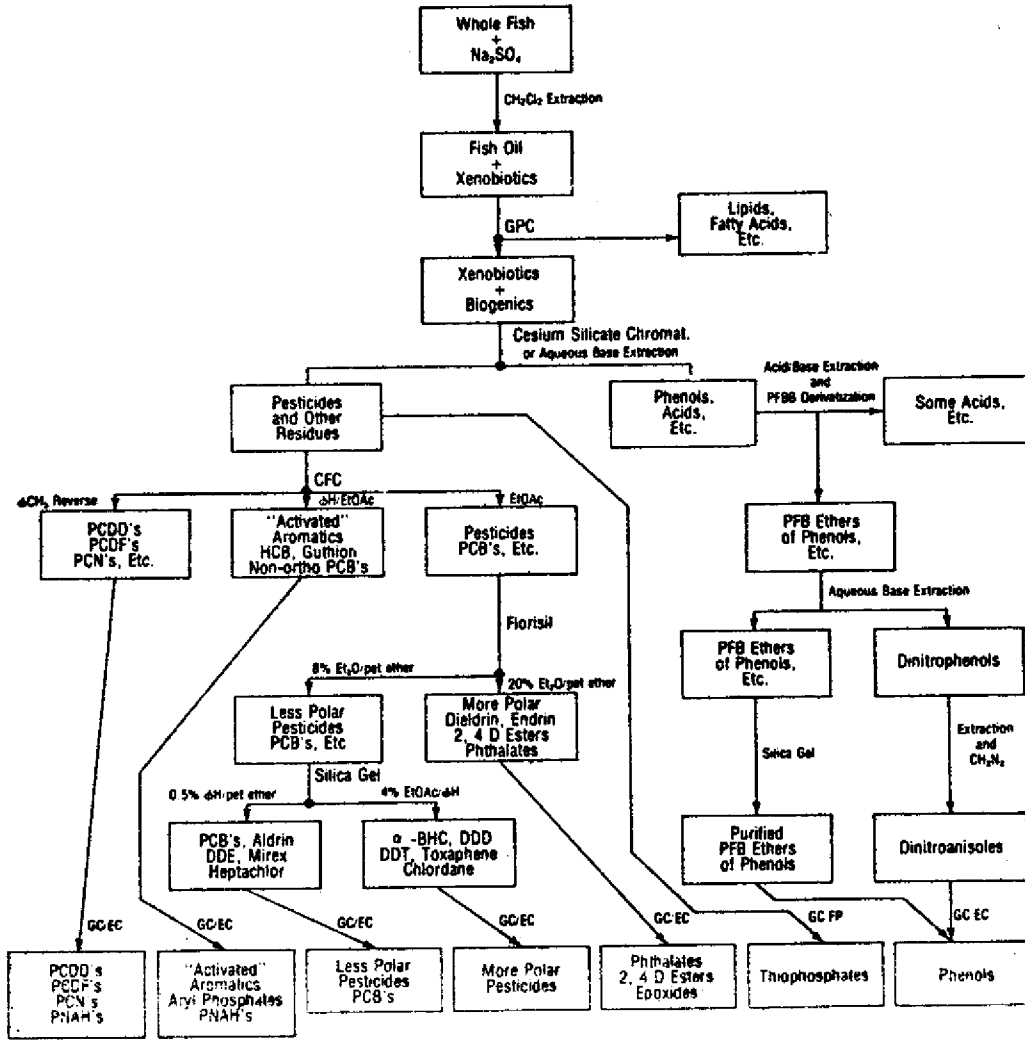


FIG. 5—*A generalized scheme for multiclass chemical residue cleanup and determination.*

aqueous sodium hydroxide and cyclohexane. The phenol residues, in aqueous solution, were carried through the previously described cleanup and derivatization procedure and analyzed by GC–EC (Fig. 6). A portion of the cyclohexane solution containing chemical residues was analyzed by GC–flame photometry to measure thiophosphate insecticides and other phosphorus-containing compounds (Fig. 7). The remaining portion of the cyclohexane solution was carried through the CFC procedure to further fractionate the contaminants into four chemical subgroups (Fig. 8): Fraction 1, the weakly adsorbed PCBs, pesticides, and re-

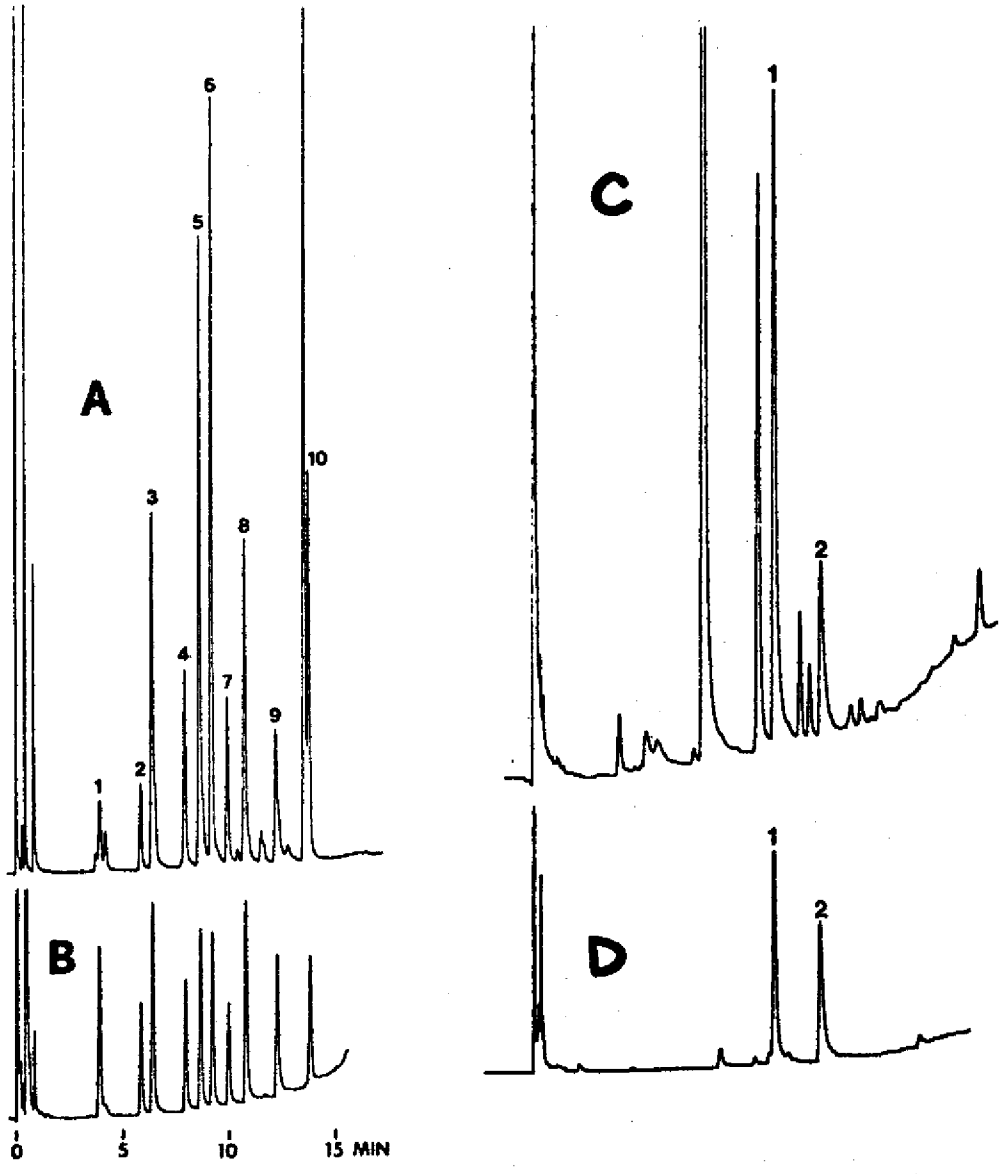


FIG. 6—(A) *GC–EC analysis of phenols as PFB ethers in fish spiked with multiclass residue.* (B) *PFB ethers standard.* (C) *GC–EC analysis of dinitrophenols as anisoles.* (D) *Dinitroanisole standard; SP2250/2401 column.*

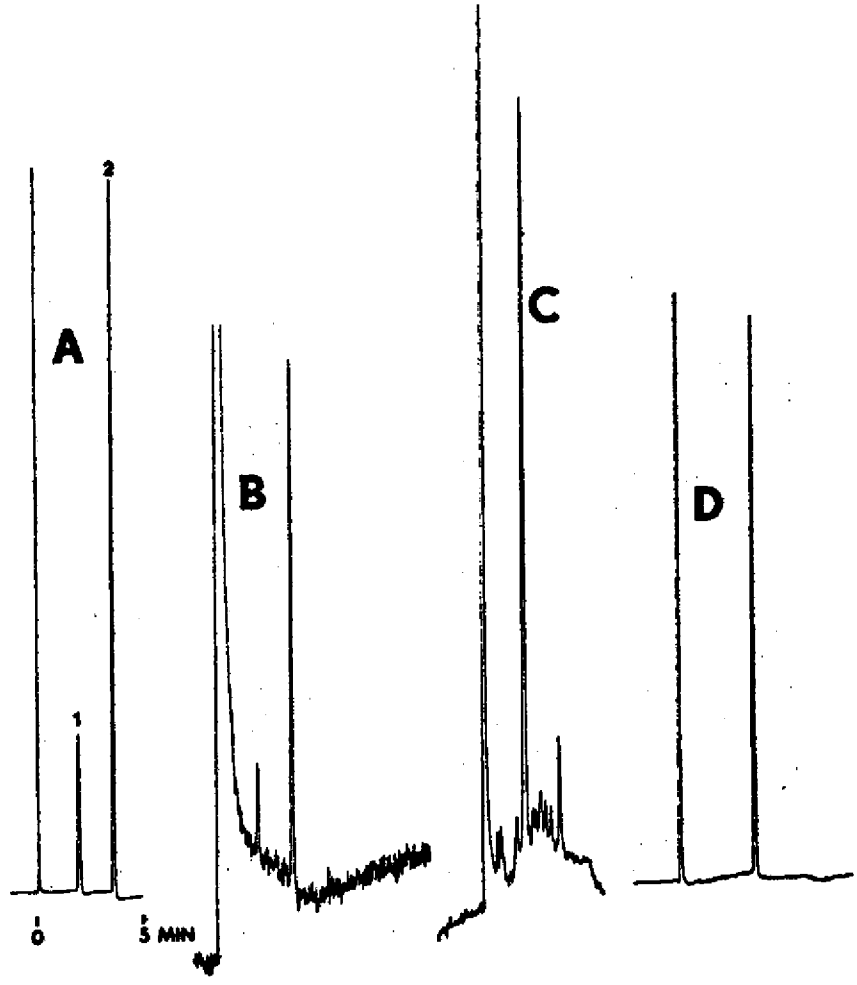
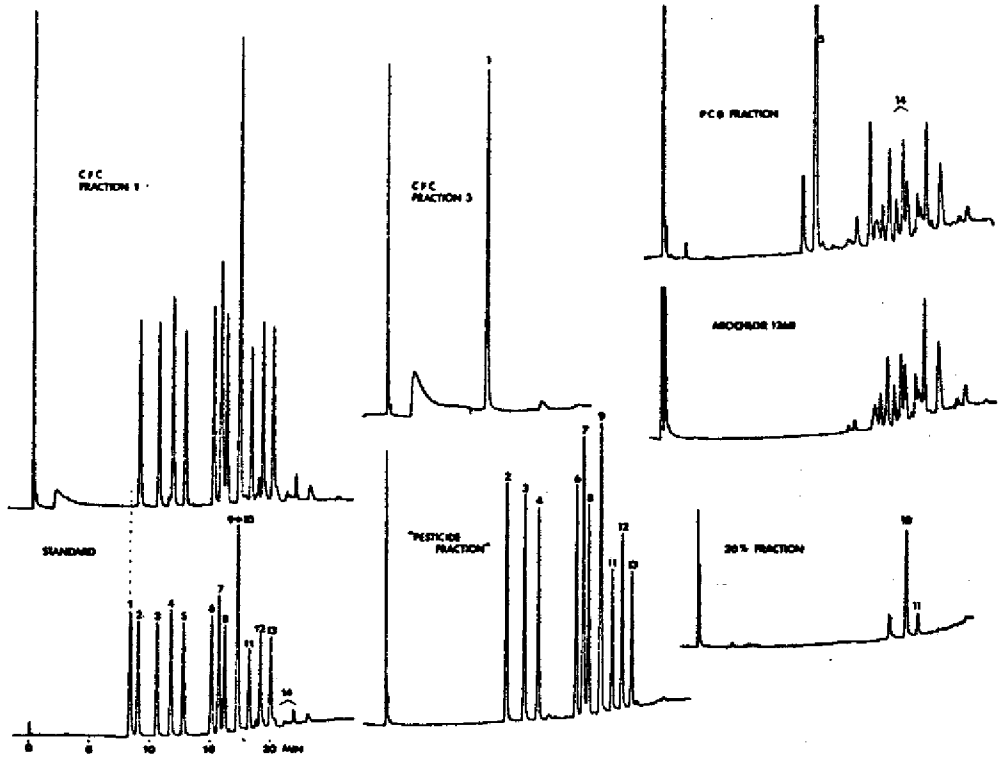


FIG. 7—*GC/FP analysis of thiophosphates in fish spiked with multiclass residue; OV-101 column.* (A) *Standard: 1. malathion plus parathion, 2. guthion.* (B) *Multiclass spiked fish (100 ppb) before CFC.* (C) *First fraction CFC.* (D) *Second fraction CFC.*

lated compounds; Fractions 2 and 3, the activated aromatic systems such as 1-chloronaphthalene, HCB, aryl phosphates, Guthion, and non-ortho-chlorine PCBs; and Fraction 4, the strongly adsorbed residues such as PCDFs, PCDDs, PCNs, and certain polynuclear aromatic hydrocarbons (PNAHs), obtained by reverse elution with toluene.

As expected, GC–EC analysis of the first fraction from CFC showed nearly quantitative recoveries of the pesticides and PCBs, except HCB (Fig. 8). HCB was recovered free of the other chlorinated pesticides in CFD Fraction 3. The last two fractions required no further cleanup before GC–EC analyses.

The GC–EC analysis of the CFC toluene fraction (Fraction 4) was straightforward. The octachloro isomers were recovered in 75 to 80 percent yields when eluted with 25 ml of toluene; however, elution with 50 ml of toluene yielded approximately 95 percent recoveries. When CFC is used, the PCDF, and PCDDs



1. HCB
2. α-BHC
3. Lindane
4. Heptachlor
5. Aldrin
6. Heptachlor epoxide
7. *trans*-Chlordane
8. *cis*-Chlordane
9. DDE
10. Dieldrin
11. Endrin
12. DDD
13. DDT
14. Arochlor 1260

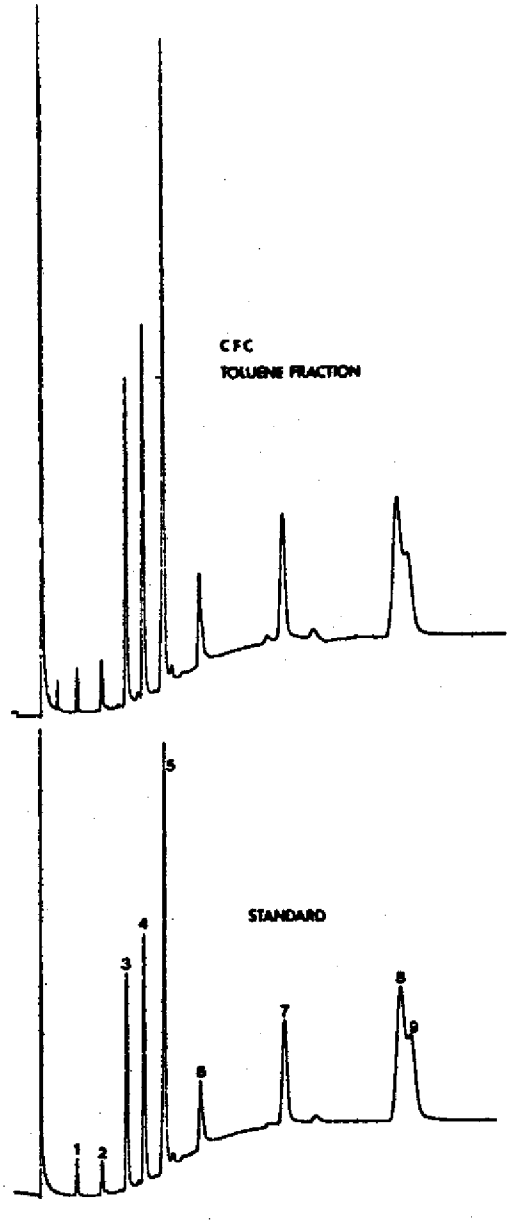
FIG. 8—*GC–EC analyses of pesticides and PCBs in fish spiked with multiclass residue; SP 2250/2401 column.*

can be recovered nearly quantitatively and with little background interference (Fig. 9).

The first CFC fraction, which contained the bulk of the PCBs and pesticides, was then fractionated further by using the cleanup methods approved by the U.S. Food and Drug Administration (Florisil followed by silica gel chromatography). These procedures provide further separation of residues into three additional groups: (1) PCBs and less polar pesticides, (2) more polar pesticides, and (3) phthalates, 2,4-D esters, and epoxides (Fig. 8).

Generally, the residue recoveries were good with the exception of several phenols (Table 2). Interferences in nearly all the final analyses were very low.

The cleanup and analysis of the phenols proceeded as expected. Before silica gel chromatography, the PFB derivatization mixture showed numerous unidentified derivatives, although the sample probably would have been satisfactory for phenol analysis by GC–MS. After silica gel cleanup, all ten phenol derivatives



1. 2,8- + 3,6-DCDF$_s$
2. 2,4,6-TrCDF
3. 2,4,6,8-TCDF
4. 2,3,7,8-TCDF + 2378 TCDD
5. 1,2,4,7,8-PnCDF
6. 1,2,4,6,7,9-HCDF
7. 1,2,3,4,6,8,9-HpCDF
8. OCDD
9. OCDF

FIG. 9—*GC–EC analysis of PCDFs and PCDDs in fish spiked with multiclass residue; SP 2250/2401 column.*

(4-iodophenol was included as in internal standard) were detected with little background interference (Fig. 6A). The dinitrophenols were detected by GC–EC analysis as the corresponding anisoles (Fig. 6C). Work is continuing toward improving the recoveries of phenolic residues.

The effectiveness of the fractionation and cleanup of the wide range of chemical residue classes demonstrated in this experiment is encouraging. These approaches appear to offer promise. This scheme represents our first step in developing a versatile and fairly comprehensive procedure for semiautomated cleanup and analysis of multiclass chemical residues present in aquatic environmental samples.

Alkali Metal Hydroxide–Silica Gel Chromatography

A method published by Ramljak et al [*51*] appeared to be useful in circumventing problems associated with the isolation of phenols and other acidic compounds from sample extracts. In this procedure, silica gel was treated with a saturated solution of potassium hydroxide in isopropanol to produce an adsorbent that can retain phenols and carboxylic acids and separate them from neutral compounds (Table 3).

TABLE 3—*Correlation of* pK_a *with* R_f *on potassium hydroxide-treated silica H plates.*[a]

Substance	Spot No.	pK_a	R_f[b]
n-Nonadecane	1	...	1.0
Naphthalene	2	...	1.0
Carbazole	3	12.10	0.73
o-Cresol	4	10.20	0.70
p-Cresol	5	10.17	0.64
m-Cresol	6	10.01	0.61
Resorcinol	7	9.81	0.54
2-Naphthol	8	9.51	0.50
1-Naphthol	9	9.34	0.46
m-Chlorophenol	10	8.85	0.45
o-Chlorophenol	11	8.49	0.38
m-Nitrophenol	12	8.25	0.37
p-Nitrophenol	13	7.15	0.32
n-Octadecanoic acid	14	...	<0.01
Benzoic acid	15	4.19	<0.01

[a]Source: Ramljack et al [*51*], pp. 1222–1225.
[b]Calculated from TLC plate A in Fig. 4 of reference. Chloroform development solvent.

When we used this material, our preliminary results indicated that the adsorbent removed many phenols from the GPC effluent (1:1, by volume, methylene chloride–cyclohexane). We further found that a small volume of methanol eluted the phenols from the potassium hydroxide–silica gel and that the column could be recycled after reequilibration with the GPC solvent. These chromatographic characteristics are considered as prerequisites for coupling this technqiue with our GPC and CFC modular procedures.

After our evaluation of the postassium hydroxide–silica gel for retention of this diverse group of phenols (Table 1), we observed variable retention of the least acidic consent decree phenol–2,4-dimethylphenol–on the potassium hy-

droxide-treated silica gel column. The other phenols were consistently retained and subsequently recovered with methanol.

In an attempt to overcome this restriction, we prepared and evaluated the alkali metal hydroxide-treated silica gels of lithium, sodium, potassium, and cesium. The silica gels treated with alkali metal hydroxide were prepared by treating 10.0 g of Silica Gel-60 (70–230 mesh) with 75 ml of anhydrous methanol containing 100 mmoles of the respective alkali metal hydroxide. The silica gel suspensions were gently refluxed for 3 h; then cooled, filtered, and thoroughly washed with fresh methanol. The treated silica gels were dried with a stream of dry nitrogen and later sieved to yield particles in the 100 to 325-mesh range. The columns were dry packed and washed with methylene chloride–cyclohexane (1:1, by volume) before use.

Of this series, silica gel treated with cesium hydroxide proved superior for retention of acidic compounds. A column of cesium hydroxide-treated silica gel consistently retained 2,4-dimethylphenol and other Consent Decree phenols–2,4-dichloro- and 2,4,5-trichlorophenoxyacetic acid and picloram–from the GPC eluate. We found that 2 ml of methanol was adequate to quantitatively recover all of the Consent Decree phenols or their cesium salts and that the columns were regenerated after washing with 20 ml of the GPC solvent mixture. Neutral compounds such as PCBs, PCDFs, PCDDs, triaryl phosphates, and chlorinated hydrocarbon pesticides were not retained. Chromatography using silica gel treated with an alkali metal hydroxide, together with GPC and CFC, form the basis for a modular contaminant enrichment chromatographic system (Fig. 10). Further studies are planned to achieve separation of organic acids from phenols to extend the utility of this chromatographic module.

Automated Chromatographic Controller System

To ensure that our analyses are reasonably comprehensive and accurate, we are developing mechanized procedures to clean up extracts and separate residues into chemical classes. Additional automation of residue cleanup procedures at our laboratory is critically needed for the analysis of the large number of samples generated by the National Pesticides Monitoring Program and our laboratory contaminant exposure studies. Space and manpower limitations reinforce the need for higher sample throughput. Also, improved routine purification capabilities made possible with such automation broadens the scope of contaminants amenable to more definitive analysis (for example, glass capillary GC or GC–MS). Three factors that have limited progress in development of automated cleanup techniques are: (1) lack of suitable method modules or adsorption materials; (2) lack of flexible electronic controllers; and (3) lack of suitable mechanical devices compatible with the variety of solvents employed in residue methods.

Hence, we have tried to develop a versatile controller system to accommodate multiple sequential chromatographic processes and extensively fractionate chemical residues. The system permits use of multiple solvents and the reversal of the

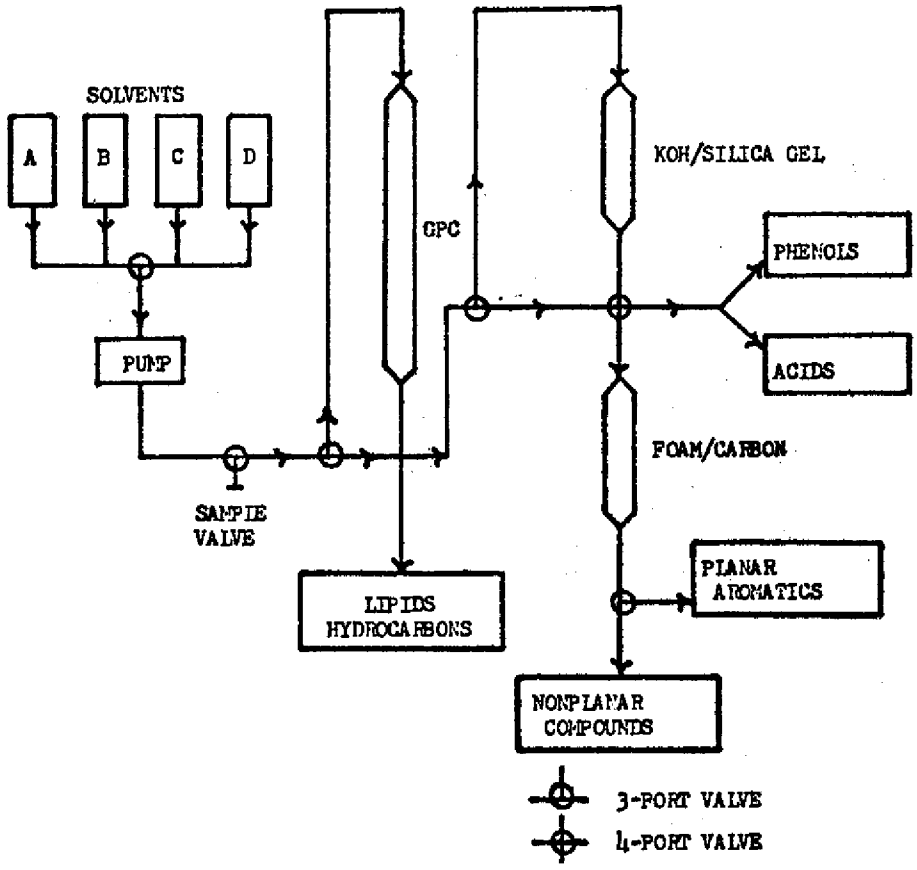


FIG. 10—*Contaminant enrichment chromatography system.* (A) *Methylene chloride–cyclohexane, 1:1;* (B) *toluene;* (C) *methanol;* (D) *methylene chloride–formic acid, 4:1.*

flow of eluting solvents through the columns. The design schematic for the chromatographic controller system is shown in Fig. 11.

This system will control solvent delivery, sample introduction, direction and selection of flow pathways, selection of chromatographic columns, and eluate fractionation with multiple solvents. The chromatography procedures supported are: (1) GPC with one or two columns in series; (2) a single adsorption or partition column or two columns in series or multiple sequential columns; or (3) combination of 1 and 2.

A microcomputer will be employed to provide the system control functions and system status display. The operator will control the systems through a display panel and keyboard. Interpretive language is planned so that the operator can program the separation pathways desired.

After developing and building this device, we plan to evaluate the utility of the system and determine its performance characteristics and extend its capabilities. The system is designed to process one sample at a time through sequential multiple chromatographic modules. After performance characteristics are deter-

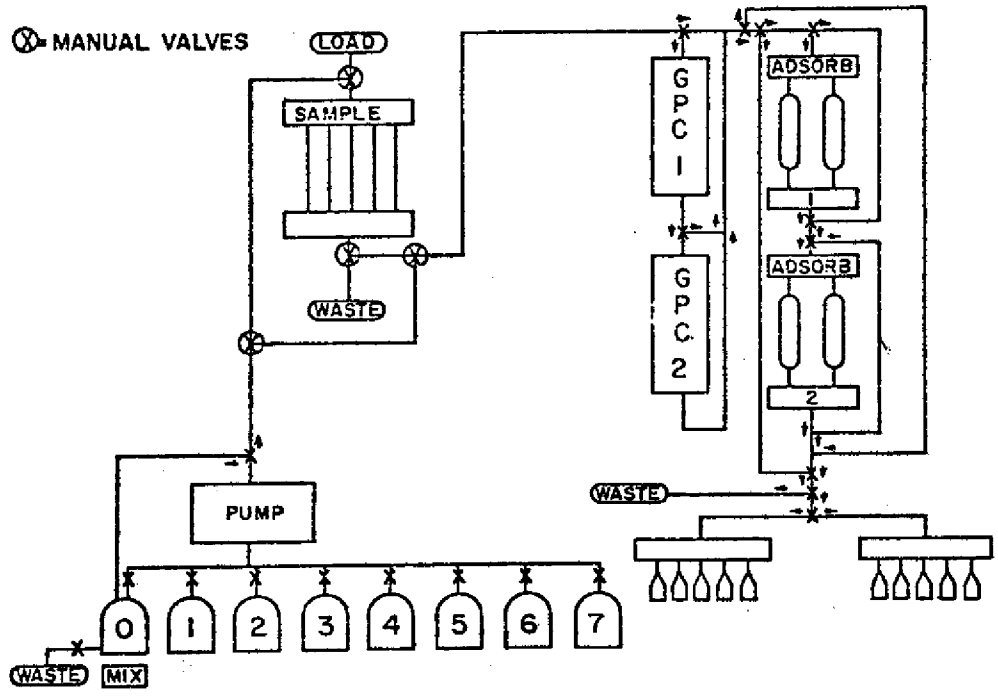


FIG. 11—*Flow system for the chromatographic controller.*

mined, the basic design can be upgraded so that up to four samples could be processed simultaneously; however, this upgrading will require revision of the microprocessor system software and controller.

Two functions remain to be developed: (1) automated solvent removal and (2) a module to transfer contaminants to a different solvent. These are important extensions needed to further applications of the automated contaminant-enrichment chromatography system.

Summary

Recent advances in instrumentation interfaced with computers indicate that increasingly automated analytical systems, such as the GC–MS computer, will continue to develop, making even more comprehensive contaminant analysis feasible. Optimum use of these sophisticated analytical systems for contaminant analysis is facilitated by effective sample processing techniques that are efficient and definitive for chemical classes.

