MAY 2 8 1974

STATEMENT OF ALAN V. NEBEKER

My name is Alan V. Nebeker. I am now a Research Aquatic Biologist with the Western Fish Toxicology Station, Corvallis, Oregon, which is a field station of the National Water Quality Laboratory, EPA, Duluth, Minnesota. I was stationed at the Duluth laboratory from 1966 to 1971, under the direction of Dr. Donald I. Mount, Director, where I conducted research dealing with the effects of temperature, low pH, and low oxygen on survival and adult emergence of aquatic insects; and the effects of PCB's on survival and reproduction of Daphnia, Gammarus, Tanytarsus (fish food organisms), the flagfish and the fathead minnow.

I attended Utah State University, B.Y.U., the University of Montana, and the University of Utah. I received the B.S.,M.S., and Ph.D. Degrees at the University of Utah. My Master's Thesis study dealt with the effects of insecticides and herbicides on the survival of Gammarus, the freshwater scud or shrimp. My doctoral study dealt with the taxonomy and ecology of winter stoneflies, with special emphasis on the effects of stream temperature on adult emergence. I also served as Instructor in Biology for six years while at the University of Utah, and two summers at the University of Montana. I have published one book and thirty scientific papers in the field of aquatic biology and toxicology.

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My testimony is taken from the following exhibits:

Ex. 1. Nebeker, A. V., and F. A. Puglisi, 1974. Effect of Polychlorinated Biphenyls (PCB's) on Survival and Reproduction of Daphnia, Gammarus, and Tanytarsus. Trans. Amer. Fish. Soc. 103(4): Oct., 1974.

Ex. 2. Nebeker, A.V., F. A. Puglisi, and D. L. Defoe, 1974. Effect of Polychlorinated Biphenyl Compounds on Survival and Reproduction of the Fathead Minnow and Flagfish. Trans. Amer. Fish. Soc. 103(3): July, 1974.

Ex. 3. Defoe, D. L., R. W. Carlson, and G. D. Veith, 1973. Exposure of Fathead Minnows to Aroclor® 1248 and 1260. In: A Summary of NWQL Research Concerning the Effects of Polychlorinated Biphenyl on Water Quality and Aquatic Life. May, 1973. EPA, National Water Quality Laboratory, Duluth, Minnesota.

Ex. 4. Moorman, Ken, and K. E. Biesinger, 1973. Polychlorinated Biphenyl Research--a static bioassay using Aroclor® 1016 and related experiments. Unpublished Report. National Water Quality Laboratory, Duluth, Minnesota.

Ex. 5. Ringer, R. K., R. J. Aulerich, and M. Zabik, 1972. .. Effect of Dietary Polychlorinated Biphenyls on Growth and Reproduction of Mink. Michigan State University, East Lansing, Michigan 48823. (a more complete citation will be here when I receive the paper from Gil Veith). Water and Waste Chemistry. 12(2):149-154.

Ex. 6. Platonow, N. S., and L. H. Karstad, 1973. Dietary Effects of Polychlorinated Biphenyls on Mink. Can. J. Comp. Med. 37: 391-400.

Ex. 7. Sanders, H.O. and J. H. Chandler, 1972. Biological Magnification of a Polychlorinated Biphenyl (Aroclor® 1254) from Water by Aquatic Invertebrates. Bull. Env. Cont. and Toxicol. 7(5): 257-263.

Ex. 8. Sanders, Herman O., 1972. Toxicity of Aroclors® to Aquatic Organisms: Invertebrates. Fish-Pesticide Laboratory, Columbia, Missouri. PCB Newsletter, 30 March, 1972, National Water Quality Laboratory, Duluth, Minnesota. pg. 18.

Ex. 9. Stalling, D. L., and F. L. Mayer, Jr., 1972. Toxicities of PCB's to Fish and Environmental Residues. Environmental Health Perspectives, April, 1972.

Ex. 10. Schoettger, R.A., B. Grant, H. D. Kennedy, F. L. Mayer, H. O. Sanders, and D. Swedberg, 1970. Special Report on Polychlorinated Biphenyls (PCB's). In: Progress in Sport Fishery Research, Fish-Pesticide Research Laboratory, Division of Fisheries Research, Bureau of Sport Fishery and Wildlife, Washington, D.C.

Ex. 11. Mayer, Foster L., 1972. Chronic Toxicity of PCB's to Fish. Fish-Pesticide Laboratory, Columbia, Missouri. PCB Newsletter, March 30, 1972. National Water Quality Laboratory, Duluth, Minnesota.

Ex. 12. Morgan, J. R., 1972. Effect of Aroclor 1242® (a Polychlorinated Biphenyl) and DDT on cultures of an Alga, Protozoan, Daphnid, Ostracod, and Guppy. Bull. Env. Cont. and Toxicol. 8(3): 129-137.

Ex. 13. National Water Quality Laboratory, 1974. Unpublished reports on toxicity of isomers of Aroclor 1242 and 1254 to Daphnia magna. National Water Quality Laboratory, Duluth, Minnesota.

Ex. 14. Johnson, Waynon, 1973. Unpublished reports on toxicity of Aroclor 1016. Fish-Pesticide Laboratory, Columbia, Missouri.

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Research on the effects of Polychlorinated Biphenyls on aquatic life was started at the National Water Quality Laboratory in 1970, when I began an initial survey of the eight Aroclors using the water flea Daphnia magna. We had to develop testing and analytical methodology before we could do any detailed bioassays. The initial Daphnia studies were static tests conducted in small covered beakers. Later Daphnia tests were conducted in continuous-flow systems, with survival and reproduction as measures of toxicity. Aroclor 1248 was the most toxic to Daphnia magna of the eight Aroclors tested in static tests (1221, 1232, 1242, 1248, 1254, 1260, 1262, 1268), the 3-week LC50 (where 50% die after 3-weeks) was 25 µg/liter. Aroclor 1254 was the most toxic PCB to Daphnia under continuous-flow conditions with a 3-week LC50 of 1.3 µg/liter (Exhibit 1). As better analytical techniques were developed it became apparent that significant losses of PCB occurred in both static and continuous-flow test systems, explaining the difference between static and continuous-flow LC50 values, due to sorption on food, glassware, and animal tissues, and loss to the air. The mid-range PCB's were most toxic to Daphnia in static tests. The toxicity of the PCB's decreased progressively from A-1248 to A-1221 and from A-1248 to A-1268 (Exhibit 1).

Two additional fish food animals were tested (Exhibit 1). Ninety-six hour LC50 values for A-1242 and A-1248 on Gammarus pseudolimnaeus in continuous-flow tests were 73 and 20 µg/liter. Survival after 60 days was 52% at 8.7 µg/liter A-1242 and 53% at 5.1 µg/liter A-1248. Reproduction and survival of young were good at 2.8 µg/liter A-1242 and 2.2 µg/liter A-1248. The midge Tanytarsus dissimilis, in continuous-flow tests, did

not emerge (as adults) in abundance above 5.1 µg/liter A-1248 or 3.6 µg/liter A-1254. The 3-week LC50 for A-1254 was 0.65 µg/liter for larvae and 0.45 µg/liter for pupae. Tissue residues in Gammarus ranged from 4.0 µg/gram A-1254 in control animals to 552 µg/gram A-1248 in animals held for 60 days in water containing 5.1 µg/liter A-1248 (Exhibit 1).

Several tests were completed with two fish species (Exhibit 2). Two 9-month continuous-flow bioassays and several intermediate length continuous-flow tests were conducted to determine safe levels of Aroclor 1242, A-1248, and A-1254 for the fathead minnow (Pimephales promelas) and A-1248 for the flagfish, Jordanella floridae. Calculated 96-hour LC50 values for newly hatched fathead minnows were 7.7 µg/liter for Aroclor 1254 and 15 µg/liter for A-1242. Three-month-old fatheads had a 96-hour LC50 of ca. 300 µg/liter for A-1242. Reproduction occurred at and below 1.8 µg/liter A-1254 and at and below 5.4 µg/liter A-1242. Newley hatched young were the most sensitive life stages. Eggs hatched better at higher PCB levels such as 15 µg/liter, due to apparent bacteriostatic properties of PCB's, though the young died rapidly after hatching. Growth of young fatheads was also affected above 2.2 µg/liter 1248, and none survived above 5.1 µg/liter after 30 days. Young flagfish did not survive at A-1248 concentrations above 5.1 µg/liter and did not grow well above 2.2 µg/liter. Tissue residues in fathead minnows ranged from 0.7 µg/gram A-1248 in control fish to 1036 µg/gram 1254 in fish held for 8 months in water containing 4.6 µg/liter A-1254.

PCB studies were continued at the National Water Quality Laboratory after I moved to Corvallis. David Defoe, et. al., conducted two complete fathead continuous-flow life cycle tests with A-1248 and A-1260 (Exhibit 3).

They showed that in 30-day acute exposures using A-1248 and fathead minnow fry (less than 12 hours old) 8 µg/l killed all the fry, 4.5 µg/l killed 50% of the fry and caused a 70% reduction in the weight of the fry, and 2.2 µg/l caused no mortality but a 20% reduction in weight. Similar studies with Aroclor 1260 showed that 9 µg/l killed all the fry and 4 µg/l caused 70% mortality and a 30% reduction in fry weight. Reproduction, measured as the number of eggs spawned per female, in females exposed to 1.1 µg/l A-1248 was only 60% of that of the controls. Aroclor 1260 did not affect reproduction at concentrations up to 2.1 µg/l (Exhibit 3).

Additional studies are presently continuing at NWQL under the direction of Dr. K. E. Biesinger and Dr. Gil Veith. Studies of individual isomers and Aroclor 1016 are being continued. Preliminary studies comparing the toxicity of A-1016 and A-1242 by Ken Moorman and Dr. Biesinger (Exhibit 4), NWQL, Duluth, have shown similar effects on Daphnia magna in static tests. No young survived after 3 weeks at 125 µg/l A-1016 or A-1242. At 100 µg/liter 100 young survived after 3 weeks in A-1016 and 36 young survived A-1242 (Exhibit 4).

Significant work with marine organisms under the direction of Dr. Tom Duke, Gulf Breeze, Florida, has been conducted but will not be discussed here. Also a great wealth of information has been published on PCB tissue levels in a wide variety of freshwater, marine, and terrestrial animals. This material also will not be discussed here, with the exception of a brief mention of the works of R. K. Ringer, R. J. Aulerich and M. Zabik, East Lansing, Michigan (Exhibit 5); and N. S. Platonow and L. H. Karstad, Guelph, Ontario (Exhibit 6). Very low levels of PCB's in Lake Michigan were magnified in salmon tissues, which were subsequently fed to mink by mink ranchers (Exhibit 5). Meat from cows fed PCBs were fed to mink to determine if mink were really susceptible (Exhibit 6).

Tissue levels of PCBs below the FDA recommended standard of 5 µg/gram in salmon caused reproductive failure and early kit mortality when fed to the mink.

Much excellent data on the toxicity of PCBs to freshwater animals has been produced by people at the Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Columbia Missouri, under the direction of Dr. Richard A. Schoettger. A wide range of fish and fish-food organisms have been tested with all the Aroclor series, including A-1016 and several individual isomers. Sanders and Chandler (Exhibit 7) demonstrated that after a 7-day exposure to 1.5 µg/liter A-1254 many late-instar mosquito larvae were unable to metamorphose into the adult form. Control organisms pupated with success. Sanders (Exhibit 8) presented data with individual isomers using Gammarus pseudolimnaeus as the test animal in static tests:

No. Cl	PCB Component	TL 50(mg/l) 24 hr.	96 hr.
2	2,4' - dichlorobiphenyl	0.15	0.12
2	4,4' - dichlorobiphenyl	0.16	0.10
3	2,3,4' - trichlorobiphenyl	0.10	0.07
5	2,4,5,2',5' pentachlorobiphenyl	0.43	0.21
6	2,4,6,2',4',6' hexachlorobiphenyl	0.20	0.15

Acute toxicity data for cutthroat trout, channel catfish and blue gills are presented by Stalling and Mayer (Exhibit 9). For Aroclor 1221-1268, 96 hr. LC50 values in static tests ranged from 1,170 to 50,000 µg/l for cutthroat trout. The acute oral toxicity of Aroclors 1242, 1248, 1254, and 1260 was greater than 1500 mg/kg in rainbow trout. Intermittent-flow bioassays of Aroclor 1242, 1248, and 1254 to blue gills resulted in

15-day LC50 values of 54, 76, and 204 µg/l, respectively. Exposures to channel catfish gave 15-day LC50 values of 107, 127, and 741 µg/l for the same Aroclors. All LC50 values decreased significantly with longer exposures. The 20-day LC50 values for Aroclor 1248, 1254, and 1260 were 10,135, and 245 µg/l for bluegills. The toxicity of the PCBs to fish was inversely related to percent chlorine (higher Aroclor number) (Exhibit 9).

The crayfish, Orconectes nais, were more susceptible to Aroclor 1242 (7-day LC50 = 30 µg/l) than they were to A-1254 (7 day LC50 = 80 µg/l). Palaemonetes Kadiakensis, the glass shrimp, were very sensitive to Aroclor 1254, having a 7-day LC50 value of 3 µg/l compared to a 5-day LC50 of 1 µg/liter for DDT (Exhibit 10). Growth of lake trout was retarded by Aroclor 1248 in the diet for 3 months. Concentrations of 1.2, 3.8, and 12 ppm decreased weight gain by 6, 10, and 28%, respectively, below that of controls. Growth of the high-exposure group was only about half that of the controls after 6 months. Conversely, thyroid activity, as indicated by 72-hr. radioiodine uptake, was stimulated by PCB exposure. Serum cortisol concentrations ranged from 33 to 46% below that of the controls. Depressed growth rates and elevated thyroid activity imply metabolic inefficiency producing a "loading" stress that can adversely affect viability and reproductive stress (Exhibit 10).

Foster L. Mayer (Exhibit 11) presented data on the chronic toxicology of PCBs to fish. Thirty-day TL50 values for 1242 were 84 µg/l for Blue gills and 87 µg/liter for channel catfish; 1248 values were 78 µg/l for Blue gills and 75 µg/l for catfish; 1254 values were 177 µg/l for Blue gills and 139 µg/l for catfish; 1260 values were 51 µg/l for rainbow trout, >400 µg/l for Blue gills and 433 µg/l for catfish.

The study by Morgan (Exhibit 12) on the effects of Aroclor 1242 on an alga, protozoan, daphnid, ostracod, and guppy presents data similar to that given above. Algal growth was inhibited at 20, 2, and .2 ppm in static tests, though they eventually reached the same cell count levels, due to loss of PCB from the static test system. There was no effect on the protozoan Tetrahymena vorax at 20ppm in the conditions it was tested at. Aroclor 1242 was toxic to Daphnia pulex however, as young daphnia died at levels as low as 0.02 ppm. At 20 ppm there was no survival of the ostracod Cypridopsis vidua. The guppy Poecilia reticulata had 100% mortality at 20 and 2 ppm, with 25% mortality at 0.2 ppm (Exhibit 12).

The National Water Quality Lab, Duluth, MN, is currently conducting PCB work. Exhibit 13 presents chronic toxicity data for isomers of Aroclor 1242 and 1254 (Exhibit 13). It will be seen that there is not much difference in toxicity between trichlorobiphenyl and tetrachlorobiphenyl isomers. The isomers were prepared and identified by Ron Webb, EPA, Athens, GA.

The table presented below illustrates a comparison of the toxicity of Aroclor 1242 and 1016, obtained from Waynon Johnson, Fish-Pesticide lab., Missouri (Exhibit 14). It is apparent that the toxicities are similar.

Comparative toxicity of Aroclor 1242 and 1016, based on data from the Fish-Pesticide Lab. Columbia, MO., in continuous-flow exposures.

Test Organism	1242 (µg/l) 96 hr. LC50 values	1242 (µg/l) LC50 values after-	1016 (µg/l) 96 hr. LC50 values	1016 (µg/l) LC50 values after-
Rainbow Trout	67 109	10 days = 39	100 (sac fry) 440 (2.5 g)	17 days = 49 23 days = 11
Bluegill	125 154	15 days - 54	46 (1.8 g) 420 (0.9 g)	35 days = 43 22 days = 14

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Exhibit 1

Effect of Polychlorinated Biphenyls (PCB's) on Survival and Reproduction of Daphnia, Gammarus, and Tanytarsus

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Abstract

Continuous-flow and static bioassays were conducted at 18 C, with survival and reproduction as measures of relative toxicity of eight PCB's, Aroclor 1221 (A-1221), A-1232, A-1242, A-1248, A-1254, A-1260, A-1262, and A-1268. Three PCB-mixture bioassays were also conducted. Aroclor 1248 was the most toxic to Daphnia magna of the eight Aroclors tested in static tests; the 3-wk LC50 was 25 µg/liter. Aroclor 1254 was the most toxic PCB to Daphnia under continuous-flow conditions with a 3-wk LC50 of 1.3 µg/liter. Ninety-six-hr LC50 values for A-1242, and A-1248 on Gammarus pseudolimnaeus in continuous-flow tests were 73 and 20 µg/liter. Survival after 60 days was 52% at 8.7 µg/liter 1242 and 53% at 5.1 µg/liter A-1248. Reproduction and survival of young were good at 2.8 µg/liter A-1242 and 2.2 µg/liter A-1248. The midge Tanytarsus dissimilis, in continuous-flow tests, did not emerge in abundance above 5.1 µg/liter A-1248 or 3.5 µg/liter A-1254. The 3-wk LC50 for Aroclor 1254 was 0.65 µg/liter for larvae and 0.45 µg/liter for pupae. Tissue residues in Gammarus pseudolimnaeus ranged from 4.0 µg/gram A-1254 in control animals to 552 µg/gram A-1248 in scuds held for 60 days in water containing 5.1 µg/liter A-1248.

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Introduction

Polychlorinated biphenyls (PCB's) are widespread in the environment, rivaling DDT in general occurrence (Risebrough and Bodine 1970). Their significance in the aquatic environment as toxicants is now being revealed. Duke, Lowe, and Wilson (1970) have documented the occurence of one of the many PCB mixtures (Aroclor 1254) in the water, sediment, and biota of Escambia Bay, Florida. Veith and Lee (1970) reviewed chlorinated biphenyl contamination in natural waters and stated that PCB's may be among the more widespread contaminants. A general review of the structural and physical properties, uses, analytical methods of analysis, concentrations found in nature, and toxicology of PCB's is given by Peakall and Lincer (1970). The acute toxicity of some of the many types of PCB's produced commercially (Monsanto Chemical Co.) has been demonstrated for a few species of fish and fish-food organisms (Stalling 1970; Zitco 1970; Nebeker, Puglisi, and DePoe 1972; Duke, personal communication).

To assess the danger of the compounds to fish-food organisms, bioassays were designed and conducted with Daphnia magna as the initial test animal. All eight commercially available PCB's-Aroclor 1221 (A-1221) A-1232, A-1242, A-1248, A-1254, A-1260, A-1262, and A-1268—were tested. Seven continuous-flow bioassays were carried out with Aroclor 1248 and A-1254, twenty-four 3-wk static bioassays with all eight Aroclors, and three bioassays with PCB mixtures. Survival and reproductive success of Daphnia magna were used as measures of toxicity. The effects of A-1242 and A-1248 on the growth, survival, and reproduction of

Gammarus pseudolimnaeus and the effects of A-1248 and A-1254 on the growth, survival, and adult emergence of Tanytarsus dissimilis were also determined. Tissue residues were also determined in Gammarus to measure uptake of PCB's.

Materials and Methods

Physical Testing Methods

Daphnia static tests—Test containers were 250-ml glass beakers which were randomly distributed and covered with glass sheets to minimize evaporation. The experiments were conducted at 18 ± 1 C; the photoperiod was set for 16 hours light per day. Raw Lake Superior water was used for all testing.

Daphnia continuous-flow tests—Daphnia, Gammarus, and Tanytarsus were tested in a continuous-flow system which consisted of 24 stainless steel and glass 7.6 liter (2-gal) aquaria and which permitted the testing of five concentrations and a control in quadruplicate. A proportional diluter (Mount and Brungs 1967) provided the necessary continuous-flow conditions. A gas syringe filled with acetone and PCB was used with the diluter system to deliver the toxicant to the mixing chamber of the diluter. Additional agitation was maintained by circulating the mixing-chamber contents through a submersible pump. Mixing boxes with four-way splits to the test aquaria provided further mixing of the PCB solutions. All tanks were randomly distributed on the test tables. Raw Lake Superior water was used for all testing. The diluter delivered 150 ml to each test tank every 3 minutes. This flow rate ensured adequate dissolved oxygen levels, replenishment of fresh toxic solutions, and a complete exchange of water in the test tanks every 4-5 hours.

Test temperatures were maintained at 18 ± 1 C. Duro-test[2] (Optima FS) and wide spectrum Grow-lux fluorescent tubes provided light for all

[2]Mention of commercial products does not constitute endorsement by the U. S. Environmental Protection Agency.

tests. The photoperiod of Evansville, Indiana, was maintained during testing, and adjustments in day length were made every 2 wks, as recommended by the Bioassay Methods Committee, National Water Quality Laboratory (Bioassay Committee MS 1971).

Biological Testing Methods

Daphnia static tests (single PCB)—Daphnia magna, obtained from the National Water Quality Laboratory cultures, were tested from young stages less than 24 hrs old through maturation, reproduction, and growth of their young (3 wks). Five animals were placed in a 250-ml beaker containing 200 ml of test solution; four duplicates gave 20 test animals at each concentration. The survival of the original animals was recorded each week, and the test solutions were renewed. The young surviving after 2 wks were counted and discarded. Young surviving after the third week were also counted, and the total number of young surviving was recorded. All tests were repeated three times.

The 3-wk LC50, that concentration at which 50% of the animals had died after 3 wks, was used as one measure of toxicity. Fifty percent and 16% reproductive impairment were used as measures of reproductive success (Biesinger and Christensen 1972). These values describe the survival of young as the percentage decrease from the highest number of young produced in either the control or one of the lower tested toxicant concentrations. The LC50, 50% and 16% reproductive impairment values, and 95% confidence limits for each were determined by the statistical method of Litchfield and Wilcoxon (1949). The terminology recommended by Sprague (1969) was used to present toxicity data.

Food for the Daphnia was prepared from 0.5 g powdered dried wheat leaves and 10 g enriched trout food (fry granules) mixed in a blender with 300 ml of Lake Superior water (Biesinger and Christensen 1972).

Daphnia static mixture tests—Different percentages of a mixture of all eight PCB's were tested to determine the percentage survival in an aquatic system containing all the PCB's at low concentrations which barely permit reproduction. Testing methods were the same as those used in previous Daphnia static tests, and 50% survival and 50% and 16% reproductive impairment were also calculated as previously. The mixture consisted of the following concentrations (in µg/liter:) A-1221, 89; A-1232, 53; A-1242, 48; A-1248, 16; A-1254, 18; A-1260, 22; A-1262, 24; and A-1268, 162.

Daphnia continuous-flow tests—Five animals, less than 24 hrs old, were placed into 7.6 liter (2-gal) aquaria; four aquaria gave 20 test animals at each concentration. All surviving original Daphnia and their young were counted at the end of each 2- or 3-wk test. Analysis of data and physical-chemical conditions were the same as for the static tests; however, the animals were fed finely powdered fish food twice a day rather than the solution used in static tests. Mean measured PCB concentrations from the continuous-flow test tanks were used for all calculations.

Gammarus continuous-flow tests—Juvenile scuds, Gammarus pseudolimnaeus, from the Eau Claire River (Wisconsin) were held in the laboratory for at least 1 wk prior to testing. Twenty animals were placed in each tank; two (A-1242 test) and three (A-1248 test) replicate

tanks for each PCB concentration gave 40 (A-1242) and 60 (A-1248) scuds in each concentration. They were fed a combination of well-soaked maple and aspen leaves; dry commercial fish food; and algae, which were permitted to grow on the tanks before introduction of the test animals. Tests were maintained for 2 mo; they were started with juvenile scuds and were continued until the animals matured, coupled, and reproduced (1 mo). Reproduction was allowed to continue for 1 mo before the test was ended. The adults were counted, and the young were counted and weighed.

Midge continuous-flow tests--The species used was Tanytarsus (Peratanytarsus) dissimilis; its original source is unknown. It was tested with Daphnia as they are compatible. The midges were reared in extra tanks in the room so that many egg-carrying females were flying in the room at the beginning of the test. These females deposited eggs in all clean test tanks when the test was started, prior to introduction of Daphnia, and within a few hours all tanks contained larvae. This continuing random ovipositing provided an abundant supply of young midges, which lived in cases on the bottom of the aquaria, independent of the Daphnia. The occurrence of mature larval midge cases and pupal cases, which the growing larvae constructed, was used as one measure of growth and survival. The additional occurrence of pupae and cast pupal skins at the water surface indicated the success of adult emergence.

All mature larval and pupal cases were counted at the end of 3 wks. Tanks were observed daily to determine when successful adult emergence occurred. The 3-wk LC50 was based on 50% reduction of test animals, with the number of control midges being considered as 100%. This

procedure was useful under the particular conditions of these tests, but it is not recommended as a routine method.

Analytical Methods

Static Daphnia tests—Test solutions were prepared by weighing the Aroclor samples on an analytical balance and transferring them to volumetric flasks with acetone and Triton X-100, a surfactant (maintained at 0.03 x PCB level) added to help keep the PCB's solution. The concentrations of acetone and Triton X-100 used were tested previously and found to be nontoxic at the levels used during testing. The Triton-PCB mixtures were diluted to 500 ml with acetone and used as stock solutions. Portions of each stock solution were diluted with acetone to give five different working stock solutions for each Aroclor. One ml of each stock solution and 1 ml of food suspension were pipetted into a volumetric flask and diluted to 1,000 ml with Lake Superior water. Control solutions were prepared with 1 ml of acetone and 1 ml of food suspension in a liter of lake water to keep the acetone concentration constant (1 ml/liter) in all test solutions.

Continuous-flow tests—Methodology for preparation of stock solutions, diluter delivery systems, and analysis of PCB concentrations (gas chromatography) was identical to that used in earlier studies by Mehrle, Puglisi, and DeFoe (in press) with fathead minnows.

Chemical characteristics of the Lake Superior test water were monitored daily or weekly, and measurements were made with methods described by the American Public Health Association (1965). Dissolved oxygen in the tanks was measured by the azide modification of the Winkler method. Chemical characteristics were: pH, 7.5-8.0; acidity, 2.4-4.0 mg/liter; alkalinity, 40-43 mg/liter (as $CaCO_3$); calcium hardness, 34-36 mg/liter; and total hardness, 44-46 mg/liter.

A standard one/two factor analysis-of-variance program was used to test for experimental differences.

Terminal residues of Aroclor 1242 and A-1248 in Gammarus were determined by the Fish-Pesticide Laboratory, Columbia, Missouri, using the methods of Stalling and Johnson (1971). Residues of PCB's in feed used in tests were also determined (Table 1).

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Results and Discussion

Daphnia Static Tests

Of the PCB's tested A-1248 was the most toxic to *Daphnia*; the 3-wk LC50 was 25 µg/liter. Fifty percent reproductive impairment occurred at 24 µg/liter, and 16% impairment was at 16 µg/liter. The toxicity of the other PCB's decreased progressively from A-1248 to A-1221 and from A-1248 to A-1268 (Table 2). Aroclors A-1254, A-1260, and A-1262 were nearly as toxic as A-1248, and A-1242 and A-1232 were about half as toxic as A-1248. Aroclor-1221 was much less toxic; the LC50 was 180 µg/liter. The least toxic was A-1268, with an LC50 of 253 µg/liter. In all tests reproduction, as judged by the number of young produced, was impaired by lower concentrations of Aroclor than was required to cause death. Values for 50% reproductive impairment of all PCB's tested were lower than the LC50 for survival. Excepting A-1221 and A-1268 the confidence limits indicate that the values are not significantly different, however.

No survival or reproduction occurred at 40% of the PCB mixture after 3 wk. At 20% of the mixture there was 17% adult survival and 13% young survival (Table 3). Fifty percent of the adults survived at 15% of the mixture. Survival of young was reduced to 50% at 14% of the mixture, and 16% reproductive impairment occurred at 11% of the mixture. Good survival, growth, and reproduction occurred at and below 5% of the mixture.

Daphnia Continuous-Flow Tests

Aroclor-1254 was most toxic to *Daphnia* under continuous-flow test conditions. There was no significant difference between the 2-wk LC50 of 1.8 µg/liter and the 3-wk LC50 of 1.3 µg/liter (Table 2). Reproductive

impairment occurred at or just below those concentrations that prevented survival of the adults. Aroclor-1248 killed 50% of the adult Daphnia at 2.6 µg/liter, and 50% of the young survived at 2.1 µg/liter. Sixteen percent reproductive impairment occurred at 1.0 µg/liter for A-1248 and for A-1254 at 0.48 (2 weeks) and 1.0 (3 weeks) µg/liter (Table 2). Almost no reproduction occurred at 7.5 µg/liter A-1248 (Table 4). No reproduction occurred at 3.8 µg/liter A-1254, and no adults survived 3.5 µg/liter. The number of young produced ranged from 0.7 young per initial adult in 7.5 µg/liter A-1248 to 76 young per surviving adult in 0.45 µg/liter A-1254 (Table 4). The Aroclors were much more toxic in continuous-flow conditions due to continual replacement of fresh PCB. Almost one-third of the PCB added to the test water was lost to the air, with some lost to bacteria, algae waste materials, test container surfaces, and test animal tissues. The difference between static- and continuous-flow results illustrates how critical test methodology can be for certain chemicals.