As the demand for more comprehensive residue analyses increases, broader-based contaminant enrichment procedures must be provided. The development and adoption of a broad and orderly chemical classification scheme is most important in guiding future methods research.

An important consideration in methodology research is the amenability of a particular procedure to automated sequential linkage with another procedure. The research described herein illustrates approaches and methodologies that

could be considered for a wide variety of residue analyses. Because halogenated organic compounds are ubiquitous in the environment and because a wealth of information is available on their analysis, these compounds are prime candidates for testing comprehensive analytical methodology.

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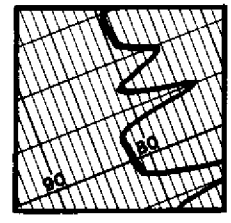
Bioconcentration of Xenobiotics in Trout Bile: A Proposed Monitoring Aid for Some Waterborne Chemicals

C. N. Statham, M. J. Melancon, Jr., and J. J. Lech



Bioconcentration of Xenobiotics in Trout Bile: A Proposed Monitoring Aid for Some Waterborne Chemicals

Abstract. *A technique is proposed for the monitoring of certain xenobiotic pollutants in suspect aquatic environments by fish bile analysis. Bile removed from rainbow trout* (Salmo gairdneri) *exposed to nine different radioactive compounds in vivo contained concentrations of radioactivity greater than those in the surrounding water. Bile-to-water radioactivity ratios as high as 10,000 : 1 were found after 24-hour exposures. The results of these experiments suggest that analysis of bile of wild or caged fish from a suspect site may be useful as a qualitative monitoring aid for certain types of xenobiotics in water.*

Studies of rainbow trout in this laboratory have established that several foreign compounds can be conjugated with glucuronic acid and excreted into bile in high concentrations (*1*). More recently, the results of other investigators have indicated that other fish species are able to conjugate certain phenols, such as pentachlorophenol, with sulfate (*2*). Although the biliary concentration of a variety of organic anions including conjugates of foreign compounds is thought to occur through a specific transport mechanism in mammals (*3*), very few studies have dealt with this process in fish (*4*). We have recently reported on the biliary concentration of several xenobiotic substances in rainbow trout (*5*). This report deals with the biliary concentration of a structurally diverse group of chemical compounds and indicates that the sampling of bile may be of potential use as an aid in monitoring water quality or as a diagnostic tool in the investigation of chemically related fish kills.

Table 1. Biliary concentration of various xenobiotics by rainbow trout (*Salmo gairdneri*). Exposures were made at 12°C for 24 hours. Water hardness was 134 parts per million, measured by the $CaCO_3$ method, and *p*H was 7.2. Radioactivities are expressed as disintegrations per minute (dpm) per milliliter; each value of the 24-hour bile radioactivity is the mean of a minimum of five animals from at least two separate exposures. Abbreviation: UL, uniformly labeled.

Compound	Concentration in H_2O (mg/liter)	Radioactivity (dpm/ml)		Ratio (bile ^{14}C)/(H_2O ^{14}C)	Metabolites
		H_2O (0 hours)	Bile (24 hours)		
2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73; chlorosalicylic acid; ring-UL-^{14}C)	0.05	3,010	30,500,000	10,100	1
Di-2-ethylhexylphthalate (DEHP; carboxyl-^{14}C)	0.5	1,070	265,000	247	5?
Methylnaphthalene (ring-UL-^{14}C)	0.005	310	796,000	2,570	?
1-Naphthyl-*N*-methylcarbamate (carbaryl; naphthyl-1-^{14}C)	0.25	1,030	975,000	947	3
Naphthalene (ring-UL-^{14}C)	0.005	305	127,000	414	2
Pentachlorophenol (PCP; ring-UL-^{14}C)	0.1	4,070	21,800,000	5,360	2?
2,5,2',5'-Tetrachlorobiphenyl (TCB; ring-UL-^{14}C)	0.5	3,640	39,000	11	2?
1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p*,*p*'-DDT; ring-UL-^{14}C)	0.1	180	22,500	124	1
3-Trifluoromethyl-4-nitrophenol (TFM; ring-UL-^{14}C)	0.5	2,020	2,150,000	1,064	1

Rainbow trout (usually 10 g of biomass per liter) were placed in a glass tank that contained 50 liters of dechlorinated water (*p*H 7.2) and the ^{14}C-labeled compound or compounds. The tanks were aerated and kept at 12°C and the system was allowed to remain undisturbed for 24 hours. The concentrations of the compounds in the exposures were below the level of acute toxicity for the times indicated and had no observable effects on the fish during the 24-hour exposures. The concentrations were chosen for convenience in metabolite detection rather than to simulate environmental levels. The amount of ^{14}C in the tank water was determined by counting suitable portions in 15 ml of ACS scintillation mixture (Amersham/Searle) in a model 6872 (Searle Analytic) liquid scintillation counter. After exposure, the fish were killed by cervical dislocation and the bile was collected by gallbladder puncture. Portions of crude bile were then placed in the scintillation mixture for counting, and the remainder was pooled for metabolite identification. The pooled bile was diluted with water and passed over a bed (5 by 15 cm) of XAD-2 resin in a glass column and washed with two bed-volumes of distilled water. The radioactive materials were eluted from the columns with three bed-volumes of methanol. The methanol was then concentrated to 30 ml. Thin-layer chromatography was performed on 0.25-mm silica gel plates. The plates were scanned for radioactivity by scraping segments (1 by 2 cm) of silica gel from them from the origin to the solvent front and counting the gel in ACS scintillation mixture.

The data shown in Table 1 indicate that the ratios of ^{14}C in bile to ^{14}C in water after the 24-hour exposures to the indicated compounds range from a low value of 11 for 2,5,2',5'-tetrachlorobiphenyl (TCB) to 10,000 for 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73). In most cases the ^{14}C in bile was associated with metabolites of the parent compounds and some of these biliary metabolites have been characterized (*1*, *2*). It is evident that the lowest bile-to-water ratios were associated with compounds (DDT and TCB) that have a high lipid solubility, and this may be due to a low rate of metabolism or conjugation related to the sequestration of these compounds by tissue lipids. Of great interest are the high bile-to-water ratios of the compounds that have comparatively lower lipid solubilities, since from a monitoring point of view, more polar compounds may have a low bioaccumulation potential (*6*). Although much attention has been given in the past several years to monitoring for chemicals that tend to accumulate in the food chain, there are few innovations in the area of monitoring for potentially hazardous chemicals that have lower bioaccumulation potentials, such as phenols and certain components of petroleum products. A recent report has suggested the use of liver benzopyrene hydroxylase activity as a monitor for petroleum pollution (*7*). The data in Table 1 concerning naphthalene and methylnaphthalene appear to be relevant, since both of these compounds are constituents of crude oil (*8*), and the appearance of metabolites of these compounds in bile in high concentrations suggests the possibility of using this technique as a tool in the monitoring of petroleum pollution.

Although more work needs to be done concerning the qualitative and quantitative aspects of the biliary concentrating system in the diverse species of fish and in the development of specific identification techniques for xenobiotic compounds and their metabolites, the value of capitalizing on this process is apparent. The careful design of monitoring methods based on bile collection from either captured fish or caged fish placed at a suspect site may well serve to provide increasingly needed environmental indices (*9*).

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Isolation of Xenobiotic Chemicals from Tissue Samples by Gel Permeation Chromatography

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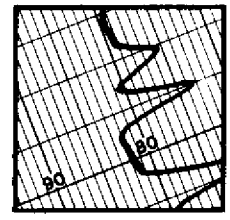

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Isolation of Xenobiotic Chemicals from Tissue Samples by Gel Permeation Chromatography

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Since automatic gel permeation chromatographic (GPC) systems were first described for the cleanup of samples with high fat content (*1*, *2*), efforts have been made to characterize (*3*) and utilize (*4*, *5*) these systems more fully. Mulder and Buytenhuys (*6*) had previously reviewed applications of GPC for the separation of a variety of organics on Bio-Beads or Sephadex LH-20 with various organic solvents. An excellent review on the fundamental gel network structure, its ability

Table I. Gel Permeation Chromatography Retention Volume and Recovery Data

Compound	CH_2Cl_2		Cyclohexane/CH_2Cl_2		Retn vol shift
	Retn vol, mL	Recovery, %	Retn vol, mL	Recovery, %	
Aroclor 1254	160-190	100	160-190	100	0
Aroclor 1016	150-186	95	150-188	72	0
Hexachlorobenzene	168-199	100	168-198	84	0
Naphthalene	168-195	100	170-195	86	2
Hexachlorobutadiene	144-168	90	148-169	87	4
p,p-DDT	144-171	100	152-178	57	8
o-Chlorophenol	162-178	100	170-198	100	8
Pentachloroanisole	156-174	100	172-196	75	16
2,4,6-Tribromoanisole	158-176	89	172-198	98	14
2,4-Dibromoanisole	152-169	84	168-202	67	16
p-Bromoanisole	156-174	95	174-200	60	18
o-Bromophenol	156-181	98	174-208	84	18
2,4-Dibromophenol	162-183	94	182-211	100	20
2,4-Dichlorophenol	170-195	100	190-218	87	20
2,4,6-Trichlorophenol	172-197	90	192-244	81	20
Pentachlorophenol	166-196	95	186-216	90	20
3,4-Dichloroaniline	170-195	94	200-255	91	30
Diphenylamine	154-180	89	184-214	83	30
1-Naphthylamine	166-192	100	206-237	60	40
m-Chloroaniline	172-200	100	216-246	84	44
2,4,6-Tribromophenol	147-161	100	194-214	80	50
5-Bromoindole	167-181	100	224-270	61	58
1,2,4-Trichlorobenzene	174-200	75	234-266	64	60
p-Chlorophenol	180-210	93	240-284	100	60
p-Bromophenol	154-176	100	220-244	95	66
Pyrene	176-200	100	252-294	87	76
Phenanthrene	168-195	99	250-284	87	82

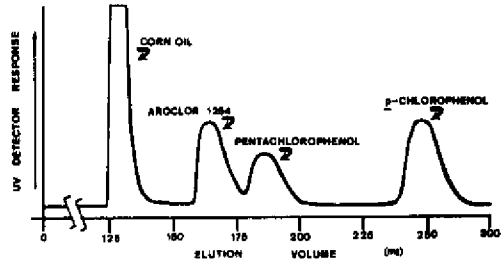

Figure 1. GPC chromatogram showing separation capabilities of methylene chloride–cyclohexane mixed solvent system on a Bio-Rad SX-2 column

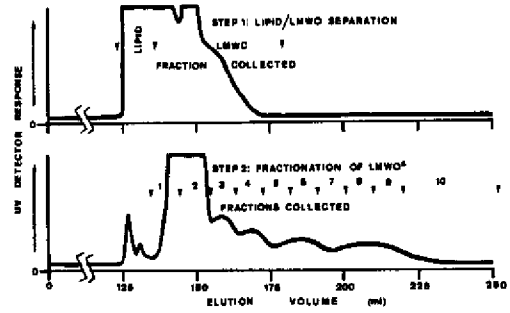

Figure 2. GPC chromatograms showing the separation of low molecular weight organic chemicals (LMWO) from lipids in the upper trace and fractionation of LMWO's in lower trace

to fractionate on the basis of steric exclusion, and the chemical contributions of chromatographic affinity has been presented by Freeman (7). Gel permeation, gel filtration, or molecular sieve chromatography are synonymous terms for separation by steric exclusion, i.e., differences in solute molecular size. In theory, inert gels have the ability to function as a sort of mass spectrometer where the degree of permeation varies inversely with solute molecular size. However, chromatographic affinity due principally to hydrogen bonding between the solute and the gel network has been shown to have a definite effect upon column performance (8). The finding of an orderly relationship between measured affinity and solute proton-donor strength suggests a new framework for studying hydrogen bonding, for measuring the proton-donor strengths of chemicals and for performing chemical separations (9).

The objective of this work was to develop an efficient, rapid method for the isolation of low molecular weight polar organics in fatty tissue for subsequent gas–liquid chromatographic (GLC) – mass spectrometric (MS) analysis. We describe a 2-step GPC cleanup procedure for samples with high fat content that uses first the steric exclusion principle and then takes advantage of the combination of the steric exclusion and chromatographic affinity phenomenon.

EXPERIMENTAL

Procedure. Blend ground fathead minnow (*Pimephales promelas*) tissue with enough anhydrous sodium sulfate to dry sample. Extract with hexane/acetone (1+1) on Soxhlet extractor for 8 h. Evaporate solvent and dilute concentrated oil to 100 mg/mL with CH_2Cl_2. Inject a series of 5-mL aliquots on a GPC/CH_2Cl_2 system, operating at 3.5 mL/min. Collect proper fraction in a Kuderna-Danish apparatus. Concentrate sample to 5 mL.

Equilibrate GPC system by recycling CH_2Cl_2/cyclohexane. Inject 5-mL sample and collect fractions.

Apparatus and Reagents. *Solvents.* Hexane, cyclohexane, methylene chloride, and acetone, redistilled pesticide grade (Burdick and Jackson, Muskegon, Mich.).

Gel Permeation System. Dual 25 cm × 2.5 cm glass columns connected in series and filled with 100–200 mesh Bio-Rad SX-2 beads. A high pressure all-Teflon sample valve (Durrum Model 24089, Palo Alto, Calif.) with a 5-mL sample loop was used for sample injection. A 254-nm ultraviolet detector (Varian Aerograph) and a recorder (Varian Aerograph model A-25) were used to monitor the chromatogram. All connections and sample transfer lines were Teflon.

Gas–Liquid Chromatograph–Mass Spectrometer. Varian Aerograph Model 1700 GC equipped with flame ionization detector and 6-ft × $^1/_8$-in. i.d. glass column packed with 3% OV-101 on 80–100 mesh Gas-Chrom Q was used for GPC recovery studies. The GLC-MS system is a Varian MAT CH-5 system, and the

Table II. Compounds Identified in Fish Tissue by GC/MS after Sample Cleanup by Two-Step GPC Method

Fractions 3 and 4	Fractions 5, 6, and 7	Fractions 8, 9, and 10
Methylnaphthylene	Chlorophenol	Anisole
C-alkylnaphthylene	Indole	Dibromoanisole
C-alkylnaphthylene	Bromoindole	Tribromoanisole
Biphenyl	Dibromoindole	Chlorobromoanisole
Dichlorobiphenyl	Tribromoindole	Tetrachloroanisole
Trichlorobiphenyl	Dibromomethylindole	Phenol
Tetrachlorobiphenyl	Trichloromethylindole	Chlorophenol
Trichlorobenzene	Pentachlorophenol	Dibromophenol
Tetrachloroanisole	Trichlorophenylphenol	Bromodichlorophenol
Tribromoanisole	Nicotinamide	Chlorodibromophenol
cis-Chlordane	1,3-Diphenylpyrazoline	Ethylphenol
trans-Chlordane	Dichlorodibenzofuran	Dibromocresol
cis-Nonachlor		Dichlorobenzene
trans-Nonachlor		Trichlorobenzene
p,p'-DDE		Chloronaphthalene
		Dichloronaphthalene
		Dibenzofuran
		Benzthiazole
		Methylbenzothiaphene
		Dibenzothiaphene
		Naphthylamine
		Methylcarbazole
		Indole
		Dibromoindole
		Tribromoindole
		Dibromomethylindole
		Tribromomethylindole
		Tetrabromomethylindole
		Pentachloroaniline
		Dibromomethylbenzothiazole

Varian MAT Spectrosystem-100 MS data system was used for data acquisition and processing.

Standards. All standard compounds used for retention volume and recovery studies (Table I) were from the Lab Assist kit (Chemservices, Inc., West Chester, Pa.). Solutions of 1 mg/mL CH_2Cl_2 were prepared.

RESULTS AND DISCUSSION

The isolation of nonpolar xenobiotic organics from fatty tissue on Bio-Rad SX-2 (copoly(styrene-2% divinylbenzene)) with cyclohexane as the solvent is a very efficient sample preparation technique for subsequent GLC-MS analysis (*4*). Recoveries of PCBs, for instance, are generally better than 95%. A disadvantage of this system, however, is the low recovery of polar organics such as pentachlorophenol, which was only 10%. On this system, however, a mixture of equal quantities of corn oil, Aroclor 1254, and pentachlorophenol can be completely separated. Excellent chromatographic resolution can therefore be obtained if low recoveries of the polar chemicals are considered acceptable. Polar solvent systems will reduce retention volumes, reduce band broadening, and increase recoveries of polar compounds to acceptable levels. Johnson et al. (*5*) observed that polar solvent systems such as mixtures of toluene and ethyl acetate will result in high percentage recoveries of both polar and nonpolar chemicals. This system, however, did not provide any chromatographic resolution of polar and nonpolar compounds. Nonpolar solvents readily elute nonpolar solutes from the gel network, whereas larger quantities of solvent are required to eventually elute polar solutes at low yields and both polar and nonpolar solutes co-elute at high yields with polar solvents.

It was necessary then to develop a GPC system that would yield high recoveries of both polar and nonpolar chemicals and would give good chromatographic resolution of polar and nonpolar chemicals. In addition, the solvent system had to be highly volatile so that the more volatile chemicals isolated from samples would not be lost during solvent removal.

Retention volume and recovery studies for various polar and nonpolar organics ranging from *p*-chlorophenol to PCBs were conducted for the following solvent systems: (a) 100% CH_2CL_2; (b) 10% CH_2Cl_2/90% C_6H_{12}; (c) 33% CH_2Cl_2/67% C_6H_{12}; (d) 50% CH_2Cl_2/50% C_6H_{12}; and (e) 75% CH_2Cl_2/25% C_6H_{12}. The 50:50 mixture was the best compromise of high percentage recoveries and compound separations. Figure 1 shows an example of the resolution obtained with the 50:50 mixture. The retention volume and percentage recovery data with the 50:50 mixture and 100% CH_2Cl_2 are presented in Table I.

All compounds tested eluted at low retention volumes in narrow bands and at approximately the same retention volume when 100% CH_2Cl_2 was the solvent. This system, therefore, can be used as a rapid and efficient technique for the bulk separation of lipids from low molecular weight organic chemicals (LMWO). The unique elution of compounds from the mixed solvent GPC system can then be used to fractionate the LMWOs into polar and nonpolar organics. This 2-step procedure has been demonstrated for an extract of fish that had previously been exposed to a bromine chloride-disinfected wastewater effluent (*10*).

Figure 2 shows a GPC trace for the lipid/LMWO separation (step 1), and a GPC trace for fractionation of the LMWO by polarity (step 2). After each sample was screened on the GC, fractions 3 and 4 were combined; 5, 6, and 7 were combined; and 8, 9, and 10 were combined for GLC-MS analysis. The qualitative results are presented in Table II. These fractions contained compound types such as phenols, anisoles, and heterocyclic aromatics (dibenzofurans, dibenzothiaphenes, indoles, etc.). Fractions 1 and 2 were analyzed separately and were basically PCBs and chlorobenzenes.

Commercially available GPC units can be used for both steps of this technique. Typically a timing unit is used to provide "waste", "collect", and "wash" cycles for repetitive processing of a single sample. Step 1 can therefore be completed, after sample loading, without operator assistance. Step 2 can also be automated by routing the effluent to a fraction collector during the "collect" cycle. However, since step 2 is done only once for each sample, and each sample may be slightly different, it may be advantageous to allow the operator to decide when each fraction should be collected.

Future developments involving automatic GPC units for the cleanup of fatty tissue are the addition of an expanded fraction-collection system, the addition of gradient solvent-elution capabilities, and the use of a variety of detectors for a more complete characterization of the GPC elution pattern of a sample.

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Organic Compounds in the Delaware River

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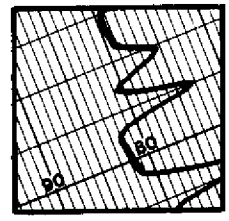

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Organic Compounds in the Delaware River

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■ Nearly 100 compounds were identified in Delaware River water samples taken in August 1976 and March 1977 between Marcus Hook, Pa. (river mile 78), and Trenton, N.J. (river mile 132). Extraction with CH_2Cl_2, liquid chromatographic cleanup, and gas chromatographic mass spectrometry were used for compound separation and identification. The observed compounds included natural products, municipal wastes, and industrial contaminates. The latter were of three types: those found in industrialized urban areas with no particular production source such as aromatic hydrocarbons and phenols; those commonly used in manufacturing processes such as plasticizers and industrial solvents; and those specific to a single source and traceable to that source such as a series of chlorinated aliphatic and aromatic polyethylene glycols which were specific to a plant in the Philadelphia area.

Thirty million people in the United States drink water taken from rivers (*1*). In many cases, these rivers also receive wastewaters from surrounding municipal and industrial dischargers. Although both drinking water and wastewater are usually treated to make them safe, many of the treatment processes are not fully effective. This has resulted in the widespread contamination of river waters and, hence, drinking waters. It is obviously of critical importance to know the identities and abundances of these contaminants in order to assess health effects and to devise rational control procedures.

There is now a growing knowledge of the organic compounds in the drinking water of many cities (*2–4*) and, to a more limited extent, in the wastewaters of several types of industries (*5–7*). There have been, however, few studies of the rivers themselves. This is unfortunate since the river is the ever-present, connecting link between wastewater and drinking water. Furthermore, the river can act as a carrier, sink, or reactor causing transformation of compounds to more or less hazardous species.

This paper reports on a detailed study of the organic compounds in the Delaware River. We selected the Delaware for several reasons: Over 120 major chemical manufacturing plants (see Figure 1) are located along its banks and many discharge wastewater, either directly or indirectly, into the river (*8*). The Delaware is a major source of drinking water for many of the cities and counties in the area; for example, it provides 50% of Philadelphia's water (*9*). The incidence of cancer is very high in the areas surrounding the Delaware River (*10, 11*).

Based on these considerations, it is logical to ask if there is a correlation among cancer incidence, organics in the drinking water, and organics in the Delaware River. This is obviously a very complex and difficult question, but the answer must begin with a complete study of the organic compounds in the river itself. Our study includes compound identification and quantitation as well as a preliminary assessment of their sources. The analytical techniques used in this study included vapor stripping of volatile organic compounds, liquid chromatographic (LC) fractionation, high-resolution gas chromatography, computerized gas chromatographic mass spectrometry (GC/MS) in both the electron impact (EI) and chemical ionization (CI) modes, and high-resolution mass spectrometry (HRMS).

River System. The Delaware River is a 350-mile-long water-way rising in central New York State, running through heavily industrialized areas of New Jersey, Pennsylvania, and Delaware to the Delaware Bay. Water flow in the lower third of the river from Trenton, N.J. (river mile 132), to the Bay, is dominated by tidal action; tidal volumes around Philadelphia are at least an order of magnitude greater than the downstream river flow (*12*). In general, any effluent discharged into the river will travel approximately 15 miles during one tidal cycle—7 miles upstream during high tide, and 8 miles downstream with ebb flow (*12*). This tidal action is important for the movement of industrial wastewaters discharged into the river both by dispersing the effluent (particularly in the upstream direction) and by prolonging residence times. Additionally, during periods of normal flow the lower 55 miles of the river is an estuary characterized by salinity gradients which limit both domestic and industrial usage (*13*). Background data provided by the Delaware River Basin Commission show that the ratio of municipal to industrial discharge is approximately 2 to 1 (*8*). Information provided on effluent source locations (*8*) aided in selecting representative sampling sites.

Experimental

In August 1976, 11 grab samples (3.5 L each) suitable for solvent extraction were collected from the center channel of the Delaware River between Marcus Hook, Pa. (river mile 78), and Trenton, N.J. (river mile 132). In October, two additional samples (7.0 L) were taken from the shore for volatile analysis.

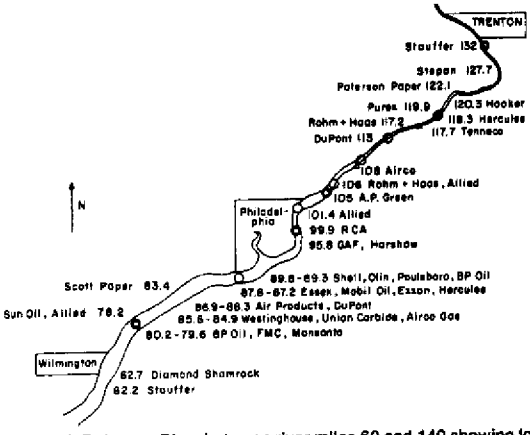


Figure 1. Delaware River between river miles 60 and 140 showing locations of chemical companies (side not significant)
Sampling sites: O = collected August 1976, Δ = collected October 1976, □ = collected March 1977

Figure 1 gives water sampling locations. This particular river segment was of interest because it is the most heavily industrialized area along the river and is a direct source of drinking water for several of the cities in the region including Philadelphia. In addition, because of high tidal flows, this segment is well mixed, but it is far enough upstream to avoid estuarine salinity effects.

Grab samples were collected in 1-gal amber glass bottles with Teflon-lined caps at depths of 0.5–1.0 m. All center channel samples were collected aboard the *Aquadelphia*, the boat used by the City of Philadelphia for its own river sampling program.

Samples for volatile analysis were packed in ice to slow biological degradation prior to analysis, while samples for solvent extraction were immediately preserved by acidifying to pH 2 with hydrochloric acid and by adding approximately 250 mL of dichloromethane. Addition of the organic solvent also started the extraction process. Sample workup was begun as soon as possible after returning to the laboratory, usually within 24 h. In all cases, samples were kept refrigerated until analyzed. Analytical techniques for concentration, separation, and identification using solvent extraction, gas chromatography, and mass spectrometry have been discussed in detail elsewhere (7).

The GC and GC/MS analyses of the initial samples indicated low levels of all organics (in the sub-ppb range) and only gradual qualitative changes in the sample composition as a function of river location. Therefore, only five grab samples were collected in early March 1977; these were larger in volume (21 L) and were more widely spaced than the first group. Their locations are given in Figure 1.

Initial results indicated that the samples were extremely complex, containing not only mixtures of industrial and natural organic compounds but also high background levels of gas chromatographically unresolvable materials. Organic background interferences were removed from the extracts using a silica gel chromatographic cleanup procedure. Dichloromethane extracts were evaporated to dryness, and transferred to a column (5 × 0.6 cm i.d.) packed with deactivated silica gel (5% water). The sample was then fractionated by successively eluting with 10 mL each of hexane, benzene, and methanol. To remove fatty acid interferences, fractionated extracts were dissolved in dichloromethane and extracted with aqueous NaOH (pH 9–11). GC/MS analyses were run on all samples prior to each of these cleanup procedures to verify that sample integrity was being maintained, that contamination was not introduced, that major compounds were not lost, and that sample components were not degraded. In addition, blanks run for all of the concentration and cleanup steps showed no significant contamination.

Identification of compounds in the river water extracts was based on coincidence of gas chromatographic retention times and on equivalence of electron impact and chemical ionization mass spectra with those of authentic compounds. Those compounds not commercially available were synthesized in our laboratory.

We should emphasize that this study was primarily qualitative; our principal goal was to identify compounds in the Delaware River rather than to exactly measure their abundance. For this reason, the concentration data are only semiquantitative. These data were based on GC peak heights measured from chromatograms of the CH_2Cl_2 extracts before any cleanup procedures were applied. The GC response factors were determined for nine representative compounds and ranged from 0.085 to 0.20 ng/mm. Since solvent extraction efficiencies were not determined, the concentrations reported below are minimum levels. Other errors in quantitation result from losses of volatile compounds (such as toluene and chlorobenzene) and from poor GC resolution due to high background interferences in some nonfractionated samples. Taking all of these factors into account, we estimate that the errors in quantitation range from ±50% for the more abundant compounds (>2 ppb) to an order of magnitude for some of the very trace level compounds (<0.01 ppb). On the other hand, the *relative* concentrations of a given compound which were measured at different river locations or times are more accurate because all samples have the same experimental bias.

One of the major experimental problems associated with GC/MS analysis of environmental samples is the possibility of artifact formation during sampling, concentration, or analysis procedures. Because we are dealing with a complex and undefined sample matrix, it is often impossible to predict reactions of individual compounds within this matrix or to run controlled experiments to test for their occurrence. However, we have taken care to both minimize these effects in terms of sampling, handling, and cleanup techniques and to consider them during data interpretation. For example, since liquid chromatographic cleanup separated sample components into three groups, the number of interactive effects which could occur was reduced. Furthermore, in an LC fraction only compounds of a given polarity should appear. If within a given fraction, a specific compound is identified which is outside of the proper polarity range, artifact formation was considered.

Results and Discussion

The organic compounds identified in the Delaware River water samples are listed in Table I. The data include the concentration range and the location of the maximum concentration for each sampling season. Structures for a selected group of compounds are given in Figure 2.

Figure 3 shows high-resolution gas chromatograms for a single water extract after sample workup. An inspection of these results demonstrates the high GC resolution and dynamic range resulting from our procedures. Without this high dynamic range, only a few of the most abundant compounds would have been identified.

The compounds listed in Table I are derived from three principal sources: natural products, municipal wastes, and industrial contaminants. Examples of each source are included in the following discussion.

Compounds 1, 2, and 3 are naturally occurring compounds resulting from the normal biological processes taking place in the river. The first compound, 6,10,14-trimethyl-2-penta-

Table I. Compounds Found in the Delaware River

compounds	vol[a]	winter: conc range, ppb	winter: river mile max	summer: conc range, ppb	summer: river mile max
isoprenoids					
1. 6,10,14-trimethyl-2-pentadecanone*	...	ND[b]	...	0.8–2 (11)[c]	106
2. α-terpineol*	...	0.5–4 (5)[d]	98	ND	...
3. chlorophyll[e]	...	4–8 (5)	98	3–16 (11)	106
steroids					
4. cholesterol	...	5–10 (5)	78	3–8 (11)	93
5. cholestene	...	ND	...	trace	93
6. cholestanol	...	4–9 (5)	78	1–2 (11)	93
fatty acids and esters					
7. stearic acid	...	NQ[f]	98	NQ	...
8. palmitic acid	...	NQ	98	NQ	...
9. methyl stearate	...	ND	...	NQ	93
10. methyl palmitate	...	ND	...	NQ	93
11. methyl myristate	...	ND	...	NQ	93
aromatic hydrocarbons					
12. benzene	D	ND	...	ND	...
13. toluene	D	ND	...	ND	...
14. C_2 benzenes	D	17	98	ND	...
15. C_3 benzenes	D	4	98	ND	...
16. C_4 benzenes	D	trace	...	ND	...
17. C_5 benzenes	D	ND	...	ND	...
18. styrene	D	ND	...	ND	...
19. α-methylstyrene	D	ND	...	ND	...
20. C_5 unsaturated benzene	D	ND	...	ND	...
21. naphthalene	D	0.7–0.9 (3)	98	NA[g]	...
22. methylnaphthalenes	D	0.4–1 (3)	78, 98	NA	...
23. C_2 naphthalenes	D	1–5 (5)	78	NA	...
24. C_3 naphthalenes	D	2–5 (5)	78	NA	...
25. C_5 naphthalenes	D	0.2–0.5 (3)	78	NA	...
26. pyrene	...	trace	...	NA	...
27. fluoranthene	...	trace	...	NA	...
28. anthracene	...	trace	...	NA	...
29. phenanthrene	...	trace	...	NA	...
30. methylphenanthrene	...	trace	...	NA	...
31. chrysene	...	trace	...	NA	...
phenols					
32. phenol	...	2–4 (2)	98	ND	...
33. cresols	...	2	98	ND	...
34. C_2-phenols	...	2	98	ND	...
35. C_3-phenols	...	2	98	ND	...
36. C_4-phenols	...	2	98	ND	...
37. C_8-phenols	...	0.4–2 (5)	105	trace	...
38. *p*-(1,1,3,3-tetramethylbutyl)phenol*	...	1–2 (5)	98	0.2–2 (11)	98
39. nonylphenols	...	1–2 (5)	105	0.04–1 (11)	98
40. phenylphenol	...	0.3	98	ND	...
41. cumylphenol*	...	ND	...	trace	98
42. methylisoeugenol*	...	ND	...	NQ	...
chlorinated compounds					
43. chlorobenzene	D	7.0	98	ND	...
44. dichlorobenzene	D	0.4	98	ND	...
45. trichlorobenzenes	D	0.5–1 (3)	98	ND	...
46. chlorotoluene	D	3	98	ND	...
47. benzyl chloride	D	ND	...	ND	...
48. dichloromethane	D	ND	...	ND	...
49. chloroethylene	D	ND	...	ND	...
50. chloroform	D	ND	...	ND	...
51. trichloroethylene	D	ND	...	ND	...
52. tetrachloroethylene	D	ND	...	ND	...
53. dichlorophenols	...	0.3	98	ND	...
54. trichlorophenols	...	2	98	ND	...
55. (trifluoromethyl)chloroaniline	...	trace-2 (2)	78	ND	...

Table I. Continued

compounds	vol[a]	winter: concn range, ppb	winter: river mile max	summer: concn range, ppb	summer: river mile max
56. (trifluoromethyl)chloronitrobenzene	...	2–3 (3)	78	ND	...
57. bis(chlorophenyl) ketone*	...	NQ	98	0.2–2 (8)	93
58. bis(chlorophenyl)methanol*	...	NQ	...	0.1–1 (8)	93
59. chlorophenylphenylmethanol*	...	NQ	98	trace	93
60. 1,1-bis(chlorophenyl)-2,2-dichloroethylene*	...	NQ	...	NA	...
61. chloromethylacetophenone*	...	ND	...	trace	...
62. $C_{10}H_{11}Cl_3OS$[h]	...	trace	78	trace	78
ethylene glycol derivatives					
63. bis(2-chloroethyl) ether*	...	trace	98	ND	...
64. 1,2-bis(2-chloroethoxy)ethane*	...	15 (2)	98	ND	...
65. 1-(2-chloroethoxy)-2-phenoxyethane*	...	3	98	NQ	101
66. 1-chloro-2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]ethane*	...	trace-2 (3)	98	0.01–0.2 (7)	101
67. 1-chloro-2-(2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]ethoxy)-ethane*	...	0.2–4 (3)	98	0.03–0.1 (7)	101
68. 2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethanol*	...	NQ	...	NQ	...
69. 2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]ethanol	...	NQ	...	NQ	...
70. 2-(2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]ethoxy)ethanol*	...	NQ	...	NQ	...
71. bis(2-[2-(*n*-butoxy)ethoxy]ethoxy)methane*	...	1–3 (5)	78	2–3 (11)	115
esters (plasticizers)					
72. tri(*tert*-butyl) phosphate	...	0.4–2 (4)	78	0.06–0.4 (10)	88
73. tri(2-butoxyethyl) phosphate*	...	0.3–3 (5)	78	0.4–2 (11)	88
74. triphenyl phosphate	...	0.1–0.3 (2)	78	0.1–0.4 (11)	93
75. dibutyl phthalate	...	0.2–0.6 (5)	...	0.1–0.4 (11)	...
76. dioctyl phthalates	...	3–5 (5)	...	0.06–2 (11)	...
77. butylbenzyl phthalates	...	0.4–1 (5)	...	0.3–0.3 (11)	...
78. dimethyl terphthalate	...	0.06	...	ND	...
79. di(2-ethylhexyl) adipate	...	0.08–0.3 (5)	...	0.02–0.3 (11)	...
80. di(isobutyl) azelate	...	NQ	98	ND	...
81. di(2-ethylhexyl) sebacate		trace	98	ND	...
82. tetraethyleneglycol di(2-ethylhexanoate)*	...	1–14 (5)	78	1–4 (11)	106
83. tetraethyleneglycol di(2-methylheptanoate)*	...	0.1–0.3 (5)	78	0.1–0.3 (11)	106
84. triethyleneglycol di(2-ethylhexanoate)*	...	0.6–1 (4)	78	ND	...
others					
85. 2-ethylhexanol	...	3–5 (2)	98	ND	...
86. 2,2,4-trimethyl-1,3-pentanediol-1-isobutyrate	...	1–6 (5)	78	ND	...
87. 2,2,4-trimethyl-1,3-pentanediol-3-isobutyrate	...	1–4 (5)	78	ND	...
88. 2-phenyl-2-propanol	...	2–3 (3)	78	ND	...
89. isophorone*	...	trace	78	ND	...
90. nitroxylene	...	0.3	98	ND	...
91. *o*-phenylanisole	...	trace	...	ND	...
92. binaphthyl sulfones*	...	NQ	98	NQ	98
93. caffeine	...	trace	78	ND	...
94. methylcyclohexane	D	ND	...	ND	...
95. methyl isobutyl ketone	D	ND	...	ND	...
96. ethylthiopyridine*	...	ND	...	trace	103
97. phthallic acid	...	ND	...	NQ	...
98. 1,1,1-triphenylethane	...	ND	...	NQ	...
99. fluorenone	...	NQ	...	NQ	...

[a] D: compound was detected in the vapor stripping analysis of the October samples; quantitation was not possible. [b] ND: compound was not detected. [c] Number indicates the number of samples out of 11 where the compound was found. When no number is shown the compound was detected in only one sample. [d] Number indicates the number of samples out of 5 where the compound was found. [e] Chlorophyll was identified from phytadienes (*16*) which are its volatile pyrolytic degradation products. [f] NQ: compound was detected but for various reasons it was not quantitated. [g] NA: analysis of the LC fraction containing these compounds was not carried out. [h] Exact structure not known. * For structure, see Figure 2.

decanone, probably results from the oxidative degradation of phytol; other workers have found this ketone in various sediments (*14*) and plants (*15*). Chlorophyll was observed in our GC/MS analyses as phytadienes which are produced in the injection port by pyrolysis of the phytol ester part of chlorophyll (*16*). Chlorophyll was abundant in the August water extracts, but it was a minor component in the winter water samples. This is not surprising since chlorophyll comes from algae and phytoplankton which are at higher levels in the river during the summer months.

Municipal waste effluents are characterized by high concentrations of sterols, fatty acids, and fatty acid esters (*17*).

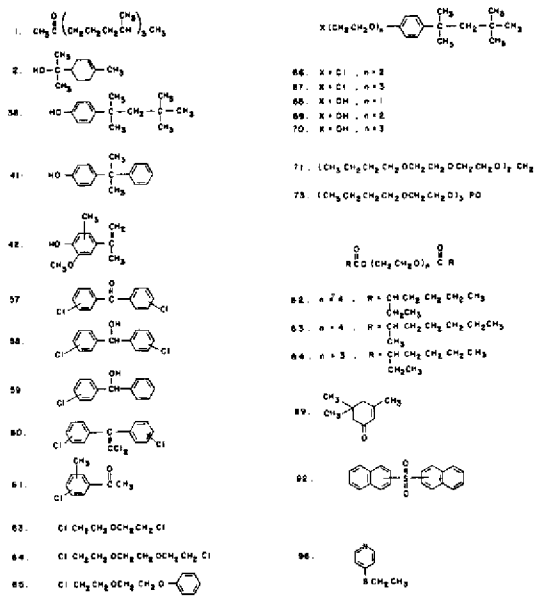


Figure 2. Structures of selected organic compounds found in the Delaware River (see Table I)

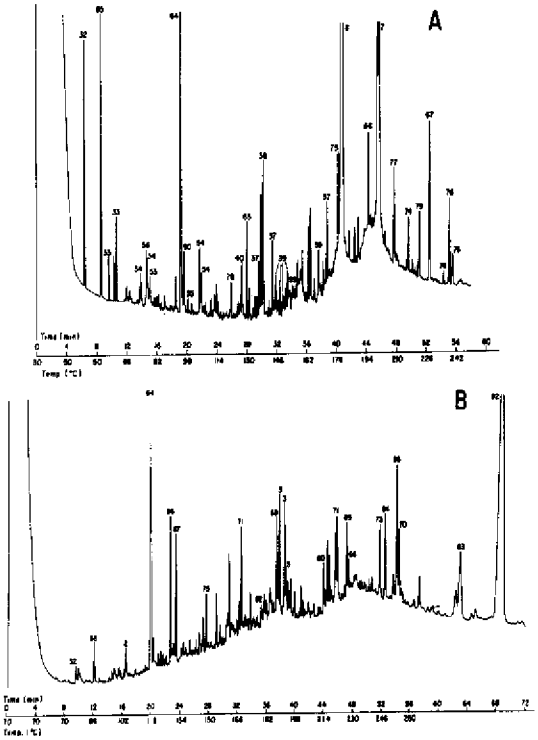


Figure 3. Gas chromatograms of benzene (A) and base extracted methanol (B) fractions of Delaware River water collected March 1977 (river mile 98).
Run on SE-52 open tubular (25 m × 0.22 mm) glass capillary column. Numbered peaks identified in Table I

These compounds (no. 4–11) were found at high levels in most of the samples from the Delaware River. For example, cholesterol was usually one of the most abundant compounds in the water. The concentration profile for cholesterol in the August water samples showed a maximum at river mile 93; this is consistent with locations of municipal sewage plants in the Philadelphia-Camden area. Fatty acids were not quantitated due to their poor chromatographic resolution, but they were present at very high levels in all samples.

The anthropogenic chemicals were by far the most numerous group of compounds and are the compounds of greatest concern to this study. In reviewing concentration and source data for these chemicals, it becomes apparent that they are of three types: those found in industrialized urban areas with no specific production source; those commonly used in manufacturing processes with multiple sources; and those specific to a single industrial site and traceable to that source.

Included in the first group of general industrial contaminants are all of the aromatic hydrocarbons (no. 12–31), most of the phenolic compounds (no. 32–42), most of the chlorinated species (no. 43–54), and some industrial solvents (no. 85, 88, 89, 95). Almost all of these compounds have been isolated and identified in urban watersheds (*18–20*); they appear to arise from automobile emissions, water chlorination, and general urban activities.

Source identification for several of the phenols, notably *p*-(1,1,3,3-tetramethylbutyl)phenol, and the nonylphenol isomers is more difficult. Concentration data show highest levels around Philadelphia, implicating general urban activity as the primary source; however, there are several high production chemical companies in the area, one of which produces these phenols commercially (*21*). Under these circumstances no definite source can be identified.

Perhaps the most ubiquitous of all environmental contaminants are the plasticizers (no. 72–84). These compounds can be found in the wastewater from a large number of industrial sources (*18*). The most common plasticizers (phthalates and adipates) show no concentration maxima along the river and may be assumed to enter from multiple locations. Some of the less common plasticizers tend to maximize in particular river segments, suggesting single point sources. For example, tri(*tert*-butyl) phosphate, and tri(2-butoxyethyl) phosphate, maximize near river mile 78 in the winter. These concentration data are consistent with commercial production sites along the river (*21*).

The plasticizer, tetraethyleneglycol di(2-ethylhexanoate), no. 82 on Figure 3, is interesting because it was both the most abundant compound in the river and the most challenging to identify. The electron impact mass spectrum for this compound (Figure 4) shows an intense ion at *m/e* 171 with less abundant ions at *m/e* 127, 99, 87, and 57. High-resolution mass spectrometry established the elemental compositions of *m/e* 171 and 127 (see Figure 4). The small fragment ion at *m/e* 45 and the large neutral loss of 44 mass units (171 to 127) are characteristic of ethylene glycol compounds. The GC retention time suggested a rather high molecular weight despite the absence of any high mass fragments in the EI mode. Methane CI gave no additional information on molecular weight; but isobutane CI showed an M + 1 ion at 447. An elemental composition for the molecular ion of $C_{23}H_{46}O_7$ was hypothesized based on the rather saturated composition of *m/e* 171. Gas chromatography using a nitrogen-phosphorus detector did not contradict this hypothesis. A search of the EPA TSCA list (*22*) and *Chemical Abstracts* for industrial compounds corresponding to this molecular composition indicated that tetraethyleneglycol di(2-ethylhexanoate) (a plasticizer patented and produced by one of the companies along the river) was a possibility. In fact, river water concentrations for this

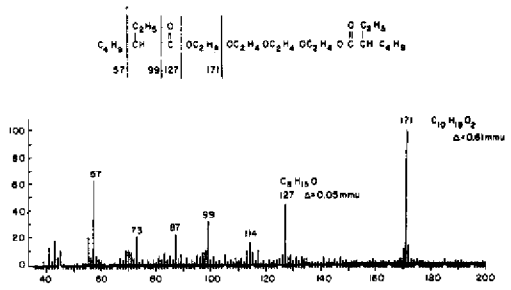


Figure 4. Electron impact mass spectrum of tetraethyleneglycol di(2-ethylhexanoate), compound 82

Elemental compositions of *m/e* 127 and 171 established by high-resolution mass spectrometry; Δ values indicate error (in milli-mass units) between measured and calculated exact masses

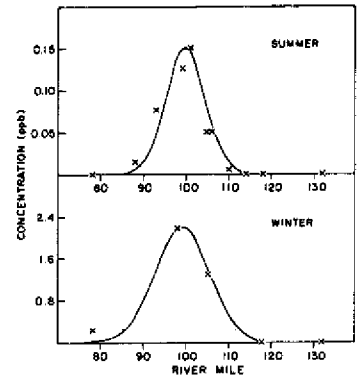


Figure 6. River water concentrations of compound 67 as a function of river mile for samples collected in August 1976 (summer) and March 1977 (winter)

compound were highest in that sample taken adjacent to the suspected discharge site (*21*). Identification and approximate concentrations of the compound were verified using the authentic commercial product. The methylheptanoate isomer and the triethylene glycol homolog (compounds 83 and 84, respectively) were identified in a similar manner.

The other polyethylene glycol compounds (no. 63–71) are also industrial chemicals which are specific to a single source and which are traceable to that source. Identification of 1,2-bis(2-chloroethoxy)ethane in the river water near Philadelphia initiated a search for a possible source. According to the 1974 U.S. Tariff Commission Report (*21*), one of the companies in the area is the sole commercial producer of this compound and holds a patent for its production (*23*). Similarily, 1-(2-chloroethoxy)-2-phenoxyethane, bis(2-chloroethyl) ether, and compounds 68, 69, and 70 are produced or patented by the same company.

Identification of two other chloroethers (no. 66 and 67) was facilitated by their spectral and structural similarity to the above compounds. Figure 5 shows the EI mass spectrum of compound 67; the elemental composition of *m/e* 285 (obtained from HRMS) is included. An electron impact fragmentation pattern of 63, 65, 107, 109, 151, and 153 is characteristic of a monochlorinated ion with 44 mass unit adducts. Previous identifications of chlorinated ethylene glycols suggested that this should be a similar compound with *m/e* 63 due to $ClCH_2CH_2$, *m/e* 107 to $ClCH_2CH_2OCH_2CH_2$, and *m/e* 151 to $ClCH_2CH_2OCH_2CH_2OCH_2CH_2$. Ions at 77, 91, and 135 are characteristic of C_3-phenolic compounds. A combination of these fragments accounts for the base peak at 285 (see Figure 5). A mass chromatogram indicated a very weak molecular ion at *m/e* 356 suggesting that a C_5H_{11} fragment should be added to the 285 ion to give compound 67. The hypothesized structure was synthesized by chlorinating the hydroxy compound (no. 70) with PCl_3 (*23*). The GC retention time and mass spectrum for the unknown compound were identical to the synthetic compound. Compound 66 was similarly identified.

We should point out that compounds 66 and 67 are not artifacts formed by the chlorination of compounds 68 to 70 during the course of sample analysis. Since these two groups of compounds were separated during LC fractionation prior to GC analysis, there was no opportunity for their interconversion.

Concentration profiles for compound 67 are given in Figure 6. Although the relative effects of dispersion due to tidal flow and of downstream movement due to the net river flow are not precisely known, it is clear from these data that compound 67 comes from a point source located near river mile 100. In fact, the company which produces the related alcohols (no. 68–70), the chlorinated ethylene glycol (no. 64), and the C_8-phenol (no. 38) discharges its effluent at river mile 104.

The presence of these compounds in the Delaware River *may* have some health implications. If the discharge site at river mile 104 is correct, then these compounds would enter the river only six miles downstream from the inlet for Philadelphia's drinking water. Tidal action is sufficient to carry these chemicals upstream to the inlet and, in fact, the volatile ethers, bis(2-chloroethyl) ether, and 1,2-bis(2-chloroethoxy)-ethane, have been found in the drinking water supply (*24*). Health effects, notably the carcinogenic activity, of these compounds are not known. It should be stressed that the higher molecular weight compounds (no. 65–70) have not yet been detected in the drinking water, nor have their health effects been evaluated.

The chlorinated compounds (no. 57–60), bis(chlorophenyl) ketone, bis(chlorophenyl)methanol, chlorophenylphenylmethanol, and 1,1-bis(chlorophenyl)-2,2-dichloroethylene (see Figure 2 for structures), represent another important group traceable to a single industrial source. Al-

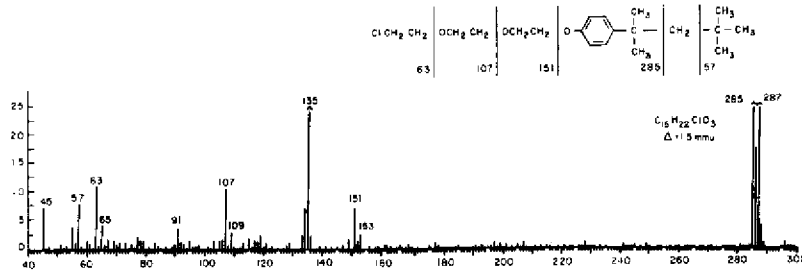
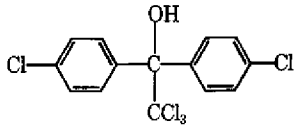


Figure 5. Electron impact mass spectrum of 1-chloro-2-(2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]ethoxy)ethane, compound 67. Relative intensities expanded by factor of 4; intensities of off-scale peaks are 135 (34%), 285 (100%), 287 (37%)

though none of these compounds is manufactured commercially, the insecticide 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol is produced by the same company which manufactures most of the ethylene glycol compounds:



We suggest that compounds 57 to 60 are either manufacturing by-products from the production of this insecticide or are its environmental degradation products. Model experiments with the commercial insecticide demonstrated that compounds 57–60 were *not* formed during our analytical procedures.

Finally, a few miscellaneous compounds which were identified in the Delaware River and which have not been previously reported as water contaminants will be discussed: (Trifluoromethyl)chloroaniline and (trifluoromethyl)chloronitrobenzene (no. 55 and 56) were identified in the water; they had maximum concentrations at river mile 78. Both compounds represent common substructures in various pesticide and dye molecules, and several of the companies located along the river have patents using these compounds (25–27). It is possible that these (trifluoromethyl)chloro compounds are actually present in the river water as such, but it is also possible that they are formed in the GC injection port by pyrolytic degradation of larger pesticide or dye molecules. All three binaphthyl sulfone isomers (no. 92) were identified in the river water near Philadelphia. Product literature for one of the companies in the area indicates production of condensed sulfonated polymers derived from naphthalene sulfonic acid and maleic anhydride. It seems likely that the binaphthyl sulfones could be formed as by-products during preparation of this commercial product.

River water samples were collected both in August and early March; this allowed us to compare results from two sampling seasons. Generally, the two data sets were qualitatively similar suggesting that pollution sources remained stable over the test period; however, two major changes were observed. First, winter samples contained high levels of volatile organics (10–20 ppb) which were not detected in the summer water. Most likely high water temperature (25–27 °C) and turbulent river flow volatilized organics from the river during the summer months.

The second change was the three- to fourfold increase in the level of almost all organics in the winter samples. This observation was corroborated by weekly data on nonspecific organic levels (COD, TOC, etc.) collected by another laboratory during the same sampling period (9). Winter samples were collected during a period of high stormwater runoff and were very turbid in nature. It is possible that high levels of particulate matter were responsible for increased organic concentrations in the water column. These particles could have been sedimentary organic compounds, or they could have provided favorable adsorption sites within the water column for dissolved organics. As an alternative, municipal and industrial waste treatment systems may have been adversely affected by the cold winter temperatures, resulting in significantly higher organic loads entering the river system.

Conclusions

The organic compounds in any river system will be a complex mixture of natural products, municipal wastes, and industrial contaminants with the predominance of any type dependent on river hydrology, discharge sources, and general river conditions. In a large river, flow volumes are usually orders of magnitude greater than incoming discharge streams; thus, the concentration of most industrial chemicals will be in the sub-ppb range. The analysis of these low levels of extremely complex mixtures is not an easy task. The analysis of organics in an industrial wastewater, for example, is usually much simpler.

We have noticed the predominance of ethylene glycol derivatives in the Delaware River. Compounds 63–71 and 82–84 are all based on ethylene glycol and are among the most abundant anthropogenic compounds in the river. Since few mass spectra of these compounds are in reference collections, their proper identification is frequently difficult and overlooked. In most cases the presence of an ion at *m/e* 45 together with abundant neutral losses of 44 amu should indicate to the mass spectral interpreter that an ethylene glycol derivative may be a good structural hypothesis.

Acknowledgments

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Sources and Movement of Organic Chemicals in the Delaware River

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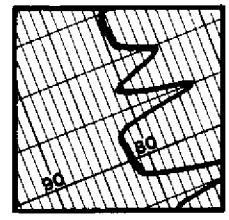

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Sources and Movement of Organic Chemicals in the Delaware River

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■ The transport of industrial organic chemicals from their source, into the Delaware River, through various treatment facilities, and into Philadelphia's finished drinking water was studied using water samples collected in August 1977. Solvent extraction, liquid chromatographic cleanup, and gas chromatographic mass spectrometry were used for compound separation and identification. Results confirmed discharge sources for many previously identified compounds. Furthermore, it was shown that many of these compounds circulated into Philadelphia's drinking water, and that the various water and waste treatment facilities had a minimal effect on the organic levels. For all chemicals, dilution processes were responsible for the largest reduction in organic concentrations. Results were substantiated by a 10-week sampling program designed to monitor seven selected waste chemicals.

Nearly 100 organic compounds of biological, municipal, and industrial origin have been identified in the Delaware River (*1*). Among the industrial contaminants, several compounds seemed to be coming from a specific plant in the Philadelphia area. Furthermore, relatively high levels of anthropogenic chemicals were observed in the river near the Philadelphia area (*1*), indicating that they may be entering the city's drinking water. We have, therefore, traced the movement of various industrial chemicals from their origin, through the river, and into Philadelphia's drinking water. We have also conducted a 10-week, continuous sampling program to monitor seven selected compounds in the aquatic system. This paper is a report on these studies.

The Sampling Area. Only a small segment of the Delaware River, lying just north of Philadelphia, was studied. A schematic diagram of the complete sampling area is shown in Figure 1. General flow and hydraulic characteristics of the river have been discussed previously (*1*). The box in the upper left-hand corner of Figure 1 represents a plant in the Philadelphia area which we will refer to as plant A. This plant does not discharge its wastewater directly into the river, but rather into the city sewer along with several other industrial users. These combined industrial wastes are treated at the City of Philadelphia's Northeast Sewage Treatment plant using classical secondary treatment methods (*2*). The treated effluent is then discharged into the Delaware River at river mile 104.

Water flow in this segment of the river is dominated by tidal movement rather than by downstream river flow; tidal volumes are an order of magnitude greater than downstream river flows. During periods of normal flow, effluents discharged into the river travel approximately 7 miles upstream during high tide (*3*). Under these conditions, water flow in the upstream direction is sufficient to transport industrial chemicals from the sewer outfall upstream to the intake pipes of Philadelphia's Torresdale drinking water facility at river mile 110 (*4*). Intake valves for this plant are open only during high tide, making industrial waste contamination of the city's drinking water not only possible but probable (*4*). Water entering the drinking water plant is treated using standard techniques (*4, 5*): prechlorination; settling; coagulation (ferric chloride, alum, and lime); disinfection; flocculation; and filtration (rapid sand filters). After a final chlorination step, drinking water is distributed throughout the city. Water from this treatment facility provides the city of Philadelphia with approximately 50% of its finished drinking water (*4*). All present drinking water standards are being met at this water treatment plant (*6*).

Experimental

Samples were collected in late August 1977 from sites a to h, as shown in Figure 1. Our purpose was to follow a 24-h slug of industrial wastes through the cycle from plant A to the finished drinking water. The sampling scheme was designed to account for retention times between the various sampling locations, as well as for tidal movement in the river (*3, 4*). Details of this sampling regime are outlined in Table I.

The composite sample from plant A was taken from a 5-gal continuous sampler after the 24-h sampling period. All other samples were composites of individual grab samples collected at a particular location. River water samples were collected approximately 100 yards from the western shore at the designated river mile and at a depth of about 0.5 m.

Another set of samples was collected weekly over the 10-week period extending from January 15 to March 28, 1978, from points c, g, and h (see Figure 1) and from the center channel of the Delaware at river mile 98. Samples from sites c, g, and h were composites of 200-mL grab samples collected every 8 h beginning Tuesday 8 a.m., Tuesday 8 p.m., and Wednesday 8 a.m., respectively. River samples were 1-gal grab samples taken every Wednesday morning.

All samples were collected in glass bottles fitted with Teflon-lined screw caps. Methylene chloride and hydrochloric acid were added to the water samples at the collection site in order to minimize biological degradation and to start the extraction process. Since waste effluents from plant A do not support microbial activity (*6*), sample preservation in the 24-h continuous sampler was not needed.

All samples were stored in the dark. Small samples were kept on ice during transport to the laboratory. Larger samples were refrigerated as soon as possible after collection.

Analytical techniques and instrumentation for the concentration, separation, and identification of sample components have been discussed in detail elsewhere (*1*). In general, analytical techniques used in this study included solvent extraction, liquid chromatographic fractionation, high-resolution gas chromatography, computerized gas chromatographic mass spectrometry (GC–MS) in both the electron impact (EI) and chemical ionization (CI) modes, mass spectrometric selected ion monitoring (SIM), and high-resolution mass spectrometry (HRMS).

For the initial phase of this study (August, 1977), concentration values were semiquantitative and were based on standard curves for selected compounds. Estimated errors in quantitation are approximately ±20% in plant A's waste effluent, ±50% in the Northeast influent and effluent and the river water, and an order of magnitude in the finished drinking water.

During the second phase of this study (January–March, 1978), experimental procedures were developed to more precisely quantitate seven previously identified compounds. Concentration values were measured using selected ion monitoring (SIM) performed on the unfractionated, combined neutral and acidic extracts for each sample. Sample concentrations were calculated by comparing the computer-integrated peak areas of selected masses with those obtained from standard solutions containing the seven compounds.

Table I. Sampling Scheme Giving Details of Timing, Volumes, Types, and Locations (See Figure 1)

location	collection period [a]	total vol, L	sampling interval, h	no. of samples	type
(a) plant A effluent	8/23 12 p.m. to 8/24 12 p.m.	0.5		1	continuous
(b) Northeast influent	8/24 2 a.m. to 8/25 2 a.m.	0.5	4	7	grab
(c) Northeast effluent	8/24 8 a.m. to 8/25 8 a.m.	1	4	7	grab
(d) river mile 106	8/25 10 a.m.	23		1	grab
(e) river mile 108	8/25 10:30 a.m.	23		1	grab
(f) river mile 118	8/25 11 a.m.	23		1	grab
(g) Torresdale influent	8/25 8 a.m. to 8/26 8 a.m.	4	4	7	grab
(h) Torresdale effluent	8/25 8 p.m. to 8/26 8 p.m.	4	4	7	grab

[a] All samples taken in August, 1977.