Gammarus Tests

Ninety-six-hr LC50 values for Gammarus juveniles were calculated for Aroclor-1242 and A-1248 (American Public Health Association 1965). Aroclor-1248 was twice as toxic as A-1242; the mean LC50 was 29 µg/liter for A-1248 (four tests, range 26-32 µg/liter) and 73 µg/liter for A-1242 (two tests, 72 and 74 µg/liter). Survival of the initial test animals after 2 mo was significantly less than survival after 96 hr (Tables 5 and 6). No live animals remained at 26 µg/liter A-1242 or 18 µg/liter A-1248. Fifty-two percent were alive at 8.7 µg/liter A-1242 (Table 5) and 53% were alive at 5.1 µg/liter A-1248 (Table 6). Good reproduction

occurred at 2.8 µg/liter A-1242 and 2.2 µg/liter A-1248. Some reproduction occurred at 5.1 µg/liter A-1248, but it was only half that of the control and only one-fourth that of 2.2 µg/liter (Table 6). Terminal residues of A-1242 and A-1248 in Gammarus exposed for 60 days are given in Table 7, with control animal residue data summarized in Table 8.

Midge Tests

Adult midges emerged at concentrations up to 9 µg/liter A-1248. Larvae were present at 18 µg/liter, but adult emergence did not occur. Abundant emergence did not occur above 5.1 µg/liter. Aroclor-1254 was more toxic to midges as no emergence occurred above 3.5 µg/liter, and abundant emergence did not occur above 3 µg/liter, even though larvae were present (Table 9). Survival and growth of the midge when tested with A-1254 were excellent in control chambers, but were reduced by 50% at the lowest test concentration of 0.45 µg/liter (Table 9). At 1.2 µg/liter the numbers of larval cases were reduced to 35% of the control, and the numbers of pupal cases were reduced to 18% of the control. No larval cases were formed at 33 µg/liter, and no pupal cases were constructed at 9 µg/liter. The calculated 3 wk LC50 for A-1254 (50% reduction based on control as 100%) was 0.65 µg/liter for larvae and 0.45 µg/liter for pupae, indicating that a safe level of A-1254 for midge well-being is below 1 µg/liter.

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Table 1. Residues in feed used in tests

	Pesticide and PCB (µg/gram)				
	p,p'-DDE	p,p'-DDD	p,p'-DDT	Aroclor 1248	Aroclor 1254
Fish food Jan., 1971	0.22	0.02	0.10	0.3	0.8
Fish food Feb., 1971	0.20	0.02	0.10	0.5	1.5

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Table 2. Calculated 2- and 3-wk LC50 and 16% reproductive impairment values for Daphnia magna subjected to Aroclor 1248 and Aroclor 1254 in continuous-flow conditions and eight Aroclors in static conditions at 18 ± 1 C

Aroclor (PCB)	3-wk LC50 (ug/liter)	Confidence limits (0.05)	Reproductive impairment (ug/liter) 50%	16%
Static test conditions				
A-1221	180	158.0-205.0	125	89
A-1232	72	62.6-82.8	66	53
A-1242	67	55.4-81.0	63	48
A-1248	25	21.4-29.2	24	16
A-1254	31	25.8-37.2	28	18
A-1260	36	27.7-46.8	33	22
A-1262	43	37.0-49.9	41	24
A-1268	253	222.0-288.0	206	162
Continuous-flow test conditions				
A-1248	2.6 (2-wk LC50)		2.1	1.0
A-1254	1.8 (2-wk LC50)		1.1	0.48
A-1254	1.3 (3-wk LC50)		1.3	1.0

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Table 3. Survival and reproduction of Daphnia magna after a 3 week exposure to various percentages of a PCB mixture of 16% impairment concentrations at 18 ± 1 C[a]

Percentage of mixture[b]	Initial number of animals	Adults alive at end of test			Mean survival of adults (%)	Total young produced			Mean young produced[c] (%)
		Test 1	Test 2	Test 3		Test 1	Test 2	Test 3	
40	20	0	0	0	0[d]	0	0	0	0[d]
20	20	2	8	0	17[d]	80	152	68	13[d]
10	20	16	20	20	93	554	792	631	87
5	20	18	20	20	97	690	795	804	100
1	20	14	19	19	87	425	670	668	77
0.5	20	15	20	20	92	566	871	701	94
0.0	20	17	18	20	92	688	757	836	99

[a]Concentrations of each Aroclor that permitted reproduction, but reduced it by 16%.

[b]Mixture consisted of the following concentrations (in µg/liter): A-1221, 89; A-1232, 53; A-1242, 48; A-1248, 16; A-1254, 18; A-1260, 22; A-1262, 24; and A-1268, 162.

[c]Percentage of the highest young produced in one of the lower tested toxicant levels (5%).

[d]Significantly different from the controls at the 0.05 level.

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Table 4. Survival and reproduction of Daphnia magna after 2 weeks' continuous-flow exposure to Aroclor 1248 and Aroclor 1254, and 3 weeks' exposure to Aroclor 1254

Mean measured concentration (µg/liter)	Initial number of animals	Total number of adults alive at end of test	Mean survival of adults (%)	Total young produced	Young produced[a] (%)	Young per initial adult	Young per surviving adult
1248 (2 wk)							
7.5	60	3	5	43	2.6	0.7	14
2.5	60	39	65	699	43	12	18
0.86	60	55	92	1441	88	24	26
0.26	60	52	87	1641	100	27	31
0.1	50	37	74	627	46	13	17
0.0	50	30	60	786	56	16	26
1254 (2 wk)							
9.0	20	0	0	0	0	0	0
3.8	20	0	0	0	0	0	0
1.7	20	12	60	239	36	12	20
0.92	15	14	93	269	53	18	19
0.37	15	12	80	504	100	34	42
0.0	15	8	53	290	58	19	36
1254 (3 wk)							
33.0	40	0	0	0	0	0	0
9.0	40	0	0	0	0	0	0
3.5	40	0	0	0	0	0	0
1.2	40	28	70	1583	76	39	56
0.45	35	24	69	1816	100	52	76
0.0	30	24	80	1154	74	38	48

[a]Percent decrease from the highest number of young produced in either the controls or one of the lower tested toxicant levels.

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Table 5. Survival and reproduction of Gammarus pseudolimnaeus after 2 months' exposure to Aroclor 1242 in continuous-flow systems at 18 C

Mean measured concentration (µg/liter)	Initial number of animals	Total number of adults alive at end of test	Mean survival of adults (%)	Total young produced	Young per surviving adult
234	40	0	0	0	0
81	40	0	0	0	0
26	40	0	0	0	0
8.7	40	21	52	0	0
2.8	40	31	77	131	4.2
0.0	40	19	48	130	6.8

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Table 6. Survival and reproduction of Gammarus pseudolimnaeus after 2 month's exposure to Aroclor 1248

Mean measured concentration (µg/liter)	Initial number of animals	Total number of adults alive at end of test	Mean survival of adults (%)	Total young produced	Young per surviving adult	Total weight of young (mg)	Mean weight of each young scud (mg)
18	45	0	0	0	0	0	0
5.1	45	24	53	169	7.0	90	0.53
2.2	45	33	73	729	22.1	287	0.39
0.54	45	32	71	627	19.6	336	0.54
0.18	45	33	73	357	10.8	154	0.43
0.0	45	29	64	330	11.4	200	0.61

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Table 7. Terminal residues of Aroclor 1242 and Aroclor 1248 in *Gammarus* exposed for 60 days

Mean measured PCB concentration (µg/liter)	Residues (µg/gram)			Concentration factor
	A	B	Mean	
A-1242				
2.8	71(14)[a]	80(17)	76	22,000
8.7	246(9)	387(10)	316	29,000
26	409(1)	—	409	14,000
A-1248				
0.18	9(33)	—	9	24,000
0.54	15(32)	—	15	14,000
2.2	127(33)	—	127	38,000
5.1	552(24)	—	552	55,000

[a]Mean of (N) individual samples.

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Table 8. Terminal residues in control Gammarus from Aroclor 1242 study

Gammarus	p,p'DDE (µg/gram)	Aroclor 1248 (µg/gram)	Aroclor 1254 (µg/gram)
A Controls	0.12(7)[a]	8.0	4.0
B Controls	0.06(12)	6.0	7.0

[a]Mean of (N) individual samples.

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Table 9. Effect of Aroclor 1254 on the growth and survival of the midge *Tanytarsus dissimilis*

Mean measured concentration (µg/liter)	Test	Number of mature larval cases: Each test	Mean	Percentage of control	Number of pupal cases: Each test	Mean	Percentage of control
33	A	0			0		
	B	0	0	0	0	0	0
	C	0			0		
	D	0			0		
9.0	A	0			0		
	B	0	0.25	0.2	0	0	0
	C	0			0		
	D	1			0		
3.5	A	3			1		
	B	7	8	7.4	0	2	6.8
	C	14			5		
	D	-			-		
1.2	A	43			7		
	B	68	37	35	5	5.2	18
	C	4			2		
	D	35			7		
0.45	A	70			4		
	B	25	56	52	27	16	55
	C	82			16		
	D	48			16		
0.0	A	94			32		
	B	108	107	100	33	29	100
	C	119			22		
	D	-			-		

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Exhibit 2

Effect of Polychlorinated Biphenyl Compounds on Survival and Reproduction of the Fathead Minnow and Flagfish.

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Abstract

Two 9-month continuous-flow bioassays and several intermediate length continuous-flow tests were conducted to determine safe levels of Aroclor 1242, 1248, and 1254 for the fathead minnow (Pimephales promelas) and Aroclor 1248 for the flagfish, Jordanella floridae. Calculated 96-hour LC50 values for newly hatched fathead minnows were 7.7 µg/liter for Aroclor 1254 and 15 µg/liter for 1242. Three-month-old fatheads had a 96-hour LC50 of 300 µg/liter for 1242. Reproduction occurred at and below 1.8 µg/liter 1254 and at and below 5.4 µg/liter 1242. Newly hatched young were the most sensitive life stage. Growth of young fatheads was also affected above 2.2 µg/liter 1248, and none survived above 5.1 µg/liter after 30 days. Young flagfish did not survive at 1248 concentrations above 5.1 µg/liter and did not grow well above 2.2 µg/liter.

Tissue residues in fathead minnows ranged from 0.7 µg/g 1248 in control fish to 1036 µg/g 1254 in fish held for 8 months in water containing 4.6 µg/liter 1254.

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Introduction

Polychlorinated biphenyl compounds (PCB's) are among the many exotic chemicals now finding their way into our waters. They are detected in fish and other aquatic organisms at concentrations much higher than those found in the ambient water. Because analytical methodology for detecting and identifying low levels of PCB's in water and aquatic organisms has only recently become available, little is known about the interactions. The acute toxicities of some of the PCB's produced commercially are presently being demonstrated at the Fish-Pecticide Laboratory, USDI, Columbia, Missouri (Stalling 1970); the Gulf Breeze Laboratory, EPA, Gulf Breeze, Florida (Duke et al. 1970); and the National Water Quality Laboratory, EPA, Duluth, Minnesota (Nebeker et al. 1972). The effects of PCB's on reproduction of Daphnia magna, Gammarus pseudolimnaeus, and the midge Tanytarsus dissimilis have been detailed by Nebeker and Puglisi (In press), but no full life-cycle information is available for fish species.

This study was begun in October 1970 to determine the effects of three PCB's, Aroclor 1242, 1248, and 1254, on the survival, growth, and reproduction of the fathead minnow (Pimephales promelas) and the effects of Aroclor 1248 on the survival and growth of the flagfish, Jordanella floridae. Further studies were conducted to determine the effects of Aroclor 1242 and 1254 on maturation, egg production, egg batch, young production, and survival of young of the fathead minnow. Tissue residues were also determined to measure uptake of PCB's.

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Materials and Methods

Physical Testing Methods

Three continuous-flow testing units were used, each consisting of five duplicated test concentrations and a control. Stainless steel and glass aquaria were used for all test chambers. Twelve 10-gallon aquaria were used for adult fish in the Aroclor 1254 testing; twelve 5-gallon aquaria were used for Aroclor 1242 testing; and twenty-four 2 1/2-gallon aquaria were used for testing Jordanella and some young fathead minnows. Young fish from spawning tanks were tested in 5- and 2 1/2-gallon aquaria. Three proportional diluters (Mount and Brungs 1967) provided the necessary continuous-flow conditions. A modification of the diluter system, a gas syringe filled with acetone and PCB, was used with a mechanical injector system to deliver the toxicant to the mixing chamber of the diluter. Agitation was maintained by circulating the mixing box contents through a submersible pump. Additional mixing boxes, with two-way and four-way splits to the duplicate test aquaria, provided further mixing of the PCB solutions. All tanks were randomly distributed on the test tables. Raw Lake Superior water was used for all testing.

The diluters delivered 350 ml (1242), 200 ml (1254), and 150 ml (1248) to each tank every 3 minutes; flow rates were set primarily to maintain adequate dissolved oxygen concentrations. These flow rates ensured a complete exchange of water in the test tanks every 4-5 hours. All tanks were cleaned daily, and excess algal and bacterial growths were scraped away weekly.

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Spawning substrates for fathead minnows consisted of inverted 3-inch-long logitudinal sections of glass quart beverage bottles. Egg hatchability studies were conducted in the same manner as previous fathead studies by Mount (1968), Brungs (1969), and others.

Temperatures were maintained at 24 ± 1 C. Durotest[2] (Optima FS) and wide spectrum Grow-lux fluorescent tubes provided light. The photoperiod of Evansville, Indiana, was maintained, with the adjustments in day length made every 2 weeks, as recommended by the National Water Quality Laboratory Committee on Aquatic Bioassays (Bioassay Committee MS 1971).

[2]Mention of commercial products does not constitute endorsement by the U. S. Environmental Protection Agency.

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Biological Methods

All fish were obtained from stocks at the National Water Quality Laboratory in Duluth, Minn. Ninety-six-hour acute tests with various ages of fish, conducted according to standard methods (American Public Health Association 1965), and chronic tests were carried out to obtain information for future calculation of application factors. Measured PCB concentrations were used for all calculations.

Full life-cycle studies with the fathead minnows (Aroclor 1242 and 1254) and the tests with Jordanella (1248) were started with newly hatched young (< 24 hours old). In the 30-day Jordanella test observations on survival were made daily, and growth was measured at the end of the test. Young fathead minnows were tested in the same diluter system, but in different tanks, at the same time to get comparative information on the two species.

Fathead (full life-cycle) testing was begun with 40 newly hatched young per concentration, 20 fish in each aquarium. They were fed twice daily with newly hatched live brine shrimp, frozen brine shrimp, Daphnia, and dry and frozen commercial fish foods. Growth was measured photogrsphically (McKim and Benoit 1971) at 60 and 90 days and at the end of the test. Prior to sexual maturation five spawning substrates were placed in the aquaria, and the fish were thinned to leave 10 fish per tank. The fish that were removed were used to study the effects of PCB's on adenosine triphosphatase activity (Cutkomp and Koch In press).

Eggs laid on the spawning substrates were removed, counted, and placed in egg cups for hatchability testing. Twenty-five or fifty eggs were placed in cups, and the number hatched was recorded. Some of the newly hatched fish were transferred to smaller aquaria at the same concentrations and held for 30 days to determine survival and growth.

Analytical Methods

The ploychlorinated biphenyls tested were Aroclor 1242, 1248, and 1254, manufactured by Monsanto Chemical Company. The stock solutions were prepared by weighing the compound and dissolving it in acetone.

Six liters of test water were collected from each test concentration to be analyzed. Three liters were siphoned into each of two 1-gallon glass bottles to provide duplicate samples. Teflon-lined screw caps were constructed for the bottles. A duplicate of the control and at least one other sample were spiked with equal amounts of the toxicant being used in the system. Ten milliliters of concentrated sulfuric acid and 300 ml of glass-distilled methylene chloride were added to each sample. The caps were then tightened and the bottles placed on a mechanical shaker for 10 minutes. The samples were allowed to settle for at least 10 minutes before the aqueous phase was decanted. The organic phase and remaining water were transferred to a separatory funnel and again allowed to settle. The organic layer was drained through solvent-washed anhydrous sodium sulfate into a Kunderna-Danish evaporator. The solvent was evaporated on a steam bath until the entire sample was contained in the 10 ml evaporator receiver. The remaining solvent was evaporated by blowing a gentle stream of dry nitrogen over it until the first sign of dryness. The receiver was stoppered with a cork wrapped in acetone-washed aluminum foil and stored for gas chromatograph (G.C.) analysis.

Analysis was done on a Tracor MT-220 gas chromatograph with a 6-foot 1/4-inch OD glass column packed with 3% OV-1 chromosorb W 80/90 mesh. A Coulson conductivity detector was used in the reductive mode without a catalyst. Sixty ml/min helium carrier gas was used with a 10 ml/min helium purge through the furnace when the column was vented. A rate of 40 ml/min of electrolytic hydrogen was used for the reductive pyrolysis. The temperatures of the inlet and all transfer lines were kept at 260 C, and the column varied from 195 C to 205 C depending on the PCB mixture being measured. The pyrolysis furnace was kept at 820 C.

The residue was dissolved in a volume of n-hexane that would give a concentration such that a 50-microliter injection would give at least 25% scale response on a 1-millivolt chart recorder. The solvent was vanted to prevent it from contaminating the furnace.

A single well-defined peak (Figure 1), present in all three of the PCB's tested, was used as an internal standard and was compared to the same peak in the "standard." This "quantitation" was done by alternately injecting standards and samples making sure the standards response (peak height) bracketed the response of the sample injected between them. The responses were plotted on linear graph paper, and the unknown quantity was taken from the plot. The calculated concentrations were corrected for recovery by using the ratio of the difference between the spiked and unspiked duplicate samples to the size of the spike. Mean measured concentrations were used for all calculations of biological data.

The extraction efficiencies of this method are presented in a previous report (Nebeker et al. 1972). The recovery values based on the single-peak evaluation show little difference in the Aroclors. This indicates that the extraction of the compound represented by the peak may be only slightly affected by the other compounds present.

The variability of this method on duplicate samples is less than 10%, regardless of concentration of toxicant. The variability of G.C. injection was less than 5% for duplicate injections of the standard or the same residue and up to 10% for injections of different volumes of the same residue.

A comparison of nominal and measured concentrations for all three Aroclors (Nebeker et al. 1972) indicated that, except for a few high values, the diluter systems were working well.

A standard, one/two factor analysis of variance program (ANOVA) written for the CDC 3300 was used to test for experimental differences.

Chemical characteristics of the Lake Superior Test water, measured daily or weekly with Standard Methods (American Public Health Association 1965), were as follows: pH, 7.5-8.0; acidity, 2.4-4.0 mg/liter; alkalinity, 40-43 mg/liter (as $CaCO_3$); calcium hardness, 34-36 mg/liter; and total hardness, 44-46 mg/liter.

Terminal residues of Aroclor 1242 and 1254 in fathead minnows were determined by the Fish-Pesticide Laboratory, Columbia, Missouri, using the methods of Stalling and Johnson (1971). Residues of PCB's in feed used in tests was also determined (Table 9).

Results

Fathead Minnow - Aroclor 1242

Concentrations of Aroclor 1242 above 10 µg/liter were lethal to newly hatched fry. Calculated 96-hr LC50 values for Aroclor 1242 were 15 µg/liter for newly hatched young fathead minnows and greater than 234 µg/liter (near 300 µg/liter) for 2-month-old fish. All minnows in the long-term study were dead after 96 hours in 51 µg/liter 1242, and none survived to the end of the test (8 months) at 15 µg/liter. Survival of fry was excellent when reared at the same concentrations their parents lived and spawned at. The final weights and total lengths of the fish did not differ significantly at the end of the 8-month test, however, growth of newly hatched fish was retarded prior to their death at the higher PCB concentrations.

Reproduction occurred at and below 5.4 µg/liter 1242 but spawning results and egg production were highly variable (Table 1) even when numbers of males and females were the same in some duplicate tanks. Egg hatchability was also variable but good hatching occurred at 5.4 µg/liter. Eggs were more resistant than fry at 15 and 51 mg/liter. Eggs produced by the controls but maintained at the higher concentrations hatched with good success, but none of the fry survived.

Terminal residues of Aroclor 1242 in fathead minnows exposed for 8.5 months are given in Table 5 (controls - Table 7).

Fathead Minnow - Aroclor 1248

Fathead minnows died rapidly at 18 µg/liter Aroclor 1248, and none was alive at the end of a 30-day test. Seventy-five percent were alive at 5.1 µg/liter, but their weight was only one-third that

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of the controls; their final lengths were not significantly different, however.

The final weight of the fish in 2.2 µg/liter was only half that of the controls; final lengths were the same. The results in the two lowest concentrations (0.54 and 0.18 µg/liter) were not significantly different from the controls.

Fathead Minnow - Aroclor 1254

Aroclor 1254 was more toxic to the fathead minnow than 1242 as they did not survive and reproduce above 1.8 µg/liter 1254.

Calculated 96-hr LC50 values for Aroclor 1254 were 7.7 µg/liter for newly hatched young fathead minnows and greater than 33 µg/liter for 2-month-old fish. All fish in the long-term reproduction study were dead after 96 hours in 15 µg/liter 1254 in both tanks. Survival at lower concentrations was not significantly different from the controls. The mean terminal weights of the fish were not significantly different in the various concentrations, but growth in length was delayed at 4.6 µg/liter. Spawning occurred at 1.8 µg/liter, but was significantly less than that at lower concentrations. Spawning was variable during the test, even between duplicate tanks (Table 2).

Egg hatchability and fry survival were good at and below 1.8 µg/liter (Table 3). Eggs produced by the controls and maintained in higher PCB concentrations (15 µg/liter) hatched readily, but all young were dead within 96 hours. Young fish which were held for 30 days in separate tanks at the same PCB concentrations at which they were spawned survived and grew well.

Terminal residues of Aroclor 1254 in fathead minnows exposed for 8 months are given in Table 6 (controls - Table 8).

Jordanella - Aroclor 1248

Fish held in 18 µg/liter Aroclor 1248 lived for about 2 weeks before they started to die. One died every 2-3 days until all were dead at the end of the 40-day test. Thirty-five percent were alive at 5.1 µg/liter at the end of the test; mean weight was only 15% that of the controls (Table 4). Mean length at 5.1 µg/liter was 21.8 mm, compared to 24.6 mm for the controls.

The fish in 18 and 5.1 µg/liter 1248 lost their fins and tail almost completely, as if they had been eroded away. The death of the one remaining fish at 18 µg/liter and three fish in 5.1 µg/liter when the temperature dropped from 25 C to 21 C indicates the precarious condition of the fish even though they were still alive.

Discussion

The results obtained from this study indicate that polychlorinated biphenyls are acutely toxic, and cause chronic toxicity at very low levels. The newly hatched larvae apparently are the most sensitive stage in the life cycle, at least for short-term exposure. The eggs were resistant or impermeable to the PCB mixtures. The inhibition by the PCB mixtures of bacterial-fungal growths on the incubating eggs resulted in a much better egg hatch at higher PCB levels. Juvenile fish, when tested for up to 30 days, were much less susceptible than very young fish. Tissue residues were very high in test fish, with some fish containing more than 200,000 times as much PCB as the water they were tested in.

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Acknowledgments

We wish to thank Henry Bell, Wesley Smith, and Allan Batterman for aid during testing, Dr. David L. Stalling for residue analysis of fish tissues, and Dr. Kenneth Biesinger and Kenneth Byram for helpful suggestions and statistical assistance. We also thank Monsanto Chemical Company for their cooperation and for making Aroclor samples available for testing.

MONS 203664

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MONS 203665

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MONS 203666

Table 1. Spawning and egg production of the fathead minnow at various concentrations of Aroclor 1242

Mean measured concentration (µg/liter)	Test tanks	Number of males	Number of females	Number of spawnings	Number of spawnings per female	Total eggs produced	Number of eggs per spawning	Number of eggs per female
51	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
15	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
5.4	A	4	5	25	5.0	1,514	61	303
	B	4	6	0	0	0	0	0
2.9	A	5	4	24	6.0	1,923	80	481
	B	4	5	9	1.8	424	47	85
0.86	A	6	4	5	1.3	138	28	35
	B*	-	-	—	—	—	—	—
0.0 (control)	A	1	8	42	5.2	5,350	127	669
	B	3	6	24	4.0	1,288	54	215

*Accidentally killed.

MONS 203667

Table 2. Spawning and egg production of the fathead minnow at various concentrations of Aroclor 1254.

Mean measured concentration (µg/liter)	Test tanks	Number of males	Number of females	Number of spawnings	Number of spawnings per female	Total eggs produced	Number of eggs per spawning	Number of eggs per female
15	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
4.6	A	4	5	0	0	0	0	0
	B	1	5	0	0	0	0	0
1.8	A	3	7	14	2.0	1,473	105	210
	B	4	7	1	0.14	22	22	3
0.52	A	4	6	30	5.0	4,142	138	690
	B	4	6	33	5.5	2,538	77	423
0.23	A	1	8	35	4.4	2,993	86	374
	B	5	5	8	1.6	346	43	69
0.0 (control)	A	3	6	27	4.5	2,838	105	473
	B	4	6	2	0.3	206	103	34

MONS 203668

minnow at various concentrations of Aroclor 1254

Mean measured concentration (µg/liter)	Test tanks	Number of eggs used	Number of live fry obtained	Egg hatchability (%)
15	A	0	0	0
	B	0	0	0
4.6	A	0	0	0
	B	0	0	0
1.8	A	300	186	62
	B	50	48	96
0.52	A	375	251	67
	B	345	205	59
0.23	A	272	150	55
	B	0	0	0
0.0 (control)	A	250	191	76
	B	150	107	71

MONS 203669

Table 4. Survival and growth of newly hatched *Jordanella floridae* after 40 days' exposure to Aroclor 1248

Mean measured concentration (µg/liter)	Initial number of animals	Mean survival (%)	Final mean weight (g)	Final mean length (mm)
18	20	0	—	—
5.1	20	35	.60	21.8
2.2	20	85	3.02	24.1
0.54	20	100	4.47	26.3
0.18	20	90	3.90	25.5
0 (control)	20	100	4.33	24.6

MONS 203670

Table 5. Terminal residues of Aroclor 1242 in fathead minnows exposed for 8.5 months.

Mean measured PCB concentration (µg/liter)	Residues (µg/g)						Concentration factor	
	A		B		Mean			
	Males	Females	Males	Females	Males	Females	Males	Females
0.86	62(2)	92(3)[a]	—	—	236	92[c]	274,000	107,000
	410[4][b]	—	—	—				
2.9	—	—	124(3)	98(3)	93	107	32,000	37,000
	—	—	62[5]	117[5]				
5.4	306(1)	364(2)	—	436(2)	345	436	63,000	81,000
	278[2]	430[4]	452[2]	514[5]				

[a] Mean of (N) individual samples.
[b] Pooled samples of [N] fish.
[c] Average of pooled and mean individual analysis for both A and B tests.

MONS 203671

Table 6. Terminal residues of Aroclor 1254 in fathead minnows exposed for 8 months

Mean measured PCB concentration (µg/liter)	Residues (µg/g)						Concentration factor	
	A		B		Mean			
	Males	Females	Males	Females	Males	Females	Males	Females
0.23	57(2)	28(1)	43(2)[a]	—	54	45[c]	235,000	196,000
	—	48[6]	61[4][b]	60[4]				
0.52	133(2)	99(3)	64(2)	104(2)	81	105	156,000	201,000
	54[3]	—	74[4]	113[5]				
1.8	204(1)	458(3)	103(1)	361(2)	196	429	109,000	238,000
	83[2]	347[6]	394[4]	553[5]				
4.6	803(1)	741(1)	1110(1)	1004(5)	1036	999	225,000	217,000
	1204[3]	1253[3]	—	—				

[a] Mean of (N) individual samples.
[b] Pooled samples of [N] fish.
[c] Average of pooled and mean individual analysis for both A and B tests.

MONS 203672

Nebeker, Puglisi and DePoe

Table 7. Terminal residues in control fish from Aroclor 1242 study

Fish (µg/g)	P,P'-DDE	Aroclor 1248	Aroclor 1260
A controls			
Males (1)[a]	0.02	—	2.2
Females (8)	0.08	3.3	2.8
B controls			
Males (3)	0.04	0.9	1.1
Females (6)	0.10	3.2	2.8

[a]Average of (N) fish.

MONS 203673

Table 8. Terminal residues in control fish from Aroclor 1254 study

Fish (ug/g)	p',p''-DDE	Aroclor 1248	Aroclor 1260
A controls			
Males (3)[a]	0.05	0.7	1.1
Females (6)	0.04	1.1	2.3
B controls			
Males (2)	0.03	0.9	1.9
Females (8)	0.04	1.6	2.7

[a]Average of (N) fish.