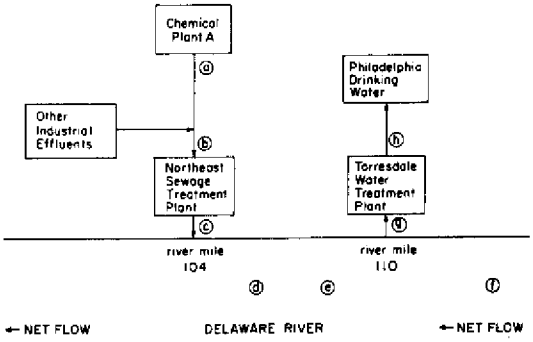

Figure 1. The sampling area, showing collection sites. River mileages are measured upstream from the mouth; net flow proceeds from right to left

Solvent extraction efficiencies were measured for these seven compounds. Preextracted water samples were spiked with a known aliquot of a standard solution. Spiked samples were extracted and quantitated using the above procedures. Tests were run in triplicate using water samples from all four sampling locations. Recoveries were better than 75% in all cases. Reported concentration values were corrected for solvent extraction efficiencies and have errors of less than ±20%, excluding sampling errors.

Results and Discussion

All of the compounds identified in the industrial wastewater, the municipal sewage effluent, the river water, and Philadelphia's finished drinking water are listed in Table II. Some structures are given in Figure 2. Estimated concentrations have been included for most of the abundant compounds. The compounds in Table II are listed according to location of first appearance. Within each of these groups, chemicals have been subdivided by compound type. This arrangement allows for both a quick identification of specific pollution sources and for a facile appraisal of the movement of these chemicals in the aquatic system.

For an overview of the occurrence and environmental significance of many of the compounds listed in Table II, the reader is referred to our previous paper on the Delaware River (*1*). During the following discussion, only those compounds which were not previously identified in the Delaware River or which gave some insight into the movement of chemicals through the various treatment processes and in the Delaware River will be considered.

Identification of Contamination Sources. The first objective of this study was to verify that plant A was the specific source for a set of previously identified compounds. These compounds included 1,2-bis(chloroethoxy)ethane (**6**), the phenyl glycols (**7–11**), the chlorinated phenyl glycols (**12** and **13**), DDE (**17**), dichlorobenzophenone (**16**), and the binaphthyl sulfones (**37**).

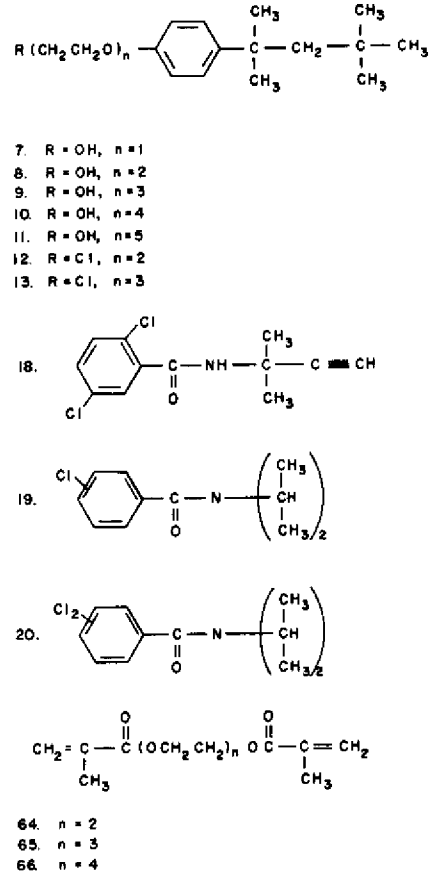

Figure 2. Structures of selected organic compounds found in the Delaware River (see Table II)

Our data (see Table II) verify that these chemicals are, in fact, being discharged from plant A along with various other phenolic compounds (**1–5**), chlorinated compounds (**18–20**), and esterified species (**25** and **26**). All of the above compounds are either commercial products manufactured at plant A or are process byproducts.

The commercial herbicide (**7**) 2,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide (**18**) was discharged in plant A's waste effluent in relatively high concentration (500 ppb). We should point out that this compound was not detected during our earlier work (*1*), but plant A operates in a batch mode (*6*) and does not consistently discharge the same mix

Table II. Compounds and Their Concentrations (ppb) Observed at the Various Sampling Sites (See Figure 1)

	plant A	NE in	NE out	RM 109	RM 108	Tor in	Tor out	RM 118
I. plant A								
A. phenols								
1. phenol	7000	60	20	0.3	0.3	t[a]	t	–[b]
2. cresol	50	un[c]	20	–	–	–	–	–
3. octylphenols[d]	5000	400	200	3	2	0.4	0.01	–
4. nonylphenols[e]	600	un	40	1	0.02	0.02	–	–
5. 4-octyl-2,6-di-*tert*-butylphenol	200	un	un	–	–	–	–	–
B. ethylene glycol derivatives								
6. 1,2-bis(2-chloroethoxy)ethane	100	un	un	t	t	t	t	–
7. 2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethanol[m]	200	50	10	un	un	un	0.02	5
8. 2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]ethanol[m]	100	un	un	0.6	0.3	un	0.02	5
9. 2-(2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]-ethoxy)ethanol[m]	400	un	un	0.4	0.2	un	0.002	t
10. 2-[2-(2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]-ethoxy)ethoxy]ethanol[m]	200	un	un	un	NA[f]	NA	NA	–
11. 2-(2-[2-(2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]-ethoxy)ethoxy]ethoxy)ethanol[m]	un	un	NA	NA	NA	NA	NA	NA
12. 1-chloro-2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)-ethoxy]ethane[m]	2000	200	80	0.6	0.4	0.3	0.2	–
13. 1-chloro-2-(2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)-ethoxy]ethoxy)ethane[m]	1500	120	50	0.6	0.5	0.3	0.2	–
C. chlorinated compounds								
14. tetrachlorostyrenes[e]	400	60	20	0.5	0.06	–	–	–
15. hexachloroethylbenzene[g]	un	un	–	–	–	–	–	–
16. dichlorobenzophenones[g]	1000	110	60	1	0.2	0.1	0.1	–
17. 1,1-bis(chlorophenyl)-2,2-dichloroethylene (DDE)	1800	200	30	0.4	0.3	un	un	–
18. 2,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide[m]	500	40	20	0.4	0.2	0.02	0.01	–
19. chloro-*N*-(1,1-diisopropyl)benzamide[g,m]	50	t	t	t	t	–	–	–
20. dichloro-*N*-(1,1-diisopropyl)benzamide[g,m]	un	un	un	0.2	0.06	0.04	0.02	–
21. dichlorobenzenes[e]	100	100	t	v[h]	v	v	v	v
22. chlorotoluene[g]	un	t	t	v	v	v	v	v
23. trichlorobenzenes[e]	200	20	10	t	t	–	–	–
24. tetrachlorobenzenes[e]	200	t	t	t	t	–	–	–
D. plasticizers								
25. bis(2-ethylhexyl) adipate	2000	90	10	0.2	0.04	0.02	0.002	–
26. dioctyl sebacate	200	–	–	–	–	–	–	–
27. tris(*tert*-butyl) phosphate	50	un	un	0.5	0.4	0.3	0.4	0.8
E. hydrocarbons								
28. C_2 benzenes[e]	1000	100	v	v	v	v	v	v
29. C_3 benzenes[e]	un	40	10	2	0.6	–	–	–
30. naphthalene	un	20	4	t	t	t	–	–
31. methylnaphthalenes	500	un	0.4	0.2	0.02	t	t	t
32. C_2 naphthalenes[e]	t	t	t	t	t	t	–	t
33. C_3 naphthalenes[e]	un	t	t	t	t	t	–	t
34. C_4 benzenes[e]	un	200	40	2	t	–	–	–
35. $C_{14}H_{28}$[i]	200	10	un	–	–	–	–	–
36. $C_{16}H_{32}$[i]	2000	30	10	–	–	–	–	–
F. others								
37. binaphthyl sulfones[e]	0.6	un	un	un	–	–	–	–
38. isophorone	un	100	10	3	0.6	t	t	–
II. Northeast influent								
A. phenols								
39. phenylphenol	–	un	un	un	un	–	–	–
40. cumylphenol	–	t	t	0.3	0.01	0.01	–	–
41. *tert*-butylmethoxyphenol	–	t	t	t	t	t	t	t
C. chlorinated compounds								
42. dichlorophenols[e]	–	un	0.4	0.4	t	t	un	–
43. trichlorophenols[e]	–	t	0.1	0.1	t	t	un	t
44. bis(chlorophenyl)methanol[g]	–	3	5	0.7	0.2	0.1	0.002	–
D. plasticizers								
45. triphenyl phosphate	–	16	2	0.3	0.2	0.2	0.03	t
46. dibutyl phthalate	–	50	25	0.6	0.4	0.1	0.1	0.3
47. butylbenzyl phthalate	–	40	100	0.6	0.3	0.3	0.1	0.4
48. bis(2-ethylhexyl) phthalate	–	200	100	1	1	0.5	0.6	0.5

Table II. Continued

	plant A	NE in	NE out	RM 106	RM 100	Ter in	Ter out	RM 116
E. hydrocarbons								
49. pyrene	–	t	t	t	t	t	–	t
50. fluoranthene	–	t	t	t	t	t	–	t
51. anthracene	–	t	t	t	t	t	–	t
52. phenanthrene	–	t	t	t	t	t	–	t
53. methylphenanthrene	–	t	t	t	t	t	–	t
54. chrysene	–	t	t	t	t	t	–	t
F. others								
55. cholesterol	–	400	200	2	1	0.6	–	0.9
56. cholestanol	–	600	300	3	2	1	–	0.9
57. α-terpineol	–	80	80	–	–	–	–	–
58. 2-phenyl-2-propanol	–	70	70	2	0.5	–	–	–
59. stearic acid	–	h[j]	h	h	h	h	–	m[k]
60. palmitic acid	–	h	h	h	h	h	–	m
61. benzil	–	t	un	0.4	0.2	un	0.02	–
62. bornyl acetate	–	100	50	1	1	0.1	0.002	–
63. *N*-(*n*-butyl)benzenesulfonamide	–	un	un	0.6	0.3	t	t	–
III. Northeast effluent								
B. ethylene glycol derivatives								
64. diethyleneglycol dimethacrylate[m]	–	–	10	0.2	t	t	–	–
65. triethyleneglycol dimethacrylate[m]	–	–	35	0.5	0.1	5	–	–
66. tetraethyleneglycol dimethacrylate[m]	–	–	700	10	3	0.5	0.02	–
67. chlorophenylphenylmethanol[g]	–	–	t	0.1	0.1	0.1	un	–
F. others								
68. menthol	–	–	8	un	–	–	–	–
IV. river								
B. ethylene glycol derivatives								
69. bis(2-[2-(*n*-butoxy)ethoxy]ethoxy)methane	–	–	–	un	1	2	t	3
70. triethyleneglycol bis(2-ethylhexanoate)	–	–	–	0.2	0.1	–	–	0.1
71. tetraethyleneglycol bis(2-ethylhexanoate)	–	–	–	2	3	t	–	2
C. chlorinated compounds								
72. dimethyl 2,3,5,6-tetrachloroterphthalate	–	–	–	un	un	un	0.03	t
D. plasticizers								
73. 2,2,4-trimethylpentane-1,3-diol-1-isobutyrate	–	–	–	0.2	0.2	t	t	–
74. 2,2,4-trimethylpentane-1,3-diol-3-isobutyrate	–	–	–	0.2	0.3	t	t	–
75. 2,2,4-trimethylpentane-1,3-dioldiisobutyrate	–	–	–	0.5	0.3	t	t	–
F. others								
76. chlorophyll[l]	–	–	–	2	7	4	–	9
77. fluorenone	–	–	–	0.04	0.02	un	un	t
78. ethylthiopyridine[g]	–	–	–	t	t	t	t	t
79. 1,1-bis(chlorophenyl)-2,2,2-trichloroethane (DDT)	–	–	–	t	t	t	un	–
V. drinking water								
C. halogenated compounds								
80. dichloroisopropenyltoluene[g]	–	–	–	–	–	–	un	–
81. bromochlorophenol[g]	–	–	–	–	–	–	un	–
82. dibromophenol[g]	–	–	–	–	–	–	un	–
83. dichlorobromophenol[g]	–	–	–	–	–	–	un	–
84. dibromochlorophenol[g]	–	–	–	–	–	–	un	–

[a] t indicates that only trace levels were detected. [b] – indicates not detected. [c] un indicates that compound was not resolved gas chromatographically and, therefore, was not quantitated. [d] The predominant species was *p*-1,1,3,3-tetramethylbutylphenol, although other isomers were present. [e] Several isomers present. [f] NA indicates that analysis for these compounds was not carried out (not analyzed). [g] Isomer unknown. [h] v indicates volatile compound; these compounds would not be retained in the water column during the summer months. [i] Structure unknown, mol wt from CI, present in hexane fraction. [j] h indicates very high concentrations; these compounds give broad unresolved peaks which could not be quantitated. [k] m indicates moderate concentration. [l] Chlorophyll was observed as a series of phytadienes (see ref 7). [m] See Figure 2 for the structure of this compound.

of waste chemicals. Concentrations of compound 18 around 0.003 ppb were found in drinking water samples during our 10-week quantitation study (see below).

An interesting case is presented by several of the multichlorinated aromatic compounds (**14**–**17**): tetrachlorostyrene, hexachloroethylbenzene, DDE, and dichlorobenzophenone. None of these compounds are produced commercially; however, plant A did manufacture the pesticide 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol. This pesticide was produced commercially using the reaction scheme outlined in Figure 3 (*8*). DDE is the unreacted starting material; tetrachlorostyrene and hexachloroethylbenzene are probably cleavage byproducts formed during the initial chlorination step or from the reaction intermediate 1,1-bis(*p*-chlorophenyl)tetrachloroethane. Dichlorobenzophenone could form during alkaline hydrolysis of either the tosylate ester intermediate or the pesticide itself. Two other structurally related compounds, bis(chlorophenyl)methanol (**44**) and chloro-

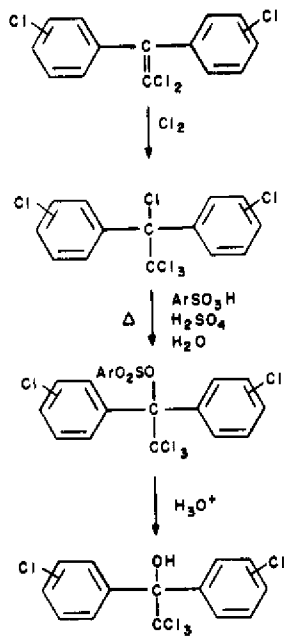

Figure 3. Reaction pathway for the commercial production of 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol (see ref *8*)

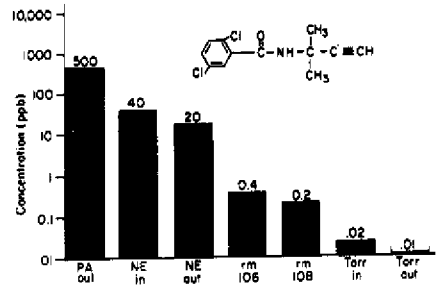

Figure 4. Concentration levels of 2,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide (18) throughout the sampling system

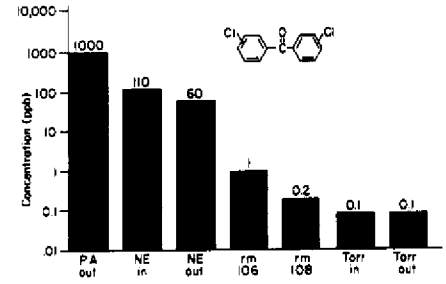

Figure 5. Concentration levels of dichlorobenzophenone (16) throughout the sampling system

phenylphenylmethanol (67), first appear in the Northeast influent and effluent water, respectively. We think that these are probably degradation products of one of the above chlorinated species. We should point out that the pesticide itself was not detected in any of the wastewater or river water samples.

Although some of the methyl substituted compounds (28–34) and chlorinated aromatics (21–24) and the solvent isophorone (38) first appear in plant A's waste effluent, they are common industrial chemicals which could also be entering the water system at various other points. This was confirmed by comparing concentration data for these compounds with the same data for the compounds specific to plant A. The former compounds show much smaller changes in concentration between sampling locations, suggesting multiple discharge sources.

Most of the compounds which appear for the first time in the Northeast treatment plant's influent (39–63) are common industrial or municipal contaminants. They are not unusual and have been discussed in detail elsewhere (*1, 9–11*). *N*-(*n*-Butyl)benzenesulfonamide (63) is interesting because it has never been identified in environmental samples. Its major commercial use is as a plasticizer for polyamide materials (*12–14*). It has also been patented as a starting material in the production of sulfonyl carbamate herbicides (*15*). The exact source of this contaminate is not yet known.

Those compounds originally appearing in the treatment plant's effluent water (64–68) were, of course, not present in the influent water; this suggests that they were formed during the treatment process. The most striking example is the poly(ethylene glycol) derivative, tetraethyleneglycol dimethacrylate (66). This particular chemical is commonly used as a copolymer in many synthetic materials (*16–18*). It seems possible that a polymer entering the Northeast treatment plant is being degraded to monomer units during treatment, or that residual monomer is being washed off polymers during treatment. This compound was the most abundant chemical discharged in the Northeast treatment plant effluent; this leads to correspondingly high river water values. The di- and triethyleneglycol homologues (64 and 65) were also identified.

Compounds first appearing in the river water (69–79) may be categorized into three groups according to source: first, those entering the river system from other industrial discharges such as various ethylene glycol derivatives (69–71) and various plasticizers; second, those compounds formed by the natural biological activity in the river, for example, chlorophyll (76); lastly, compounds which enter the river via rainwater runoff, most notably the herbicide dimethyl 2,3,5,6-tetrachloroterphthalate (72) (*19*).

In the finished drinking water a series of halogenated compounds appears which were previously undetected. It seems logical that these compounds, especially the halogenated phenols (81–84), are formed during the chlorination process (*20*).

Movement of Compounds through the System. It is easiest to assess concentration changes as various compounds travel from industrial wastewater to finished drinking water if the data are presented graphically. Figures 4 to 7 are a series of bar graphs showing concentration data for several compounds at each of the seven sampling locations. These particular compounds were chosen because: (a) they are unique chemicals entering from a single, well-defined source, and (b) they complete the sample loop and were found at all sample locations. This second characteristic makes it possible to assess the effects of all treatment processes and of dilution during upstream river movement.

Figures 4 to 7 indicate several trends. Large changes in concentration (approximately four orders of magnitude) were observed between plant A's effluent and the finished drinking water. Obviously, this large decrease in organic concentration is important when considering allowable discharge levels and treatment processes. For all four compounds, a definite concentration pattern developed over the sample system. The greatest concentration decreases occurred between plant A's effluent and the Northeast Treatment plant's influent (sites a to b) and between the Northeast Treatment plant's effluent and the first upstream river sampling location (sites c to d). It is interesting that these large decreases in concentration are

Table III. Median Concentrations [a] and Relative Concentrations for the 10-Week Study (January to March, 1978) and Grab Sample Concentrations (August 1977)

	10-week concn, ppb				relative concn				grab concn, ppb			
compd [b]	NE eff	river [c]	Torr inf	Torr eff	NE eff	river [c]	Torr inf	Torr eff	NE eff	river [d]	Torr inf	Torr eff
3	200	8	0.4	0.2	100	4	0.2	0.1	200	3	0.4	0.01
7	8	0.3	0.03	0.03	100	4	0.4	0.4	10	un	un	0.02
8	10	0.5	0.05	0.06	100	5	0.5	0.6	un	0.6	un	0.02
12	20	0.3	0.02	0.04	100	2	0.1	0.2	80	0.6	0.3	0.2
13	20	0.5	0.07	0.04	100	3	0.3	0.2	50	0.6	0.3	0.2
17	20	0.3	0.04	0.02	100	2	0.2	0.1	30	0.4	un	un
18	4	0.2	0.003	0.003	100	5	0.08	0.08	20	0.4	0.02	0.01

[a] The range of the individual measurements is usually a factor of 3 above and below the median; for example, for a median of 20 ppb, the range is 7 to 60 ppb. [b] See Table II. [c] River mile 98. [d] River mile 106.

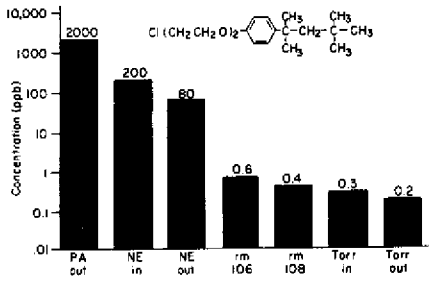

Figure 6. Concentration levels of 1-chloro-2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]ethane (**12**) throughout the sampling system

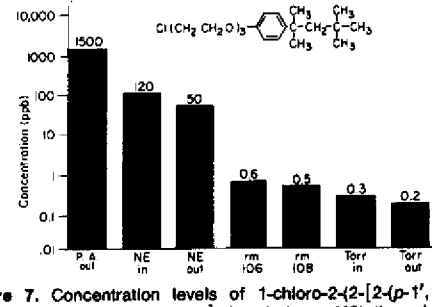

Figure 7. Concentration levels of 1-chloro-2-(2-[2-(*p*-1′,1′,3′,3′-tetramethylbutylphenoxy)ethoxy]ethoxy)ethane (**13**) throughout the sampling system

caused solely by dilution. In the first case, plant A's effluent was diluted with other industrial wastewaters; in the second case, the municipal waste effluent was diluted with river water. In the two areas where treatment was performed, namely between the Northeast Treatment plant's influent and effluent (sites b to c) and between the Torresdale drinking water plant's influent and effluent (sites g to h), only small concentration decreases occurred. For these four compounds, at least in this system, dilution is the most effective treatment process.

On the other hand, the data in Table II show that there are several compounds where treatment processes, especially at the Torresdale plant, are effective. These include the hydrocarbons, sterols, palmitic and stearic acids, some of the ethylene glycol compounds, the phenols, and chlorophyll. Unfortunately, it appears that the compounds of greatest environmental significance may be the least affected by the waste treatment processes.

Table III presents *median* concentration values for seven compounds which were found in plant A's effluent. These data were collected over a 10-week period using selected ion monitoring GC/MS techniques. Table III also lists the concentration data for the summer grab samples (see Table II) and *relative* concentration values for the 10-week study. A comparison between the concentration values for the 10-week study and the summer grab measurements shows good agreement (within the estimated error) for the two data sets. Data on the relative concentration levels in the 10-week samples demonstrate again that dilution is the most important treatment process for reducing industrial waste levels in this aquatic system. No more than a 50% reduction in concentration is achieved at the Torresdale water treatment plant for any of the compounds. For the phenyl glycols (**7** and **8**), a chlorinated phenyl glycol (**12**), and 2,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide (**18**), the treatment process appeared to have no effect at all.

Acknowledgments

The cooperation of the Delaware River Basin Commission and the help of the Water Department of the City of Philadelphia are gratefully acknowledged.

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Chloro-organic Compounds in the Lower Fox River, Wisconsin

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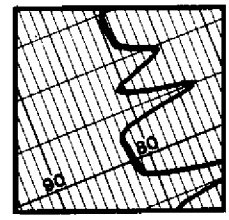

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CHLORO-ORGANIC COMPOUNDS IN THE LOWER FOX RIVER, WISCONSIN

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ABSTRACT

The Lower Fox River, Wisconsin is one of the most densely developed industrial river basins in the world. During 1976-77 about 250 samples were analyzed by GC and GC/MS including biota, sediments, river water and wastewaters from 15 pulp and/or paper mills and 12 sewage treatment plants. A total of 105 compounds were identified in selected extracts by GC/MS with another 20 compounds characterized but not conclusively identified. Twenty of the 105 compounds are on the EPA Priority Pollutant List. Other compounds identified in pulp and paper mill wastewaters, including chloro-guaiacols, chlorophenols, resin acids and chloro-resin acids have been reported toxic to fish by other investigators. Several compounds apparently not previously reported in wastewaters are chloro-syringaldehyde, chloroindole, trichlorodimethoxyphenol, and various 1-4 chlorinated isomers of bisphenol A. Concentrations of the various compounds, when present in final effluents, ranged from 0.5 to ca. 100 µg/L. An exception was dehydroabietic acid, a toxic resin acid not found on the EPA Priority Pollutant List. It was frequently found in pulp and paper mill effluents in concentrations ranging from 100 to 8500 µg/L. PCBs were found in all of the matrices sampled. Sixteen of the 35 fish exceeded the FDA limit of 5 mg/kg while 31 of the 35 exceeded the Canadian limit of 2 mg/kg. Concentrations of PCBs and other chloro-organics were related to point source discharges. There was a direct correlation of the concentrations of these compounds in wastewater with suspended solids values.

INTRODUCTION

Concern over the sources, distribution and fate of organic compounds in natural waters has increased considerably in recent years. With the development of GC/MS/DS instrumentation, thousands of compounds have been identified in industrial and municipal wastewaters, receiving waters and biota (Donaldson, 1977). Also, due to the extensive use of chlorination, numerous chlorinated organic compounds are being formed and these are now a matter of interest to researchers and regulatory officials (Jolley, 1976).

Because of this interest, the U.S. Environmental Protection Agency (Great Lakes Program Office, Region V) contracted with the State of Wisconsin (Department of Natural Resources and Laboratory of Hygiene) to assess the sources and distribution of organic compounds, particularly polychlorinated biphenyls (PCBs) and other chloro-organics, in the 64 km Lower Fox River in northeastern Wisconsin (Figure 1). This river drains into Green Bay-Lake Michigan and is one of the most densely developed industrial river basins in the world. Pulp and paper mills predominate; many of these use extensive amounts of chlorine. Five of the paper mills

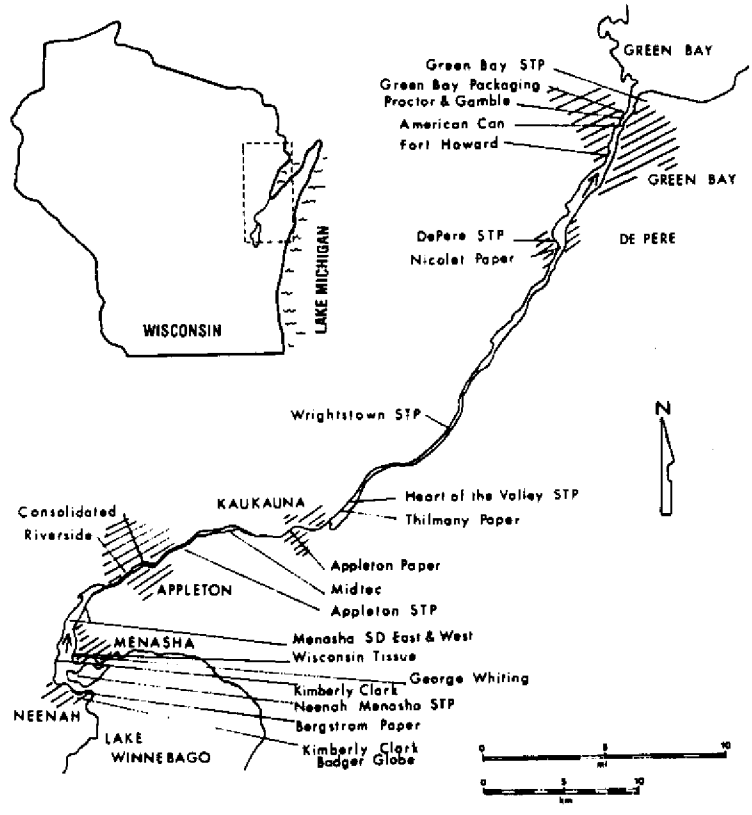

Figure 1. Effluent discharges to the Lower Fox River.

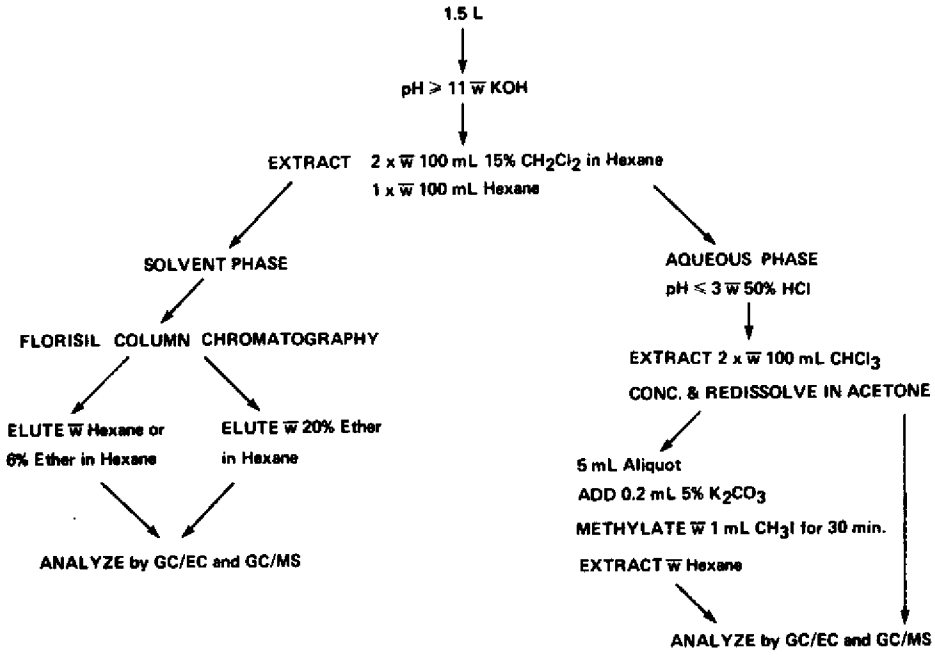

Figure 2. Water and wastewater extraction sequence.

de-ink and recycle paper to produce pulp, leading to the discharge of PCBs in their wastewaters.

EXPERIMENTAL

During 1976-77, ca. 250 samples were analyzed, including river and lake bottom sediments, snowmelt, biota (seston, clams and fish), river water and wastewaters from 15 pulp and/or paper mills and 12 municipal sewage treatment plants. Four of these municipal plants also treat pulp and/or paper mill wastewaters. Wastewaters comprised the majority of the samples received and were analyzed as described in Figure 2. Wastewater samples (1.5 L) were extracted at pH $\geq$ 11 with methylene chloride/hexane, fractionated on Florisil and screened by gas chromatography with electron capture detection (GC/EC) using procedures for chlorinated base-neutral compounds (USEPA, 1973). The remaining aqeuous phase of each sample was acidified to pH 3 and extracted with chloroform. The solvent was evaporated and the residue dissolved in acetone. This fraction was analyzed for chlorophenols, chloroguaiacols, and related chlorinated compounds. Selected extracts were derivatized with methyl iodide to facilitate analysis of acidic compounds and to confirm compounds identified by GC prior to methylation. Fractions whose GC/EC chromatograms exhibited significant unknown peaks were analyzed with a Finnigan 3100D Gas Chromatograph/Mass Spectrometer (GC/MS) and 6000 Data System.

Electron impact mass spectra ranging from m/z 35-500 were acquired every 3-4 sec. at an emission current of 0.35 ma, electron energy of 70 eV, amplification of 10^{-7} amp/V and electron miltiplier setting of 2.10 kV. Calibration with perfluorotertiarybutylamine was carried out according to the instrument manual and to specifications given by Carter (1976). PCBs and other base-neutral compounds were chromatographed on glass columns (1.8 m x 2 mm i.d.) packed with 3% SE-30 or 3% SP-2100 and temperature programmed from 100 to 220° C. Methylated and non-methylated acid fractions were analyzed on 1.8 or 3 m x 2 mm glass columns packed with Ultra-Bond 20M and programmed either from 90 to 210°C or 110 to 250°C, respectively.

Compounds were identified by (a) comparison of retention time and mass spectrum of a suspected constituent with those of a standard of that compound; (b) comparison of the full or partial mass spectrum (8 peaks) of a constituent with published spectra (e.g. Eight Peak Index, 1974; or (c) by interpretation of the mass spectral fragmentation pattern. Many of the compounds were available commercially, while some were provided by other researchers.

One compound that was unavailable from any source was chlorosyringaldehyde. Therefore, an experimental chlorination of syringaldehyde was performed. A commercial standard of syringaldehyde was added to a solution of 5.25% sodium hypochlorite (commercial bleach) in aqueous acetic acid. The reaction proceeded 16 hours, after which time the reaction product was extracted with methylene chloride, evapo-concentrated to dryness with a gentle stream of air, redissolved in acetone, and then injected into the GC/MS. A total ion chromatogram indicated both unreacted syringaldehyde and newly formed chlorosyringaldhyde. A very small amount of dichlorosyringaldehyde was also detected in the reaction product.

RESULTS AND DISCUSSIONS

Chlorosyringaldehyde was one of the 105 compounds identified by GC/MS (Table 1). Compounds in final effluents which were detected several times by GC/MS were quantitated by GC/MS, GC using flame ionization detection (FID) or GC/EC and listed in Table 2. Various effluents and extraction efficiencies were experienced, therefore only concentration ranges are given. The concentrations of these compounds generally corroborate earlier investigations of pulp and paper mill effluents (Rogers, 1973; Keith, 1976). Compounds detected and quantitated by GC/EC in fish, clams, river water, seston and sediments are also included in Table 2. A complete set of these data appears in a technical report (WI DNR, in press).

Table 1 Compounds Identified but not Quantified in Samples from the Lower Fox River System

Acetone, Tetrachloro-
c Acetovanillone
Aniline, Trichloro-
Benzene, Dichloro-diethyl-
Benzoate, Dimethyl-
Benzoate, Methyl-methoxy-
Benzoic Acid
Benzoic Acid, Isopropyl-
Benzophenanthrene, Methyl- or (Benzanthracene, Methyl-)
Benzophenone
c Benzyl Alcohol
Biphenyl
Biphenyl, Methyl-
Bisphenol A
Bisphenol A, Chloro-
Bisphenol A, Dichloro- (2 isomers)
Bisphenol A, Tetrachloro-
Bisphenol A, Trichloro-
Borneol, Iso-
c Caffeine
Camphor, Oxo-
Carbazole
a Chlordane
a DDD
a DDE
a DDT
c Dodecane

FATTY ACIDS AND THEIR METHYL ESTERS

c Heptadecanoic Acid
c Lauric Acid
c Myristic Acid
b Oleic Acid
c Palmitic Acid
c Stearic Acid
c Methyl Palmitate
c Methyl Stearate
c Guaiacol
b Guaiacol, Dichloro- (3 isomers)
Heptadecane
a Hexachlorocyclopentadiene
c Hexadecane
Indole, Chloro-
p-Menth-4-ene-3-one
Naphthalene, Isopropyl-
Naphthalene, Methyl-
Nonadecane
Octadecane
c Pentadecane
Phenanthrene, Methyl-
a Phenol
Phenol, p-Tertiary Amyl-
a Phenol, Chloro-
Phenol, p-(∝ - chloroethyl)-
Phenol, Decyl-
c Phenol, Ethyl-
Phenol, Nonyl- (3 isomers)
Phenol, Trichloro-dimethoxy-
Phenol, Undecyl-
Phenyl Decane
Phenyl Dodecane
Phenyl Undecane
Phosphate, Tributyl-

PHTHALATES

a Dibutyl Phthalate
a Diethyl Phthalate
a Dioctyl Phthalate
c Propan-2-one, 1-(4-hydroxy-3-methoxy phenyl) or guaiacyl acetone

RESIN ACIDS

b 6,8,11,13-Abietatetraen-18-oic Acid
b 8,15-Isopimardien-18-oic Acid
b Oxo-dehydroabietic Acid
b Pimaric Acid
b Sandaracopimaric Acid

RESIN ACIDS, METHYL ESTERS

b Methyl Dehydroabietate

(Continued next page)

Table 1 (Cont.)

RESIN ACIDS, CHLORINATED

b Chlorodehydroabietic Acid (2 isomers)
b Dichlorodehydroabietic Acid

RESIN ACID METHYL ESTERS, CHLORINATED

Methyl Chlorodehydroabietate
Methyl Dichlorodehydroabietate

c Salicyclic Acid
c Syringaldehyde
Syringaldehyde, Chloro-
Tetradecane
Toluene, Dichloro-
Toluene, Trichloro-
c Vanillin
c Vanillic Acid
c Veratrole, Dichloro-
c Veratrole, Trichloro-
Xylene, Dichloro-
Xylene, Trichloro-

a Compounds on EPA Consent Decree Priority Pollutant List
b Compounds in paper mill wastewaters reported toxic to fish
c Other compounds previously reported in paper mill wastewaters

To assess the significance of the compounds detected in this study, certain classifications were assigned. Twenty of the 105 compounds, including PCBs, appear in the EPA Consent Decree Priority Pollutant List (USEPA, 1977). Although the commercial use of Aroclar 1242 and other forms of PCBs in printing inks and carbonless copy paper apparently ended in 1972, PCBs are still being released into the Lower Fox River Basin. Deinking-recycling processes of five paper mills are some of the main sources. PCBs were detected in all of the various matrices sampled. All 35 fish fillet samples, consisting of both rough and sport fish, contained detectable levels of PCBs which were correlated to their fat content. Sixteen of the 35 fish exceeded the U.S. Food & Drug Administration tolerance limit of 5 mg/kg, while 31 of 35 exceeded the Canadian Food & Drug Directorate tolerance limit of 2 mg/kg.

PCBs are lipophilic and accumulate in fat tissue. Clams seeded in the Lower Fox River for 9 - 28 days showed that PCBs can rapidly bioaccumulate (WI DNR, in press). The mean uptake rates varied from 10 to 24 µg/day. The higher PCB uptake rate in the

Table 2 Compounds Identified and Quantified in Samples from the Lower Fox River System

	Compound	Environmental Matrix	Concentration Range	Units
	Anisole, Pentachloro-	Wastewaters	0.05 - 0.38	µg/L
		River water	0.002 - 0.02	µg/L
		*Seston	0.02 - 0.05	µg/L
		Fish	0.005 - 0.06	mg/kg
	Anisole, Tetrachloro-	Wastewaters	0.04 - 0.08	µg/L
	Benzothiazole	Wastewaters	10 - 30	µg/L
	Benzothiazole, Hydroxy-	Wastewaters	10 - 30	µg/L
c	Benzothiazole, Methylthio-	Wastewaters	10 - 40	µg/L
b	Dehydroabietic Acid	Wastewaters	100 - 8500	µg/L
		Sediment	2.7	mg/kg
a	Dieldrin	Fish	0.008 - 0.022	mg/kg
b	Guaiacol, Tetrachloro-	Wastewaters	10 - 50	µg/L
b	Guaiacol, Trichloro- (3 isomers)	Wastewaters	10 - 60	µg/L
a	Hexachlorocyclohexane (Lindane)	Wastewater	0.04	µg/L
a	Phenols, Dichloro- (2 isomers)	Wastewaters	15 - 40	µg/L
a	Phenol, Pentachloro-	Wastewaters	0.1 - 40	µg/L
		Sediments	0.22 - 0.28	mg/kg
	Phenol, Tetrachloro-	Wastewaters	2 - 20	µg/L
a	Phenols, Trichloro- (2 isomers)	Wastewaters	5 - 100	µg/L
a	Polychlorinated Biphenyls (Aroclor 1242, 1248 and 1254)	Raw wastewaters	0.2 - 8200	µg/L
		Final effluents	0.1 - 56	µg/L
		River water	0.05 - 0.85	µg/L
		*Seston	0.002 - 0.029	µg/L
		Sediments	0.05 - 61	mg/kg
		Clams seeded	0.26 - 0.74	mg/kg
		Fish	0.5 - 90	mg/kg
a	Polycyclic Aromatic Hydrocarbons (Acenaphthene, Anthracene, Chrysene, Fluoranthene, Pyrene)	Wastewaters	0.5 - 10	µg/L

* Entire aqueous sample filtered
a Compounds on EPA Consent Decree Priority Pollutant List
b Compounds in paper mill wastewaters reported toxic to fish
c Other compounds previously reported in paper mill wastewaters

clams occurred at locations having relatively high PCB concentrations in the river sediments. These locations were downstream from discharges containing PCBs.

Other compounds identified in pulp and paper mill wastewaters were those found to be toxic to fish by other investigators (Rogers and Keith, 1974; Leach and Thakore, 1977). These toxicants included chloroguaiacols, resin acids, chloro-resin acids and oleic acid. Our observations of chlorophenols corroborates work by Lindström and Nordin (1976). The source of the chlorophenols in the mill wastewaters investigated in this study has not yet been determined. Chlorophenols may have been used by paper mills for slime control or been present as wood preservatives. Phenolic compounds could also have been chlorinated in the bleaching or wastewater treatment stages. Chlorocatechols may have been present, but the analytical method employed did not appear to give any significant recovery of these compounds.

Previous investigations of toxicants in paper mill wastewater have always involved a pulp mill that derives its pulp from wood, thus releasing wood extractives and lignin-derived compounds such as resin acids, guaiacols and other phenolics, some of which become chlorinated in the bleach plant. In our survey, the highest levels of chloroguaiacols occurred at a sulfite pulp mill which produces mostly bleached pulp. These compounds eluted cleanly from an unmethylated acid extract on the Ultra-Bond 20M column which resolved 3 apparent trichloroguaiacol isomers. Chloroguaiacols were not detected in some Fox River paper mill wastewaters, presumably because these mills either do not bleach their wood-derived pulp, or else they bleach purchased pulp and/or deinked recycled paper. The last two should contain lesser amounts of the wood extractives and lignin-derived compounds.

The GC/MS analysis of a sample extract of a paper mill which bleached either purchased bleached pulp and/or deinked recycled paper is shown in Figure 3. The total ion chromatogram (TIC) of a methylated acid extract of the mill's final effluent shows 39 identified compounds, most of which are methylated derivatives. Thus, chloroanisoles were originally present as chlorophenols, chloroveratroles as chloroguaiacols and the dimethyl ether derivatives as various bisphenol A isomers. Likewise, the fatty and resin acid methyl esters were originally present as the corresponding acids in the acid extract. The scale of the TIC has been limited to 30% of full scale to better show the compounds at lower concentrations. For reference, 75 ng of aldrin (peak 15) were injected as an external standard. Peak 8, representing tetrachloroguaiacol, was quantitated by GC/EC at 14 µg/L, while peak 25, representing the resin acid dehydroabietic acid (DHA), was quantitated by GC/FID at 3200 µg/L. This concentration approached that of 8500 µg/L which was seen in the aforementioned sulfite pulp mill's

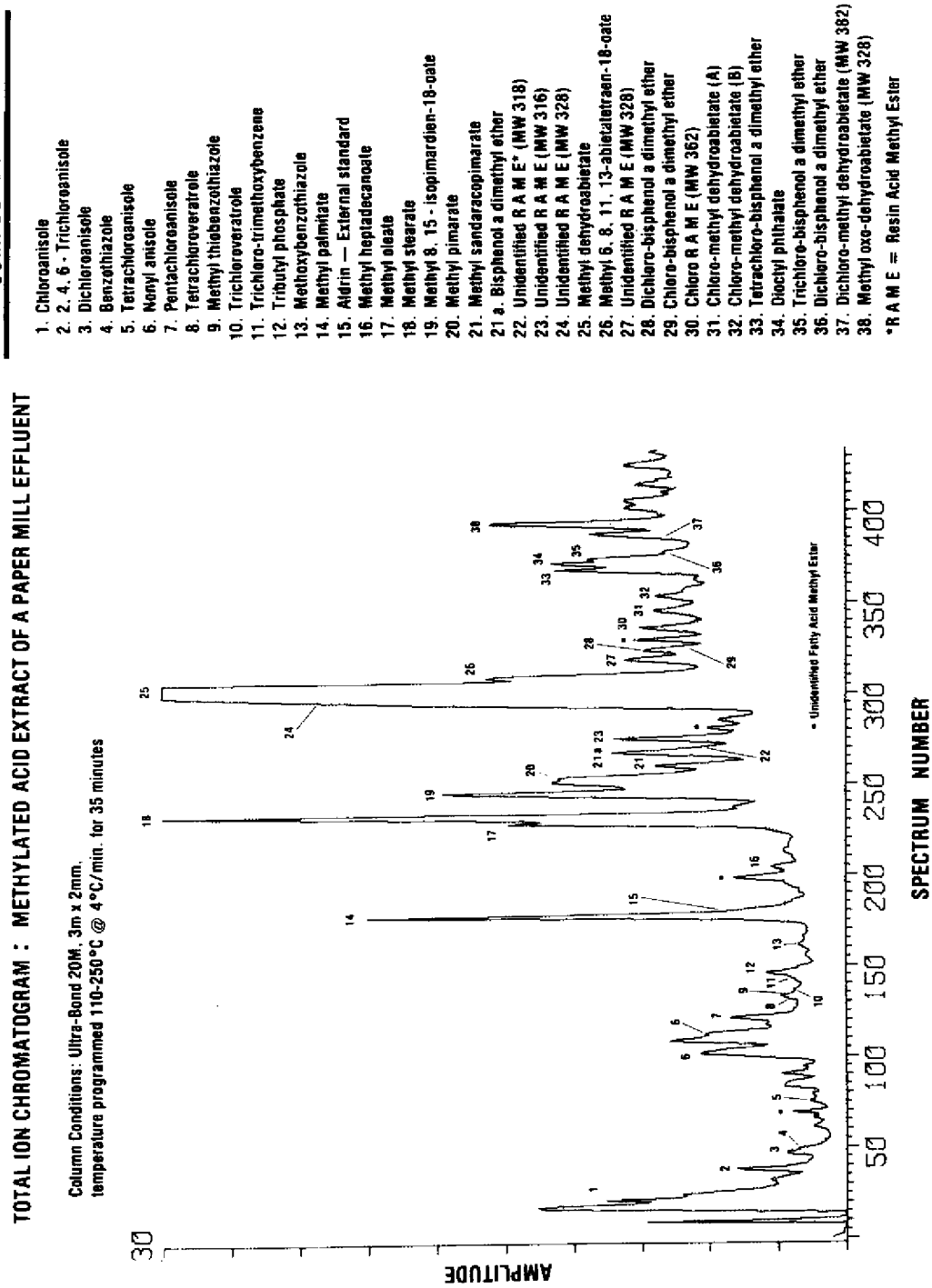

Figure 3. Total ion chromatogram of a methylated acid extract of a paper mill effluent

final effluent. Since the mill whose effluent is represented in Figure 3 lacks a wood pulping process, the relatively large amounts of fatty and resin acids present, especially DHA, could have come from its use of resin sizing (Merck Index, 1976) in the papermaking process. This water-intensive process could have diluted the available chlorine, thereby reducing the effectiveness of formation of chloro-resin acids (peaks 31, 32 and 37, Figure 3).

DHA appears to be the most stable of the resin acids (Brownlee and Strachan, 1977; Fox, 1977). The toxicity of resin acids to fish has been known since 1936. The 96 hour LC_{50} concentrations of DHA for young Sockeye Salmon are 2000 µg/L (Rogers, 1973) and 750 µg/L for Coho Salmon (Leach and Thakore, 1977). The latter investigators also reported even lower 96 hour LC_{50} concentrations for mono- and dichlorinated DHA.

Other compounds previously reported in paper mill wastewaters were also found in this study including acetovanillone, guaiacol, methyl thiobenzothiazole, syringaldehyde, vanillin and vanillic acid. Several compounds commonly used in industry that were identified including benzothiazole, an antioxidant; bisphenol A, a fungicide or an intermediate in the production of epoxy resins; and nonyl phenol, present in surfactants (Merck Index, 1976).

Several compounds which apparently have not been reported before in the environment are chlorosyringaldehyde, 5 separate chlorobisphenol A isomers, and trichloro-dimethoxyphenol, while chloroindole was found apparently for the first time in a sewage treatment plant effluent. Chlorosyringaldehyde was identified together with syringaldehyde in a semi-chemical pulp and paper mill untreated wastewater, as seen in another TIC (Figure 4). Syringaldehyde, a hardwood lignin degradation product would be anticipated to come from a pulp mill using hardwood. The apparent chlorination reaction within the plant compares with similar examples of the chloroguaiacols and chloro-resin acids in other plants. Chlorosyringaldehyde was identified in an acid fraction without derivatization when chromatographed in an Ultra-Bond 20M column. The mass spectrum of chlorosyringaldehyde has isotopic molecular ions of m/z 216 and 218 which are consistent for a compound with one chlorine atom (Figure 5). These two ions as well as the fragment ions m/z 215, 201, 173, 145, 130, 127 and others have been shifted 34 mass units higher than for syringaldehyde which is also consistent with the addition of a chlorine atom to the benzene ring. In addition, the laboratory chlorination of syringaldehyde described in the Experimental Section yielded a mono-chlorinated compound which matched not only the identical retention time but also the mass spectrum of the apparent chlorosyringaldehyde in the untreated wastewater (Figure 4).

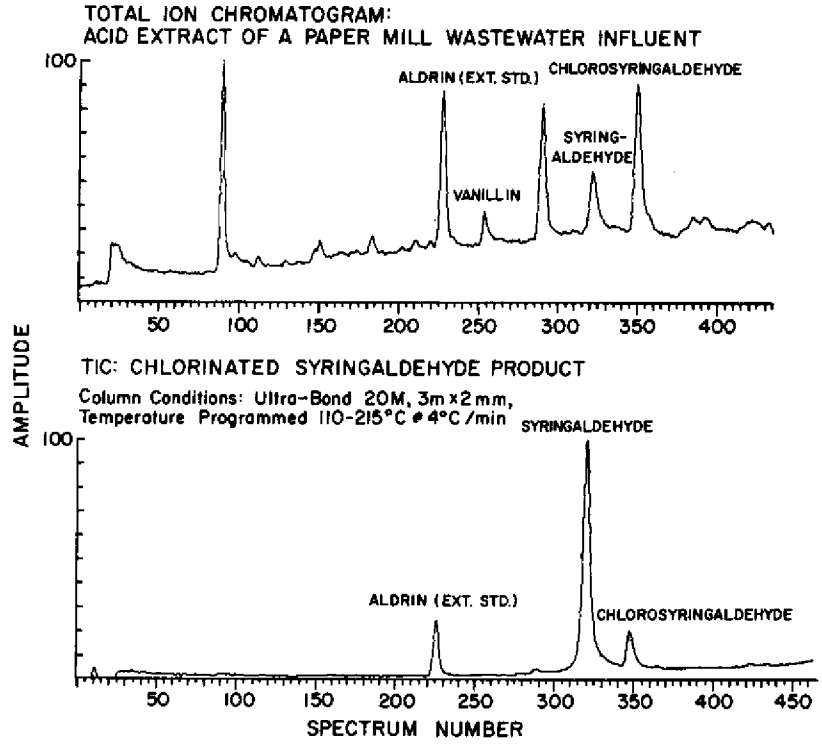

Figure 4. Total ion chromatograms: syringaldehyde and chlorosyringaldehyde

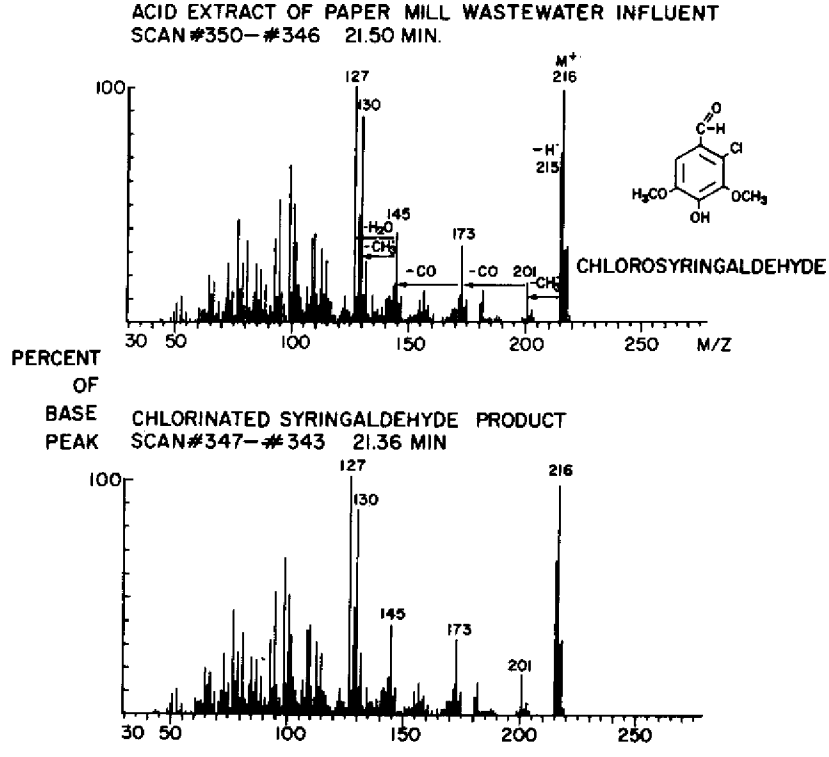

Figure 5. Mass spectra of chlorosyringaldehyde

A group of chlorinated bisphenol A compounds was identified in the same extract as that shown in Figure 3, which also contained chloroguaiacols and chloro-resin acids. Despite its widespread industrial use, bisphenol A has apparently only been recently identified in the environment (Matsumoto et al, 1977). The mass spectrum of its dimethyl ether derivation is compared to that of peak 21a (Figure 3) and included here (Figure 6) because it was apparently not available in the literature. The mass spectrum of its derivative shows the molecular ion at m/z 256 and the base peak $(-CH_3)^+$ at m/z 241 shifted 28 mass units higher than that of bisphenol A, which is consistent for methylation of both hydroxyl groups. The mass spectra of the 1-4 chlorinated isomers (peaks 28, 29, 33, 35 and 36, Figure 3) show similar upward shifts of the two main ions 34 mass units for each additional chlorine atom. The mass spectra of these compounds also show the respective isotopic clusters corresponding to the number of chlorine atoms present. Using ^{35}Cl, the molecular ion and the base peak of monochlorobisphenol A dimethyl ether were, respectively, 290 and 275; those of both dichlorobisphenol A dimethyl ether isomers were 324 and 309; those of trichlorobisphenol A dimethyl ether were 358 and 343; and those of tetrachlorobisphenol A dimethyl ether were 392 and 377. A pure standard of tetrachlorobisphenol A was commercially available, as it is apparently used as a flame retardant. The mass spectrum of its methylated derivative is compared with

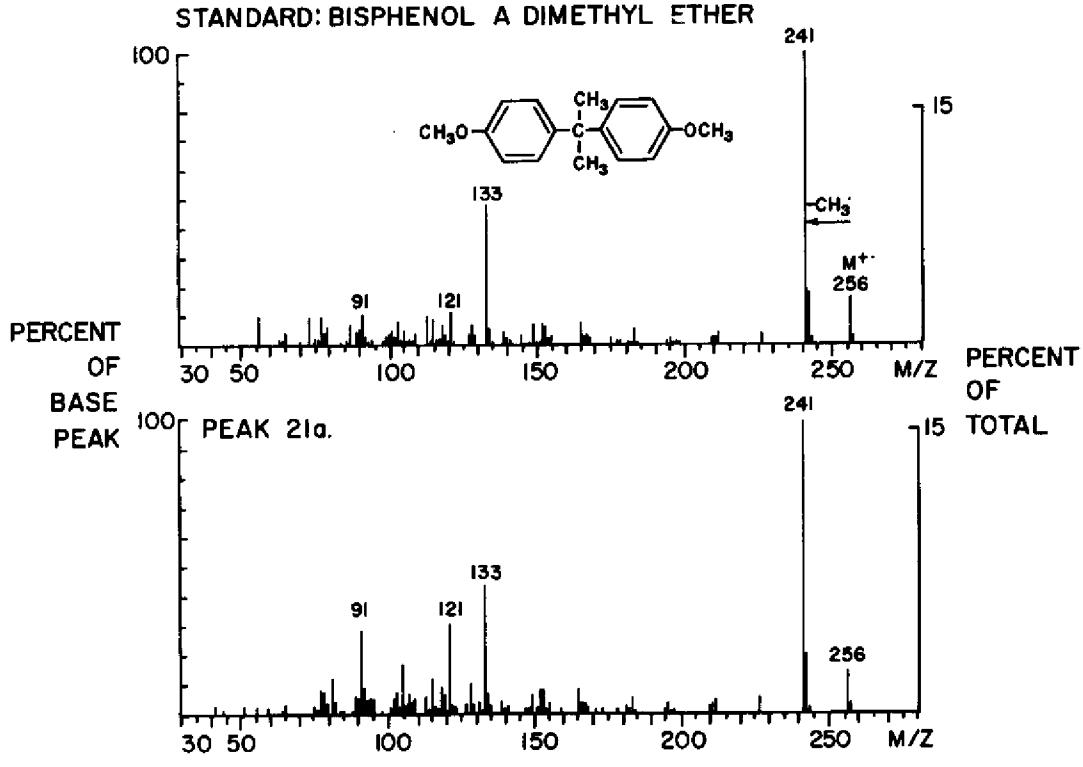

Figure 6. Mass spectra of bisphenol A dimethyl ether.

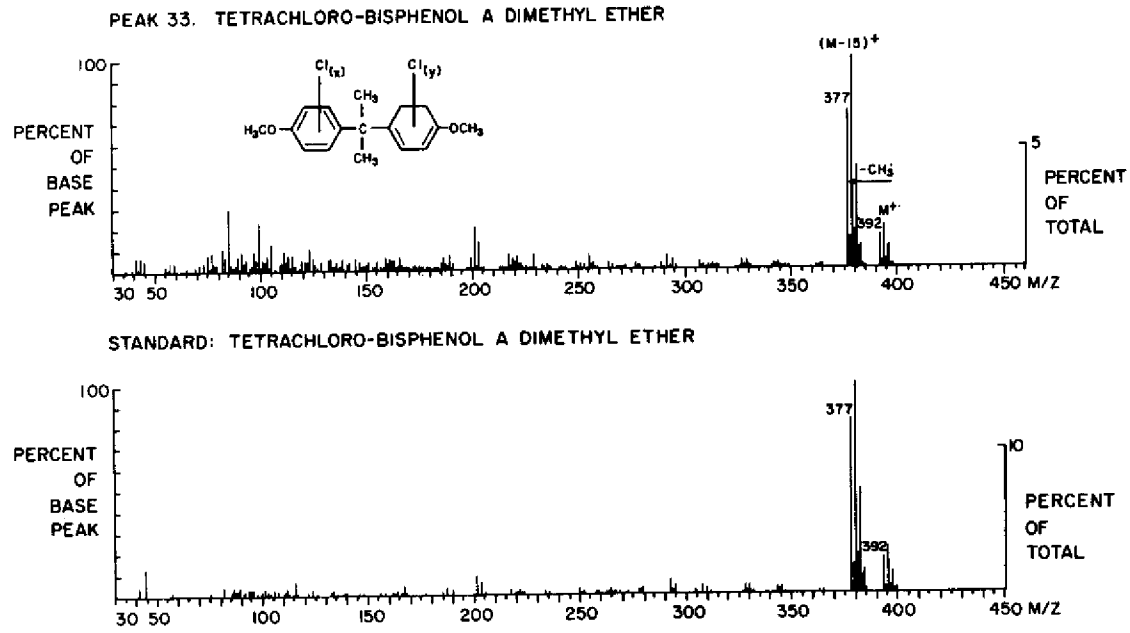

Figure 7. Mass spectra of tetrachlorobisphenol A dimethyl ether.

that of peak 33 (Figure 3) in Figure 7. In a laboratory chlorination of bisphenol A performed similarly to that of syringaldehyde, it was shown that 2,4,6-trichlorophenol was the main product formed, although various chlorinated bisphenol A isomers having 1-4 chlorines were also formed.