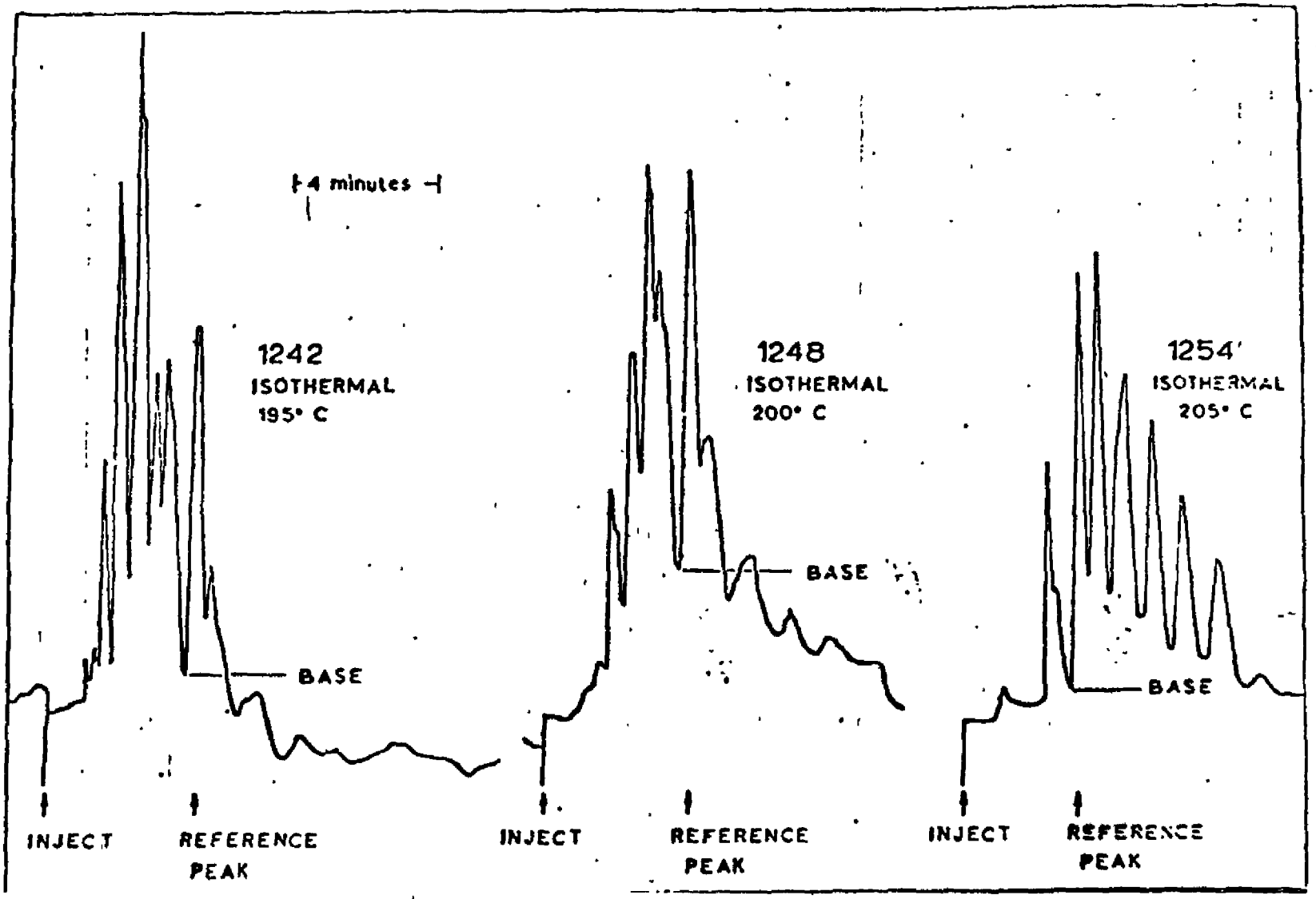
Table 9. Residues in feed used in tests

	Pesticide and PCB (µg/g)				
	p,p'-DDE	p,p'-DDD	p,p'-DDT	Aroclor 1248	Aroclor 1254
Fish food Jan., 1971	0.22	0.02	0.10	0.3	0.8
Fish food Feb., 1971	0.20	0.02	0.10	0.5	1.5

MONS 203675

Figure 3. Single peak used as reference for quantitation of Aroclors 1242, 1248, 1254.

MONS 203676


4 minutes
1242
ISOTHERMAL
195° C
1248
ISOTHERMAL
200° C
1254
ISOTHERMAL
205° C
BASE
BASE
BASE
INJECT
REFERENCE PEAK
INJECT
REFERENCE PEAK
INJECT
REFERENCE PEAK

MONS 203677

Exhibit 3

Defoe

A Summary of NWQL Research Concerning the Effects of Polychlorinated Biphenyl on Water Quality and Aquatic Life

May 1973

Environmental Protection Agency

National Water Quality Laboratory

Duluth, Minnesota

MONS 203678

Exposure of Fathead Minnows to Aroclor® 1248 and 1260

D. L. Defoe, R. W. Carlson and C. D. Veith

Chronic exposures of PCB Aroclors® 1248 and 1260 to the fathead minnow have been completed. The data is being analyzed and prepared for publication.

The data presented herein which represent mean values of duplicate bioassays are normalized to the results obtained with the control fish exposed only to the acetone carrier. No significant differences were observed for the reproductive success of the control fish exposed to acetone and control fish which were not exposed to acetone. More detailed studies to determine the effect of carriers on the bioassay results are underway.

The 30 day acute exposures using Aroclor® 1248 and fathead minnow fry (less than 12 hours old) has shown that 8 µg/l of A 1248 killed all the fry, 4.5 µg/l killed 50 percent of the fry and caused a 70 percent reduction in the weight of the fry, and 2.2 µg/l caused no mortality but a 20 percent reduction in weight. Similar studies with Aroclor® 1260 demonstrated that 9 µg/l killed all the fry and 4 µg/l caused 70 percent mortality and a 30 percent reduction in fry weight.

The reproduction, measured as the number of eggs spawned per female, in females exposed to 1.1 µg/l Aroclor® 1248 were only 60 percent of that of the controls. Aroclor® 1260 did not affect reproduction at concentrations up to 2.1 µg/l.

The second generation fry from adults previously exposed to the PCB mixtures were exposed to the respective mixtures for 30 days after hatching. Preliminary data analyses indicated that 3 µg/l of Aroclor® 1248

caused no mortality and no reduction in the weight of the fry. Similarly, Aroclor® 1260 caused no mortality when the fry were exposed to 2.1 µg/l; however, the weight of the fry was reduced by 33 percent.

The concentration of both Aroclor® 1248 and 1260 in the male fathead minnows reached an apparent steady-state after the first 100 days. The concentration of the PCBs in the female fish was more than double that in the male when expressed on a wet weight basis. However, the concentrations expressed on a lipid basis indicate that the females contained slightly less PCB per gram of lipid than did the males. In addition, the PCB concentration in the female on a wet weight basis was much more variable than the male, presumably due to the variable lipid content of the female.

The fathead minnow accumulated Aroclor® 1260 concentrations (lipid basis) approximately 270,000 times that in the water, whereas Aroclor® 1248 was accumulated to lipid concentrations approximately 120,000 times that in the water.

Data analyses and studies concerning the 60 day growth, delays in spawning, egg hatchability, body burden of PCBs, and the elimination of PCBs from the fish are continuing.

Exhibit 4

(4)

POLYCLORINATED BIPHENYL RESEARCH
a Static Bioassay using Arochlor® 1016 and Related Experiments

by Ken Moorman
Bemidji State College
Bemidji, Minnesota

Intern at
National Water Quality Laboratory
Duluth, Minnesota

MONS 203681

1st wk count Nov. 14
2nd wk count Nov. 21
3rd wk count Nov. 28

		1			10ppb	2			25ppb	3			50ppb	4			75ppb
9101	1st wk	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5
	2nd wk	4	3	0	4	4	5	5	5	3	4	1	5	5	5	5	5
	[illegible]	12	23	0	6	17	21	22	21	0	21	0	43	0	10	18	10
	3rd wk	4	3	0	4	3	3	5	5	3	4	1	5	5	5	5	5
	[illegible]	82	72	0	78	60	65	83	90	33	55	35	79	38	67	73	65
	T. Ad	11				16				13		1		20			
	T. Y	273				370				288				271			

		5			100ppb	6			125ppb	7			150ppb	8			200ppb
9101	1st wk	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
	2nd wk	5	3	3	4												
	[illegible]	9	2	1	0												
	3rd wk	5	3	3	3												
	[illegible]	64	29	8	41												
	T. Ad	14			1	0				0				0			
	T. Y	154				0				0				0			

		9			10ppb	10			25ppb	11			50ppb	12			75ppb
9102	1st wk	5	5	4	1	5	5	4	5	5	5	4	5	5	4	4	5
	2nd wk	4	2	0	0	3	0	1	0	5	5	4	4	4	0	4	4
	[illegible]	1	0	0	0	0	0	0	0	0	33	2	0	12	0	0	0
	3rd wk	4	2	0	0	3	0	0	0	5	5	4	3	4	0	4	3
	[illegible]	38	11	0	0	11	0	17	0	38	66	33	2	61	0	42	2
	T. Ad	6				4				17				11			
	T. Y	50				28				179				122			

		13			100ppb	14			125ppb	15			150ppb	16			200ppb
9102	1st wk	5	5	5	5	0	4	3	0	0	0	0	0	0	0	0	0
	2nd wk	5	4	5	5	0	2	3	0								
	[illegible]	8	0	0	0	0	0	0	0								
	3rd wk	1	4	5	4	0	2	0	0								
	[illegible]	9	4	13	2	0	0	0	0								
	T. Ad	14				2				0				0			
	T. Y	36				0				0				0			

17

Acetone Cont.	1st wk	5	5	5	5
	2nd wk	2	4	4	0
	[illegible]	7	0	22	0
	3rd wk	1	3	4	0
	[illegible]	19	66	77	0
	T. Ad	8			
	T. Y	194			

18

Water Cont.	1st wk	5	5	5	5
	2nd wk	4	5	5	5
	[illegible]	14	28	14	29
	3rd wk	2	5	5	5
	[illegible]	84	87	109	85
	T. Ad	19			
	T. Y	451			

MONS 203682

Chronic toxicity of the isomers of Aroclor 1242 and 1254 to Daphnia magna tested in standing jars. Concentrations are based on the nominal concentration and expressed in µg/liter. (National Water Quality Lab. unpublished data)

Isomers	Aroclor (PCB)	100% Adult Mortality (µg/l)	Reproduction[a] No significant Impairment (µg/l)	Reproduction[a] Significant Impairment (µg/l)
Parent mixture	1242	< 50	25	50 µg/l
2,4,4' trichlorobiphenyl[b] 2,5,4' trichlorobiphenyl 3,4,2' trichlorobiphenyl	1242	< 50	25	50 µg/l
2,3,2',3' tetrachlor - 2,5,3',4' tetrachlor - 2,4,3',4' tetrachlor -	1242	< 50	12.5	25 µg/l
Parent mixture	1254	> 50	50	> 50 µg/l
2,5,2',5' tetrachlor - 2,4,2',5' tetrachlor - 2,3,2',5' tetrachlor -	1254	100	25	50
2,3,6,2',5' pentachlor - 2,4,5,2',5' pentachlor - 2,4,5,2',4' pentachlor - 2,4,5,2',3' pentachlor -	1254	100	50	100

a. As determined from an Analysis of Variance and Dunnett's Test(p=.05)

b. Isomers prepared by Ron Webb, EPA, Athens, GA.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL WATER QUALITY LABORATORY
6201 CONGDON BOULEVARD
DULUTH, MINNESOTA 55804

April 2, 1974

Dear Dr. Nebeker:

Enclosed you will find the information on PCB's which you requested last week. All research work was done by LaVarre Uhlken while he was a presidential intern here last year, excluding isomers on 1242 which Leroy Anderson and I have recently started.

Since your call last week, I have reiewed LaVarre's records, and after doing so, have compiled all the raw data and performed statistical analysis on it and arranged it in table form. As I have typed the tables myself, you may want to change the table forms and/or have a secretary retype them if you find the information useful.

Sincerely yours,

Arlene Shelhon
Biological Lab. Technician

Enclosures:

MONS 203684

145

United States Department of the Interior
FISH AND WILDLIFE SERVICE
BURFAU OF SPORT FISHERIES AND WILDLIFE
Fish-Pesticide Research Laboratory
Route No 1
Columbia, Missouri 65201

IN REPLY REFER TO:

March 29, 1974

Dr. Alan Nebeker
Western Fish Toxicology Laboratory
Environmental Protection Agency
1350 S. E. Goodnight
Corvallis, Oregon 97330

Dear Al:

Enclosed find the toxicity data we have available on Aroclor 1016. The work was accomplished both at this lab and at our LaCrosse, Wisconsin field station by Mr. Wilbur Mauck. As indicated previously, we have no specific isomer data nor any longer term, chronic toxicity test data.

Static and flow-through toxicity tests were conducted under conditions generally as outlined in APHA (1971), Lennon and Walker (1964), and Mount and Brungs (1967). Values for LC50 (lethal concentration estimate for 50% of the test organisms) were calculated according to the method of Litchfield and Wilcoxon (1949). Time independent LC50 and LC10 values (concentration estimate at which 50% (90%) of the test organisms can live for an indefinite period of exposure) were calculated by the method of Green (1965).

I am quite interested in following the progress of your action relative to Aroclor 1016 and would appreciate receiving copies of the information you accumulate on it. I would especially like to know the outcome of your efforts and what the future applications and production of 1016 is predicted to be.

If you have other questions concerning our work please feel free to call me.

Sincerely,

W. Waynon Johnson
Fishery Biologist

sm

Enc.

MONS 203685

Literature Cited

APHA, et al. (1971). Standard methods for the examination of water and wastewater. Amer. Public Health Assoc., Washington, D. C. 13th ed. 562-577.

Green, R. H. (1965). Estimation of tolerance over an indefinite time period. Ecology, 46(6):887.

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MONS 203686

Table 1. Toxicity of Aroclor 1016 to selected species of freshwater fish by the static technique.

Test Organism		Test Conditions				LC Values (µg/l) and 95% Confidence Limits	
Species	Size (g)	Temp (°C)	pH	Alkal (mg/l)	Hard. (mg/l)	24 hr LC50	96 hr LC50
Bluegills	0.9	12	7.4	35	40	> 1200	1000 540-1850
Channel catfish	0.8	20	7.4	35	40	> 100,000	> 100,000
Chinook salmon	green eggs[1/]	12	7.4	35	40	> 1000	> 1000
Coho salmon	green eggs	12	7.4	35	40	> 1000	> 1000
Rainbow trout	0.5	12	7.4	35	40	800 470-1370	135 114-160
Yellow perch	0.8	12	7.4	35	40	-	240 153-375

1/ Fertilized eggs less than 48 hours old at initiation of test.

MONS 203687

Table 2. Toxicity of Aroclor 1016 to selected species of freshwater fish by the flow-through technique.

Test Organism		Test Conditions				LC Values (µg/l) and 95% Confidence Limits				
						96 hr.	Terminal Value		TI	TI
Species	Size (g)	Temp (°C)	pH	Alkal (mg/l)	Hard. (mg/l)	LC50	Day	LC50	LC10	LC50
Atlantic salmon	5.6	17	7.6	250	310	134 113-160	16	60 52-70	24	34
Bluegill	0.9	12	7.6	257	314	420 240-720	35	43 28-65	8.2	28
Bluegill	1.8	20	7.4	237	272	46 39-54	22	14 12-16	7	8
Brown trout	4.6	12	7.6	257	314	138 108-175	17	81 64-102	36	65
Rainbow trout	sac fry	12	7.6	257	314	440 366-527	17	49 33-72		
Rainbow trout	2.5	10	7.4	237	272	100 64-156	23	11 10-12		1.2

MONS 203688

MAY 2 8 1974

STATEMENT OF ALFRED J. WILSON, JR.

My name is Alfred J. Wilson, Jr. My address is 3221 Rothschild Drive, Pensacola, Florida 32503. I am a research chemist at the Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida. I am in charge of the Analytical Services Program at this facility. I received my Bachelor of Science Degree from the University of New Hampshire in 1950.

I have been analyzing and/or supervising the analysis of pesticide residues in the marine environment since 1962. During that time, the Gulf Breeze Laboratory has analyzed over twenty thousand samples for the presence of pesticides and related organics in various substrates of the marine environment such as water, sediment, oysters, crabs, fish, birds, and mammals. I have utilized the methods which were current with the state of the art for the detection of these compounds. These techniques include paper and thin-layer chromatography, gas-liquid chromatography and mass spectrometry.

My statement will describe the analytical procedures used by the Gulf Breeze Environmental Research Laboratory for the detection of polychlorinated biphenyl compounds (PCB's) in the marine environment. In addition the statement will be supplement to, and verification of, data given in the following reports which have been submitted as exhibits. Appendix A

"A Polychlorinated Biphenyl (Aroclor 1254) in the Water, Sediment and Biota of Escambia Bay, Florida." T. W. Duke,

J. I. Lowe, and A. J. Wilson, Jr. Bulletin of Environmental Contamination and Toxicology 5(2):171-180. 1970

" Polychlorinated Biphenyls Absorbed from Sediments by Fiddler Crabs and Pink Shrimp." D. R. Nimmo, P. D. Wilson, R. R. Blackman, and A. J. Wilson, Jr. Nature, Vol. 231, pp 50-52, May 7, 1971

"Chronic Toxicity, Uptake, and Retention of Aroclor 1254 in Two Estuarine Fishes." D. J. Hansen, P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson. Bulletin of Environmental Contamination and Toxicology (2):113-119, 1971.

"Toxicity and Distribution of Aroclor 1254 in the Pink Shrimp Penaeus Duroarum. D. R. Nimmo, R. R. Blackman, A. J. Wilson, Jr. and J. Forester. Marine Biology 2(3): 191-197. 1971

"Effects of Aroclor 1254, a PCB, on Oysters, Crassostrea Virginica (Bivalvia: Protobranchia: Ostreidae). P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and J. M. Patrick, Jr. The Association of Southeastern Biologists Bulletin (Abstract) Vol. 19, No. 2 p. 90, 1972.

The polychlorinated biphenyl compounds (PCB's) are industrially prepared by chlorination of biphenyl. Several fractions of different degrees of chlorination are manufactured by the Monsanto Company under the trademarks Aroclor 1221, 1232, 1248, 1254, 1260, 1262 and 1268. The last two digits designate the percent chlorine content of each

each formulation. The Aroclors are relatively inert, chemically resistant compounds which are practically insoluble in water but appear to be soluble in lipoid material. PCB's have been widely used for both consumer goods and industrial applications for more than 4 decades.

The methods used for the detection of PCB's in the marine environment are similar to those techniques used for the analysis of pesticides. The analytical procedures for the detection of PCB's vary with the type of substrate (water, sediment or biological material) and to some extent with the size of the sample. The usual technique, however, consists of extracting the substrate with a suitable organic solvent, purification (clean-up) of the resulting extract to remove lipoid and other interfering compounds, and finally, the identification and measuring of the compound.

PCB's are usually identified and quantified initially through gas liquid chromatography (GLC). A gas chromatograph equipped with an electron capture detector is used to identify and quantify chlorinated hydrocarbons in a cleaned-up extract. Sample components enter a detector, which in turn effects a response on a recorder; this results in peaks on a gas chromatogram for each component which is isolated. Pure materials of expected components of a sample have been previously injected into the chromatograph for a determination of the retention time, i.e., the time required for elution in the gas chromatograph. That retention time is than compared with those of the unknown components so as to identify, or qualify, them. Confirmation of results is sometimes undertaken by

using a different component (column) on the gas chromatograph to produce a different identifying retention time.

Quantiation is accomplished by measuring either the peak area or peak height and comparing it to previously determined known amounts of standards or reference materials. Peak area or peak height reflects the concentration of the component which elutes at a given time.

In addition to gas liquid chromatography, thin layer chromatography (TLC) and mass spectrometry are sometimes used to check GLC results. In thin layer chromatography, PCB's interact with an organic solvent to move up a glass sheet, with the amount of movement identifying the particular compound. In mass spectrometry, the compound is broken into its components and thereafter identified on an oscillograph or through computer analysis.

The identification and measurement of PCB's are complicated for the following reasons:

1. If PCB's are present in samples along with the commonly occuring organochlorine pesticides, i.e., DDE, DDD, and DDT, a separation of the PCB's from the pesticides must be performed before a reliable analysis can be done.
2. Each of the Aroclor series contain several compounds depending on the degree of chlorination and the position of the chlorine molecule on the biphenyl ring. Consequently, each of these compounds (isomers) elute from the gas chromatograph with different retention time producing a multiple peak chromatogram for each Aroclor. In addition

these isomers may be present in more than one Aroclor. For example, some of the isomers present in Aroclor 1254 are also present in Aroclors 1248, 1260 and 1262. Since environmental residues of PCB's are subject to varying degrees of alterations due to solubility, metabolism and other environmental factors, each Aroclor may not matintain its "integrity" in the field over long periods of time. Due to this multiplicity of peaks and isomers common to more than one Aroclor, it is difficult in some field samples to specifically identify what Aroclor is present and to accurately quantitate the amount present.

In laboratory PCB toxicity and bioaccumulation studies at the Gulf Breeze Laboratory these problems have not been encountered for the following reasons:

1. Test animals are analyzed prior to exposure to assure they are uncontaminated with organochlorine compounds. During exposure, water samples are analyzed to evaluate the validity of the exposure level and to assess water quality.
2. Since the identity of the Aroclor being evaluated is known and the exposure period is relatively short, all of the Aroclors tested to date can be identified and measured with reasonable accuracy. In some tissues we have observed differences in the proportion of Aroclor peaks with respect to a standard.

In samples where we have detected the presence of PCB's and interfering organochlorine pesticides we have separated these compounds by column chromatography with silicic acid as described by Armour-Burke (1). Currently we are quantitating the Aroclors by comparing the total

peak height of all discrete peaks with that of a standard. In addition, laboratory samples are fortified with an internal standard just prior to analyses. Samples are fortified with mirex, a compound which behaves analytically similar to PCB's. That is, it elutes from a Florisil column in the same fraction as PCB's and has similar, although not identical, recovery rates. This technique is valuable in assessing the validity of our results and is commonly practiced in analytical quality control. For example, if you only retrieve 50% of your fortification compound when normally over 90% is expected, your results for the compound of interest is also low. This warrants a new sample or discarding the results. The analysis of control tissue (from animals not exposed to PCB's) offers an additional check on possible in-house laboratory contamination.

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Alfred J. Hilson Jr.

MONS 203694

Nov 28 1974

Statement of Gilman D. Veith

My name is Gilman D. Veith. I am a research chemist at the Environmental Protection Agency National Water Quality Laboratory in Duluth, Minnesota. My duties involve the development of analytical methodology for isolating and identifying persistent organic chemicals in natural waters and aquatic organisms, and research concerning the bioconcentration of organic chemicals in aquatic organisms. I have been employed by the National Water Quality Laboratory since 1972.

I received a B.S. cum laude in chemistry from Augustana College in Sioux Falls, South Dakota, in 1966 and spent the following academic year in graduate studies in chemistry at Cornell University as a Woodrow Wilson Fellow. I completed the M. S. requirements in Water Chemistry at the University of Wisconsin at Madison in 1968 and received a Ph.D. in Water Chemistry from the University of Wisconsin - Madison in 1970.

From 1970 to 1972, I was an Assistant Professor in Water Chemistry at the University of Wisconsin - Madison. My teaching duties included graduate courses in water chemistry, water analysis and advanced techniques in water analysis. The research I have been engaged in during the past six years has centered largely around the occurrence of chlorobiphenyls (PCBs) in the environment. In particular, I have completed studies of the sources of PCBs in selected Wisconsin rivers in the Lake Michigan watershed and the identification and measurement of PCBs in fish from Lake Michigan and Lake Superior.

My testimony will be taken from completed and ongoing research, as well as pertinent results of related studies in the literature. My testimony will

discuss the analytical limitations associated with PCB measurements and show that PCBs are bioconcentrated in fish by factors greater than 10^5, that the residues resulting by bioconcentration from less than 0.01 ug/l in water have decreased the value of the food resources in Lake Michigan, and that recent evidence confirms the presence of polychlorinated dibenzofurans in PCBs mixtures manufactured in the United States.

ANALYSIS OF CHLOROBIPHENYLS

The identification and measurement of PCBs in environmental samples is inherently difficult because multi-component mixtures are involved. The PCBs include mixtures of hydrophobic chemicals which vary considerably in chlorine content, chemical reactivity, bioconcentration potential, and biological activity. The analytical problems associated with developing methodology for the measurement of complex mixtures are not unique to PCBs but are encountered in numerous other analyses such as chlordane and toxaphene. The need to measure PCBs in large numbers of fish from a bioassay or the Great Lakes where biological variation is subtracted dictates that the methodology permit the necessary numbers of samples to be analyzed, and the interpretation of the residue data is based on more detailed analyses of a small number of representative samples.

A. Isolation of PCBs

The PCBs are generally extracted from water, tissue, or sediment with organic solvents such as hexane, acetonitrile, and acetone. Although the PCB mixtures contain chlorobiphenyls with varying water solubilities (Exhibit 1 summarizes estimates of vapor pressures and solubilities for PCBs and indicates the solubility is a function of composition and varies

by more than 100, the extraction of PCBs in water with immiscible solvents can be designed to assure the extraction is essentially quantitative.

The extraction of large quantities of water (i.e. more than 10 liters) to measure small concentrations of PCBs may require the use of large quantities of solvents which increase the possibility of sample contamination unless high purity reagents are used. To minimize reagent blanks, reverse liquid-liquid partitioning using supports coated with hydrocarbons (Exhibit 2) and adsorption on polymeric foams (Exhibit 3) have been used to extract up to 200 liters of water.

The isolation of the PCBs from the bulk of the co-extractable materials has been based largely on group separations using adsorption differences on florisil, partitioning differences with acetonitrile water systems, and chemical stability to oxidizing acids (Exhibit 4). Although the PCBs are separable from the co-extracted lipids and related materials, the PCBs are more difficult to isolate from interfering chlorinated pesticides, and additional isolation steps are necessary. A frequently encountered interference in PCB measurements is the presence of DDT and its analogs in the sample. One of the more commonly used methods for isolating the PCBs from DDT is silicic acid column chromatography (Exhibit 5) in which the PCBs are eluted with a non-polar solvent and DDT is eluted with a more polar solvent. However, the adsorption of the many PCBs and DDT analogs may not be restricted to two distinct groups, and incomplete isolation is observed. Exhibit 6 discusses some of the commonly observed problems in using this group separation technique and specifically points out that DDE is often found in the PCB fraction whereas some lesser chlorinated PCBs are eluted in the polar solvent. Thus, the isolation procedure may

alter the composition of the PCB residue, and detailed studies of the separation must be included in the discussion of results.

In addition to the possibility that the PCB residue may be altered, other chemicals or mixtures of chemicals may not be separated from PCBs under conventional methodology. Exhibit 7 illustrates that polychlorinated naphthalenes behave similar to the PCBs on silicic acid. Although analyses using gas chromatography/mass spectrometry (GC/MS) may provide the best estimate of the significance of interferences, other chromatographic techniques such as reverse-phase thin-layer chromatography have been used to complement GLC analyses when GC/MS facilities are not available (Exhibit 8).

B. Identification and Measurement of PCBs

Gas chromatography has served as the basis for PCB measurements due to the capability of separating mixtures and providing quantitative and some qualitative information about the mixtures (Exhibit 9). When the gas chromatograph is interfaced with a mass spectrometer, the mixtures can be resolved and identified with respect to chlorine content and carbon chelation skeleton.

The extent to which the PCB mixtures are resolved in GC/MS analyses is controlled by the GLC conditions. For example, Exhibit 10 illustrates that the PCB mixture, Aroclor 1254, containing 54 percent chlorine can be resolved into at least 69 components. Structures for the components have been proposed; however, since these analyses are prohibited on a routine basis due, in part, to the time required, the chromatograms of the PCB found in residues are often related to those of commercially available PCB mixtures under lower resolution GLC conditions. Under these conditions, the mixtures are resolved

into a series of multi-component peaks which provide characteristic chromatograms for most of the commercial mixtures. The composition of these components have been studied (Exhibit 11) and approximately 75 percent of the PCBs in Aroclor 1254 is represented by the six observed peaks having retention times of 70, 84, 98, 104, 125, and 146 relative to DDE=100. The PCB peak at RRT = 70 is comprised of tetrachlorobiphenyls (25 percent) and pentachlorobiphenyls (75 percent). The identification of the components in a residue can be unambiguously identified as the respective PCB components by GC/MS using isotopic abundance ratios as outlined in Exhibit 12.

The quantitation of the PCBs requires numerous analytical considerations. The total PCB content is difficult to measure by integrating total area in the mixture chromatograms because of possible interferences and because the electron capture (EC) response of the EC-GLC analysis varies widely with degree of chlorination and substitution (Exhibits 13 and 14). Webb and McCall (Exhibit 11) have discussed the rationale for the selection of methodology for PCBs. When studying a bioassay system in which a single commercial PCB mixture is added or an effluent which contains principally one PCB mixture, reliable estimates can be made by selecting several interference-free component peaks and comparing sample chromatograms to standard curves prepared from the PCB mixture present.

The same technique has been applied to samples from major watersheds and the oceans where the PCB residues resemble one of the commercially available PCB mixutes. For example, Exhibit 15 summarizes the concentration of PCB mixtures resembling Aroclor 1254 in Lake Michigan fish. It was observed that DDT and DDD interferences could be removed from the PCBs using the silicic acid technique; however, DDE could not be reproducibly removed.

Therefore, the quantitation of the major components of PCBs were estimated by summing the peak heights of the peaks at 70, 84, 125, 146 and 174 while omitting the doublet at 98 and 104 (relative to DDE = 100) because of the DDE interference. These estimates give a conservative estimate of total PCBs unless attempts to account for other possible mixtures of PCBs present are made.

For samples which contain mixtures of various PCB mixtures, only estimates of the various mixture fractions of the total PCBs present may be possible. The approach to these analytical problems is to use standard curves prepared from mixed PCB mixtures. Exhibit 11 demonstrates the feasibility of dividing complex chromatograms into three major regions corresponding to three PCB mixtures as standards--using selected peaks as the basis of comparison. Exhibits 16 and 17 discuss other methods of using mixed standards and mixtures of isolated components. The majority of monitoring studies reported to date appear to prefer the use of commercially available PCB mixtures as standards due, in part, to the fact that much of the studies of biological activities are conducted with the commercial mixtures rather than pure isomers.

The perchlorination of all PCB components to decachlorobiphenyl has been used successfully to estimate total PCB concentrations as well as confirm the presence of PCBs. The technique (Exhibits 18 and 19 using $SbCl_5$ or SCl_3AlCl_4 to convert all components of the mixutres to one component greatly enhances the analytical detection limit and, if the average percentage of chlorine of the original mixture can be estimated, the amount of decachlorobiphenyl can be used to calculate the original PCB concentration.

Despite all of the possible errors in the extraction of PCBs and the lack of standard techniques for quantitation, Holden (Exhibit 20) has reported that collaboratove studies among 17 laboratories indicated the coefficient of variation for six pesticides and PCBs requiring full processing ranged from ± 10 to ± 18 percent.

BIOCONCENTRATION OF CHLOROBIPHENYLS

An understanding of the bioconcentration of chemicals in organisms is essential to understand the dynamics of the chemicals in the environment. Considerable confusion exists regarding the relative significance of various uptake mechanisms due, in part, to the absence of detailed definition of the systems studied. Much of the early literature assumed that bioconcentration in the aquatic environment was based on the trophic level or food chain concepts.

However, exposures of fish to water containing many hydrophobic chemicals demonstrated that fish can bioconcentrate the chemicals directly from the water. Uptake can be approximated as a first order rate process and that the process appeared to be that of chemical partitioning from the water and the lipids in the tissue. This has led to proposals such as that in Exhibit 21 where the food chain bioconcentration model is rejected in favor of the chemical partitioning model. As with many kinetic models of dynamic systems, multiple pathways are often observed and the relative significance of the various rate expressions is dependent on the conditions imposed. Therefore, the selection of either the food chain or the partitioning model for the aquatic environment requires that the conditions of the ecosystem being considered are defined.

The bioconcentration of PCBs under laboratory conditions in which the mixtures are added only to the water has been studied for a number of aquatic organisms and the observed bioconcentration factor for PCBs in the aquatic environment may differ in different organisms and conditions. For example, Exhibit 21 illustrated that bioconcentration is inversely related to the water solubility (assuming relatively constant lipid solubility). Exhibits 21 and 22 demonstrate that not only does bioconcentration increase with increasing chlorine content, but also that the lipid variations between male and female fish may produce substantial sex-related differences if only the wet weight concentrations of PCBs are considered.

Exhibits 22 and 23 show that the bioconcentration factor of Aroclor 1254 in fathead minnows ranged from 1.1×10^5 to 2.4×10^5 and that the bioconcentration factor for the PCBs to the fathead minnow (where the tissue is generally less than 10 percent lipid) at 25° C ranges from about 0.3×10^5 to 2.5×10^5 with the more highly chlorinated PCBs accumulating to a greater extent. Exhibit 24 shows that Aroclor 1254 is accumulated in pinfish approximately 2×10^4 times that in the water after 35 days of exposure at a salinity of 14 - 34%. Exhibit 25 suggests that increasing salinity decreases the rate of uptake or lipid-soluble chemicals.

Exhibit 26 shows that the nymph E. danica accumulated Clophen A 50 by an approximate first order process to bioconcentration factors of 3×10^3. Exhibit 27 presents evidence that the bioconcentration factor of Aroclor 1254 in the scud, Gammarus pseudolimnaeus, (was approximately 2.2×10^4), 4.5×10^4 after 48 hours in Daphnia magna, and 1.3×10^4 in mosquito larvae, Culex tarsalis after only 24 hours. After 60 days of exposure to Aroclor 1242 and Aroclor 1248, the maximum bioconcentration factor in Gammarus was found to

be approximately 3×10^4 and 5×10^4 respectively (Exhibit 28). Exhibit 27 also reports bioconcentration factors ranging from 2×10^4 to 6×10^4 for bluegills and catfish exposed to Aroclor 1248 and 1254.

Some evidence exists that the bioconcentration factors measured in the laboratory are essentially the same as that found in the river environment. For example, Exhibit 22 shows that the laboratory derived bioconcentration factor for Aroclor 1248 in fathead minnows (~10 percent lipid) ranged from 1×10^5 to 2.4×10^5. Exhibit 29 and 30 report that goldfish in the lower Milwaukee River accumulated Aroclor 1248 approximately 0.7×10^5 to 2×10^5 times (depending on the lipid content) the estimated PCB concentration in the river water. Exhibit 31 presents evidence that the maximum concentration of Aroclor 1254 in Saginaw River shed was 165 ug/gm whereas typical water concentrations averaged 1.1 ug/l to a maximum of 2.9 ug/l. This is equivalent to a bioconcentration factor of 0.6×10^5 to 1.5×10^5, in good agreement with other measurements.

In summary, for river systems and in laboratory studies many of the aquatic organisms studied appear to bioconcentrate PCB mixtures containing 3, 4, 5, and 6 chlorine atoms per molecule approximately 3×10^3 to 2×10^5 times the concentration in the water they are exposed to.

Occurrence of PCBs in Lake Michigan

The concentration of PCBs in Lake Michigan fish has been studied near Milwaukee in 1969 (Exhibit 9) and throughout the lake in 1971 (Exhibit 5). The data from 1969 indicated that trout and salmon caught near the Wisconsin shore contained from 6 to 25 ug/gm PCBs resembling Aroclor 1254. The more detailed study presented in Exhibit 15 shows that the PCBs resembling Aroclor 1254 were found at mean concentrations ranging from 2.7 ug/gm in smelt to 15.5 ug/gm in lake trout. The predominant PCB components present in the fish were the tri-, tetra-, penta-, hexa-, and heptachlorobiphenyls by GC/MS studies of com-

posite samples.

Exhibit 30 reported concentrations of PCBs similar to Aroclor 1242 in the Milwaukee River, a major tributary to Lake Michigan, up to 2.8 ug/l. Exhibit 32 shows that Aroclor 1242 was found in Illinois tributaries at 0.14 to 1.8 ug/l in 1971 and from 0.01 ug/l to 0.7 ug/l in 1972. Aroclor 1254 was found in the same tributaries at concentrations up to 0.8 ug/l. In an extensive study of PCBs in municipal wastewater effluents in Michigan, the overall mean PCB concentration is all eight-hour composite samples collected was 2.6 ug/l. However, only one of the 50 effluents averaged over 10 ug/l and 44 of the 50 effluents averaged less than 1 ug/l PCB. Exhibit 33 reports similar results for eleven municipal effluents in southeastern Wisconsin in that only one of the eleven effluents contained an average PCB concentration greater than 1 ug/l.

Exhibit 31 provides evidence that the concentrations of PCBs in the intake water of municipalities along the Michigan shore are, with one exception, all below 10 ng/l (ppt). This indicates the minimum bioconcentration factor for smelt in the lake (mean concentration of 2.7 ug/gm) is approximately 3×10^5 and that the minimum bioconcentration factor for the large trout is approximately 15×10^5.

The PCB residues present in the Lake Michigan biota have adversely affected the value of the biological resources of the lake as a food supply for animal feeds. Exhibit 34 is an early indication that Great Lakes fish were suspected of interfering with normal reproduction of mink feeding on fish. Exhibit 35 showed that the effect on mink could not be correlated to the degree of oxidative rancidity or mercury concentration in the fish. Exhibit 36 presents evidence that 5 ug/gm of Aroclor 1254 in the coho salmon diet of mink significantly reduced reproduction and that 1 ug/gm of Aroclor 1254 in the diet caused a slight decrease in reproduction. Exhibit 37 presents similar evidence for mink feed diets enriched with Aroclor 1254. The data indicate that 0.64 ug/gm of the

PCB in the rations resulted in only one of 12 mink producing three kits, all of which dies on the first day. Exhibit 15 has shown that the mean concentration of PCBs in coho salmon in Lake Michigan was 11.5 ug/gm in 1971, well above the FDA tolerance level.

Occurrence of PCBs in Lake Superior

Exhibit 38 presents data concerning the mean concentrations of PCBs measured in Lake Superior fish captured in 1972 and 1973 from western Lake Superior. The data indicate that mean PCB concentrations in the smaller, low-lipid content fish contain up to approximately 1.5 ug/gm (on a wet tissue basis) whereas the larter trout contained up to 5.6 ug/gm. Exhibit 39 presents corroborating data for the PCB concentrations in Lake Superior trout obtained by the Michigan Department of Agriculture.

There have been some estimates of PCBs in western Lake Superior water. Exhibit 40 presents estimates of PCBs in the water intake and a bioassay control tank of the National Water Quality Laboratory in Duluth. The estimates were made using the foam plug extract to extract 20 liters of water in triplicate, perchlorinating the extracts with $SbCl_5$, and measuring the decachlorobiphenyl formed. The data indicate that approximately 1-2 ng/l PCBs (ppt as Aroclor 1254) are present in western Lake Superior nearshore water.

The mean concentration of PCBs in the smaller fish is approximately 0.9 ug/gm. This indicates the overall bioconcentration factor in western Lake Superior fish is approximately 9×10^5.

In summary, the data from the Great Lakes demonstrate that PCBs are present in the waters at concentrations below 10 ng/l (ppt), that the concentration of PCBs in the fish are more than 10^5 times that measured in the water, and that the PCB residues which bioconcentrate in fish from water concentrations below 10 ng/l have affected the use of the Lake Michigan resources as a food resource.

Chlorodibenzofurans in Aroclor[R] PCB Mixtures

Exhibit 41 presents confirmations tetra-, penta- and hexachlorodibenzofurans in the Aroclor[R] brand of PCBs prepared in the United States. The chlorinated dibenzofuran concentrations decrease with increasing chlorine content of the PCB mixture. Aroclor 1254 contained eleven chlorodibenzofurans comprised of two tetra, four penta and five hexachlorodibenzofurans.

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PCB Conference II
Stockholm 1972

PROBLEMS IN THE QUANTITATION OF PCB IN BIOLOGICAL MATERIAL

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Special Analytical Laboratory

Seldom have analysts faced greater problems than those encountered in the quantitation of PCB!

We have therefore found it important to draw especially our biologists attention to the problems attached to the results obtained from a PCB analysis. It is maybe necessary to pause for a moment and discuss the complexity of the number of substances involved which are to be identified and quantitated with the help of a gas chromatograph.

As well known PCB consists of a large number of homologues and isomers of chlorinated biphenyls. The number of chlorine atoms per molecule usually varies from 4 to 6. A technical product like Clophen A 50 contains 50% chlorine and the corresponding gas chromatogram, shown in Fig. 1a, results in 11 peaks. However, we do know that the number of isomers is much larger. It has now been shown, through the use of capillary columns, that the number in common PCB formulations is at least 69.[1] The normally used gas chromatographic columns are not only good enough because they can not separate the PCB s, but also because DDT, and its metabolites DDE and DDD, normally present in biological material, are not separated completely from the PCB s. If the presence of the o,p isomers is also considered, there are another 6 substances, whose peaks appear within the same retention times as those of the ten main components of PCB.

Fig. 1 shows (a) a chromatogram of PCB (Clophen A 50), (b) DDT and its metabolites and (c) a mixture of PCB and DDT with its metabolites, the last illustrating a most complicated chromatogram.

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Fig. 2 shows two chromatograms of biological samples. PCB components in the herring sample are difficult to quantitate due to the high content of DDT and its metabolites, while the contrary is true for the plankton sample.

The problems we meet during the analysis of PCB in biological material fall within two groups. Firstly, the samples contain besides PCB other chlorinated substances of different origin. Secondly, the complexity of the PCB chromatogram can be responsible for uncertain results even in those cases where a pure PCB extract is obtained.

An analysis of PCB consists of two stages 1) working up of the sample for injection into the gas chromatograph. 2) Quantitation of the PCB chromatogram.

The work before a sample is ready for injection into a gas chromatograph can be divided into four steps: extraction of the homogenized sample, determination of the fat contents, clean-up, and separation. The last step - separation - can in certain cases be omitted, since the gas chromatograph besides quantitation also carries out a separation.

Extraction of the lipophilic substances are performed by different methods which may vary from laboratory to laboratory. Two risks are involved: a) the extraction is not sufficiently effective. A conventional recovery experiment is not satisfactory as it is built on a post mortem addition of a standard. The extraction is then assumed to be as effective as that of pure biological samples. This is of course not true. It is easier to extract the former than the latter. Only if radioactive marked test substances have been added to the animal alive, a relevant recovery experiment can be carried out.

The next problem is: b) the fat weight determination. When the amounts of PCB have been estimated, they have to be related to the weight of the sample. Normally this is done on a fresh tissue basis, but from an ecological point of view, a calculation based on the fat weight is valuable.

The risk is that different fat determination methods give different results. This implies that results can vary from laboratory to laboratory, since a factor, in the fat weight determination, will result in an error of the same

where the extraction is performed using a mixture of chloroform and methanol. The solvents normally used for extraction have earlier mainly been ether-hexane mixtures. They have tended to give lower fat weight values than the above mentioned Torrey method.

Nowadays a dehydrating solvent, for example, acetone or acetonitrile is also added, which serves to open the cells for extraction.

Theoretically, the fat determination can be carried out as an independent analysis. However, it is common to use a gravimetric method, that is to say, the solvent is evaporated from the extract and the rest, which is defined as fat, is then weighted.

When small amounts of fat are to be determined, the errors in a gravimetr method become so large, that a more advanced method has to be used (e.g. a spectrophotometric method).

The clean-up methods can be divided into two groups, partly methods, which result in a separation at the same time - chromatographic methods - and partly those where the fat is removed by a single chemical treatment.

The concepts "clean-up" and "separation" sometimes become synonymous because the same step is often responsible for both clean-up and separation. When for example a fat extract is to be purified on a silica gel column, this constitutes a clean-up, that is, the removal of substances that are not to be studied, but at the same time a separation of substances occurs (like PCB from DDT), each of which are to be evaluated.

Two types of chemical treatment for the removal of fat are employed at our laboratory, the one of alkaline hydrolysis, the other by treating the sample with sulphuric acid[2]. In the alkali treatment a conversion of DDT to DDE and DDD to DDMU takes place, besides hydrolysis of the fat. This type of clean-up is of course not suitable when the sample is to be quantitated both with respect to DDT and its metabolites as well as PCB

Shaking the extract with concentrated sulphuric acid (containing 7% sulphur trioxide) has proved to be a quick and simple method. As already mentioned that certain pesticides (dieldrin among others) are destroyed by this treatment, consequently this method can not be used for the analysis of PCB

and such pesticides. Neither DDT nor its metabolites are effected by the sulphuric acid treatment, and since these pesticides are of major importance in samples from Sweden, we normally use this method because of its quickness and simplicity.

The non destructive methods used for clean-up (and separation) consist of chromatographic methods, usually column chromatography or thin layer chromatography. The different principles which have come to use are adsorption chromatography, partition chromatography and gel permeation chromatography.

A comparison between the technical procedures in adsorption and partition chromatography suggests that thin layer chromatography is in most cases more suitable than column chromatography. Better separation, quicker techniques, simpler procedures are some of the advantages of thin layer chromatography.

The solid phase in adsorption chromatography usually consists of silica gel, aluminium oxide, celite, Florisil, etc. or mixtures of these. It is important here to achieve good reproducibility. In adsorption chromatography the substances are separated in order of their polarity. Using a column, this means that non-polar substances are eluted first (like PCB and DDE). This is normally done using hexane, heptane, etc. Changing to a slightly more polar solvent results in the elution of the more polar pesticides (like DDT) while the fat remains on the column. Using thin layer chromatography this implies a high R_f-value for PCB and DDE, while low values for the fat components.

One disadvantage with adsorption chromatography is that the reproducibility and separation is affected by varying fat contents. To obtain the best possible separation it may therefore be necessary to remove the fat components by another method e.g. partition between a non-polar and a polar solvent (usually hexane-acetonitrile).

Partition chromatography is less usual. Paraffin or a silicon polymer is normally used as the stationary phase. Another method involves acetonitrile-impregnated silica gel and hexane or the elution solvent. The fat is here first eluted, and then the chlorinated hydrocarbons.

Gel permeation is a new method for clean-up based on the separation of molecules according to their size.[3] Here the larger fat molecules are eluted first and then the lower molecular compounds (PCB, DDT, etc.). An advantage with this method is that the column can be re-used, with high reproducibility. High fat contents create no problems.

A specific problem is the separation of PCB from DDT and its metabolites. If we look at Fig. 1c, 2a and 2b, we observe that DDT, DDD and DDE interfere with certain PCB peaks. PCB can be separated from DDT and DDD, through adsorption chromatography.

To separate DDE from PCB by purely chromatographic methods seems on the other hand very complicated. Different methods have been suggested in litterature, but we have not found them suitable for use in routine analysis. It has been reported that PCB can be separated from DDE on highly activated silica gel.[4] We have not succeeded, after repeated trials at our laboratory, to separate DDE from all PCB substances according to this method. In the DDE fraction some PCBs are still found. Unfortunately, in the gas-chromatographic analysis DDE will interfere with these PCBs.

However, methods exist whereby PCB can be obtained free from DDE. Treatment with potassium dichromate, dissolved in sulphuric acid, has been reported to remove DDE, without altering the PCBs.[5] Another oxidation method involves the use of chromic trioxide in acetic acid. This will convert DDE to dichloro-benzophenone, which can be separated from PCB by adsorption chromatography.[6]

After the extract is properly cleaned-up and interfering compounds have been removed, the next problem arises:

How is the extract, containing only PCB, to be quantitated, using a gas-chromatograph with an electron capture detector?

The widely different methods, used for the quantitation of PCB, illustrate well the complex nature of the problem of calculating the PCB content in biological samples. There are several reasons as to why there does not exist an universally accepted method. The main problem is that no standard formulation, can be directly used to estimate PCB in biological material. It has been shown that the PCBs, present in different types of biological samples, do not correspond in every detail to commercial formulations.

Another difficulty is that most gas chromatographic columns used do not yield complete resolution of the PCB components. Different columns also result in different chromatograms.

In view of these complications, analysts use different quantitation methods. A few of the practiced methods are:

1) Quantitation of the individual components from the PCB core pattern

In this method a standard formulation is used where each component of the standard, separated on a gas chromatograph, is quantitated [illegible] individually.

A commercial formulation that most resembles the PCB pattern in biological material is choosen as the standard. This formulation is quantitated using a mass spectrometer and a microcoulometer. With a mass spectrometer coupled to the gas chromatographic system, the number of chlorine atoms for the components in each peak is determined. With the same gas chromatographic system attached to a microcoulometric detector the amount of chlorine in each peak is estimated. By combining the qualitative and quantitative information thus obtained, the concentration of each component in the standard is determined. Fig. 1a shows a Clophen A 50 solution injected into a column, comprising of 67 parts 8 % QF 1 and 33 parts 4 % SF 96, mechanically mixed. This chromatogram contains 14 peaks.

The peak height of each component in the standard is compared to the respective peak height in the sample and the amount of each component calculated. The amounts are then added to give the total PCB content of

The above method is used for routine analysis at our laboratory.[?] It is advantageous since individual components are estimated. It is surprising that hardly any publications give data on the individual components determined. Only total PCB contents are named. This seems accepted while on the other hand DDT and its metabolites are nearly always reported individually.

A disadvantage with this method is that quantitations are restricted to the same column as that on which the standard formulation has been determined. The use of other columns result in different chromatograms, comprising of more or less than 14 peaks.

Good gas chromatographic separation is demanded. This implies that support material and stationary phase must be handled well to give a high number of theoretical plates. The procedure needs the measurement of many peaks. This difficulty can be solved by reliable automatic integration, coupled to a data system, to speed up quantitation.

2) Quantitation on the assumption that the PCB amounts can be estimated by using the response of DDE as a reference. Here the total peak area of the sample is directly compared to that of p,p'-DDE standard. Another version consists of multiplying each individual peak height by the corresponding retention time and summing all the products so obtained. This sum is then divided by the product of the peak height and retention time for p,p'-DDE to give the amount of PCB.[7]

The electron capture response for chlorinated biphenyls with 4-9 atoms of chlorine per molecule (normally found in biological samples) is reported not to exceed the value 1.6 relative to p,p'-DDE.[8] The assumption of the similarity in response of each PCB component to that of p,p'-DDE is questionable. Although this method is said to give reliable values for the total PCB content, it must be erroneous when individual component amounts are desired.

3) Quantitation based on an average of certain peaks when similarity to a certain PCB formulation is assumed. In this quantitation the chromatogram of the sample is studied, and the best resemblence to a standard PCB formulation is examined. Once this standard is decided, a peak from the sample is compared to the corresponding peak in the standard, and thus the whole PCb content is estimated. If several peaks are considered an average is then calculated. This method is rapid and ideal when the PCB chromatogram of the sample is identical to that of the standard. It is, however, well known that the chromatograms of the biological samples seldom resemble those of the standard formulations used. This maybe explained by assuming that the samples contain a mixture of PCBs from different sources. The fact that degradation of certain components can occur and is suspected to be true for some of the chlorinated biphenyls gives this method less reliability. Selective accumulation of different chlorinated biphenyls constitutes also a source of error. The choice of representative peaks can be problematic.

4) Quantitation based on the single peak obtained after total chlorination of PCB. Recently, it has been reported[9] that samples analyzed for PCB have, after clean-up, been treated with antimony pentachloride. The PCB components are thus chlorinated to deca chloro-biphenyl. The chromatogram, consisting of a single peak, is then compared to a standard similarly treated.

This method calls for effective clean-up and separation prior to gas chromatography. It demands a reproducible chlorination procedure. The reported yields are stated to be around 85%. With this method, quantitation of the individual PCB components is not possible.

We have here dealt with different methods for the separation and quantitation of PCB. These can, of course, be combined to attain the highest possible certainty in the PCB determination. However, it is questionable whether the highest degree of certainty always is desirable. An analytical method involving many stages will restrict the number of analysis a laboratory can carry out. It may therefore be necessary to choose between analyzing a few samples with the highest degree of certainty, or to have a large number of analysis performed with a slightly less certainty. This will naturally depend on the aim of the analysis.

We know that biological accumulation can vary a great deal for animals under identical conditions. This variation must be even larger in nature where animals live under different conditions. It is therefore important, from a statistical point of view, that a sufficiently large number of analysis are performed. This may imply that the highest degree of certainty can not be maintained for each sample.

Lastly, we would like to stress the importance of publishing detailed information of the methods used for PCB determination. Astonishingly, very few reports describe fully the quantitation methods used. A direct comparison of PCB values in different reports is thus rendered difficult.

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Regarding the Figures

The gas chromatographic conditions were
Injector temp: 210°C
Column: 160 cm x 1,8 mm, filled with 2 parts 8 % SP 1 + 1 part 4 % SF 96 on silanized Chromosorb W 100/120 mesh.

Columntemp: 165°C
The detector (electron capture Ni 63) was held at 220°C.

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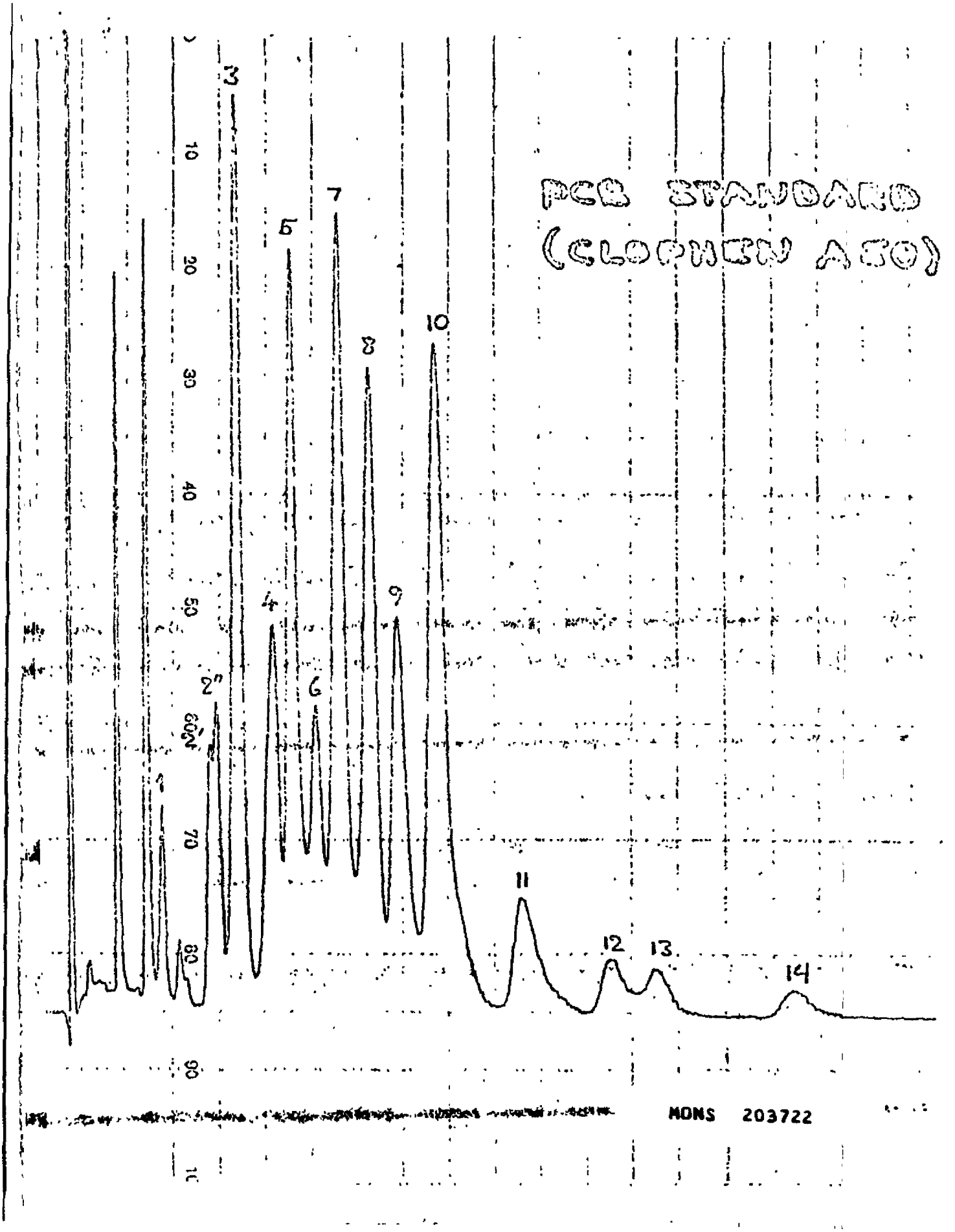
PCB STANDARD
(CLOPHEN A30)
1
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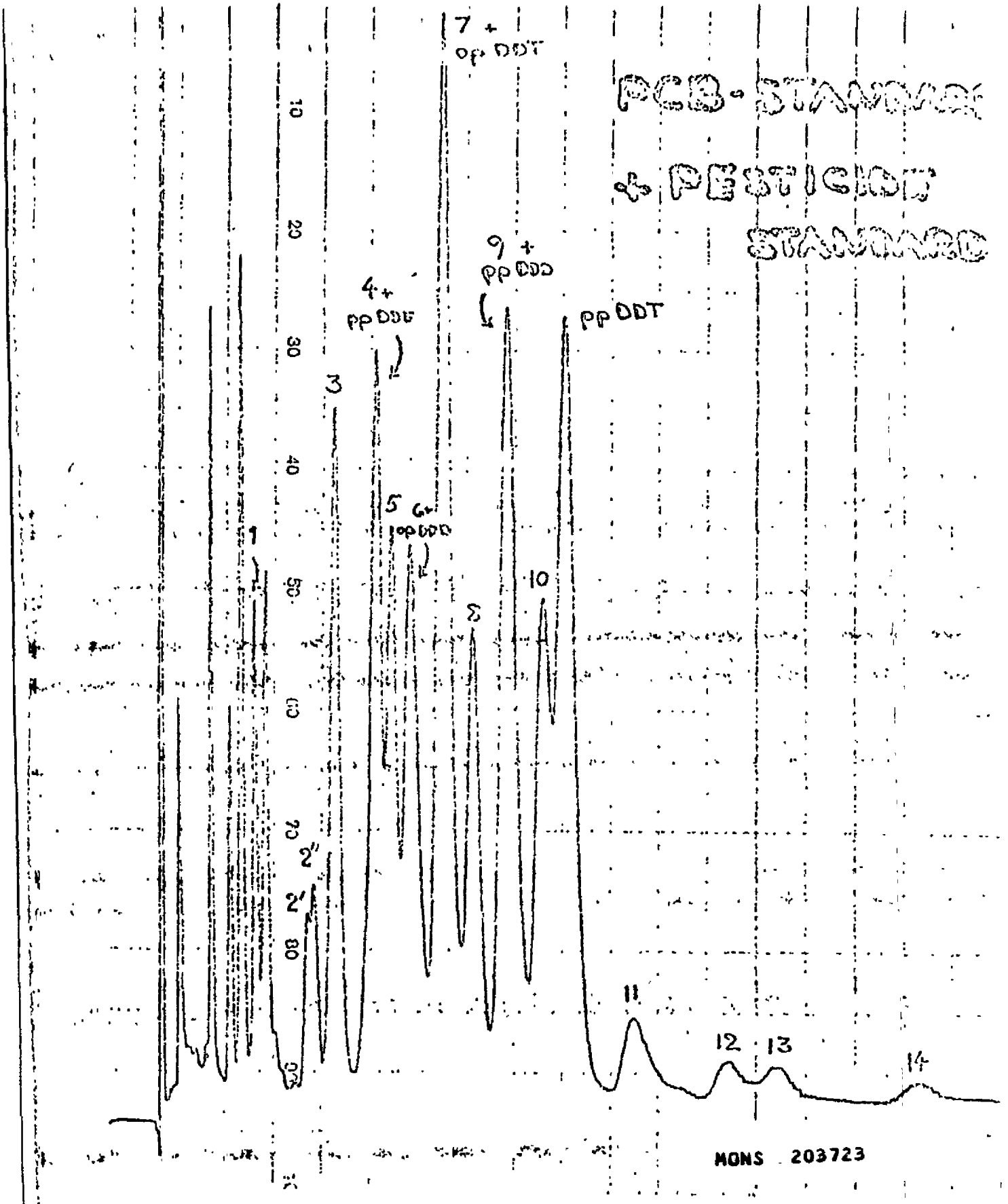
MONS 203722