Trichloro-dimethoxyphenol was tentatively identified in the acid extract (Figure 3). Its methylated derivative was peak 11 which eluted just after a close congener, trichloroveratrole (peak 10). Trichloro-dimethoxyphenol was another compound which was detected in the acid extract chromatographed directly without derivatization on an Ultra-Bond 20M column. It eluted just after tetrachloroguaiacol. Its mass spectrum showed abundant molecular ions at m/z 256-260 consistent for 3 chlorine atoms, a similar cluster at ions m/z 241-245 $(M-15)^+$ and m/z 198-202, $(M-58)^+$. The mass spectrum of the methylated derivative, peak 11 (Figure 3) showed abundant isotopic molecular ions now at m/z 270-274 with subsequent fragments $(M-15)^+$, $(M-43)^+$ and $(M-58)^+$.

Chloroindole apparently has not been previously detected in wastewaters, but it has been isolated from a bacterium (Pseudomonas pyrrocinia) (Neidleman, 1975) and from an acorn worm (Ptychodera flava laysanica) from the Pacific Ocean (Higa and Scheuer, 1975). In our study of untreated wastewater from a municipal sewage treatment plant, this compound was detected in the 20% ether in hexane Florisil eluate at a concentration of ca. 30 µg/L. The mass spectrum showed abundant isotopic molecular ions m/z 151 and 153, base peak of m/z 89, and less abundant ions at m/z 124, 116

and 63. In comparing the compound from our sample with a commercial standard of 5-chloroindole, both mass spectra matched well but their retention times differed by several minutes.

In addition to the 105 compounds identified in this study, another 20 or so compounds were detected but not conclusively identified to date. A group of related compounds was consistently detected. The most prominent were two apparent isomers with a molecular weight of 196. In wastewater samples of the paper mill represented in Figure 3, these two isomers were followed by about nine chlorinated isomers with apparent molecular weights of 230, 264 and 298. The mass spectra of all of these are included in a technical report (WI DNR, in press). Mass spectra of the two non-chlorinated isomers are similar to diphenylacetaldehyde and trans stilbene oxide. Although the compounds with molecular weight of 196 have been detected in various extraction fractions, they and the chlorinated isomers primarily have been found in the first Florisil eluate (6% ether in hexane). Their concentrations have been sufficiently high to mask some of the PCB peaks detected with GC/EC. This mill's extensive deinking, recycling and bleaching processes could conceivably release the compounds with molecular weight 196 which ultimately become chlorinated.

This GC/MS study was aided by the use of a low-loaded, Ultra-Bond 20M column packing (ca. 0.3% Carbowax 20M) similar to that first discovered by Aue (1973). Elutions were characteristically sharp, with polar phenolic compounds eluting quite well. Baseline separation of pentachloroanisole from tetrachloroveratrole was achieved, contrary to the case for 3% SP-2100, 3% OV-17, or the mixed phase packing 4% SE-30/6% OV-210 designed for pesticide analyses. Very low bleed on temperature programmed analyses aided background substraction resulting in optimum mass spectra.

The fate and long-term health and ecological implications of many of the 105 compounds identified requires further research. For PCBs and some other chloro-organics, sampling data and laboratory experiments show a direct correlation of their concentrations in wastewaters with suspended solids concentrations. Suspended solids reduction in wastewater treatment plant also reduces the chloro-organic concentration in the final effluent (WI DNR, in press). For example, the untreated wastewater of a paper mill which deinks and recycles paper contained 25 µg/L PCBs and 2,020 mg/L suspended solids. Following primary clarification, concentrations were reduced to 2.2 µg/L PCBs and 72 mg/L suspended solids. After secondary treatment the final effluent contained only 1.4 µg/L PCBs and 10 mg/L suspended solids. Now the final disposal of the treatment plant sludge containing PCBs must be resolved.

ACKNOWLEDGEMENTS

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The Global Distribution of Polycyclic Aromatic Hydrocarbons in Recent Sediments

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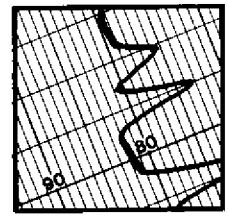

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The global distribution of polycyclic aromatic hydrocarbons in recent sediments

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Abstract—Polycyclic aromatic hydrocarbons (PAH) and their alkyl homologs are distributed in sediments throughout the world. The qualitative PAH pattern is remarkably constant for most of the locations studied, and the quantitative PAH abundance increases with proximity to urban centers. These findings are consistent with anthropogenic combustion's being the major source of these compounds. Two non-combustion sources of PAH have also been noted: retene coming from abietic acid and perylene probably coming from various extended quinone pigments.

INTRODUCTION

THE PRESENCE of complex mixtures of polycyclic aromatic hydrocarbons (PAH) and their alkyl homologs in soils and sediments from the New England region has been well established (BLUMER and YOUNGBLOOD, 1975; HITES and BIEMANN, 1975; YOUNGBLOOD and BLUMER, 1975). For example, sediment from the Charles River Basin contains PAH with at least eleven different aromatic ring structures and abundant alkyl substituted derivatives thereof, in some cases containing up to 15 carbon atoms in the alkyl groups (HITES and BIEMANN, 1975). The qualitative composition of the PAH in this sediment is best illustrated by Fig. 1, which is a high resolution gas chromatogram of the PAH in the Charles River sediment. In all of the other sediments examined, the qualitative distributions of PAH have resembled that shown in Fig. 1. In addition, the relative abundances of the alkyl homologs within a given PAH series are very similar for these sediments (HASE and HITES, 1976a). The complexity and widespread distribution of these PAH mixtures has been a surprise, revealed only through the application of very sophisticated analytical techniques centered around mass spectrometry. This finding is even more interesting when one remembers that many PAH are known chemical carcinogens (ARCOS and ARGUS, 1975).

The general goal of this paper is to develop an understanding of the sources of PAH in recent sediments. Before reporting on our results, however, we will review some of the characteristics of PAH mixtures produced by different sources and methods of distinguishing among them.

Sources of PAH

There have been suggestions that at least some PAH can be synthesized by algae (BORNEFF *et al.*, 1968), by plants (GRAEF and DIEHL, 1966; HANCOCK *et al.*, 1970), or by various bacteria (BRISOU, 1969; KNORR and SCHENK, 1968; DE LIMA-ZANGHI, 1968; MALLET and TISSIER, 1969; NIAUSSAT *et al.*, 1969, 1970; ZOBELL, 1959). Thus, it was suggested (HITES and BIEMANN, 1975) that the complex mixtures of PAH found in the Charles River sediment could originate from the biosynthetic activity of anaerobic bacteria. Selected experiments in our laboratory (HASE and HITES, 1976b), however, have indicated that this is unlikely; bacteria accumulate PAH but do not synthesize them.

Polycyclic aromatic hydrocarbons may also originate from petroleum. PAH mixtures from petroleum are quite deficient in the unsubstituted species; the most abundant alkyl homolog usually contains three or four carbon atoms (SPEERS and WHITEHEAD, 1969). Since the PAH homolog distribution in sediments is monotonically decreasing with increasing number of alkyl-carbons, the PAH in sediments could not originate directly from petroleum (YOUNGBLOOD and BLUMER, 1975).

In situ chemical aromatization of naturally occurring cyclic compounds can also produce PAH. For example, the geological diagenesis of certain terpenoids and pigments is thought to produce specific PAH (BLUMER, 1965; MAIR, 1964). This situation is characterized by the observation of one or two specific compounds rather than a complex mixture of PAH and their alkyl homologs. Most, but not all, of the sedimentary data show complex PAH mixtures; therefore, chemical aromatization is not a major source of PAH in most sediments.

Combustion is one of the most common sources of polycyclic aromatic hydrocarbons in nature (NATIONAL ACADEMY OF SCIENCES, 1972). Figure 2 shows the high resolution gas chromatogram of PAH produced by the combustion of kerosene (LEE *et al.*, 1977). Comparison of the qualitative pattern of PAH produced in this combustion system with that found in the Charles River sediment indicates a high degree of correlation in certain molecular weight regions.

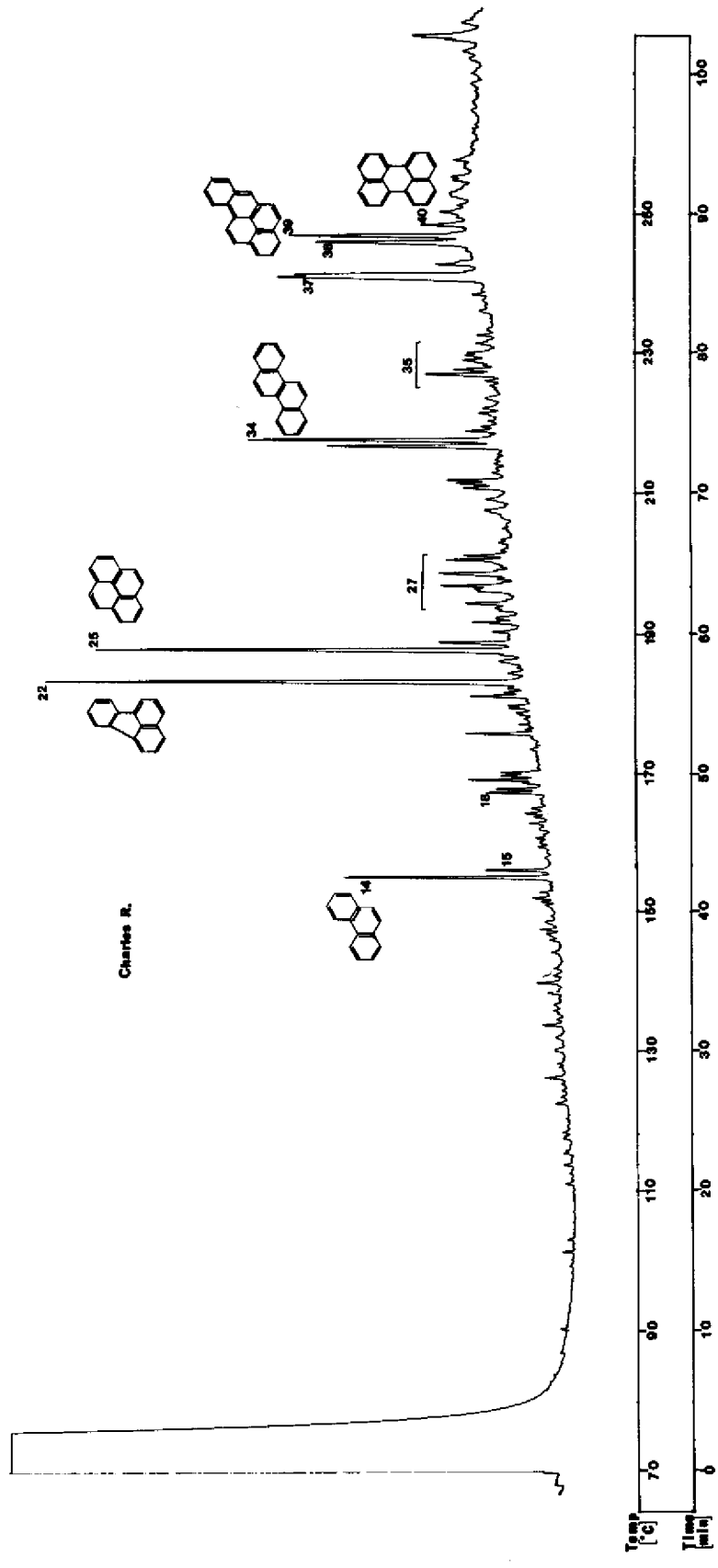


Fig. 1. High resolution gas chromatogram of PAH in Charles River sediment. See text for GC operating conditions. See Table 1 for peak identities.

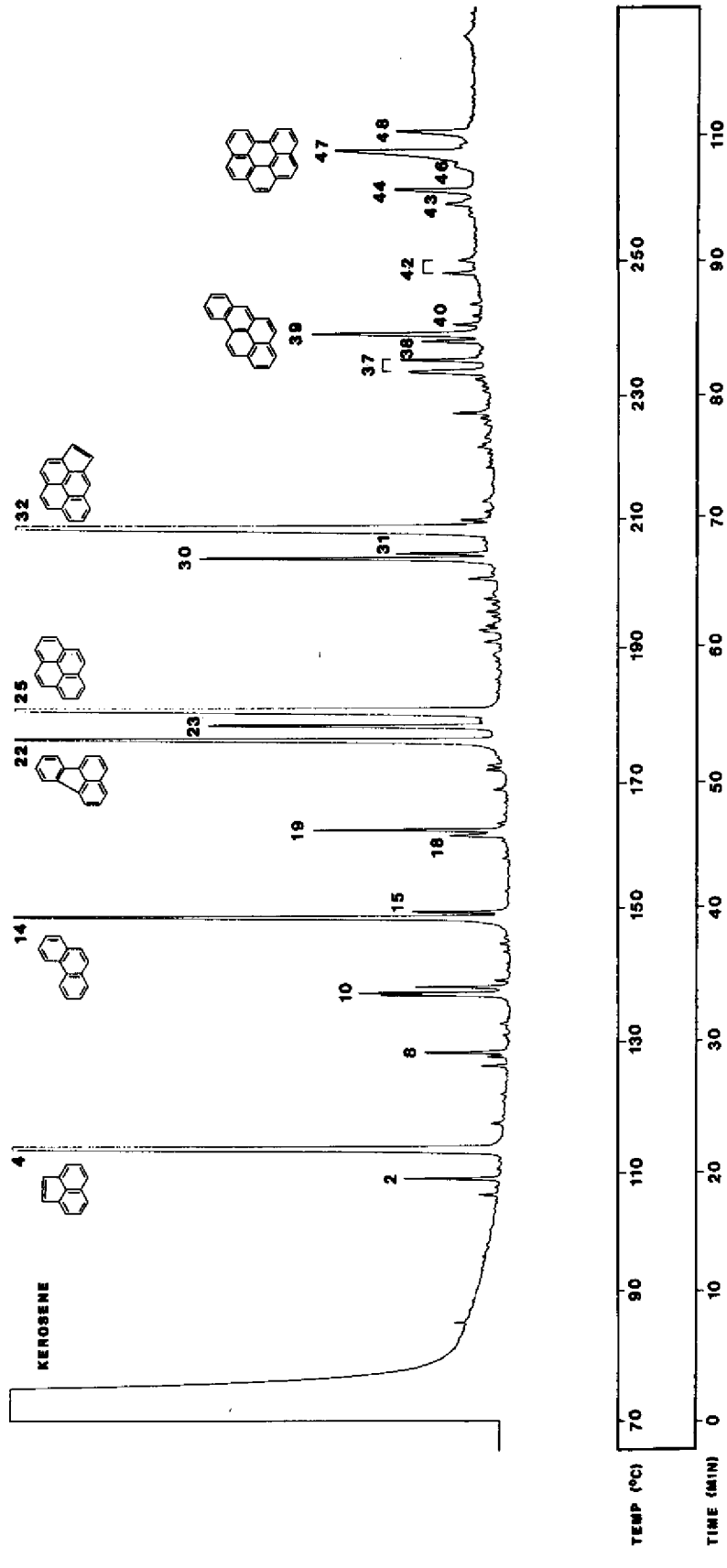


Fig. 2. High resolution gas chromatogram of PAH produced by the combustion of kerosene. See text for GC operating conditions. See Table 1 for peak identities. Taken from LEE *et al.* (1977).

Table 1. PAH identified by GC–MS in sediments and combustion effluents (see Figs. 1 and 2)

Peak No.	Compound	Peak No.	Compound
2	Biphenyl, $C_{12}H_{10}$, Z = −14, 154	30	Benzo[*ghi*]fluoranthene, $C_{18}H_{10}$, Z = −26, 226
4	Acenaphthylene, $C_{12}H_8$, Z = −16, 152	31	$C_{18}H_{10}$ (Unknown), Z = −26, 226
8	Fluorene, $C_{13}H_{10}$, Z = −16, 166	32	Cyclopenta[*cd*]pyrene, $C_{18}H_{10}$, Z = −26, 226
10	Cyclopent [*bc* or *fg*]-acenaphthylene, $C_{14}H_8$, Z = −20, 176	34	Chrysene, $C_{18}H_{12}$, Z = −24, 228
14	Phenanthrene, $C_{14}H_{10}$, Z = −18, 178	35	Methylchrysene or methyl benz[*a*]anthracene, $C_{19}H_{14}$, Z = −24, 242
15	Anthracene, $C_{14}H_{10}$, Z = −18, 178	37	Benzofluoranthene, $C_{20}H_{12}$, Z = −28, 252
18	Methylphenanthrene, $C_{15}H_{12}$, Z = −18, 192	38	Benzo[*e*]pyrene, $C_{20}H_{12}$, Z = −28, 252
19	4*H*-cyclopenta[*def*]phenanthrene, $C_{15}H_{10}$, Z = −20, 190	39	Benzo[*a*]pyrene, $C_{20}H_{12}$, Z = −28, 252
22	Fluoranthene, $C_{16}H_{10}$, Z = −22, 202	40	Perylene, $C_{20}H_{12}$, Z = −28, 252
23	Benz[*e*]acenaphthylene, $C_{16}H_{10}$, Z = −22, 202	42	$C_{21}H_{12}$ (Unknown), Z = −30, 264
25	Pyrene, $C_{16}H_{10}$, Z = −22, 202	43	$C_{22}H_{12}$ (Unknown), Z = −32, 276
27	Methylfluoranthene or methylpyrene, $C_{17}H_{12}$, Z = −22, 216	44	Indeno [1,2,3,-*cd*]pyrene, $C_{22}H_{12}$, Z = −32, 276
		46	Dibenz[*a,c*]anthracene, $C_{22}H_{12}$, Z = −32, 276
		47	Benzo[*ghi*]perylene, $C_{22}H_{12}$, Z = −32, 276
		48	Anthanthrene, $C_{22}H_{12}$, Z = −32, 276

Each entry represents the compound name, molecular formula, Z number (C_nH_{2n+Z}), and molecular weight.

Note the phenanthrene/anthracene (14, 15), fluoranthene/pyrene (22–25) and benzofluoranthene/benzopyrene regions (37–40). There is, however, a large quantitative difference: There are much lower relative abundances of alkyl homologs in the combustion produced PAH mixture; note the region between pyrene and the C_{18} group (peaks 27). The significance of these differences, if any, will be addressed below.

Although we have been discussing anthropogenic combustion here, we should point out that there are also natural combustion sources of PAH such as forest and prairie fires. In fact, assigning the PAH in sediments to either natural or anthropogenic sources is difficult and is the crux of much controversy in this field of research.

Methods of distinguishing among PAH sources

There are several different experimental approaches one can use in order to assign sources for the PAH mixtures found in soils or sediments, and we will review these here.

Alkyl homologs. The general formation mechanism of PAH in combustion systems has been thoroughly investigated, and there is now agreement that qualitatively similar PAH mixtures are produced almost regardless of the fuel type and the combustion conditions (HASE *et al.*, 1976; LINDSEY, 1960). Quantitatively, however, the distribution of alkyl homologs can be quite different depending on flame zone temperature. Low temperatures (such as 1100°K, as in a cigarette) will yield a soot with quite abundant alkyl substituted PAH. Very high temperatures (such as 2200°K, as in a carbon black furnace) will yield soot devoid of alkyl PAH (LEE and HITES, 1976). Extended periods of time (10^6 yr) at low temperatures (450°K) will yield PAH mixtures in which the unsubstituted species is not at all abundant; for example, in petroleum (MAIR, 1964).

It is thus clear that the slope of the PAH alkyl homolog distribution curve can give information on the formation temperature of these compounds (given that there has been no subsequent modification). Based on these considerations, BLUMER and YOUNGBLOOD (1975) concluded that the PAH in recent sediments were produced at modest combustion temperatures and that the fuel giving rise to such temperatures was wood (as in a forest fire); thus sedimentary PAH were of natural origin.

There is a fallacy in this line of thought; namely, wood is not unique in its combustion temperature. Other exclusively anthropogenic fuels also burn at these moderate temperatures, coal being a noteworthy example. For example, the alkyl homolog distributions of the pyrene (Z = −22)* series for PAH produced by the combustion of coal, kerosene and wood are shown in Fig. 3, along with the homolog distributions for this series in the Charles River sediment and in the Boston urban atmosphere. It is apparent from these data that the burning of wood and kerosene gives a distribution of PAH alkyl homologs more similar to that of urban air particulates from Boston than to that of the sediment; whereas the burning of coal gives a distribution of PAH alkyl homologs which is quite similar to that of the sediment.

Furthermore, it seems reasonable that there are natural mechanisms which can modify a PAH homolog distribution after the mixture has been deposited in the soil or sediment. One such mechanism could be differential water solubility of the higher alkyl homologs vs the unsubstituted species. MCAULIFFE (1966, 1969), SUTTON and CALDER (1974, 1975), EGANHOUSE and CALDER (1976) and BOEHM and QUINN (1973) have shown that the logarithm of water solu-

* PAH are frequently described by Z numbers which are calculated from the generalized molecular formula C_nH_{2n+Z}. The number of rings and double bonds in a molecule is 1 − (Z/2). Typical Z values, elemental compositions, molecular weights, and example compounds are given in Table 1.

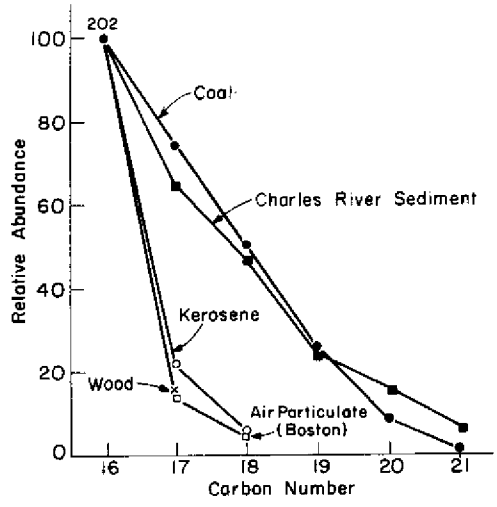


Fig. 3. Alkyl homolog distribution plots for the pyrene-type series (Z = −22) in the combustion products of coal, wood and kerosene, in air particulate matter from Boston, and in the Charles River sediment. The abundance of the parent compound in each series was normalized to 100. Data replotted from LEE *et al.* (1977) and from HASE and HITES (1976a).

bility is a negative linear function of the number of carbon atoms for several alkyl benzenes and other hydrocarbons. Extrapolating somewhat, we would expect that the various alkyl homologs of PAH systems would have water solubilities distributed in a similar fashion. We, therefore, suggest the following mechanism for transition of a typical combustion generated PAH homolog distribution into that observed in sediments: after airborne particulate deposition on soil or in water, the lowest homologs (including the unsubstituted species) continuously fractionate into the water phase to an extent inversely proportional to their carbon number as described above. The remaining species, which accumulate on the particulate matter and in the sediment, are therefore devoid of the lowest homologs, thus increasing the relative abundance of the higher homologs.

Of course, there are processes other than differential water solubility which could also modify PAH homolog distributions. These include the preferential bioaccumulation of higher homologs, preferential biodegradation of lower homologs, or the preferential vaporization of lower homologs.

Clearly, if one understood these PAH homolog distribution modification mechanisms, one could correct observed homolog distributions and thus obtain a first order indication of the PAH source. If these modification mechanisms were small or non-existent, the homolog distribution may be a sensitive indicator of the PAH source.

Azaarenes. Other experimental information pertaining to the source of sedimentary PAH has been the recent finding of complex mixtures of azaarenes in sediments from Buzzards Bay by BLUMER, DORSEY and SASS (1977). These investigators assume that these azaarenes are produced by the incomplete combustion of plant material, and thus by inference, that the PAH in these sediments are also produced by the combustion of plant material, such as in a forest fire. This is not a completely safe assumption. Azaarenes are found in automobile exhaust and in other anthropogenic combustion effluents (SAWICKI *et al.*, 1965a, 1965b). Thus, the presence of azaarenes, although interesting, does not definitely establish the PAH source.

Sediment core studies. Still another experimental approach is to search for an historical record of PAH in the environment. Man's input of DDT (HOM *et al.*, 1974), lead (CHOW *et al.*, 1973), and trace metals (BRULAND *et al.*, 1974) has been recorded in the recent sedimentary record. Obviously, fossil fuel utilization patterns have also changed considerably over time and, if the anthropogenic combustion sources of PAH are substantially larger than natural sources, this change should be reflected in the sedimentary record. We have recently undertaken the analysis of PAH in three sections of a dated sediment core from Buzzards Bay in an attempt to establish such an historical record of PAH production and deposition, and the results are reported elsewhere (HITES *et al.*, 1977). These data suggest an anthropogenic source for PAH at the location studied. Clearly, an evaluation of the sedimentary record at a number of locations and under a variety of depositional environments should give considerable information about PAH sources.

Geographical distribution studies. Obviously much information about PAH sources can be obtained by analyzing soils and sediments from different locations. A variety of sampling sites may well indicate specific sources at different locations. For example, sites near to and remote from urban locations should distinguish between anthropogenic and natural sources. Sites in the northern and southern hemispheres should show large differences due to different levels of industrial activity and population. Areas remote from trees should not be subjected to large PAH inputs due to forest fires. Samples taken from sites on a transect from a point source (such as a city or group of cities) should show uniformly decreasing PAH concentrations. Samples taken on remote islands should show PAH inputs due only to long range airborne transport.

Certainly, the study of the qualitative composition and quantitative concentration of PAH taken at many different geographical locations is a powerful tool in establishing the source of these compounds. Unfortunately, at present, most of the reported sedimentary PAH data are from the highly industrialized New England area. In addition to local industry, recent ozone measurements suggest that urban air from metropolitan New York is transported to the Boston area (CLEVELAND *et al.*, 1976). These facts would make it difficult to find an environmental sample in the northeast United States, either soil or sediment, which was free from anthropogenic in-

fluence. A few other European sediments have also been analyzed for PAH, but these are also from highly industrialized locations [the Greifen See, Switzerland (GIGER and SCHAFFNER, 1975), the Grosser Ploener See, Schleswig-Holstein, Germany (GRIMMER and BOEHNKE, 1975), and Lake Constance, Germany (MULLER *et al.*, 1977)]. In these cases too, anthropogenic influences would be quite high.

Thus, before finalizing any conclusions concerning natural vs anthropogenic sources of sedimentary PAH and the effect of the compounds on the environment and man, we must first assess the distribution of these compounds in relatively pristine sediments obtained on a *worldwide* basis. We have begun analyzing such sediments, and this paper is a report of our early findings.

EXPERIMENTAL METHODS

Sample preparation

All samples (25–250 g) were Soxhlet extracted (without drying) with methanol for 24 hr followed by methanol/benzene (2:3) for an additional 24 hr. The benzene and methanol were removed under vacuum. The remaining water was extracted with five volumes of hexane followed by one volume of methylene chloride. The extracts were dried, combined, and redissolved in cyclohexane–nitromethane (1:1) and extracted with five volumes of nitromethane (HOFFMAN and WYNDER, 1960). The nitromethane was removed at 40°C under vacuum. The extract was then subjected to column chromatography on 2.0 gm of silicic acid with elution by 300 ml of hexane. The hexane was removed and the eluate dissolved in methylene chloride and analyzed by gas chromatographic mass spectrometry.

Gas chromatography–mass spectrometry

Gas chromatography was performed on six foot glass columns (2 mm ID, 0.25 in. OD) packed with 3% OV-17 on 80/100 mesh Supelcoport. Temperature was programmed from 70 to 310° at 8°/min. Gas chromatography–mass spectrometry was done on a Hewlett–Packard 5982A quadrupole mass spectrometer with an HP 5933A data system.

The mass spectrometer was used in three modes of operation: total ion monitoring, selected ion monitoring, and direct probe distillation. In the total ion monitoring mode, the mass spectrometer continuously scans a wide mass range, usually m/e 50–350 in approximately 3–4 sec. Each scan is recorded by the computer and stored on a disc where data can be retrieved for inspection and interpretation. The total ion voltage accumulated during each scan is also recorded and stored. A plot of this total voltage (ionization) vs time corresponds to the output of a flame ionization detector of a gas chromatograph.