PCB-STANDARD
& PESTICIDE
STANDARD
7 + op DDT
4 + pp DDT
9 + pp DDD
pp DDT
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11
12
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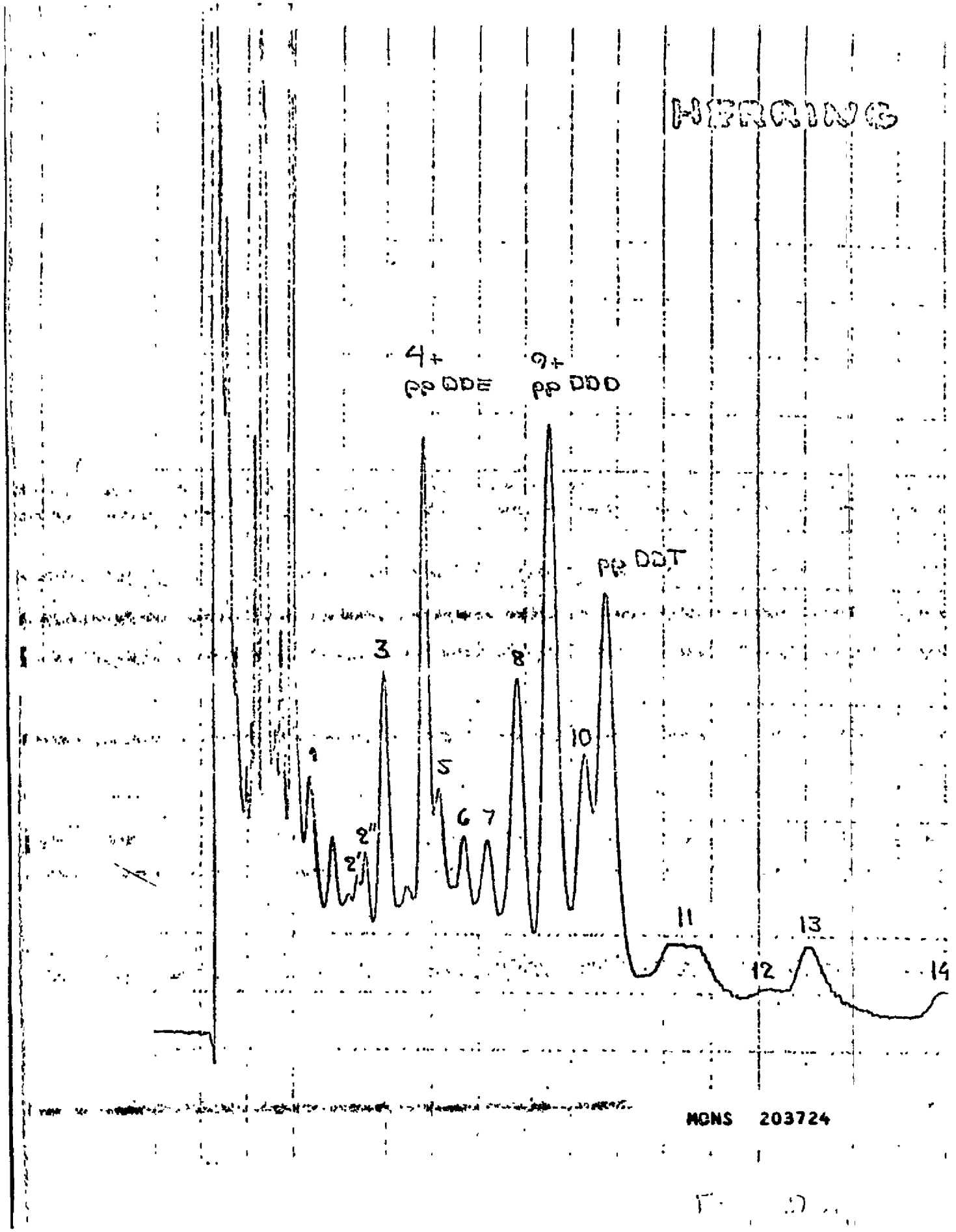
HERRING
4+
pp DDE
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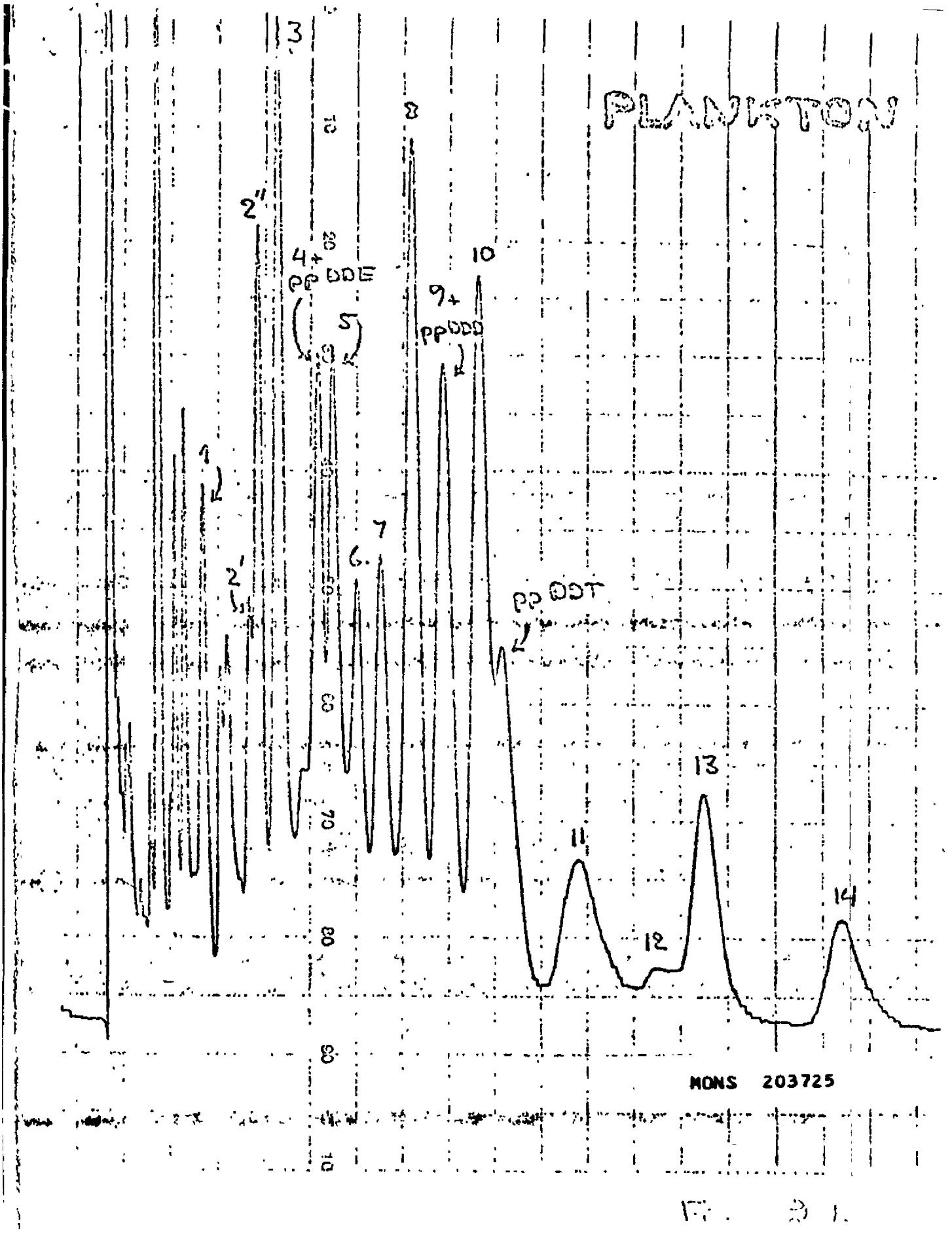
PLANKTON
1
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2'
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4
pp DDE
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pp DDT
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14
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Fig. 21.

MAY 28 1974

CHLORINATED HYDROCARBONS IN FISH FROM LAKE MICHIGAN

by

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ABSTRACT.

Lake Michigan fish representing 13 species from 11 regions were captured in the fall of 1971 and analyzed for chlorobiphenyls (PCBs) and DDT and analogs. The presence of the major chlorinated hydrocarbons was confirmed by GC/MS, and additional confirmations of PCBs were obtained through perchlorination. The major PCBs in Lake Michigan fish are the tetra-, penta-, hexa- and heptachlorobiphenyls although lesser chlorinated PCBs were present in fish from nearshore waters. Mean concentrations of PCBs in the fish expressed as Aroclor 1254 (wet weight) ranged from 2.7 μg/gm in smelt to 15 μg/gm in lake trout. Essentially all trout and salmon greater than 12 inches in length contained PCBs at concentrations greater than the FDA tolerance level of 5 μg/gm. The concentrations of DDT (sum of analogs) ranged from less than 1 μg/gm in suckers to approximately 16 μg/gm in large lake trout.

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SECTION I

CONCLUSIONS

1. The predominant chlorinated hydrocarbons in Lake Michigan fish on a weight basis are chlorobiphenyls (PCBs) and DDT and its analogs.

2. The PCB mixtures are comprised primarily of the tetra-, penta-, and hexachlorobiphenyls. although fish from the nearshore waters often contain appreciable quantities of di- and trichlorobiphenyls.

3. The data reported as total DDT is comprised of approximately 50 to 60 percent p,p'DDE, 20 to 30 percent p,p'DDT, and lesser amounts of o,p'DDT and pp'TDE.

4. Mean concentrations of PCBs in the 1971 populations of fish averaged over the entire lake range from 2.7 μg/gm in smelt to 15 μg/gm in salmon. In general, trout and salmon greater than 12 inches in length contain PCBs at concentrations greater than the FDA action level of 5 μg/gm in food for human consumption.

5. Large variations in the concentration of PCBs were observed and were dependent on species, lipid content, length, and season of sampling. All of these factors must be recognized in the design of future monitoring work on Lake Michigan fish.

6. The concentration of DDT ranged from less than 1 μg/gm in suckers to approximately 16 μg/gm in large lake trout. In general, large trout and salmon contained from 7 to 12 μg/gm DDT, also exceeding the FDA tolerance level of 5 μg/gm DDT in human food.

7. Although the mobility of fish precludes the possibility of explicitly determining the variation of chlorinated hydrocarbons with region of Lake Michigan, the trend of higher concentrations of the PCBs in the southern end of the lake was observed in most of the species analyzed. This trend was more pronounced in the analysis of bottom fauna as presented in Appendix B in which the concentrations of PCBs varied from 0.06 μg/gm in

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Pontoporeia captured in 100 feet of water near Sturgeon Bay, Wisconsin, to 0.45 ug/gm in those from Waukegan, Illinois. Decreasing concentrations of PCBs were also observed in fauna from greater depths along each transect.

8. The present methodology for the analysis of PCBs in tissue is adequate for the determination of PCBs containing four or more chlorine atoms per molecule. However, the silicic acid separation procedure does not allow explicit determination of PCBs with fewer numbers of chlorines due to their greater polarity.

9. The use of perchlorination as an analytical method for PCBs provides rapid confirmation and data similar to direct GLC analyses when the predominant PCB mixture is known.

SECTION II

RECOMMENDATIONS

1. Luecchoi (1972) recommended that the concentrations of PCBs in Lake Michigan fish should be determined. This study has satisfied this recommendation for the 1971 fish populations and has provided "baseline" data for future work aimed at detecting trends in the PCB and DDT concentrations. Because of the recent restrictions on PCB and DDT uses, the Lake Michigan system provides a unique opportunity to demonstrate the effect legislative and other regulatory actions may have on a large body of water. It is recommended that comprehensive sampling of selected fish species be conducted at two-year intervals and that these fish be analyzed for PCBs and DDT. Such studies should be modeled after the limited studies presently underway at the Great Lakes Fishery Laboratory in Ann Arbor, Michigan.

2. Detailed information is needed regarding the quantities of PCBs in use after the restrictions Monsanto Company has voluntarily imposed. It is recommended that the Environmental Protection Agency initiate appropriate investigations to assure that the "open system" use of PCBs is, in fact, curtailed and that significant quantities of PCBs are not being imported.

3. Further studies concerning the impact of PCBs and DDT on the biological resources of Lake Michigan are needed and should be stimulated by Federal funding.

4. Detailed searches for potentially hazardous chemicals heretofore unidentified should be conducted on Lake Michigan fish in order that the significance of such chemicals can be evaluated as early as possible and appropriate regulatory action be considered before the concentrations become sufficient to further jeopardize the usefulness of the Lake Michigan resources.

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SECTION III

INTRODUCTION

The literature is replete with data concerning the occurrence of chlorinated hydrocarbons in the environment and the effects of these synthetic chemicals on test animals. Concomitant with such studies are attempts to correlate the composition of organochlorine chemicals in organisms (or their environment) to observed effects in the biological communities and to formulate regulatory policies in accordance with existing evidence. However, the complex nature of ecosystems and of the mixtures of potentially hazardous chemicals found in the ecosystems often preclude explicit interpretation of data, as has been exhibited in the numerous hearings before the U.S. Senate and state agencies concerning DDT and related compounds. Thus, from the outset, large-scale field studies such as this effort which provide data on a few variables run the risk of producing data of limited value and adding to an already superfluous literature.

The purpose of this study is to identify and quantitate the major organochlorine compounds in the 1971 populations of fish from different regions of Lake Michigan. The study was recommended by the four states surrounding Lake Michigan in the Lake Michigan pesticide report which was requested by the Lake Michigan Interstate Pesticide Committee and prepared by L. A. Luecchow of the Wisconsin Department of Natural Resources. The forthcoming results are put in perspective by the following brief review of factors which produced the need for this study.

It has been established that Lake Michigan is unique in that it contains much higher concentrations of agricultural pesticides than the other Great Lakes due, in part, to the large acreage in the watershed in comparison to the flushing period for the lake and to the relatively low biomass density. Previous studies have shown that the fish from Lake Michigan approached the FDA action levels for dieldrin in 1969 and that a major fraction exceeded the action levels for DDT (Reinert, 1970). More recent data (Reinert, 1972) from carefully designed monitoring (Reinert, 1971)

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indicates that the average DDT concentration in Lake Michigan fish remained essentially the same between 1967 to 1970 and suggested that the levels have begun to decrease.

Although not realized in the early analyses by Reinert, other work (Veith, 1970) has shown that the concentration of PCBs (similar to Aroclor 1254) were greater than 15 µg/gm in large fish collected from Lake Michigan in 1969. Thus, it is apparently in Lake Michigan rather than the other Great Lakes that the potentially hazardous and persistant chemicals from our industrialized technology are most concentrated. It follows, then, that consideration must be given to the effect these chemicals have on the biological communities sustained by Lake Michigan and the possibility of stemming or even reversing the trend of increasing levels of organochlorine compounds in the Lake Michigan system.

Despite the comparatively high levels of DDT, dieldrin and PCBs in Lake Michigan, there is no direct evidence that the chemicals are endangering the aquatic life in the lake. The concentration of each of the chemicals appears to be below 10 ng/l in the pelagic waters and less than 100 ng/l in the nearshore waters, all of which are below the "no effect" level for the respective chemicals for most aquatic organisms. However, the lack of direct evidence of damage may simply reflect the difficulty in adequately measuring such effects in this environment.

There is considerable indirect evidence which suggests that the build-up of organochlorine compounds may be a threat to the future of the biological resources of the lake. It is beyond the scope of this introduction to review the literature concerning the pesticides and PCBs, and the reader is referred to existing comprehensive reviews (Zitko and Choi, 1971; NIH, 1972; Quinby, 1972; Task Force, 1972).

Consider first the major effort of the states surrounding Lake Michigan to stock the lake with brown, lake and rainbow trout and with coho and chinook salmon. Between the years of 1963 and 1970, over 600,000 rainbow trout have been released by Wisconsin alone (Kernen, 1970). In 1971, the State of Michigan stocked 3.2 million coho, 2.1 million chinook, 2.1 million lake trout and 200,000 rainbow trout. The first widespread spawning of lake trout in Lake Michigan since 1940 occurred in 1971 (GLFL, 1972). This

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massive stocking program has produced an unparalleled sport fishery and provides a valuable protein source for human food as well as animal feeds. Essential to the establishment and maintenance of this fishery is the successful reproduction of the adult fish. However, Reinert (1970) has previously pointed out that DDT and dieldrin levels in eggs are similar to those which inhibited reproduction in the studies of Burdick et al. (1964) and Hoek (1968). In addition, Jensen et al. (1970) have shown that 15 µg/gm of PCBs (lipid basis) in salmon eggs produced 50 percent mortality and complete mortality can be expected when the eggs contain 25 µg/gm PCBs (lipid basis). All of these data concerning the effects of organochlorine compounds on reproduction were obtained individually, and little is known of possible combined effects when the tissue contains mixtures of the toxicants.

Even though the continued stocking of fish may maintain a food resource in spite of possible adverse effects of the residues on reproduction in Lake Michigan fish, many fish contain body burdens which make the fish unfit as a food resource for humans. The U.S. Food and Drug Administration has established a tolerance level of 5 µg/gm, 5 µg/gm and 0.3 µg/gm for DDT, PCBs and dieldrin, respectively, in food to be sold for human consumption. Since the lake supports a sizable commercial fishery in addition to the sport fishery, the presence of these chemicals below the "no effect" level in water may still endanger these industries due to the large partition coefficient for lipid/water systems. Indeed, numerous catches of commercial fish being shipped to markets have been seized, and tons of large salmon have been withdrawn from state give-away programs in Michigan because the DDT and PCB residues exceeded the 5 µg/gm concentration.

Concern has also arisen with respect to the impact the organochlorine compounds in the Lake Michigan biota (principally fish) is having on other animals which use this food supply. Most notably is the effect of Lake Michigan fish on mink reproduction in the North Central states. Before DDT and dieldrin levels in these fish were well documented, Hartsough (1965) indicated that Lake Michigan fish were suspected as the factor leading to poor mink reproduction. Aulerich et al. (1970) clearly demonstrated the fish had been the cause of the reproductive failure. Aulerich

and Ringer (1970) found that DDT and DDD did not have significant adverse effects on mink. Furthermore, dieldrin was lethal at 2.5 µg/gm in the food for extended periods but did not appear to effect reproduction at twice this level during the gestation period. Aulerich et al. (1971) concluded that coho salmon per se did not cause reproduction problems but that the disorder is associated with other species of fish and "appears to be dependent upon the species of fish and its environment." Finally, after the earlier reports that PCBs were present in Lake Michigan fish, Ringer et al. (1972) demonstrated that 10 µg/gm of Aroclor 1254 in coho salmon produced 71 percent mortality in mink and that a mixture of 10 µg/gm PCBs and 0.5 µg/gm dieldrin in coho food produced 100 percent mortality. No kits were born alive when the diet contained 5 µg/gm or greater Aroclor 1254 alone. This evidence clearly indicates that the biological resources of Lake Michigan may seriously endanger other species even though the levels of toxicants are not sufficiently great to produce a dramatic effect within the aquatic communities.

Equally important is the coincidence of high levels of chlorinated hydrocarbon in herring gull and other bird populations with reproductive failures and subsequent population decline. Ratcliffe (1970) and Anderson (1970) have clearly shown that major changes in eggshell thickness have occurred since 1945 and that the phenomenon may be a major factor in the decline, if not extinction, of many species of birds. The effect is found in predators and most notably in fish-eating birds. In general, egg shell thinning of 10 to 15 percent (relative to pre-1940 eggs) may produce an effect in the population, 15 to 20 percent is associated with readily observable egg breakage, and 50 percent thinning has been associated with virtually total reproductive failure (Risebrough et al., 1970; Keith et al., 1970).

Anderson (1970) found the Great Lakes Herring Gulls contained the highest chlorinated hydrocarbon levels in eggs ever reported for that species. He also found that the Lake Michigan gull, which declined drastically in the early 1960's but recovered in the late 1960's, had egg shell thinning which varied from nine percent in 1953-1956 to 18 percent in 1965. However, since 1965, the degree of thinning has decreased to eight percent in 1969. In comparison, gulls on Lake Huron and Lake Superior have

exhibited thinning of seven and eight percent, respectively, and those from the east coast have remained essentially unchanged from earlier eggs.

Anderson (1970) also showed that double-crested cormorants from Wisconsin had 20 percent thinner eggs and produced eggs with the highest DDE concentrations of this species from interior North American. Golden eagles which primarily feed on mammals do not show as dramatic egg shell thinning as do the Bald Eagles which feed on fish. Despite the lack of detailed data in the literature, it is not unreasonable to suggest that the disappearance of Bald Eagles from the shores of Lake Michigan may also be linked by correlation to the chlorinated hydrocarbons in the food resources of the lake.

The Lake Michigan system is also unique in that the use of the major persistent chemicals have been curtailed in the watershed by a variety of actions. Although it is likely the use of the chlorinated pesticides in agriculture was diminishing in the late 1960's, the Lake Michigan Enforcement Conference recommended regulatory actions on many uses in 1968. This recommendation led to restrictions on DDT in Illinois, Michigan and Wisconsin, including the sale of this compound. A more detailed summary is presented by Lucchesi (1972).

The realization that the widespread use of PCBs has resulted in the widespread occurrence of these potentially hazardous chemicals in the environment moved Monsanto Company, the sole producer of PCBs in the United States, to restrict the sales of PCBs in 1970 and by April, 1971, only "close system" uses were able to purchase PCBs from Monsanto. However, there is no data to confirm the fact that PCB useages are significantly reduced.

As previously discussed, the FDA has placed a 5 µg/gm action level on PCBs in food for human consumption. Moreover, on March 18, 1972, this restriction was extended to food packaging materials. On May 2, 1972, the Environmental Protection Agency announced that all discharges to the aquatic environment involving PCBs will be restricted to the lowest possible level and that the desired limit will be 0.01 µg/l PCBs. These restrictions were re-enforced by the recommendation of the joint task

force headed by the Council on Environmental Quality and the Office of Science and Technology (Task Force, 1972) which called for all current uses of PCBs, except capacitors and transformers, to be discontinued.

In summary, the use of chlorinated hydrocarbons in the agriculture and industry in the Lake Michigan watershed over the last few decades has resulted in the contamination of this valuable, mesotrophic body of water. Indirect evidence exists that these chemicals are having subtle detrimental effects on the species near the top of the food chain, and some of the food resources have been deemed unfit for human consumption. However, the Lake Michigan watershed has also realized unparalleled legislative action regarding the use of these hazardous, persistent chemicals in the last two years. At the recommendation of the Lake Michigan Interstate Pesticide Committee, this study was funded to establish detailed data on PCBs and *DDT* in Lake Michigan fish which will serve as a 1971 baseline in determining future trends of the levels of these chemicals in the lake and the impact cooperative legislative actions have had in managing the quality of large lakes.

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SECTION IV

MATERIALS AND METHODS

This section describes the procedures used in collecting samples for the study and in analyzing the tissue for major chlorinated hydrocarbons. Special emphasis has been placed on preparation of reagent chemicals used in the study.

A. Sample Collection

The intent of the field sampling phase of this study was to collect 20 samples of yellow perch (10-12 inches), white sucker (10-12), carp (12-25), chub (8-10), lake trout (20-25), coho salmon (20-25) and alewife (6-8) from each of the following regions of Lake Michigan: central basin--north, central basin--south, Green Bay, Sheboygan - Manitowoc, Milwaukee - Racine, Chicago - Indiana Harbor, Benton Harbor, Saugatuck, Grand Haven, Muskegon and Grand Traverse Bay. The above field objectives suggested by the Lake Michigan Interstate Pesticide Committee were not explicitly attainable due, in part, to the availability of each species in the regions of interest and to the availability of fishing vessels in the respective regions of the lake. Thus, the majority of the fish were captured as available during September and October of 1971 through the use of gill nets, pond nets and trawls. To minimize variations in the concentration of chlorinated materials with size of fish, the length of the fish taken for analysis was limited as much as possible to the size ranges suggested by the Committee. Figure 1 is an illustration of Lake Michigan in which the sampling areas are indicated by dashed lines.

Where possible, the samples were transferred directly from the ship to insulated boxes lined with acetone-rinsed aluminum foil which contained dry ice. However, many of the samples which were collected by research biologists from state agencies were placed in cardboard cartons or polyethylene bags and frozen immediately. When grinding these samples, the containers were sampled to determine whether significant amounts of

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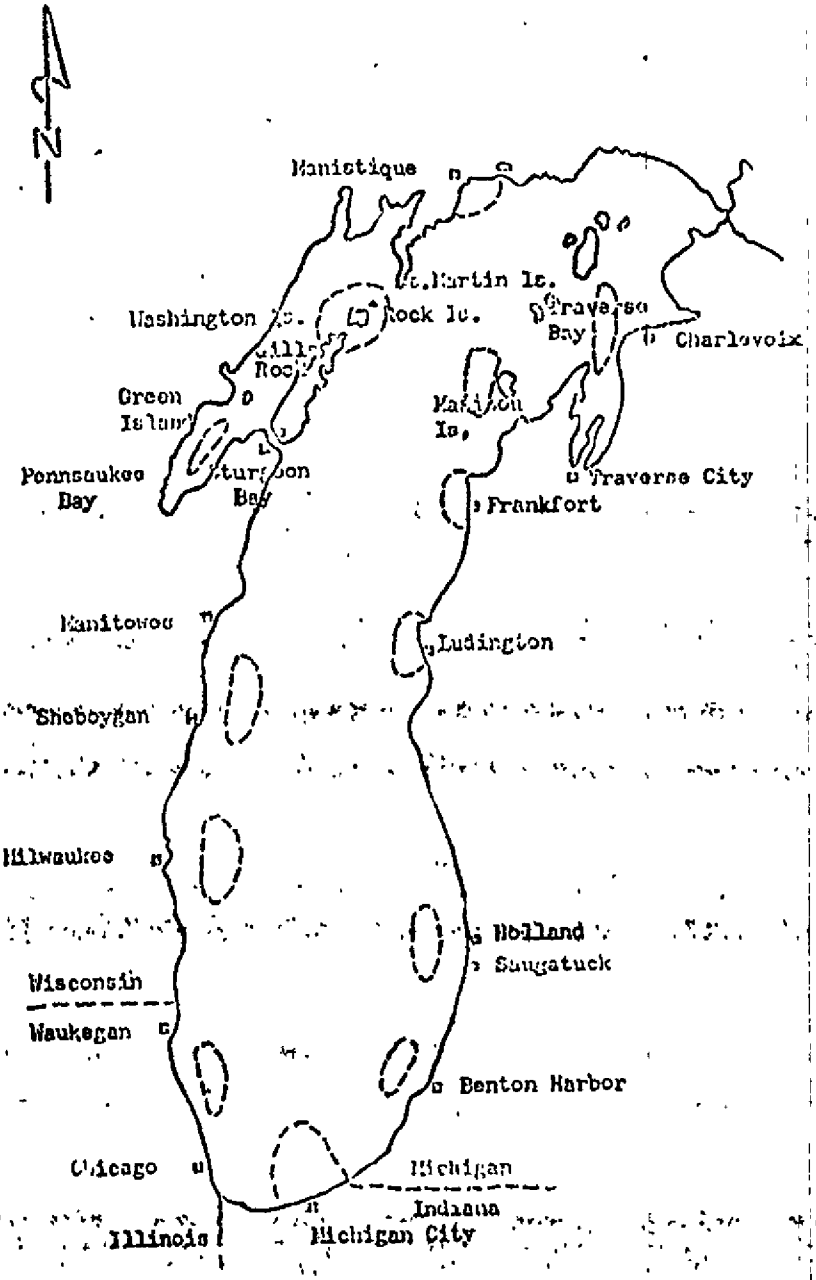
FIGURE 1. SAMPLING REGIONS OF LAKE MICHIGAN



contaminants were present in the materials in contact with the frozen fish. All samples were transported directly to the University of Wisconsin at Madison.

B. Reagents

Spurious gas chromatograph peaks which arise from the contamination of the sample in the laboratory are not an uncommon problem in trace analyses. These contaminants not only add to the complexity of the GLC interpretation but also often make true quantitative analyses an impossibility. Previous work by the author has indicated that most reagents used in pesticide analysis contain contaminants which may interfere with the analysis. Moreover, contamination of the sample may arise from objects worn or brought into the laboratory by personnel such as adhesive bandages, order forms, etc. and the laboratory is regulated accordingly. Also, the following reagent cleaning procedures were established to minimize the possibility of sample contamination during the analyses.

1. The Na_2SO_4 (Fischer Scientific Co.) was washed with three volumes of 1:1 hexane/acetone and dried at 130° C. To prevent further contamination from cap liners or containers, the Na_2SO_4 was stored in large glass bottles with Al foil liners in the cap.

2. The Florisil media (Kensington Organic, Fisher Scientific) was extracted in an all-glass Soxhlet extractor for 24 hours with the azeotrope of hexane and acetone in an effort to remove traces of organic impurities. The solvent was evaporated from the Florisil at 100° C, and the solid was heated at 650° C for 2.5 hours for activation. If not used immediately after heating, the Florisil was warmed to 105° C prior to its use.

3. The silicic acid (Mallinckrodt Chemical, Adsorbo, Ramsey and Patterson) was used directly from the reagent bottle.

4. The hexane (Skelly B) was redistilled in glass from Dri-Sodium (Fisher Scientific).

5. The acetone (Fisher Scientific, ICS) was redistilled in glass from Dri-Sodium.

6. The ethyl ether and methylene chloride (Mallinckrodt Chemical) were pesticide quality solvents and were used directly out of the reagent bottle after periodic checks showed that interferences were not present for the experimental conditions of this study.

7. Glass wool was soaked in acetone and then rinsed with an acetone in hexane mixture (1:1).

8. Glassware was washed thoroughly with hot detergent and rinsed with hot water, twice with distilled water and with a mixture of acetone and hexane (1:1).

C. Sample Preparation

The fish were stored frozen at -20° C until analysis. The storage period for the majority of the fish was restricted to several weeks but did not exceed two months in order to minimize desiccation and possible chemical changes in the tissue during storage. The frozen fish were fragmented with a cleaver and homogenized while frozen using a Hobart meat grinder. The frozen tissue was first passed through the grinder to obtain a free-flowing, granular product in which bone, fat and muscle tissue could be recognized. The entire sample was thoroughly mixed and passed through the grinder twice more to yield a uniform light pink sample. The homogenate was sampled by quartering several times and removing aliquots for immediate analysis and for bulk samples which have been stored for possible future work.

During the homogenizing operation, considerable effort was expended to assure that the tissue came in contact only with metal surfaces which were rinsed with acetone. The grinder was cleaned with hot water after each fish was ground. The bearing and seal of the grinder was inspected periodically to make certain no lubricants could enter the grinding chamber. Since several of the assistants on this project worked only part-time between classes, care was taken to clean hands thoroughly to assure that contaminants were not introduced from outside sources of PCBs such as carbon papers, inks, etc.

D. Extraction

Because of the large number of samples which were to be extracted for this study, the 12 extractor unit in Figure 2 was built at the Water Chemistry Laboratory. The unit featured two rows of six extractors, each heating mantle independently spring-loaded such that the extractors could be set up or removed individually by pressing down on the mantle and lifting the extractor off. Since many of the organic solvents require less than 115 volts on the heater for refluxing, pairs of the heaters were connected in series, thus dropping approximately 60 volts across each heater. This arrangement precluded the need for voltage transformers and provided enough power to maintain about eight extractions each hour in the extractors. The main power cord was connected to a timed relay, allowing the extraction to be carried out unattended.

Approximately 10 gm of frozen tissue was weighed into a tared beaker and then ground with 50 gm anhydrous Na_2SO_4 until the mixture appeared dry. The mixture was tamped into a glass extraction thimble fitted with an extra coarse glass frit and placed into the Soxhlet extraction apparatus. The receiver flasks were filled with 170 ml of hexane/ethyl ether (1:1), and the samples were extracted for three to four hours.

E. Column Cleanup

Sample cleanup was conducted as indicated in Figure 3. Briefly, the extracts were concentrated to 20 ml and 2 ml were withdrawn for the non-volatile residue analysis. After further concentration of the extract, the bulk of the oil and fat was removed through liquid chromatography on Florisil as shown in Figure 4.

The Florisil column consisted of a 25 mm O.D. glass column which was filled with 19 gm of Florisil (Reynolds, 1969). The column was fitted with a glass frit and Teflon stopcock at the bottom and was topped with 10 gm anhydrous Na_2SO_4 to prevent deactivation from water in the extract. The extracts (15 ml) were placed on the dry column and eluted with six percent ethyl ether in hexane (200 ml) at 3 to 5 ml/min to obtain PCBs, DDE, DDD, DDT, heptachlor, aldrin and toxaphene, if present. The receiving flask was changed, and the column was eluted with 15 percent ethyl ether

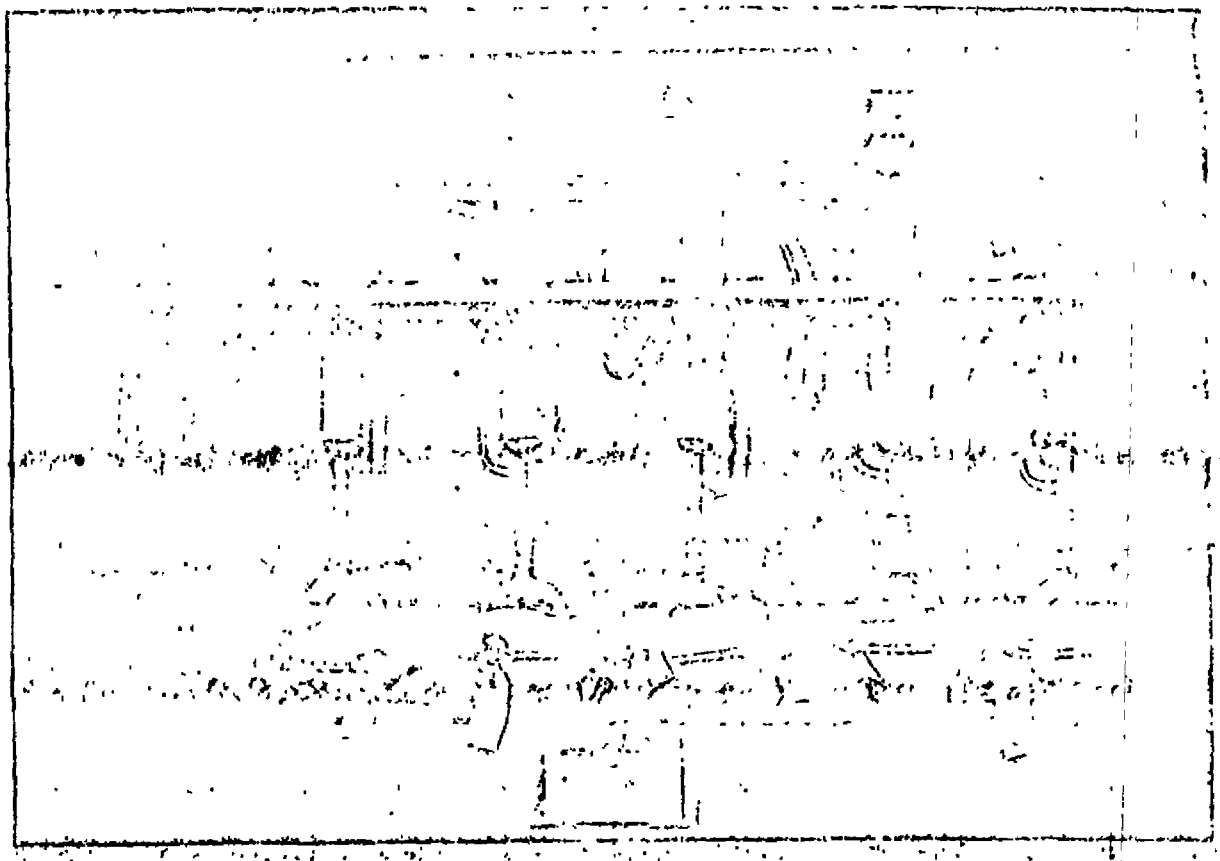
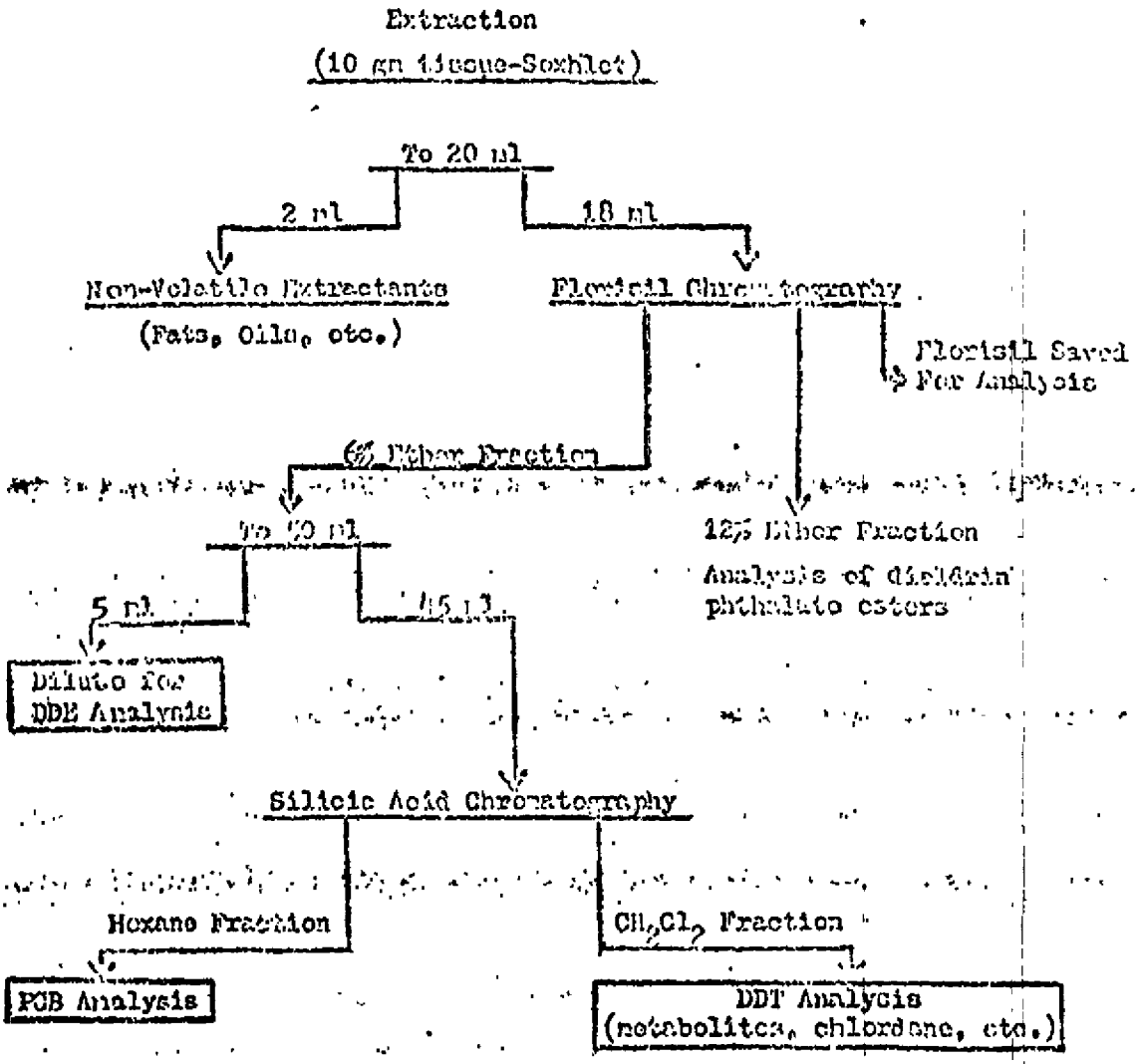


Figure 2. Apparatus for Multiple Extraction Fish Tissue Using Soxhlet Extractors

Each unit is held in place by spring tension on the heating mantles. Samples are added or removed by pressing down on the individual mantles.

Figure 3. Analysis Scheme for Lake Michigan Fish



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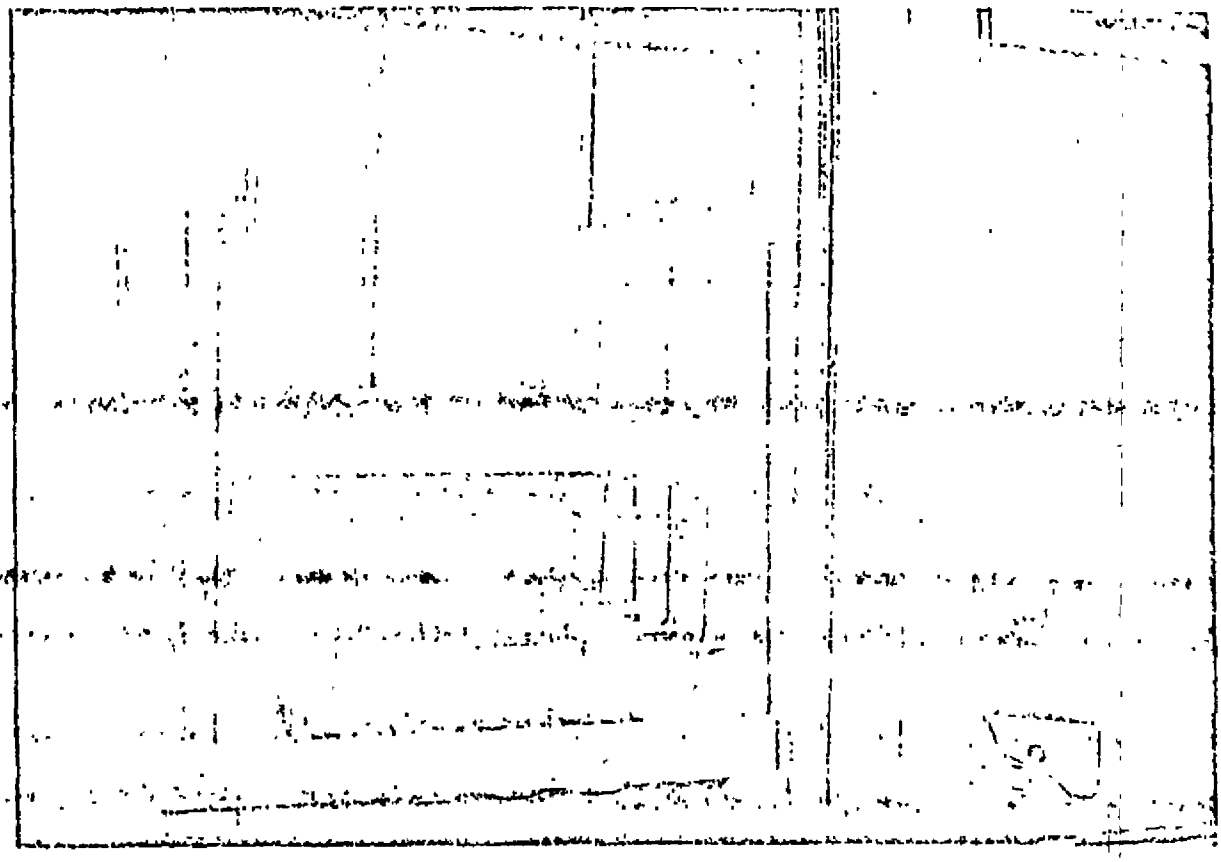


Figure 4. Apparatus for Multiple Cleanup of Extracts by Florisil Chromatography

Approximately 32 extracts were passed through this primary fractionation step each day.

in hexane (200 ml) to obtain dieldrin, heptachlor epoxide, lindane and some of the phthalate esters, if present. The samples were concentrated to appropriate volumes for GLC analysis. In general, the 19 gm Florisil column was suitable only for removing the bulk of the co-extracted non-volatile materials, dieldrin and related compounds from the chlorobiphenyls and DDT family of pesticides.

F. Liquid Chromatography on Silicic Acid

The six percent (ether in hexane) fraction from the Florisil column contained primarily PCBs and DDT and derivatives thereof (Veith, 1970). Because a mutual interference exists in GLC analyses of samples containing residues of both these groups of compounds, separation of the PCBs from DDT was necessary prior to analyses.

Although PCBs can be separated from DDT on alumina, silicic acid (Armour and Burke, 1970) and charcoal (Berg et al., 1972), previous work by the author has demonstrated that the separation of DDE from the PCBs, particularly those with three or less chlorine atoms per molecule, cannot be consistently attained in routine analyses. However, due to the comparatively large concentration of p,p'DDE with respect to other chlorinated hydrocarbons in lake Michigan fish, the extracts can be diluted and analyzed for DDE without significant error (see example in Figure 5). Therefore, prior to the fractionation of the six percent ether eluate from the Florisil column, an aliquot (nine percent of sample) was diluted approximately tenfold and analyzed directly for DDE. The remainder of the sample was subjected to further separation on silicic acid.

The silicic acid separation was conducted according to the procedure of Armour and Burke (1970) with minor modifications. Because more than 20 samples were passed through silicic acid each day, large quantities of silicic acid of uniform deactivation were necessary to assure consistent separations. To accomplish this, 500 grams of silicic acid (Mallinckrodt, 100 mesh, AR) were weighed into flasks in five 100 gram portions after the powder was dried at 130° C overnight. Water (2.1 to 2.7 percent) was added by buret to deactivate the media, and the individual flasks were sealed and shaken vigorously in a wrist-action shaker for four hours. The media in the flasks were then combined and blended in a sealed bottle and

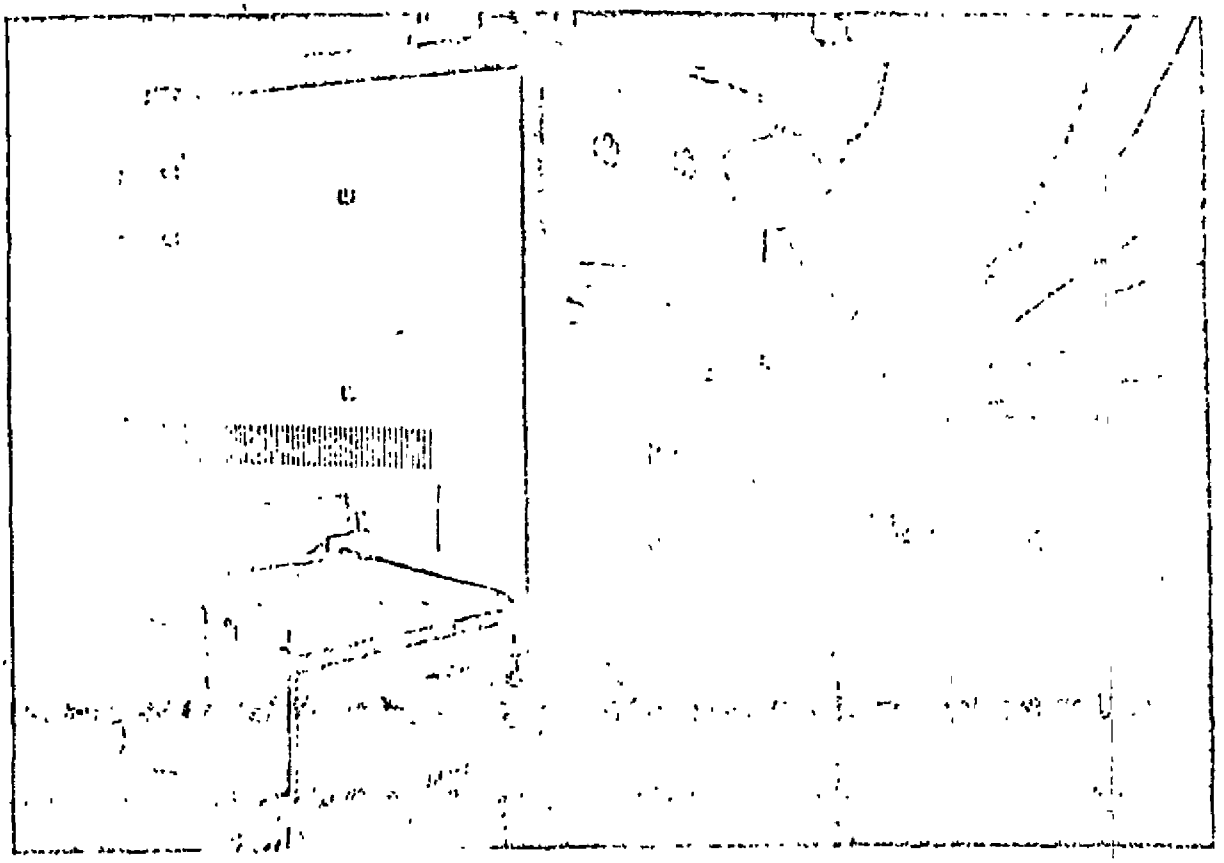
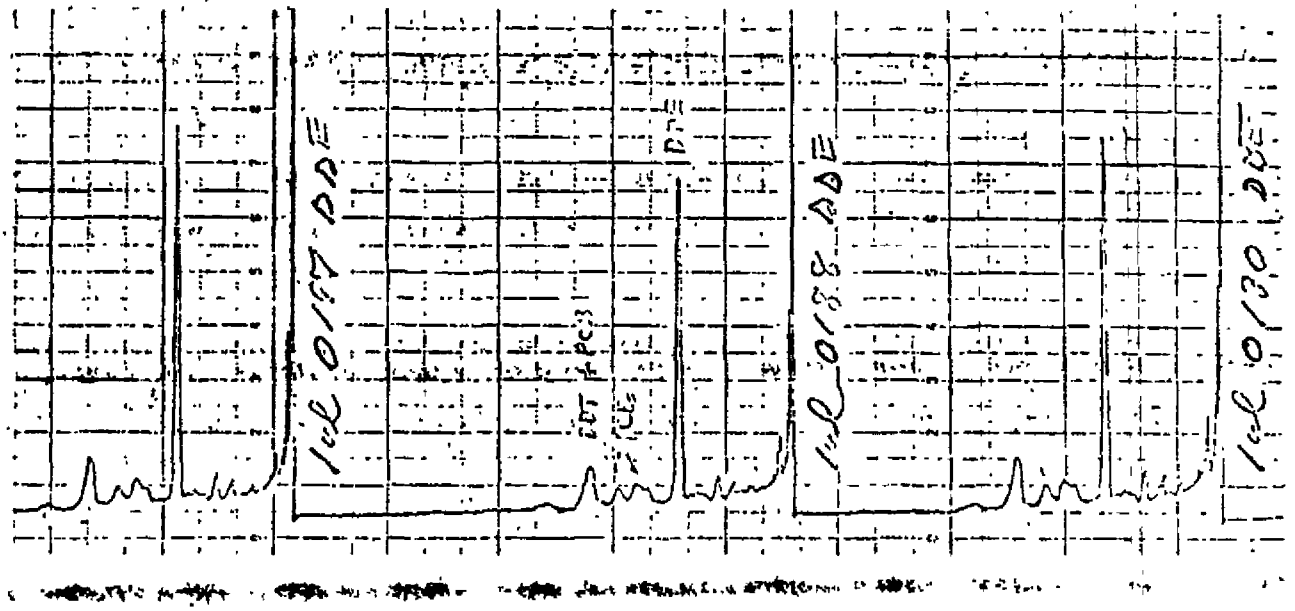


Figure 5. GLC Analysis of DDE on a Varian 1745 Gas Chromatograph. Below-A typical chromatogram for Lake Michigan fish with interference-free response of DDE in the presence of PCBs.



allowed to equilibrate approximately 18 hours in a desiccator. Celite 545 was not used in the procedure because of the impurities present in the commercially available material.

The chromatography columns were prepared by pouring a slurry of 20 grams of the deactivated silicic acid and hexane into a 22 mm (I.D.) shell column fitted with pressure fittings on top. After the sample was placed on the column, it was eluted first with 250 ml of hexane and then with 250 ml of a 1:1 mixture of hexane and methylene chloride. Since the samples were analyzed immediately by GLC, it was generally possible to adjust the water content daily in order that the DDT and DDD remain in the methylene chloride fraction but the trichlorobiphenyl isomers remain in the pure hexane fraction.

C. Instrumentation

Quantitative gas chromatographic analyses were conducted on an Aerograph 1745-20 gas chromatograph equipped with dual concentric-tube electron capture detectors (^{3}H, 250 mc) and illustrated in Figure 5. The columns were 2.0m x 1.8 mm (I.D.) glass coils packed with ov-101 (three percent) on 120/140 mesh Gas Chrom Q. The carrier gas (purified N_2) was maintained at 20 ml per minute; and the injector, column and detector temperatures were 240° C, 180° C and 220° C, respectively. The chromatograms were recorded on a Varian Model A-25 dual pen recorder.

D. Quantitative Analysis

Previous work (Veith, 1971) has shown that the fish from Lake Michigan contain mixtures of PCBs which closely resemble Aroclor 1254 produced by Monsanto Company, although PCBs both heavier and lighter than those most abundant in Aroclor 1254 were also present. Figures 6a and 6b compare chromatograms of Aroclor 1254 and a typical Lake Michigan fish extract, respectively. The fish extract contains predominantly those PCBs which elute at 70, 84, a doublet near 100, 125, 145 and 176 (relative to pp'DDE). In addition, the chromatogram shows several of the lighter PCBs and the 203, 232 and 280 PCBs (relative to DDE).

Because the majority of the fish from Lake Michigan contain apparent Aroclor 1254 primarily and a large number of samples were to be analyzed

Figure 6. Comparison of PCB Mixtures in Fish and Commercial Mixtures (Standard)

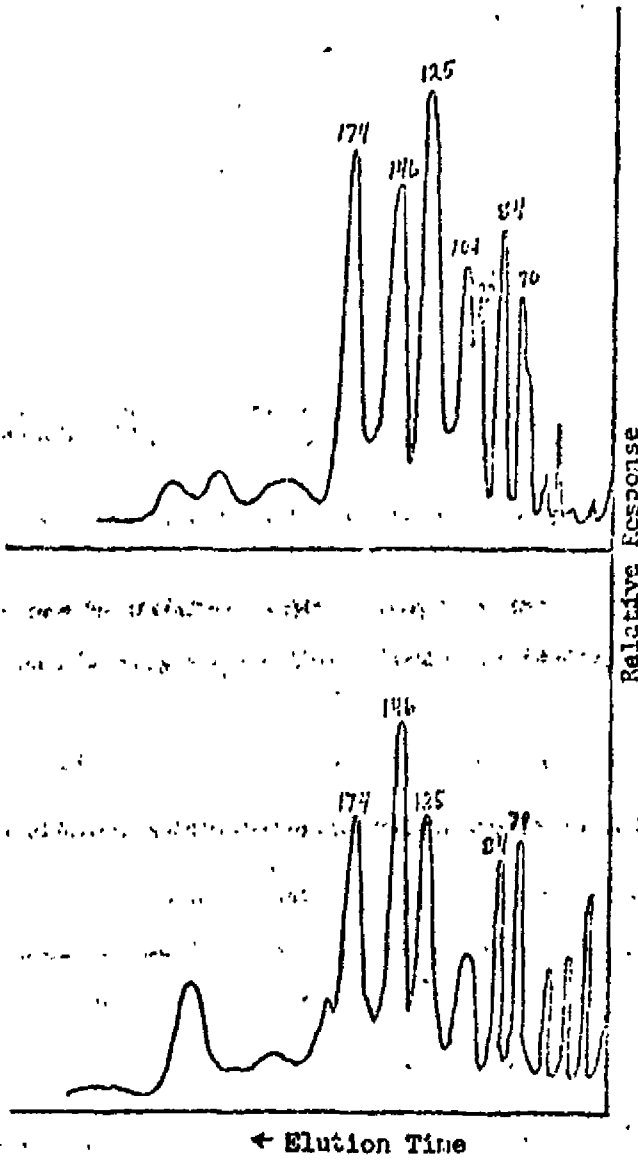


Figure 6a. Chromatogram of Aroclor 1254. Peak Number Relative to p,p'-DDE = 100.

Figure 6b. SA Chromatogram of Lake Michigan Fish.

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in this study, all of the fish were analyzed for Aroclor 1254 only. Thus, in the absence of evidence of other major, unknown contaminants in the PCB (hexane) fraction from the silicic acid separation, the data is likely to be conservative; and those fish from nearshore regions which have some Aroclor 1242 and/or 1248 present may have more PCBs in the tissue than is reported by this study.

As stated previously, pp'DDE cannot be consistently removed from the PCBs and was analyzed separately. The interference of pp'DDE to the quantitation of the PCBs was overcome by excluding the doublet at 98 and 104 from the calculations. The heights of peaks at 70, 84, 125, 146 and 174 were summed to give a number which, when compared to a standard curve prepared similarly, produced the mass estimation. With this method, the amount of pp'DDE in the sample could vary considerably without affecting the PCB analysis. Figure 7 illustrates a standard curve prepared from the peak height data.