In the selected ion monitoring (SIM) mode, the mass spectrometer scans only certain preselected masses, and the computer stores the intensity of each mass. The plot of the intensity vs time results in a mass chromatogram. In the total ionization mode, 300 mass units were scanned in approx 3 sec, which means the mass spectrometer spends less than 10 msec on each mass per scan. In the SIM mode, the operator can choose a dwell time of up to 500 msec. Our experiments utilized a 250 msec dwell time. Thus, SIM allows greater sensitivity; however, because of the greater dwell time, not all masses can be scanned during a gas chromatographic run. The operator can choose up to four masses to be scanned at any one time. Five sets of four masses can be chosen and instructions as to which of these sets are to be scanned at any given time can be given manually or automatically. This allows up to 20 masses to be monitored during a chromatographic run. This type of monitoring is especially suited to PAH since their mass spectra are dominated by their molecular ions and show very few fragment ions. The masses chosen for a SIM run are generally the molecular ions of approximately twenty alkylated and non-alkylated species. The data obtained by SIM are particularly well suited to quantitative analyses. Mass chromatograms of each of the masses scanned during a run are plotted, and the area under each curve is calculated with the aid of the computer. These areas can then be compared to the responses obtained from standard compounds for PAH of the same molecular weight and degree of alkyl substitution.

The third mode is direct probe distillation; in this method, the mixtures of PAH are introduced directly into the ion source of the mass spectrometer via a glass capillary tube on a probe which passes through a vacuum lock. The heat of the ion source distills the PAH from the capillary tube into the ionization chamber. Scanning a very limited mass range (m/e 150–350) in 4 sec results in a dwell time of 20 msec per mass. The increased dwell time and the increased sensitivity accompanying direct introduction into the ion source allows one to detect molecular ions of PAH too weak to be detected using the total ionization mode. By plotting mass chromatograms of parent PAH and their alkyl derivatives and computing the area under these curves, one obtains the relative quantity of alkylated PAH.

The reproducibility of our PAH measurements is better than ±30%. Blank analyses were carried out, and all values have been corrected for these blank levels. A typical blank value was 0.2 ppb, assuming a 200 g sample. Recovery experiments were also carried out, and these showed acceptable values (80–100% recovery of PAH spiked at a level of 10 ppb).

High resolution gas chromatography

High resolution gas chromatography was performed on 19 m × 0.26 mm ID, SE-52, wall coated, glass capillaries. Temperature was programmed from 70 to 250°C at 2°C/min. The column was prepared using a static coating method described by LEE (1975).

SAMPLING SITES

1. Charles River Sediment, Boston, MA: anoxic sediment previously described by HITES and BIEMANN (1975).

2. Maine Soil, No. Anson, ME: soil from a wooded area 100 yards from a paved rural road, two miles off routes 8 and 201A, north of the center of No. Anson. This is similar to the location described by YOUNGBLOOD and BLUMER (1975). The sample was collected by shovel at 10–20 cm below the surface.

3. Gulf of Maine: obtained from J. Farrington, Woods Hole Oceanographic Institute (WHOI). This sample was the 2–3 cm section of a core labelled Oceanus 1/1 which was collected November, 1975, under 214 meters of water at approximately 43°N, 70°W (FARRINGTON *et al.*, to be published).

4,5,6. Buzzards Bay Sediment, MA: obtained from J. Farrington (WHOI). The core sample was obtained from Buzzards Bay, Massachusetts (Station P, 41°29.0'N, 70°52.5'W, 17 m water) from an area where measurements of Pb-210 (FARRINGTON *et al.*, 1977), Cs-137 and Pu-239 + 240 (BOWEN, 1975) provide a means of estimating the sedimentation rate. An earlier study (FARRINGTON, *et al.*, to be published) of alkanes, cycloalkanes and phenanthrenes in another sediment core from this location showed an interesting trend of decreasing concentrations between the upper 2 cm and 54–58 cm which pointed towards fossil fuel combustion as the principal source of hydrocarbons in these surface sediments. The core was collected in August 1975 with a 21-cm diameter, 1 m long

sphincter corer (BURKE, 1968). Three sections of the core were used for this study: Sample 4, the top 4 cm; Sample 5, 20–24 cm; and Sample 6, 38–42 cm. There was no sulfide present in the top 8 cm. An oxic, bioturbation zone of about 4 cm is indicated by the Pb-210 depth profile and benthic ecology studies (FARRINGTON *et al.*, 1977; RHOADS, 1974).

7. New York Bight, NY: obtained from J. Farrington (WHOI). It is a grab sample taken in 1975 at water depth of 28 m at 40°25.7'N, 73°48.1'W, on the R/V *Knorr* cruise 47-1.

8. Abyssal Plain: obtained from J. Farrington (WHOI). The sample is from a core obtained on the R/V *Knorr* cruise 32-2 September–October 1973 at 32°25'N, 70°13'W.

9. South Carolina Soil, Berkely County, S.C.: soil from a marshy area fifty feet off an unpaved road numbered 220A in the Wambaw–Hunting Unit of the Francis Marion National Forest. The sample was collected by a cleaned shovel at a depth of 10–20 cm.

10, 11. Nebraska Soils, Knox Country, Nebraska: collected by a cleaned shovel. Sample 10 was obtained 10 miles west of Crofton by digging into a recently eroded stream bank so that the depth of sample from surface was 1.5 m. Soil was exposed at the surface during the early 1900s. Sample 11 was obtained at a depth of 2–10 cm, 14 miles northwest of Bloomfield in a grassy meadow where there has been no farming.

12, 13. Mono Lake Sediment, Lee Vining, California: obtained using a small hand operated dredge (Benthos Inc.). Sample 12 was taken about 1 mile offshore under 8–10 m of water. Sample 13 was obtained next to a cove near Niget Island under 5–8 meters of water. Mono Lake is a strongly alkaline desert lake in Eastern California which has a combination of high organic productivity with almost no scavengers (HENDERSON *et al.*, 1971).

14. Mono Soil, Lee Vining, California: obtained with a cleaned shovel at a depth of 10–20 cm ½ mile from route 395. The soil was taken from an area that had been uncovered in the last decade by the shrinking of the lake.

15. Yosemite Soil, Yosemite National Park, California: obtained with a cleaned shovel at a depth of 10–20 cm. The site was located in a wooded area (various species of pines) behind Summit Meadow (elevation 8000 ft) approx 500–1000 yards from a paved road.

16. Nevada Soil, Nevada: obtained with a cleaned shovel at a depth of 10–20 cm. The site was located 40 miles east of Lee Vining, California, 1 mile off Nevada route 31, and 50 m from an unpaved road. This is a sandy, desert environment.

17. Alaska K-30: obtained from H. Hertz of the National Bureau of Standards (NBS). This was an intertidal surface sediment taken in the vicinity of the Katalla River (144°35'W, 60°11'N) downstream from a known oil seep.

18. Alaska H-24: obtained from H. Hertz (NBS). This was an intertidal sediment taken near Hitchinbrook Island (146°41'W, 60°21'N), an area considered free from anthropogenic or oil contamination.

19. Walvis Bay, Africa: obtained from J. Farrington (WHOI). Sample is the top 4 cm of core No. 21, Station 14 R/V *Atlantis II* cruise 93/3, from the Namibian Shelf of Africa in an upwelling area at 22°11.5'S, 13°51.4'S.

20. Cariaco Trench: obtained from J. Farrington (WHOI). This was the top 10 cm of an anaerobic organic-rich ooze from the Cariaco Trench at 10°42.5'N; 65°10.5'W. This was a core sample.

21. Flood Plain, Amazon River, Brazil: obtained from J. Edmond, Department of Earth and Planetary Sciences (MIT). The sample is from last year's (1976) flood plain deposit taken on the downstream tip of isle Santa Helena No. 1 which is 15 km upstream of Leticia, Columbia. It is very recently deposited sediment, less than one month old.

22. Station No. 7, Amazon River: obtained from J. Edmond (MIT). The sample was collected with a dredge from station 7 (02°03.3'S, 55°23.1'W) in the Amazon River between Santarem and Obidos. This is a sample from an underwater dune, and its sandy nature is characteristic of the Amazon's main channel.

23. Coari River, Amazon River system: obtained from J. Edmond (MIT). The sample was collected with a dredge from station 5 (04°04.1'S, 63°09.1'W) which is 1 km from Rio Solimoes. The sample smelled of H_2S.

24. Rio Ica, Amazon River system: obtained from J. Edmond (MIT). The sample was collected with a dredge approximately 12 miles upstream from the Amazon; this is one of the least inhabited large tributaries in the Amazon River Basin. Sample was obtained 30 June, 1976 at 03°14.2'S, 68°04.1'W. The sample was a soupy, gray clay which seemed to be anoxic.

25. Obidos 16, Amazon River: obtained from J. Edmond (MIT). The sediment was taken from station 16 which is 500 m from shore in a 60 m deep, scoured hole near the city of Obidos. This sample was taken with a dredge. This sample was black and probably of Tertiary age.

RESULTS AND DISCUSSION

Worldwide PAH distribution

Table 2 summarizes the results of the analyses of the samples from New England (numbers 1–6), the continental United States (numbers 7–16), and other sections of the globe (numbers 17–25). Concentrations are given for non-alkylated PAH with three to five rings. All quantities were determined using the selected ion monitoring mode of the mass spectrometer.

The data in Table 2 indicate that the presence of PAH in the environment is indeed worldwide. Complex mixtures of PAH have been found in all but two samples analyzed. A detailed examination of the data in Table 2 shows the same qualitative distribution of unalkylated PAH for most of the samples studied. The ratio of fluoranthene to pyrene is near one (within experimental error) for all samples reported. With a few exceptions, which will be discussed below, all samples have a relative abundance of phenanthrene of about 12%, of fluoranthene 16%, of pyrene 15%, of $C_{18}H_{12}$ species 23%, and of $C_{20}H_{12}$ species 35%. Comparison of samples from the Gulf of Maine (No. 3), from Walvis Bay (No. 19), and from the flood plain of the Amazon River (No. 21) shows that all have the same relative PAH pattern even though the absolute abundances differ by 10^2. In general, the absolute concentrations are highest for samples taken close to human activity (New England) and lowest for samples taken from more pristine locations (Alaska). Every sample shows, not only the same parent PAH pattern, but also a complex mixture of alkylated PAH species similar to that reported for the Charles River sediment. All of these data agree with the PAH distributions observed in combustion effluents.

There seems to be little doubt that the major source of PAH in most of these samples is combustion. What is not clear, however, is the type of combustion. In the case of the Buzzards Bay core (HITES *et al.*, 1977),

Table 2. Concentration of non-alkylated PAH in sediments and soils from around the world

Sample	Phen.	(%)	Fluor.[a]	(%)	Pyr.	(%)	$C_{18}H_{12}$[b]	(%)	$C_{20}H_{12}$[c]	(%)
1. Charles River	5000	(5.8)	15000	(17)	13000	(15)	21000	(24)	33000	(38)
2. Maine Soil	70	(10)	120	(18)	100	(15)	140	(21)	250	(37)
3. Gulf of Maine	43	(8)	120	(22)	100	(18)	80	(15)	200	(37)
4. Buzzards Bay 1	53	(7)	130	(16)	120	(15)	160	(20)	340	(43)
5. Buzzards Bay 2	42	(5)	130	(15)	120	(14)	200	(23)	380	(44)
6. Buzzards Bay 3	8	(13)	11	(17)	7	(11)	12	(19)	25	(41)
7. New York Bight	740	(13)	1200	(21)	1300	(22)	890	(15)	1700	(29)
8. Abyssal Plain	N.D.		4		4		13		34	
9. South Carolina Soil	78	(51)	26	(17)	14	(9)	22	(14)	13	(8)
10. Nebraska #1	4		5		5		31		N.A.	
11. Nebraska #2	8	(10)	8	(10)	8	(10)	17	(22)	37	(47)
12. Mono Lake #1	91		13		24		29		N.A.	
13. Mono Lake #2	110	(28)	74	(19)	65	(16)	93	(23)	57	(14)
14. Mono Soil	10	(16)	9	(14)	9	(14)	26	(41)	10	(16)
15. Yosemite Soil	7	(58)	1	(8)	1	(8)	2	(17)	1	(8)
16. Nevada Soil	N.D.		N.D.		N.D.		N.D.		N.D.	
17. Alaska K-30	67	(59)	4	(3.5)	8	(7.1)	23	(20)	11	(9.7)
18. Alaska H-24	2	(42)	0.6	(12)	0.6	(12)	1	(21)	0.6	(12)
19. Walvis Bay	8	(12)	10	(15)	15	(22)	10	(15)	25	(37)
20. Cariaco Trench	18	(1.0)	9	(0.5)	16	(0.9)	13	(0.8)	1700	(97)
21. Flood Plain Amazon River	2	(12)	1	(5.9)	2	(12)	6	(35)	6	(35)
22. Station #7 Amazon River	N.D.		N.D.		N.D.		N.D.		N.D.	
23. Coari River Amazon River	9	(1.7)	3	(0.6)	4	(0.7)	4	(0.7)	520	(96)
24. Rio Ica Amazon River	6	(1.2)	3	(0.6)	3	(0.6)	13	(2.5)	500	(95)
25. Obidos 16 Amazon River	6	(0.6)	6	(0.6)	6	(0.6)	16	(3.0)	510	(94)

N.A. = not analyzed.
N.D. = not detected.
All concentrations are in parts per billion, dry weight basis. *a*: % is per cent of component relative to sum of tabulated components. *b*: Includes chrysene, triphenylene, and other $C_{18}H_{12}$ isomers. *c*: Includes benzofluorenes, benzopyrenes, and perylene.

depth distribution data give valuable insight into the possible source of PAH. No data of that type are currently available for most of the samples listed in Table 2. The alkyl homolog distribution has been proposed as a method of distinguishing anthropogenic from natural sources (YOUNGBLOOD and BLUMER, 1975), but there is some question as to the validity of such a method until mechanisms which could modify the homolog distribution *after* deposition of the airborne particulates (HASE and HITES, 1976a; HITES, 1976) have been considered. The question of modification of the homolog distribution is currently being investigated in our laboratory; therefore, data necessary to draw conclusions from the alkyl homolog distributions for the samples listed in Table 2 are not yet complete.

At present we can conclude that complex mixtures of PAH can be found in sediments and soils outside of the northeastern United States and in areas far removed from any apparent anthropogenic influence. We can also conclude that these PAH are the result of a common source: the deposition of airborne particulates formed by combustion.

Anomalous cases

In the discussion so far, a single source (combustion) has been associated with the presence of PAH in recent marine and nonmarine environments. It is reasonable to postulate that there is an additional source of sedimentary PAH if the PAH distribution pattern differs significantly from that normally encountered. Dual sources of PAH have been reported in a sediment and in a fossil (YOUNGBLOOD and BLUMER, 1975; BLUMER, 1965). In the former, fallout of airborne particulates as well as input from an oil spill could be recognized. In the latter, high molecular weight PAH due to chemical aromatization were found along with PAH corresponding to a combustion origin. Our analyses have resulted in the discovery of two anomalies that can most easily be explained by two natural sources.

The PAH distribution of the sample from South Carolina (No. 9) was significantly different from the distributions normally observed. Figure 4 shows a high resolution GC of the PAH isolated from this South Carolina soil sample; note the anomalous nature of these data (compare with Fig. 1). The predominance of the alkylated phenanthrenes, particularly the C_2 and C_4 substituted species, is most remarkable. Although the alkyl homolog distribution (obtained by high resolution mass spectrometry) of the phenanthrene series resembles that of petroleum, the other series of alkyl homologs show no such resemblance. This fact and the remoteness of the sample site from any source of oil suggest that the unusual PAH pattern in the South Carolina soil is not due to oil contamination.

If the PAH in the South Carolina Soil are not a

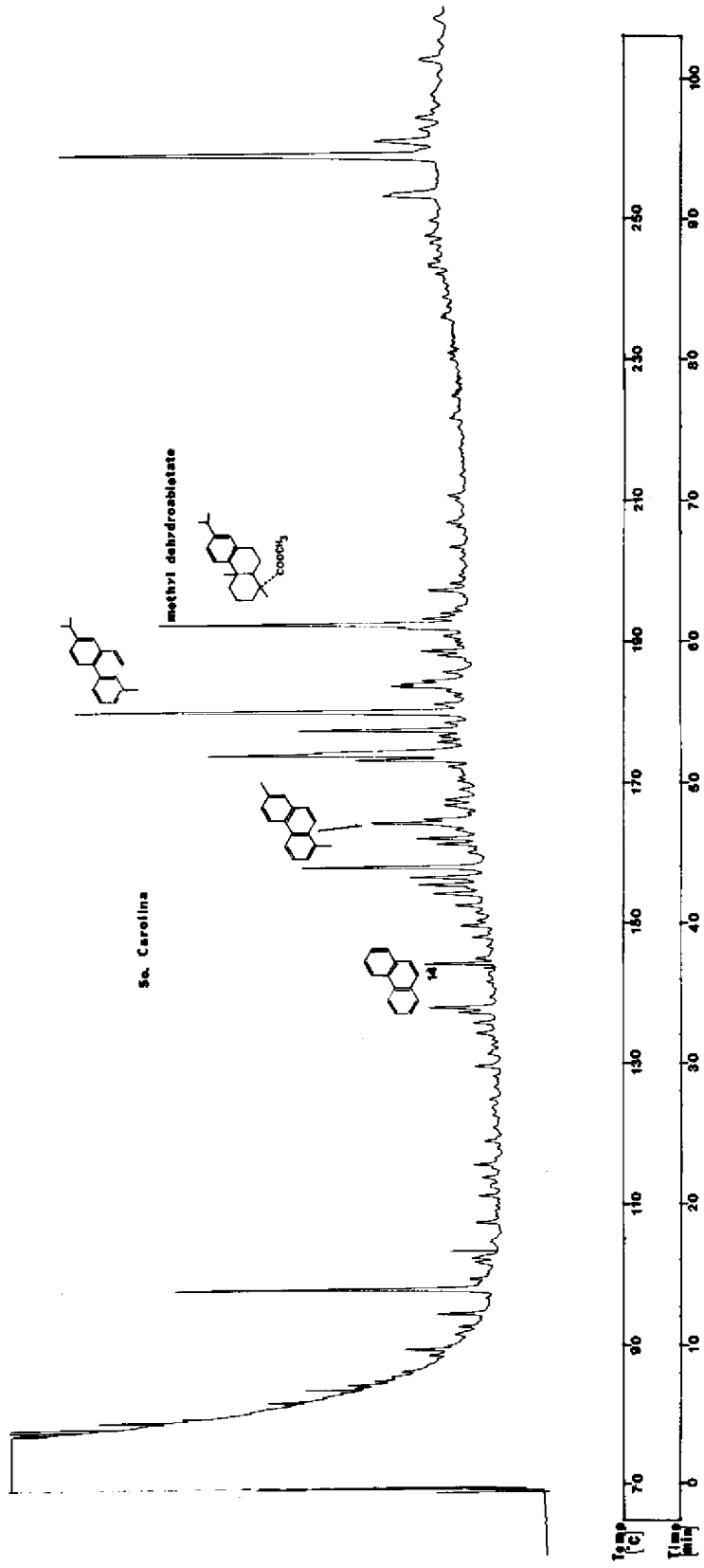
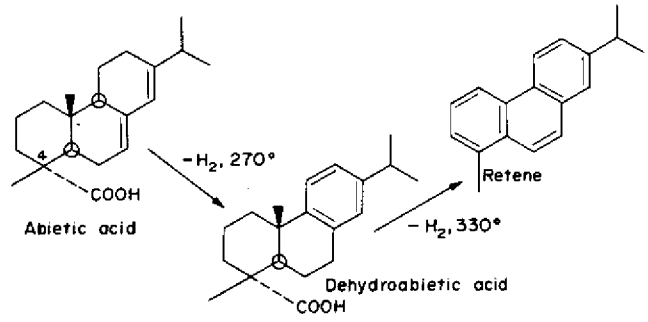
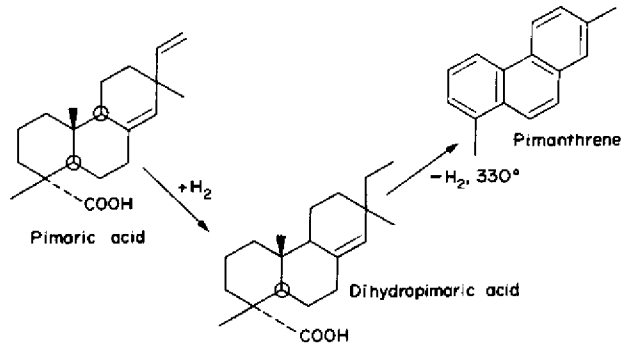


Fig. 4. High resolution gas chromatogram of PAH in a South Carolina soil. See text for GC operating conditions. Unlabeled peaks are not yet identified. Peak at retention time of 94 min is tentatively identified as a Δ^{14}-taraxerene derivative.

result of combustion nor oil pollution, then a natural source seems likely. Examination of the mass spectrum and high resolution mass spectral data of the largest peak in Fig. 4 confirms that the fragmentation pattern is identical to one reported (API, No. 302 m) for 1-methyl-7-isopropyl-phenanthrene which is also known as retene. It occurs in pine tar, in fossilized pine, and in high boiling tall oils and is a result of the dehydrogenation of abietic acid which is a major component of pine rosin (STONECIPHER and TURNER, 1970). The C_2 phenanthrene is probably the 1,7-dimethyl isomer (pimanthrene) which results from the dehydrogenation of pimaric acid, another component of pine rosin (STONECIPHER and TURNER, 1970). Since methyl dehydroabietate was also identified by its mass spectrum in this same sample, we have further evidence that the source of the retene and pimanthrene is rosin from the surrounding pine forest (see sample site description). These compounds have also been found in deep-sea sediments by SIMONEIT (1977).

Given the source of these compounds, it seemed likely that soils from other pine forests would also exhibit the presence of retene and other phenanthrene homologs; therefore, an analysis of soil from Yosemite National Park in California was carried out (see





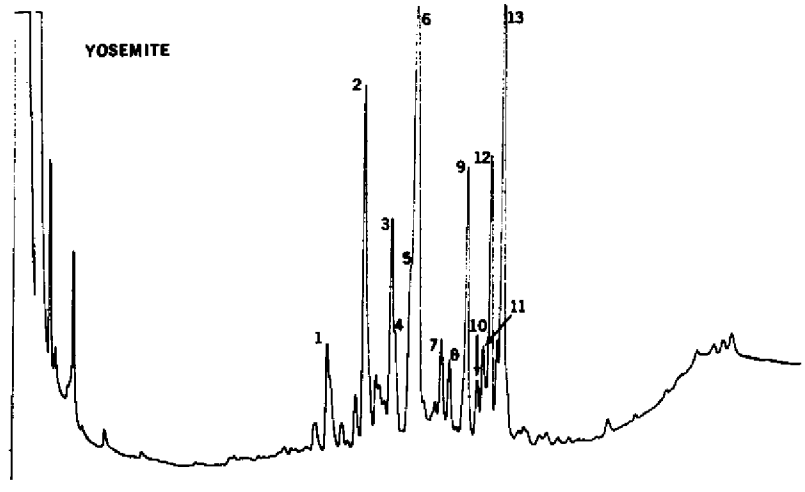


Fig. 5. Gas chromatogram of a soil extract from Yosemite National Park. Peaks 1 and 2 are fatty acid methyl esters; 3, 7 and 9 are not known. For peak identifications see Fig. 6.

site description). Figure 5 is the gas chromatogram of the Yosemite Soil extract. Mass spectrometric analysis and comparison with literature spectra give the interpretations listed in Fig. 6.

It seems clear that resin aldehydes, esters and hydrocarbons are all present. Of particular interest is the presence of the cyclohexane carboxylate (peak 8) and the epidehydroabietal (peak 5). Both appear to represent degradation products of original rosin constituents since neither have been reported to be present in pine rosin itself. Both are of interest diagenetically because they involve racemization at C-4 in one instance and the breaking of the *B* ring in the other. Furthermore, many of the compounds found in this Yosemite Soil can be linked in a degradation scheme (see Fig. 7) which accounts for the conversion of abietic acid to retene. This scheme is clearly hypothetical, but it does explain the presence of most of the observed compounds. It is also interesting that the two samples from pine forested regions are so different. In the Yosemite case we have a complex mixture, but the South Carolina case shows one resin ester (methyl dehydroabietate) and two alkyl phenanthrenes.

We feel that a thorough study of the environmental chemistry of abietic acid and its degradation products should be quite rewarding. Apparently, we have here a rare opportunity to observe the intermediates of an environmental reduction and to precisely follow the mechanism of the geochemical diagenesis of these compounds.

In Table 2, the percentage of $C_{20}H_{12}$ PAH usually ranges from 25 to 45% of the total unsubstituted

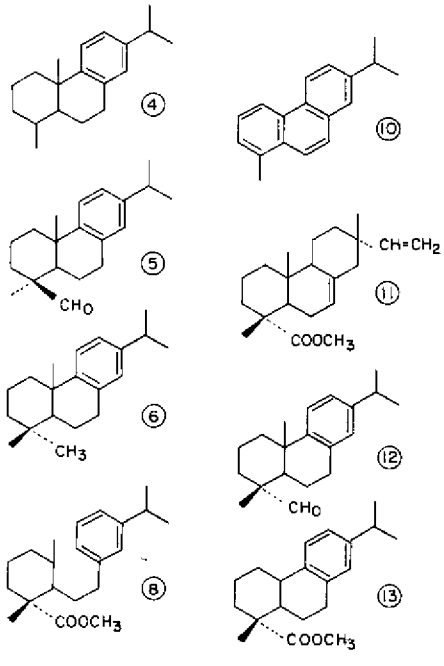


Fig. 6. Peak identifications for compounds found in a Yosemite soil. See Fig. 5.

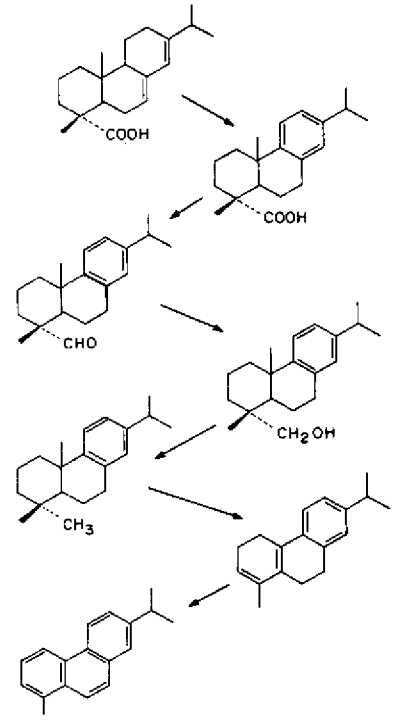


Fig. 7. Hypothetical degradation scheme of abietic acid to retene.

PAH. The composition of the $C_{20}H_{12}$ PAH isomers is largely benzofluoranthenes and benzopyrenes (confirmed by gas chromatographic retention times). There are four exceptions: Coari River sediment (No. 23), Rio Ica sediment (No. 24), Obidos 16 sediment (No. 25) (all from the Amazon River tributary system), and the Cariaco Trench sediment (No. 20). The percentage of $C_{20}H_{12}$ PAH in these sediments ranges from 94 to 97% of total PAH. The benzofluoranthenes and benzopyrenes comprise only 3% of this total. The rest is perylene as confirmed by mass spectra, gas chromatographic retention times, and ultraviolet fluorescence spectra. Figure 8 shows a comparison of the fluorescence spectra for two of the above samples (Coari River and Cariaco Trench sediments) with standard perylene. We conclude that nearly all of the PAH found in these four cases is due to the single species, perylene. The presence of a single PAH in high abundance cannot be accounted for by combustion. Certainly, there must be another source.

Other authors have also found perylene in sediments (ORR and GRADY, 1967; BROWN *et al.*, 1972; AIZENSHTAT, 1973; ISHIWATARI and HANYA, 1975; WAKEHAM, 1977). The areas where the perylene has been found include Saanich Inlet, British Columbia; Vema Fracture Zone; Santa Barbara Basin, California; Lake Biwa, Japan; and Lake Washington, Washington. Our findings add several tributary rivers of the Amazon River system and a sediment from Cariaco Trench to this list.