I. Evaluation of Analytical Methods

The methods used throughout this study are well proven (with the possible exception of work on the silicic acid column). In general, the methods will account for greater than 85 percent of the residues in the tissue and exhibit less than 10 percent variability. Thus, when analyzing a sample from the environment where the sample history is largely unknown, there may be more significant error in interpreting chromatograms than in the manipulation of the extract.

The extraction of hydrophobic chlorinated hydrocarbons from tissue using ethyl ether and hexane is quantitative, even under mild conditions. For example, Stalling (1971) found that 97 ± 3 percent of the chlorinated pesticides added to fish tissue was extracted by rinsing the tissue with 200 ml of cold solvent. The exhaustive extraction with Soxhlet extractors as used in this study is essentially 100 percent. Previous work by the author (Veith, 1970) has shown that the recovery of PCBs for extraction and clean-up was 87 ± 3 percent. Thus, approximately 10 percent of the residues are lost on the clean-up columns or in the transferring of samples between vessels. Both of these losses can be minimized by rinsing with sufficient amounts of solvents.

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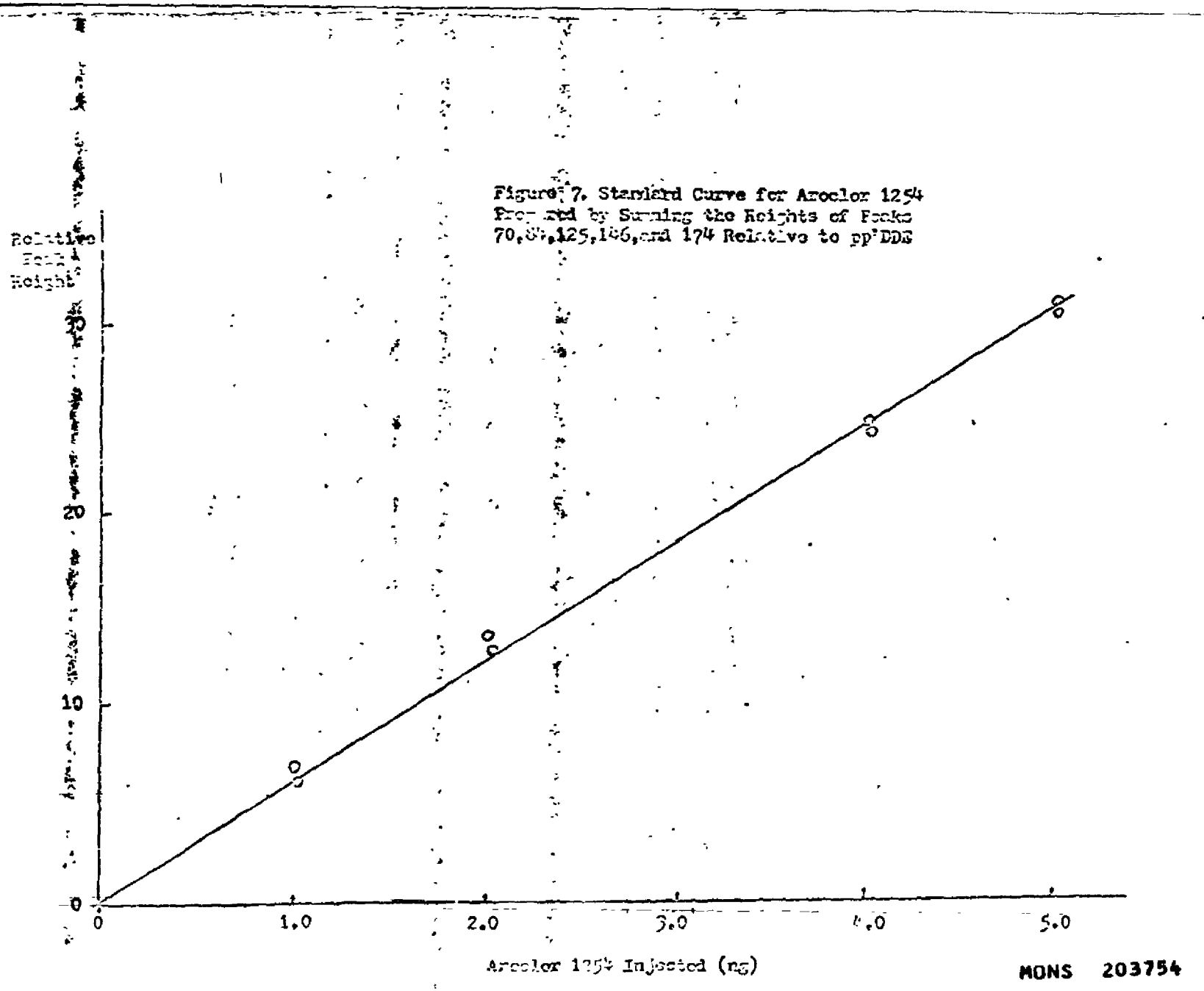

Figure 7. Standard Curve for Aroclor 1254 Prepared by Summing the Heights of Peaks 70,81,125,146,and 174 Relative to pp'DDE

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The analysis of PCBs in spiked tissues of six fish from northern Wisconsin (contain negligible quantities of PCBs) indicated that the present method permitted recovery of 85.1 $\pm$ 4.3 percent. The recovery of DDT was likely the same as PCBs while that of DDE was near 90 percent.

More important than what can be recovered under spiking conditions is the reproducibility with which a sample can be prepared, cleaned up and analyzed. Of particular concern in this study is the sampling of a large fish which, because of the large quantity of tissue to be homogenized, may give rise to considerable variability. Table 1 presents the results of multiple analyses of selected fish during this study. The data indicate that the relative standard deviation for the PCB analyses ranged from five percent in the smaller fish to 14 percent in the large coho salmon. It is likely that the decrease in precision in the coho is due to the difficulty of homogenizing the larger fish in addition to the inherent variability of interpreting the chromatograms of the hexane fractions from the silicic acid columns. The analyses with the poorest precision was that of the DDT analyses where the relative standard deviation ranged from eight percent to 23 percent and appeared to average approximately 14 percent. It is likely that this reduced precision results from losses during silicic acid chromatography which is necessary to quantitate the DDD and DDT isomers. The precision of the DDE analyses were lower than that of DDT which would be anticipated because of the fewer manipulations of the extracts. The relative standard deviation ranged from seven percent to 18 percent, but the average deviation was approximately 10 percent. The fat analyses, due to the relatively simple procedure, were the most precise and exhibited relative standard deviations ranging from five to seven percent.

To further evaluate the quantitative analysis of PCBs, replicate samples were sent to Analytical Bio Chemistry Laboratories, Inc. in Columbia, Missouri, for analysis. Table 2 presents the results of this collaborative study. In general, the concentrations of PCBs in the fish as determined by the two laboratories were in close agreement, although several of the concentrations determined by ABC Labs were a factor of two lower than that determined by this study. Of particular interest, however, is the fact that the analyses of total PCBs (ABC Labs) did not

Table 1. Precision of Chlorinated Hydrocarbon Determinations for PCBs, DDE, DDT and Fat in Selected Lake Michigan Fish.

Species	Replicates	Aroclor 1254 (ug/gm)	p,p'-DDE (ug/gm)	p,p'-DDT (ug/gm)	Fat (percent)
Coho Salmon (25 inches)	6	15.2 (2.2)[a]	7.5 (0.6)	3.2 (0.6)	4.2 (0.2)
Coho Salmon (27 inches)	6	13.1 (1.3)	5.5 (0.4)	1.8 (0.4)	10.0 (0.6)
Whitefish (13 inches)	6	4.1 (0.2)	0.35 (0.04)	0.56 (0.04)	16.2 (0.9)
Chub (10 inches)	6	5.7 (0.4)	3.4 (0.3)	2.2 (0.3)	18.2 (1.3)
Alewife (8 inches, composite)	6	4.0 (0.2)	1.1 (0.2)	1.0 (0.2)	5.7 (0.3)

[a] Standard deviation in parentheses

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Table 2. Collaborative Determination of PCBs in Lake Michigan Fish

Species	This Study Aroclor 1254 (µg/gm)	ABC Labs.[a] Total PCBs (µg/gm)
Chub	3.7	3.9
Chub	6.1	1.6
Chub	5.7(6)	2.9
Chinook Salmon	11.5	11.3
Coho Salmon	12.3	6.5
Coho Salmon	11.8	10.2
Lake Trout	7.0	7.8
Perch	5.2	5.0

[a] Analytical Bio Chemistry Laboratories, Inc. Columbia, Missouri

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result in concentrations significantly greater than that determined in the analysis of Aroclor 1254 alone. This observation will be discussed further in the next Section V of the report.

J. Confirmation of the PCBs

In addition to preliminary analysis of the extracts with a Varian Mat CH-5 mass spectrometer, the presence of PCBs in selected samples from each sampling area were confirmed by the perchlorination of the PCBs to decachlorobiphenyl and the subsequent analysis of the product by GLC (Berg et al., 1972). Briefly, the aliquots of the hexane fraction of the silicic acid columns were evaporated to dryness in a 5 ml vial fitted with a Teflon-lined screw cap. Antimony pentachloride (0.2 ml) was added to the residue, and the vial was sealed and held at 180° C for six hours. Approximately 1 ml of 6N HCl was added to the products to destroy the $SbCl_5$, and the solution was extracted with five 1 ml portions of hexane. The hexane was passed through a disposable pipet containing anhydrous Na_2SO_4 to remove traces of the aqueous solution. The sample was collected in a graduated centrifuge tube and was diluted to the proper volume for GLC analysis. The GLC analyses were conducted on a Micro-Tec MT-220 gas chromatograph equipped with a ^{63}Ni electron capture detector. The column was 1/4 in. x 3 ft, and was packed with three percent Dexsil 300 on 80/100 Chrom Q. The column, injector and detector were held at 225° C, 250° C and 265° C, respectively.

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SECTION V

QUALITATIVE ANALYSIS OF LAKE MICHIGAN FISH

A substantial effort has been made in this study to examine the extracts of Lake Michigan fish on a qualitative basis. The purpose of this effort is to confirm the presence of the PCBs and DDT (and metabolites) and to determine whether significant quantities of unrelated materials are present and interferring with the quantitative analyses. The presence of PCBs in the extracts were confirmed by perchlorinating the extracts and subsequently analyzing the decachlorobiphenyl formed by GC/MS analyses of selected extracts.

A. Perchlorination

Perchlorination is a particularly useful technique because it gives both qualitative and quantitative information concerning the extract. A group of fish extracts comprising approximately five percent of the total number of samples and representing all regions of Lake Michigan were perchlorinated according to the procedure in Section IV. Without exception, the perchlorination of the extracts produced decachlorobiphenyl, and the quantity of PCBs indicated by the decachlorobiphenyl formed (after correcting to 54 percent chlorine) was generally within 25 percent of that found by the direct analysis of the extract by GLC. Figure 8 illustrates a series of chromatograms of the perchlorination products of the extracts. The large peak eluting at approximately ten minutes is the decachlorobiphenyl and the unreacted PCBs (if present) elute as a small peak on the tail of the solvent.

Although it is often difficult to determine the amount of the various PCB mixtures present in the extract from the amount of the decachlorobiphenyl formed during perchlorinating, good agreement is expected between the estimates from GLC and perchlorination when the fish extract contains predominately one PCB mixture. Figure 9 presents data obtained from the perchlorination corrected to 54 percent chlorine and plotted against the

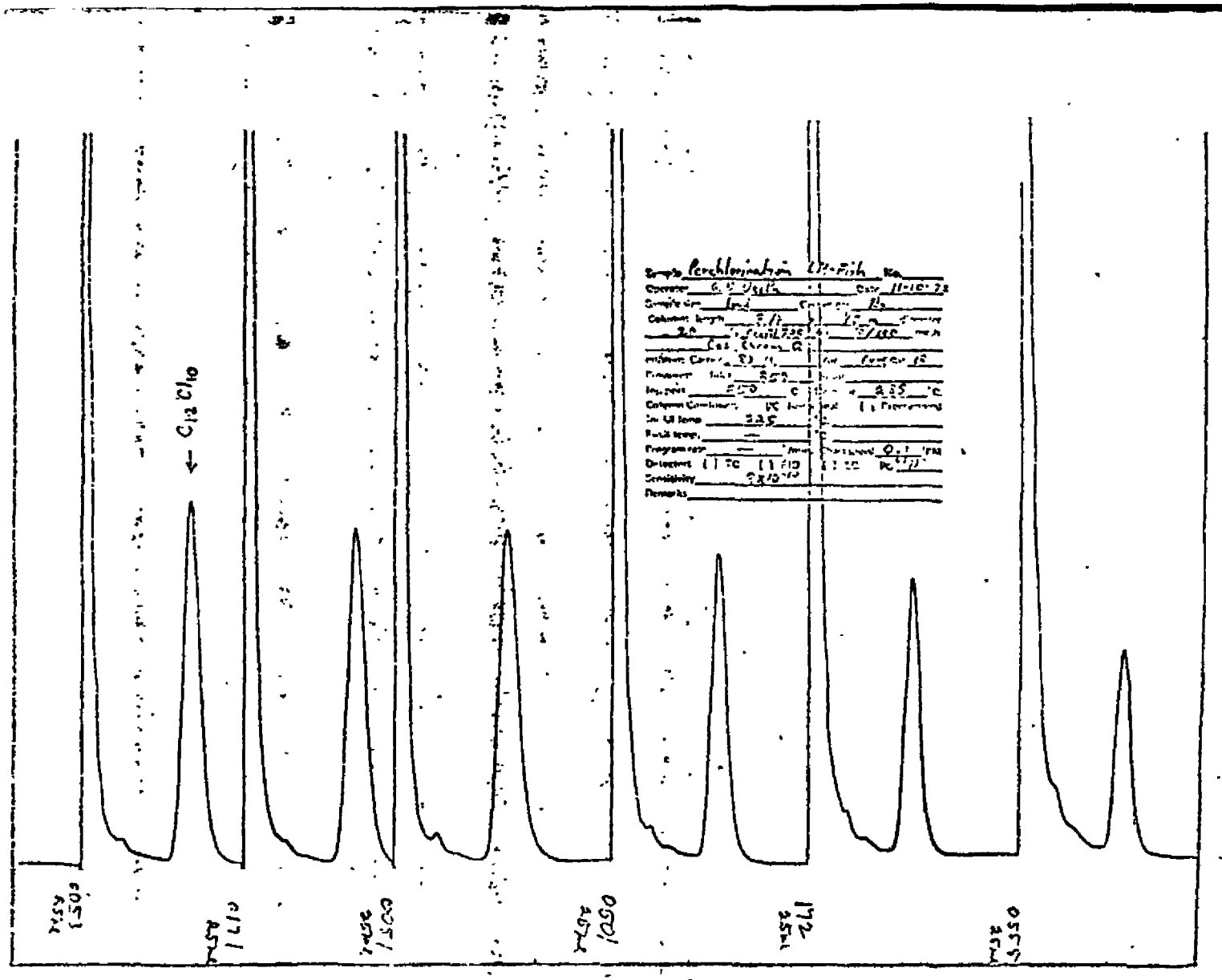

Figure 8. GLC Analysis of $C_{12}Cl_{10}$ from the Perchlorination of Lake Michigan Fish Extracts

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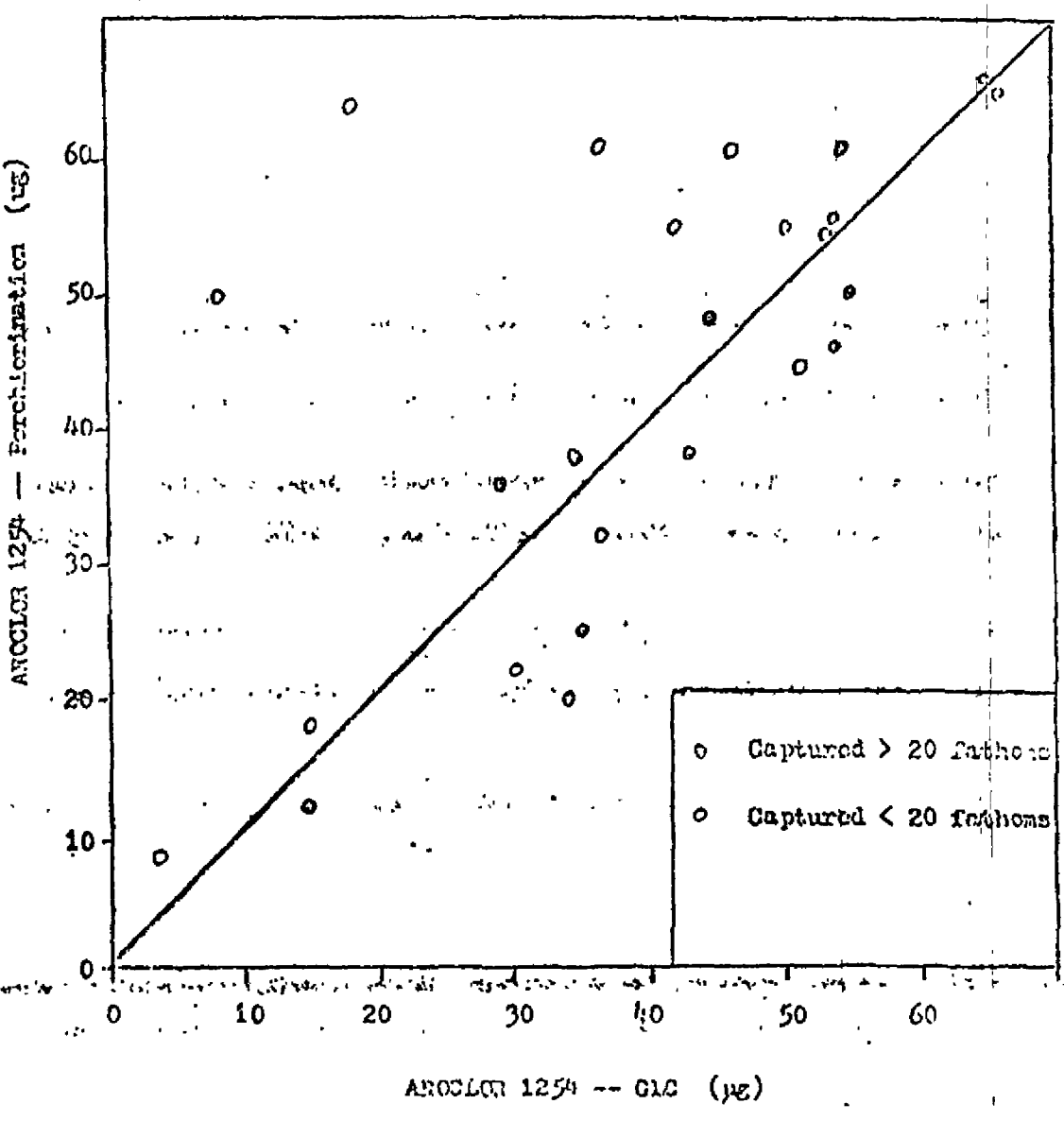
Figure 9. Comparison of PCB Analysis via Direct GLC and Perchlorination-GLC



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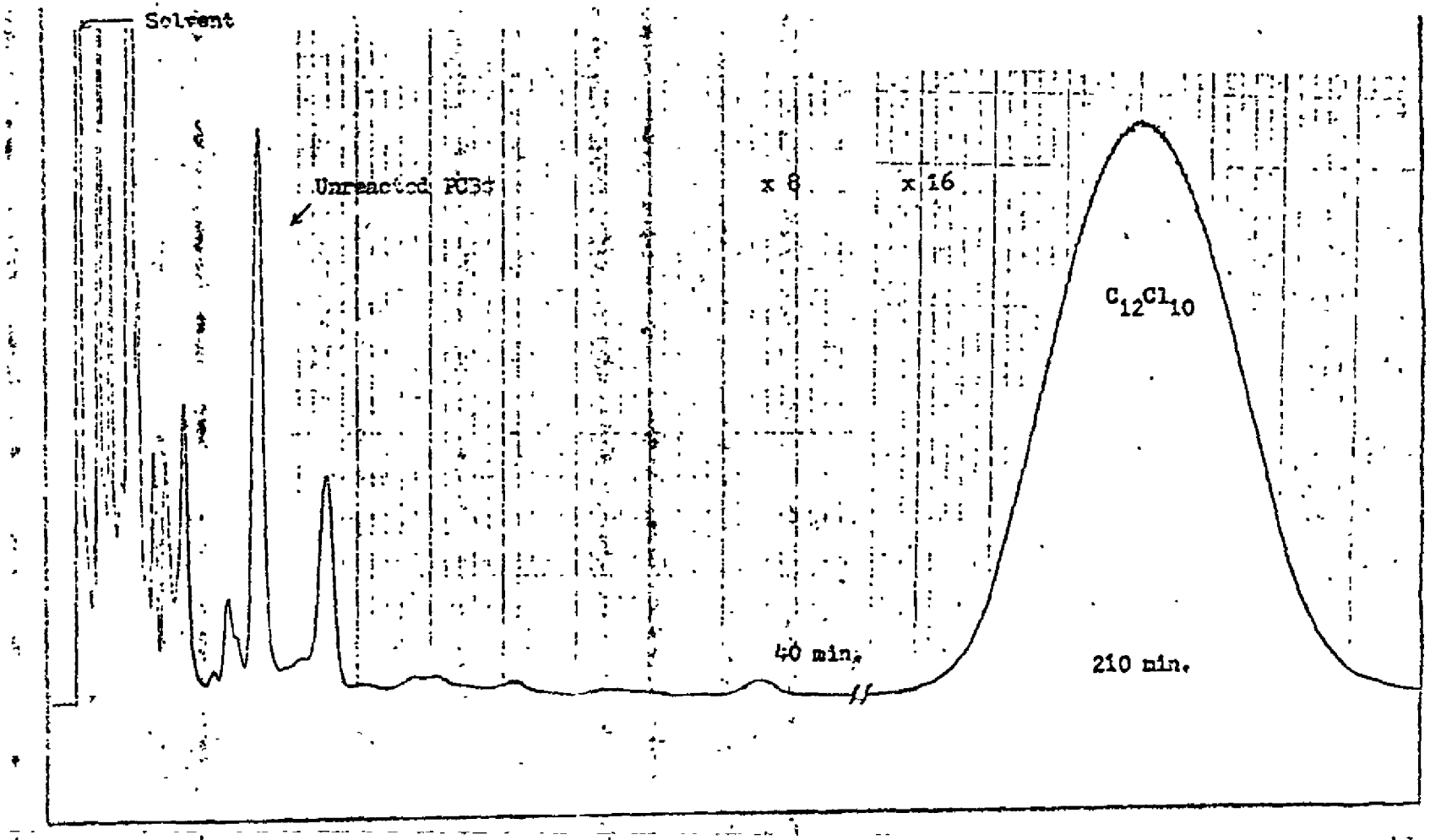
obtained from the gas chromatograph prior to perchlorination. The data are expressed as total micrograms in the aliquots of the sample extracts. Figure 9 indicates that much of the data from the two methods agree well but that a number of the perchlorination estimates differ widely from the GC analysis.

These data were examined more closely to determine the cause of the discrepancy. For the majority of points in Figure 9 where the PCBs determined by perchlorination was much lower than that by GLC, it was generally found that the perchlorination products contain small amounts of unreacted PCBs as illustrated in Figure 10. Thus, it is likely that the data below the line in Figure 9 resulted from incomplete chlorination, presumably due to varying amounts of residual oil in the extract which consumed some of the $SbCl_5$. This difficulty could be overcome by increasing the quantity of $SbCl_5$ and by perchlorinating replicates.

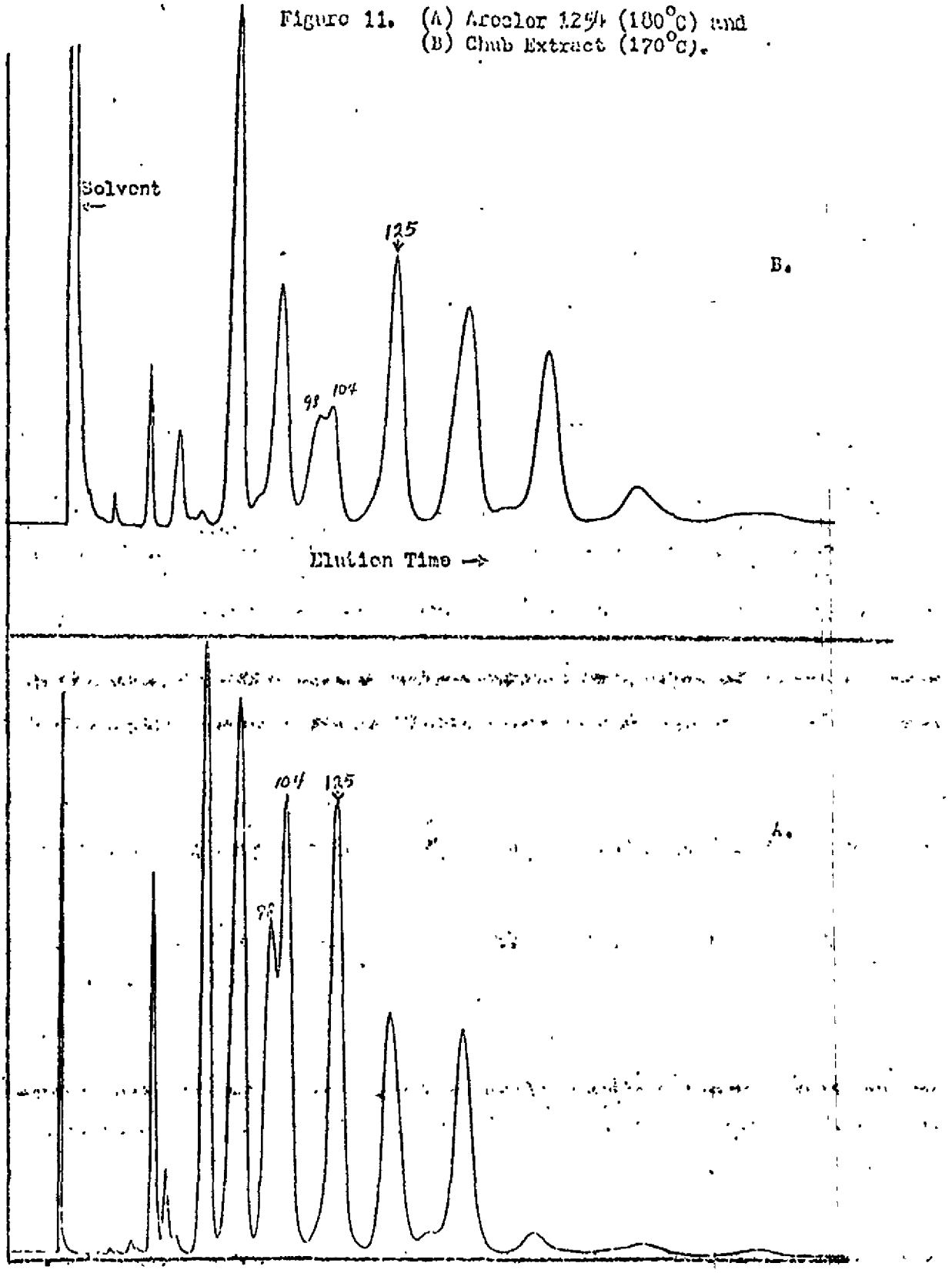
The points above the line represent fish which generally contained appreciable quantities of PCBs other than Aroclor 1254. For example, Figures 11a and 11b compare chromatograms of Aroclor 1254 and a chub captured from 50 fathoms of water off Saugatuck, Michigan. It can be seen that the fish extract resembles Aroclor 1254 closely. The results of the perchlorination indicated that 48 µg PCBs were present in the extract as does the direct analysis indicated 45 µg (as Aroclor 1254). However, the data from the smelt caught in nearshore waters off Door County, Wisconsin, contained a substantial amount of the early-eluting PCBs as shown in Figure 12. Accordingly, the GLC estimate of PCBs was lower than that of the perchlorination method as evidenced by the two points in the upper left corner of Figure 9. It must be pointed out, however, that the apparent error in the analysis of the smelt is not as great as indicated in Figure 9 if the decachlorobiphenyl formed is expressed as Aroclor 1221 (the early peaks) rather than as Aroclor 1254. For example, the smelt contained some apparent Aroclor 1221 and 19 µg Aroclor 1254 but produced approximately 84 µg $C_{12}Cl_{10}$ (64 µg as Aroclor 1254). If the 45 µg of $C_{12}Cl_{10}$ (as Aroclor 1254) not accounted for by the GLC determination is expressed as Aroclor 1221, the GLC analysis was low by 17 µg (approximately 1.7 µg/gm on a whole fish basis). Thus, the perchlorination of the extracts has confirmed without exception the

Figure 10. Chromatogram of Products from an Incomplete Perchlorination of Fish Extracts on a 6 ft. Column at 180° C



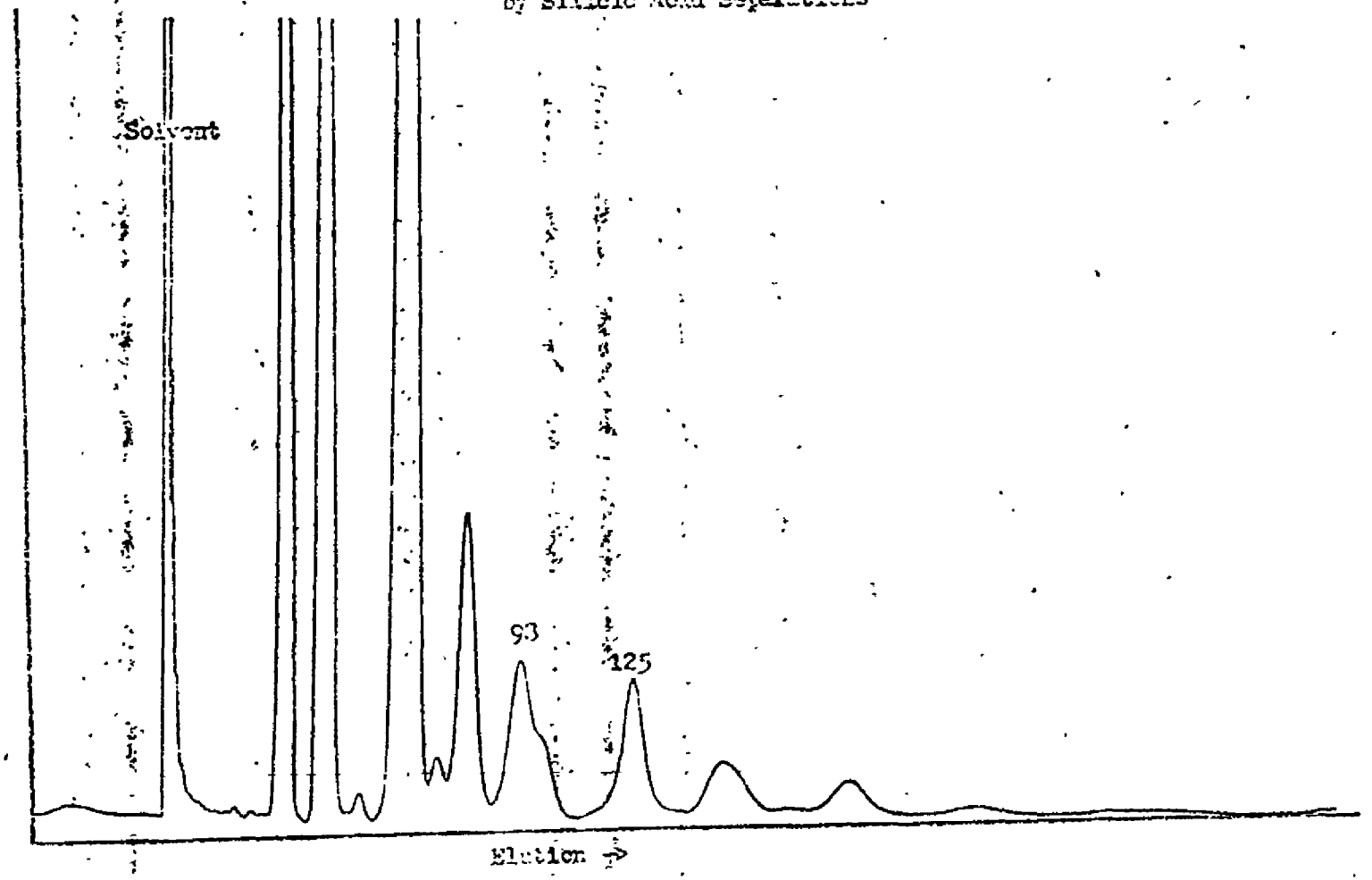
NONS 203763

Figure 11. (A) Aroclor 1254 (180°C) and (B) Chub Extract (170°C).