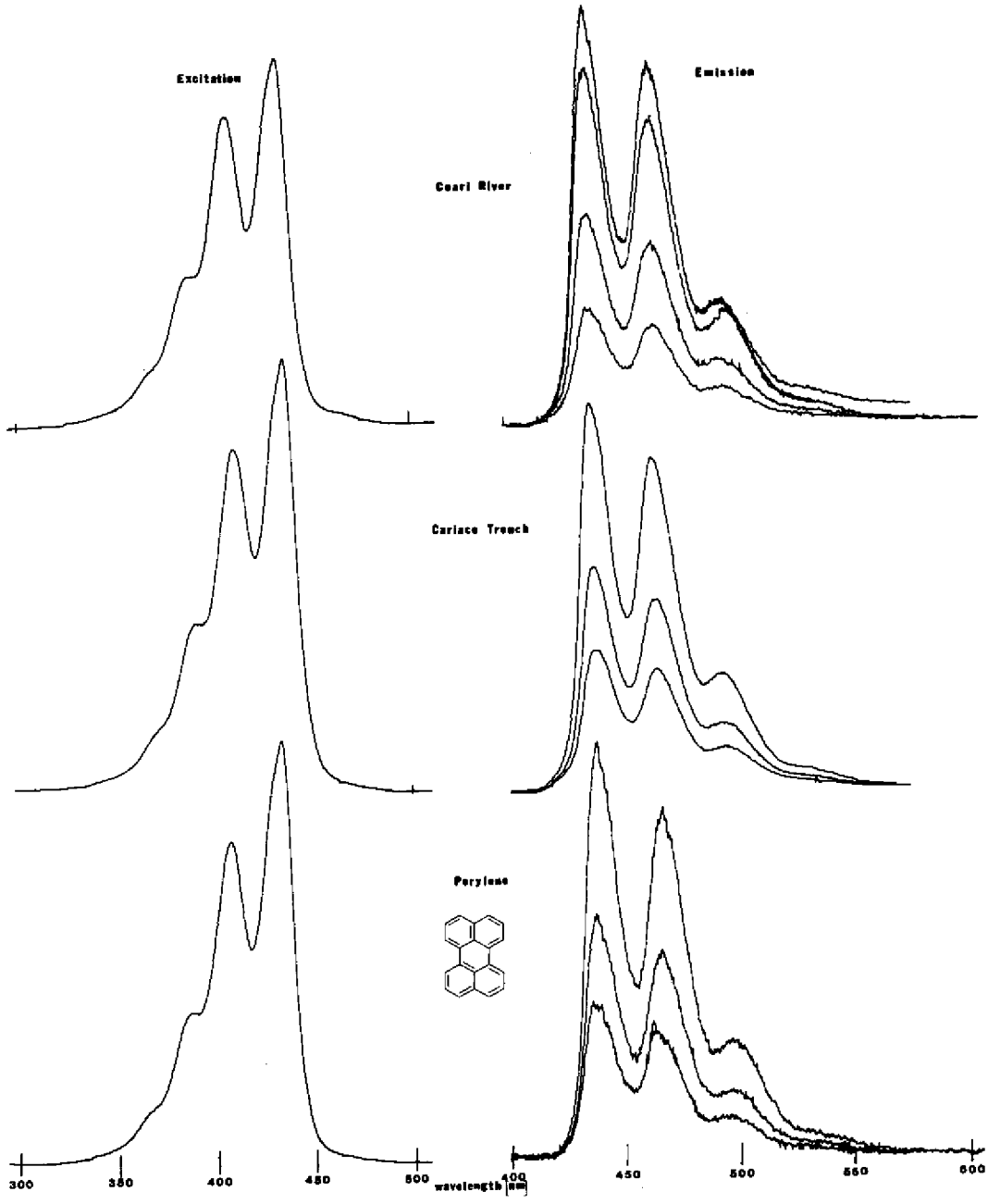


Fig. 8. Fluorescence excitation and emission spectra of two sediment samples and standard perylene. Excitation spectra were obtained by monitoring at 460 nm. Emission spectra were obtained by monitoring wavelengths of 350, 360, 370 and, in the case of the Coari River, 380 nm. All samples were dissolved in methylene chloride. Sediment samples were the silicic acid eluates used for GC-MS analyses without further fractionation.

AIZENSHTAT (1973) attributes the perylene in the Vema Fracture Zone to the deposition of sediment from the Amazon River 1200 km away. Our finding of perylene in the Amazon River system seems to support his hypothesis. AIZENSHTAT (1973), ORR and GRADY (1967), BROWN *et al.* (1972), and WAKEHAM (1977) all report that perylene concentrations increase with depth, suggesting an *in situ* formation mechanism. ISHIWATARI and HANYA (1975) report no such increase in a core ranging from 11 to 196 m in depth. This suggests either a different mode of perylene formation, or a completion of perylene synthesis by the 11 meter depth.

Where does the perylene come from? ORR and

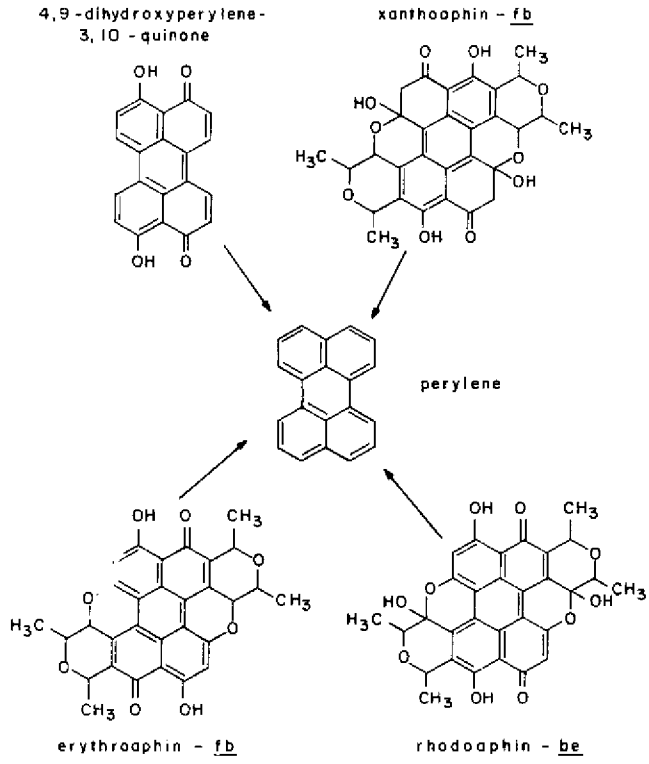


Fig. 9. Structures of some extended quinone pigments which may be possible precursors of perylene in sediments. Sterochemistry is not given.

GRADY (1967) and AIZENSHTAT (1973) suggest that the reduction of pigments such as erythroaphin pigments and 4,9-dihydroxyperylene-3,10-quinone (see Fig. 9) would produce perylene. These extended quinone pigments have been found in insects (CAMERON *et al.*, 1964) and fungi (THOMPSON, 1971; ALLPORT and BU'LOCK, 1960). Because these pigments are sensitive to oxidation, ORR and GRADY (1967) and AIZENSHTAT (1973) hypothesize that environmental transformation into perylene requires their rapid deposition into a reducing sediment. We can test this hypothesis on the Amazon River system. Besides the samples showing high perylene concentrations, we have analyzed two additional samples from this system: Flood Plain (No. 21) and Station 7 (No. 22). Neither of these sediments is reducing (see sample site description) (STALLARD and EDMOND, 1977). In addition, the Flood Plain sample should be composed of land runoff and sediment which has not substantially begun diagenetic processes. There was no abundance of perylene in either of these samples. These preliminary data support the above hypothesis.

WAKEHAM (1977) has calculated rates of formation based on core analyses and sedimentation rates. He finds a rapid rate of formation (4 ppb/yr) in Lake Washington sediments. He has also analyzed surrounding environments such as river sediments, stormwater runoff, humus and plankton. No perylene was found in any of these samples. This provides further evidence for an *in situ* formation mechanism rather than a deposition of perylene directly into the sediment.

Our data and those of the investigators mentioned above seem to support the above hypothesis. Some discrepancies do exist, however. The lack of increasing concentration of perylene with depth in Lake Biwa (ISHIWATARI and HANYA, 1975) is one. Among isoprenoid quinones, the extended quinones such as those proposed as precursors of perylene are not very abundant (THOMPSON, 1971). However, the high concentration of perylene found by all investigators seems to require a very common and abundant precursor. Obviously, a more extensive investigation into the proposed formation mechanism must be carried out before an accurate picture of the presence of perylene in marine sediments can be presented.

CONCLUSIONS

PAH and their alkyl homologs are distributed in sediments throughout the world. The qualitative PAH pattern is remarkably constant for most of the locations studied, and the total PAH abundance increases with proximity to urban centers. This is consistent with anthropogenic combustion's being the major source of these compounds. Two non-combustion sources of PAH have been noted: retene coming from abietic acid, and perylene probably coming from

various extended quinone pigments. Important questions, such as identification of sources, modes of transport, environmental alterations, and mechanisms of diagenesis are now being addressed. Nevertheless, much work remains to be done on this interesting class of compounds.

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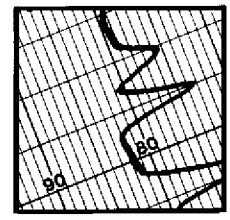

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Analysis of Polynuclear Aromatic Hydrocarbons by Glass Capillary Gas Chromatography Using Simultaneous Flame Ionization and Electron Capture Detection

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Key Words:

Gas Chromatography
Capillary, glass
Environmental specific trace analysis
FID/ECD response ratios for PNA's
PNA identification evidently improved

Summary

The possibility of simultaneous application of an electron capture (ECD) and a flame ionization detector (FID) connected to a glass capillary column for analyzing polynuclear aromatic hydrocarbons (PNA) has been investigated. The ECD/FID ratio is determined for 46 PNA compounds. The ratios vary from 0.02 to 117 with relative standard deviations better than 20 percent determined from 10 replicate analyses. The results suggest that the method may be used for obtaining additional evidence in identifying PNA in environmental samples.

Impurities and transformation products in the standard were identified by computerized glass capillary gas chromatography/mass spectrometry. Quinones and diones are responsible for the high EC-response determined in some trace components in the standard.

An application of the method is shown for PNA from particulates in urban atmospheres.

Introduction

Polynuclear aromatic hydrocarbons (PNA), formed by combustion of carbonaceous materials, are ubiquitous pollutants of the environment [1]. These pollutants are found in samples of air, water, and food as well as biological samples. The presence of PNA in these samples is of considerable interest since many of these hydrocarbons are known to exhibit carcinogenic activity [2].

Due to the complexity of the PNA-fraction, characterization of these compounds in environmental samples represents an analytical problem. A large number of analytical techniques have been used to determine PNA, including column chromatography and spectrophotometry [3,4], fluorescence [5], paper- and thin-layer chromatography [6, 7], gas chromatography [8], high pressure liquid chromatography [9], a combination of these techniques [10, 11], or gas chromatography/mass spectrometry [12]. Recently, the technique of glass capillary gas chromatography (GC^2) has been brought to a level where excellent reproducibility, high sensitivity and resolution may be obtained [13], and applications of this technique have been demonstrated on analysis of PNA in environmental samples [14, 15].

However, assignment of a gas chromatographic peak to a compound on the basis of retention time only may not be sufficient, and additional information is often required, e.g., by connecting the gas chromatograph to a mass spectrometer. Such instruments are, however, very expensive and in many cases do not provide the required information since mass spectra of isomeric PNA compounds may be indistinguishable. Further contribution to the identification of PNA may be obtained by simultaneous use of a general and a specific detector, such as a flame ionization (FID) and an electron capture detector (ECD), respectively.

Simultaneous, multiple detection in capillary gas chromatography has been reported by several authors. *Bertsch et al* [16] used a FID and a flame photometric detector (sulfur-mode) in connection with a high resolution nickel open tubular column to identify sulfur-containing organics in urine. Grob [17] applied a FID and a micro-ECD in analysis of a nonpolar fraction of an extract from a sewage plant. In this case, the detector responses were strongly complementary, demonstrating that the combined results from simultaneous recording are much more informative than the sum of independent FID and ECD runs. Recently, the application of an all-glass splitter to a glass capillary column was demonstrated for some chlorinated hydrocarbons and acetone using FID and ECD [18]. The results revealed that FID/ECD relation varied with column temperature, but replicate determinations showed very good precision at constant elution temperature.

The aim of the present paper is to demonstrate the use of simultaneous detection of PNA in a GC^2-system using FID and ECD, and to investigate the possibility of using the ratios of the FID and ECD signals for identifying PAH in environmental samples.

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the most convenient one. What are the precautions which can be taken? Large sample volumes should be preferred, but in capillary GC their size is very limited (overloading of the injector). The injector temperature ought to be high in order to heat the needle strongly, but is again seriously limited by the degradation of components on the hot metal surfaces of the needle. The most important factor to care about is the handling of the syringe needle. Experimentally the "hot needle" technique gave the best results. We explain this fact by assuming an explosion-like evaporation of a relatively small portion of sample along the walls of the needle, thus creating a high pressure which shifts the bulk of the sample as a liquid into the vaporizer (may be compared to distillation without a boiling stone). As can be seen from the heaviest part of the sample, with this technique approximately 20 to 25% of the sample is evaporated and at least 75% are expelled (beyond C40 probably very little material leaves the needle through evaporation). Components between C20 and C30 still have sufficient vapour pressure at 350° to get partly carried out of the needle by the last vapours coming from the part of the needle near the septum. The "hot needle" technique requires (a) a maximum of heat accumulated in the syringe needle when the sample enters it (there is probably a considerable cooling effect due to the evaporation) and (b) a rapid transfer of the sample into the needle in order to hinder evaporation of the sample from the front of its plug and to favour the formation of a vapour bubble on the rear side of the plug which will have an optimum expelling effect.

The alternative method to be considered is the "solvent flush" technique, as it is used in many laboratories. It is to be preferred for samples containing components which degrade on hot metal surfaces. However, it should be emphasized that it is not simply a question of the plug of pure solvent shifting the plug of liquid sample, since the injection cannot be carried out rapidly enough to avoid vaporization in the needle. The remaining sample components in the needle suggest that a portion of the sample evaporates along the needle walls, leaving behind some low boilers and forming a cushion of vapour over which subsequent solvent glides without having the desired washing effect. On the one hand the coldest possible needle temperature would seem to be desirable in order to minimize evaporation. However, experiments do not support this clearly enough. On the other hand we found, as other authors have done, that rapid movement of the plunger reduces selective evaporation out of the needle and hence the discrimination of the high boilers.

A factor not considered thus far is the temperature gradient through the axis of the injector. The temperature set for the heating block does not apply to the parts near the septum. Therefore in a substantial part of the syringe the temperature is insufficient, thus favouring a strong discrimination. However, the use of septa leads necessarily to making compromise with regard to the temperature of the upper part of the injector, although this cool zone could be shortened in the case of many commercial injectors. A similar source of increased discrimination is the part of the needle attached to the barrel of the syringe since the heat is dissipated into the cold parts. The scope for improvement in these areas may be considerable.

Acknowledgement

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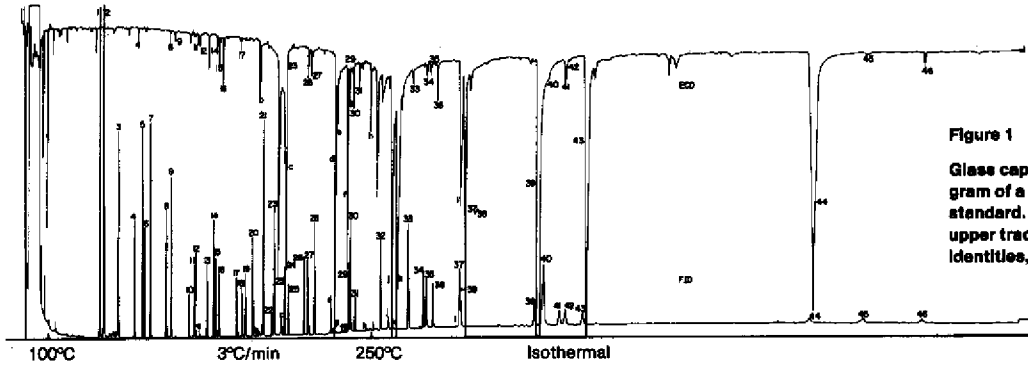

Figure 1

Glass capillary gas chromatogram of a prepared PAH standard. Lower trace – FID, upper trace – ECD. For peak identities, see Tables 1 and 2.

Experimental

Chemicals

Solvents were purified by distillation in a glass apparatus with a one-meter-high column filled with steel chips. The PNA compounds were supplied commercially. The PNA standard mixture was prepared by dissolving weighed amounts in cyclohexane. When not in use, the standard mixture was stored at 4°C in the dark.

Gas Chromatograph

The gas chromatographic analysis was carried out on a Hewlett Packard model 5730 gas chromatograph equipped with glass capillary column, FID and ECD. About 2 µl of cyclohexane extract were injected using the splitless injection technique [19]. The chromatographic conditions were as follows:
column, glass capillary 50 m x 0.35 mm i.d. coated with SE-54 (H. & G. Jaeggi, 9043 Trogen, Switzerland);
carrier gas, helium at 4 ml min^{-1};
injector temperature, 275°C;
splitless period, 30 sec;
initial temperature, 100°C;
programmed temperature, 3 deg min^{-1};
final temperature, 250°C;
detector temperature, 300°C.
The chromatographic peaks were displayed on a Perkin Elmer 50 two-pen recorder.

The split of the column effluent was made by means of Pt/Ir capillary tubing (Antech GmbH, D-6702 Bad Dürkheim 1, W. Germany) according to *Etzweiler* and *Neuner-Jehle* [20]. The splitting ratio ECD/FID was 1.07 measured at room temperature, and no significant deviation was detected at higher column temperatures. The ECD required make-up gas; 40 ml min^{-1} of Ar with 10 percent methane and the FID had N_2 as make-up gas; 60 ml min^{-1}.

Gas Chromatograph – Mass Spectrometer – Computer System

The gas chromatograph – mass spectrometer – computer system is a combination of a Carlo Erba Fractovap 2101 gas chromatograph and a Varian-MAT 112 mass spectrometer with a Spectro system 100 MS computer. The gas chromatographic conditions were the same as those described above with the exception that the flow rate which was about 2 ml min^{-1} at ambient temperature. Details concerning the GC/MS system are reported elsewhere [21].

Mass spectra were recorded in the mass range m/e 50-500 at a scan rate of 1 sec. per decade, with automatic repetitive scanning. There was a programmed delay of 0.5 sec. after each scan and a spectrum was recorded approximately every 1.5 sec. The spectra were stored on a magnetic disk.

The following experimental conditions were chosen:
ionization energy, 70 eV;
emission current, 1.5 mA;
acceleration voltage, 800 V;
multiplayer voltage, 2 kV;
ion source temperature, 260°C;
interface temperature, 300°C;
resolution, 700.

Sampling

The actual sample consisted of particulates from urban air (Oslo, Norway) collected in February, 1978. The sample was collected by pulling air through a glass fiber filter by means of a high volume sampler. The sample cleanup was carried out as described previously [22].

Results and Discussion

Standard Mixture

A typical chromatogram of the FI and EC-trace of a standard mixture consisting of 46 PNA compounds is shown in **Figure 1.** The FI-trace demonstrates the separation efficiency of the capillary column. Baseline separation is achieved for phenanthrene from anthracene, benz(a)anthracene from chrysene, and benzo(a)pyrene from benzo(e)pyrene. Partial separation is achieved for picene and benzo(ghi)-perylene, while 3-methylcholanthrene and m,m'-tetraphenyl do overlap. The EC-trace shows a somewhat larger peak

Table 1
Ratios of ECD and FID Responses for PNA'S

Peak Number	Compound	ECD/FID	RSD[a] (Percent)
1	2-Methylnaphthalene	(b)	–
2	1-Methylnaphthalene	(b)	–
3	Biphenyl	(b)	–
4	Acenaphthylene	0.089	6.4
5	Acenaphthene	0.029	8.5
6	4-Methylbiphenyl	(b)	–
7	Dibenzofuran	(b)	–
8	Fluorene	0.083	11.4
9	9-Methylfluorene	0.052	15.2
10	9, 10-Dihydroanthracene	(b)	–
11	2-Methylfluorene	0.125	10.1
12	1-Methylfluorene	0.107	10.4
13	Dibenzothiophene	(b)	–
14	Phenanthrene	0.090	19.8
15	Anthracene	0.348	15.3
16	Acridine	0.724	2.9
17	Carbazole	0.208	7.4
18	2-Methylanthracene	(b)	–
19	1-Methylphenanthrene	(b)	–
20	9-Methylanthracene	(b)	–
21	3,6-Dimethylphenanthrene	(b)	–
22	Dihydropyrene	(b)	–
23	Fluoranthene	1.664	10.0
24	Pyrene	9.154	12.6
25	9,10-Dimethylanthracene	(b)	–
26	Benzo(a)fluorene	0.322	7.3
27	Benzo(b)fluorene	0.310	6.4
28	1-Methylpyrene	(b)	–
29	Benz(a)anthracene	0.073	9.7
30	Chrysene/triphenylene	0.371	12.0
31	Naphthacene	0.874	8.0
32	β,β-Binaphthalene	(b)	–
33	9,10-Dimethylbenz(a)-anthracene	0.119	16.2
34	Benzo(e)pyrene	0.272	10.4
35	Benzo(a)pyrene	0.241	3.7
36	Perylene	0.859	3.9
37	3-Methylcholanthrene	(c)	–
38	m,m'-Tetraphenyl	(c)	–
39	o-Phenylenepyrene	48.5	5.3
40	Dibenz(a,h)anthracene	0.067	21.7
41	Picene	1.75	6.0
42	Benzo(ghi)perylene	0.162	15.4
43	Anthanthrene	116.8	6.1
44	1,2:3,4-Dibenzopyrene	48.0	9.1
45	Coronene	0.463	11.9
46	3,4:9,10-Dibenzopyrene	4.68	9.9

(a) Relative standard deviation.
(b) No or very small EC-response, ratio less than 0.01.
(c) Peak overlap, no meaningful ratio can be evaluated.

width than the FI-trace and hence a lower resolution. This is in accordance with experiences previously reported by *Grob*, who found a smaller separation number for ECD than for FID when analyzing l-bromoalkanes [17].

The arithmetic mean values of the ECD/FID ratios for the PNA compounds as well as the relative standard deviations determined from ten replicate injections are given in **Table 1.** As shown in the table, the ECD/FID ratio varies within wide ranges indicating that the ECD/FID ratio combined with retention time represents a way of assigning chromatographic peaks with a relatively high degree of certainty. In all cases, the relative standard deviation is less than 22 percent. The standard deviations seem to be low when the EC and FI peaks have the same order of magnitude (ratio is close to 1), and have higher values for the extreme ratios. The lower value of the standard deviation probably reflects the precision of the injection and the detector responses.

Several of the PNA compounds have a relatively high EC-response, such as fluoranthene, pyrene, 3-methylcholanthrene/m,m'-tetraphenyl, o-phenylene pyrene, anthanthrene and dibenzo (a, l) pyrene. High EC-responses for some of these compounds have been reported previously [23]. Of particular interest is the EC-response of the least volatile PNA, since these compounds may be difficult to transfer from a GC to a mass spectrometer and hence would make identification more difficult.

The use of simultaneous EC/FI-detection for identifying PNA compounds may also be precluded for a number of reasons. It has been shown that the EC-response is a function of column temperature and pulse intervals in the detector [23]. It is therefore mandatory to use the same chromatographic conditions while analyzing standard mixtures and real samples. The ECD/FID-ratio also depends on the split-ratio and long-term variations in the detector performance. Finally, impurities in the sample may contribute significantly to the EC-response of a PNA compound. As a result, a correct ECD/FID-ratio may be used to support the assignment of a chromatographic peak, while a higher value might indicate the presence of an impurity in the peak or a misassignment.

Impurities and Transformation Products

The standards also contain several impurities, some of which have very high EC-response. The impurities have been numbered with letters (see Figure 1) to be distinguishable from the parent compounds. These impurities may have originated from the PNA-standards or may be transformation products of PNA formed during storage. To investigate this further, the standard mixture was analyzed by GC^2/MS. The results of this investigation are shown in **Table 2.** Only impurities giving interpretable mass spectra and having significant EC-response are included in the table. Particularly high responses are obtained for anthraquinone and benz(a) anthracene-dione, which are usual oxidation products of the parent hydrocarbons [1].

It is therefore plausible that these compounds are transformation products formed by photo-oxidation or reaction with oxygen [1].

It was previously reported that atmospheric PNA are transformed to quinones and diones [24]. The concentration of the oxygenated fraction is usually about 10 percent of the PNA concentration and determination of the quinones is therefore connected with some detection problems. The findings presented in Table 2 also suggest that simultaneous ECD/FID may be used for detecting low concentrations of oxygenated transformation products of PNA.

Real Sample

To test the feasibility of the method, the PNA fraction of urban air particulates was analyzed. The ECD- and FID-traces are shown in **Figure 2**. The FID-trace demonstrates the presence of PNA; the main compounds are given in **Table 3**. The ECD-trace shows a large number of compounds with strong EC response, by far exceeding the number of PNA in urban atmospheres.

The ratios of the ECD/FID peaks are given in Table 3. For the sake of completeness, the table also contains the ratios obtained for the pure standard samples. In most cases, the agreement between the ratios determined for the sample and the standard mixture is better than one standard deviation. This shows that despite the many compounds with high EC response present in the sample, the high resolution of the glass capillary column makes it possible to obtain ratios in accordance with those previously observed for the PNA standards. In some cases, there are obvious disagreements between the ratios obtained for the standard and the real sample, indicating an overlap between a PNA and another compound with strong EC-response.

Table 2

Ratios of ECD and FID Responses for Some PNA Degradation Products (or Standard Impurities)

Peak Number	Compound[b]	ECD/FID	RSD[c] (Percent)
a	Dimethylbiphenyl	(d)	–
b	Anthraquinone	91.5	14.2
c	M = 208	23.2	16.4
d	Aldehyde	13.2	6.7
e	Naphthothionaphthene	28.8	12.7
f	Fluorenone	125.6	8.2
g	Naphthothionaphthene	21.5	16.5
h	M = 261	36.0	15.0
i	M = 254	113.0	11.1
j	Benz(a)anthracene-dione	31.3	15.3
k	Not Identified	158.8	12.6
l	M = 266	99.0	4.9

(a) Peak numbers correspond to the notations in Figure 1.
(b) Compounds identified by mass spectrometry, see text.
(c) Relative standard deviation.
(d) No EC-response observed.

The simultaneous detection of PNA by FID and ECD may also represent a way of overcoming a problem recently stressed by *Zitko* [25]. Determination of PCB in biological material by ECD may be precluded by PNA. The simultaneous detection by FID and ECD will disclose the peaks in the ECD-trace containing a contribution from PNA. Based on data from standard samples, the contribution can be calculated to reveal if it is of major or minor importance. Based on existing glass capillary technology and multiple detection systems, it is therefore possible to obtain more accurate data both for PNA and PCB in environmental samples.

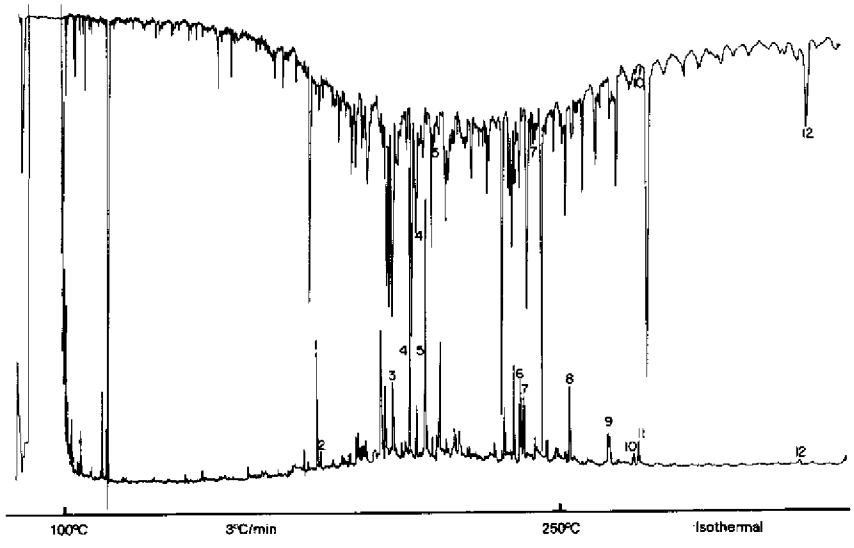

Figure 2

Glass capillary gas chromatogram of PAH in urban air particulates. Lower trace – FID, upper trace – ECD. For peak identities, see Table 3.

Table 3

Ratios of ECD and FID Responses for PNA's in Urban Air Particulates (Oslo, Norway)

Peak Number[a]	Compound	ECD/FID	Determined From Standard[b]	Comment
1	Phenanthrene	(c)	0.090	
2	Anthracene	(c)	0.348	
3	3,6-Dimethylphenanthrene	(c)	(c)	Internal Standard
4	Fluoranthrene	1.51	1.664	
5	Pyrene	0.17	0.154	
6	Benzo(a)anthracene	(d)	0.073	(e)
7	Chrysen/Triphenylene	0.34	0.371	
8	β,β-Binaphthalene	(c)	(c)	Internal Standard
9	Benzo(b&k)fluorantene	12.3	(d)	
10	Benzo(e)pyrene	0.26	0.272	
11	Benzo(a)pyrene	47.1	(d)	(e)
12	o-Phenylenepyrene	50.0	48.5	

(a) See Figure 2.
(b) See Table 1.
(c) No or very small EC-response.
(d) Not determined.
(e) Possibly interference from another compound with strong EC-response.

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Appendix B
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Appendix C
Workshop Agenda

Tuesday, April 1, 1980

8:45 a.m.	Register at Great Lakes Research Facility
9:00 a.m.	Welcome (GLRF/CGLS staff)
9:10 a.m.	Workshop Overview (J. Delfino)
9:30 a.m.	Workgroup Sessions

Title	Chairperson
PCBs	M. Mullin
Pesticides	J. Lech
PAHs & Petroleum	K. Alben
Industrial Chemicals	R. Hites

10:30 a.m.	Break
10:45 a.m.	Workgroup Sessions (continued)
12:00 p.m.	Lunch
1:00 p.m.	Tour GLRF (GLRF/CGLS staff)
2:00 p.m.	Workgroup Sessions (continued)
3:30 p.m.	Break
3:45 p.m.	Workgroup Sessions (continued)
5:15 p.m.	Adjourn

Wednesday, April 2, 1980

9:00 a.m.	Workgroup Sessions (final small-group gathering: summarize previous discussions and select key items to share with entire group)
10:30 a.m.	Break
10:45 a.m.	Workgroups report to entire group (summary of workgroup discussions by chairpersons or designees)
12:15 p.m.	Lunch
1:00 p.m.	Workgroup Reports (continued as necessary)
2:00 p.m.	Adjourn