MONS 203764

Figure 12. Chromatogram of Smelt (Door County) Showing Early-Eluting PCBs. Note: Some Trichlorobiphenyls are Removed by Silicic Acid Separations



presence of PCBs in all samples examined and substantiated the estimates of total PCBs for the fish captured in deep water. Those fish taken from the near-shore environment are more likely to have the lighter PCBs present due to the runoff from the watershed (Veith; 1970, 1971). Therefore, the data presented in this report may be considered conservative estimates of the total PCBs present.

B. Mass Spectrometry

To further examine the extracts on a qualitative basis, composites of five extracts of trout and salmon from Green Island, Manitowoc, Milwaukee, Michigan City, Saugatuck, Ludington, and Grand Traverse Bay were prepared, and the composites were examined by GLC/mass spectrometry at the National Water Quality Laboratory in Duluth.

Figure 13 presents a chromatogram of the fish extract from Michigan City, Indiana. After each sample was introduced into the gas chromatograph, the solvent was vented for 45 seconds, and the mass spectrometer was set to the repetitive scan mode. The data system acquired 50 to 600 a.m.u. spectra approximately every seven seconds, providing 300 to 500 spectra per sample. The mass-converted spectra were examined by several methods for PCBs, DDE and related materials. The first was using the data system to compile ion intensity data for a specific m/e to form a mass chromatogram. For example, in the search for PCBs, the typical mass chromatograms included those of m/e = 256, 290, 324, 358, 392 which represent molecular ions for the tri-, tetra-, penta-, hexa- and heptachlorobiphenyl, respectively. In addition, searches for the molecular ions from the ^{37}Cl isotopes such as m/e = 325, 360, etc. were plotted.

Figure 14 compares mass chromatograms for m/e 292, 326 and 360 for the sample from Michigan City. The data show the composition of PCBs in the mixture in that the early peaks contain tetrachlorobiphenyl, and the 5-6 and 6Cl peaks elute later in the mixture. All of the trout and salmon composite samples gave similar results, thus substantiating that the PCBs present were those found predominantly in Aroclor 1254.

Mass chromatograms for molecular ions of other pesticides and related materials produced no evidence for compounds which were present in

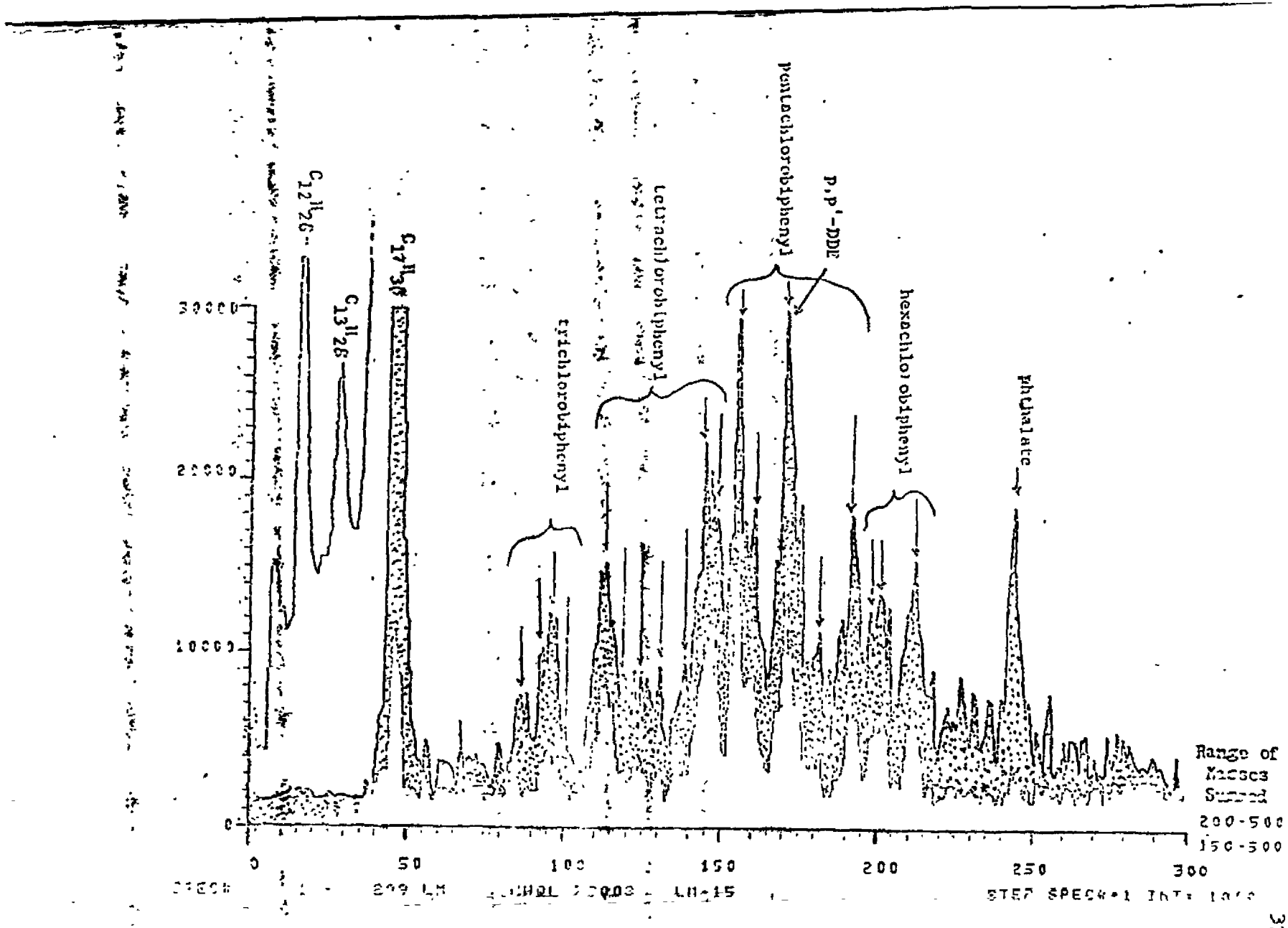
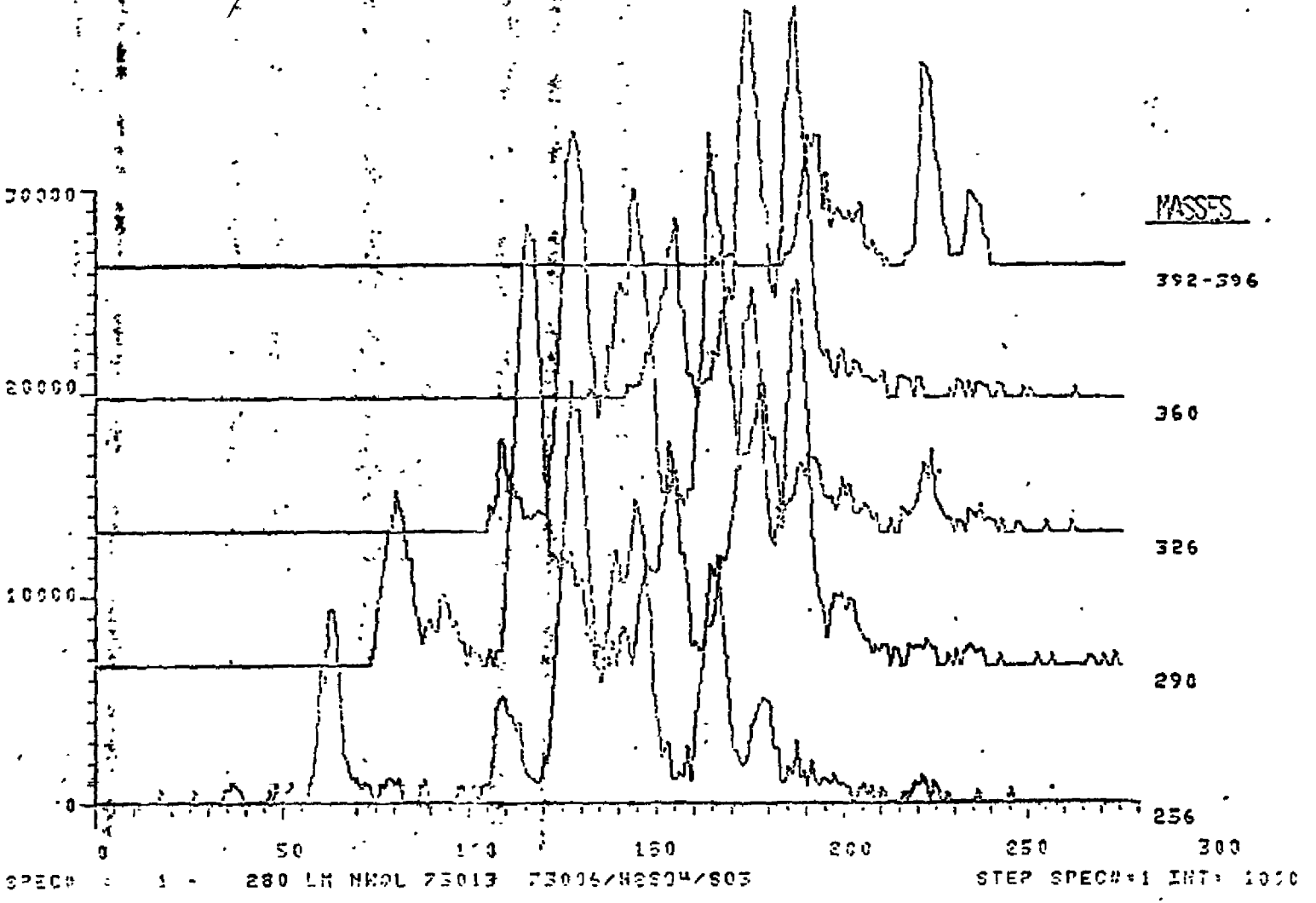


Figure 13. Chromatogram of Composite Sample of Lake Trout From Michigan City, Indiana, on GC/MS System

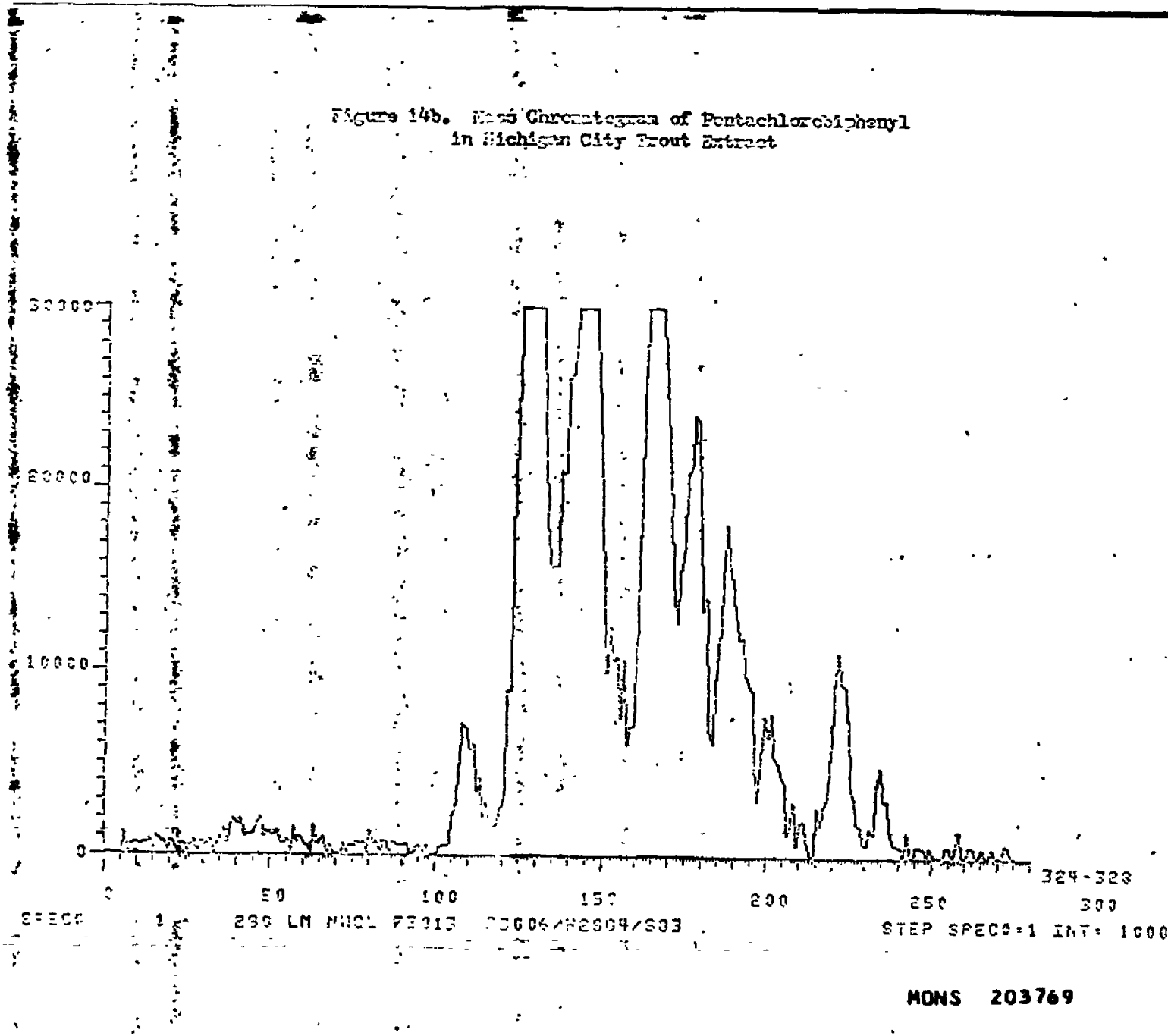
MONS 203767

Figure 142. Mass Chromatogram of PCB Components of Michigan City Trout Extract



MONS 203768

Figure 14b. Mass Chromatogram of Pentachlorobiphenyl in Michigan City Trout Extract



MONS 203769

quantities sufficiently great to cause errors in the quantitative analyses.

Locating the major components of the PCBs in the mixture also allowed for the selection of spectra which were obtained when the greatest quantity of material was eluting from the gas chromatograph. These spectra were plotted for numerous points throughout each run, and the spectra were analyzed to make certain the data arose from PCBs. A summary of the data is presented in Figures I through VI in Appendix B for the major regions of Lake Michigan. Figure Ia is a spectrum of an early-eluting peak which has a molecular ion at m/e = 256. This corresponds to a trichlorobiphenyl, and the characteristic isotope effect between m/e = 256 to 262 as well as the presence of an M - 35 peak at 221 indicates the presence of chlorine. Similarly, Figure Ib shows the presence of a M = 290 for a tetrachlorobiphenyl, and figures Ic and d show the presence of M = 392 indicating heptachlorobiphenyls. Figures II through VI further substantiate the presence of the PCBs common to Aroclor 1254 through Lake Michigan.

In addition to examining many of the spectra obtained in the GC/MS, the spectra were put in a format suitable for a library search in the computer facilities at Battel Memorial Institute in Columbus, Ohio. Tables 3 and 4 present the results of a typical library search in which the spectra of interest was found to best resemble spectra of the various components of the Aroclor mixtures. Table 3 shows that spectrum 150 in the analysis of trout from Milwaukee (LM-1) produced 21 "hits" and, although only five were called for, all five have similarity indexes between 0.48 and 0.52 (generally considered a close match), and all are pentachlorobiphenyls. Table 4 shows that spectrum 230 of the Milwaukee trout extract matched (similarity indexes of 0.24 to 0.53) the spectra of the pentachlorobiphenyls also.

MONS 203770

Table 3. MS Library Search for
5Cl - PCBs in the Milwaukee
Lake Trout Composite Sample

```
SAMPLE ID?
      150
PAPER TAPE?Y
FN= DEK:LM-1,150/LM;%
TT=LAKE MICH-1;%
134,8;145,8;146,16;149,43;162,7;171,7;184,60;186,22;193,6;72;%
194,4;205,7;219,22;220,22;254,100;256,100;258,22;289,22;291,38;77;%
324,52;326,87;328,60;330,21;34;%
END
OPTIONS? N
DATA FOR TOP OF GC PEAK?Y
 21 HITS
PRINT SIM. INDEX?Y
POLYCHLORINATED BIPHENYL (AROCLOR 1254-6) (SEWL) 324 C12-H5-CL5 BSR 01
66
FILE KEY= 10766
SI=0.5241

POLYCHLORINATED BIPHENYL (AROCLOR 1254-9) (SEWL) 324 C12-H5-CL5 BSR 01
69
FILE KEY= 10769
SI=0.5137

POLYCHLORINATED BIPHENYL (AROCLOR 1254-7) (SEWL) 324 C12-H5-CL5 BSR 01
67
FILE KEY= 10767
SI=0.5125

PCB (JA04C,53,251,1970,1254III,PK6) 324 C12-H5-CL5 BSR 0069
FILE KEY= 10669
SI=0.5099

POLYCHLORINATED BIPHENYL (AROCLOR 1254-8) (SEWL) 324 C12-H5-CL5 BSR 01
68
FILE KEY= 10768
SI=0.4785
```

Table 4. MS Library Search for
6 Cl - PCBs in the Milwaukee
Lake Trout Composite Sample

```
SAMPLE ID?
      230
PAPER TAPE?Y
FN= DWK:LM-1,230/LM:%
TT= LAKE MICH-1;%
144,50;145,81;146,42;147,28;162,10;169,10;183,27;184,30;193,7;76;%
206,8;218,85;220,60;254,43;256,41;255,13;288,84;290,100;324,30;7/;%
326,78;328,30;329,14;360,95;362,80;43;%
END
OPTIONS? N
DATA FOR TOP OF GC PEAK?Y
 10 HITS
PRINT SIM. INDEX?Y
POLYCHLORINATED BIPHENYL (AROCLOR 1254-15) (SEWL) 358 C12.H4.CL6 MSR 0
172
FILE KEY= 10772
SI=0.5394

POLYCHLORINATED BIPHENYL (AROCLOR 1254-13) (SEWL) 324 C12.H5.CL5 MSR 0
171
FILE KEY= 10771
SI=0.3632

POLYCHLORINATED BIPHENYL (AROCLOR 1254-17) (SEWL) 358 C12.H4.CL6 MSR 0
173
FILE KEY= 10773
SI=0.2792

POLYCHLORINATED BIPHENYL (AROCLOR 1254-5) (SEWL) 324 C12.H5.CL5 MSR 01
65
FILE KEY= 10765
SI=0.2365

5,8-DITHIAINDENO(2,1-C)FLUORENE 290 C18.H10.S2 APM 0139
FILE KEY=  6143
SI=0.0726
```

MONS 203772

The use of computer library searches, however, should be used only to assist the analyst and cannot be regarded as a strict confirmation of a particular chemical. Not only may the computer library searches produce misleading information, but also useful data may be lost. For example, Table 5 represents searches for spectra 167 and 208 in the sample from Manitoboc (the search for spectrum 147 was aborted due to a lack of a base peak.) Neither of these searches produced useful results due to the fact that the spectra are abbreviated to eliminate interfering peaks from hydrocarbons. However, inspection of the data in Table 5 or in Figure 15 reveals the presence of 5-Cl and 6-Cl PCBs by respective molecular ions.

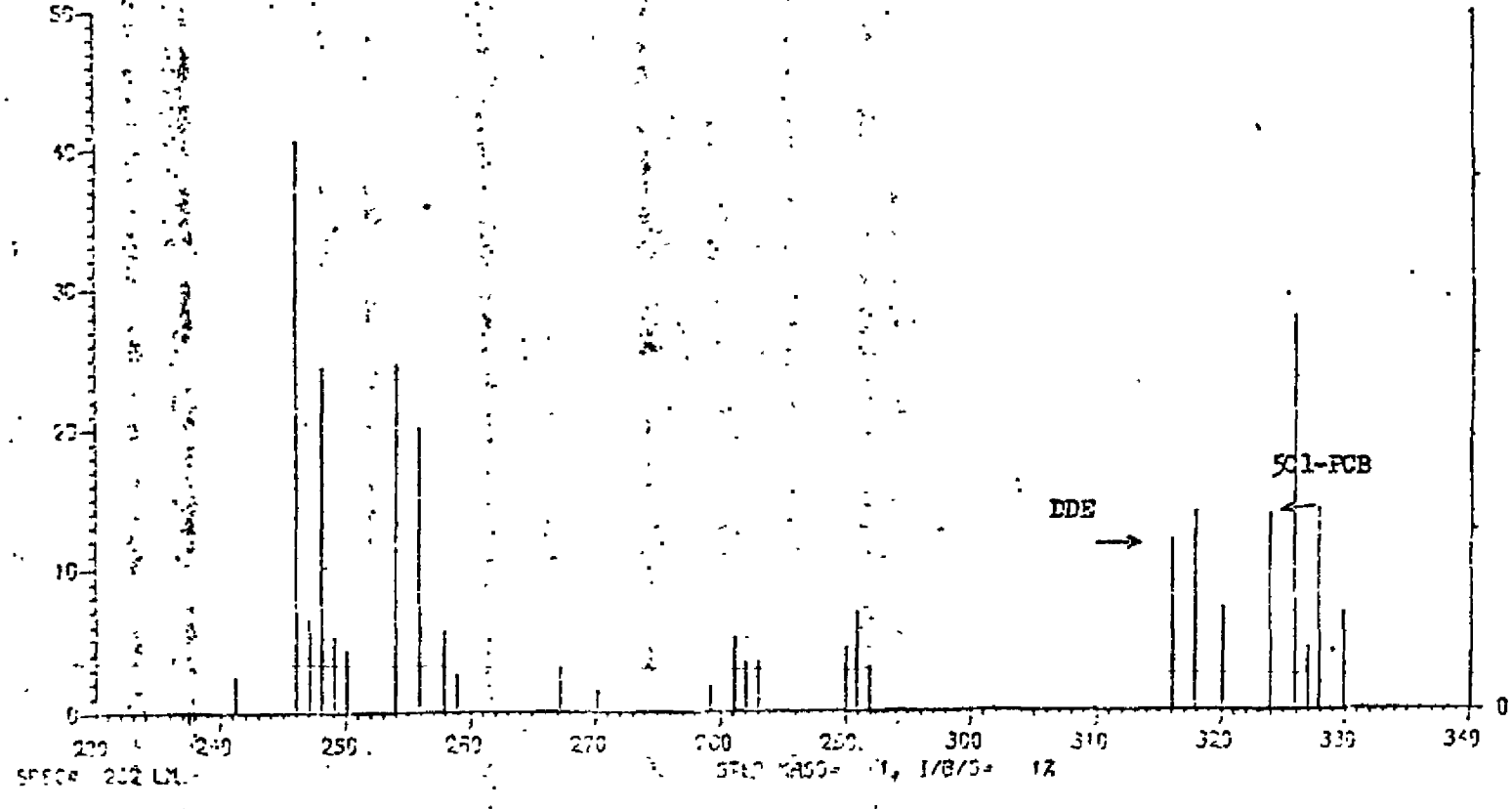
Moreover, computer library searches are of limited value in detecting minor components evident in mixed spectra obtained when several compounds elute simultaneously from the GLC. Figure 15 is a spectrum of a pentachlorobiphenyl (M^+ = 324) in which the molecular ion for DDE at m/e = 316 is observed. This is expected since the PCBs which elute with DDE contain five chlorines and are commonly found as evidenced by Figures IIIB and VIB. As discussed previously, however, the presence of DDE in some of the pentachlorobiphenyl components did not introduce an error in the quantitative analysis because the doublet (5-Cl ions) arising at 98 and 104 relative to DDE were omitted from the calculations.

Table 5 MS Library Search for Spectra 167 and 208 of the Manitowoc Lake Trout Composite Sample.

```
SAMPLE ID?
      167
PAPER TAPE?Y
FN= DMK:LM-4,167/LM:Z
TT= LAKE MICH-4:Z
146,3:149,8:163,4:185,16:207,4:218,4:219,5:254,19:256,18:71:Z
258,7:289,4:291,8:324,13:326,22:328,13:330,4:55:Z
END
OPTIONS? N
NO HITS
S, E, OR P?S
SAMPLE ID?
167
PAPER TAPE?Y
FN= DMK:LM-4,146/LM:Z
TT= LAKE MICH-03Z
149,10:150,15:184,5:185,5:220,30:222,19:256,2:257,2:290,25:73:Z
292,30:7:Z
END
UNDEC. INPT: CHAR= ABORT
S, E, OR P?S
SAMPLE ID?
      167
PAPER TAPE?Y
FN= DMK:LM-4,167/LM:Z
TT= LAKE MICH-4:Z
146,3:149,8:163,4:185,16:207,4:218,4:219,5:254,19:256,18:71:Z
258,7:289,4:291,8:324,13:326,22:328,13:330,4:55:Z
END
OPTIONS? N
NO HITS
S, E, OR P?S
SAMPLE ID?
      208
PAPER TAPE?Y
FN= DMK:LM-4,208/LM:Z
TT= LAKE MICH-4:Z
183,3:184,6:218,5:220,3:254,9:256,6:258,3:286,5:290,6:66:Z
324,7:326,12:328,6:330,3:360,6:362,5:45:Z
END
OPTIONS? N
NO HITS
```

MONS 203774

Figure 15 Mass Spectrum 282 of the Manitowoc Lake Trout Sample Illustrating the Presence of Both DDE and a Pentachlorobiphenyl in the Peak



MONS 203775

C. Other Confirmations

Several composite samples were prepared from the methylene chloride fractions of the silicic acid separation, and the samples were examined by GC/MS. Figure 16 shows the "total"-ion-current chromatograph for the extract of trout from Michigan City, Indiana. The detection of components of this mixture is enhanced by plotting the sum of ion intensities for each spectrum as illustrated in Figure 17. Many of the components appear to be materials with considerable hydrocarbon characteristics--presumably triglycerides. The contribution of these components to the mass chromatogram is removed by considering only the sum of ion intensities within the range of m/e 150 to 400 as plotted in Figure 18. All of the components which eluted prior to spectrum 115 are trichlorobiphenyls which are separated from the heavier PCBs on silicic acid and appear in the fraction with DDT. The retention volume of DDE, DDD, and DDT isomers in the temperature program used corresponds to the region between spectrum 120 and 160. The presence of these chlorinated hydrocarbons in the extract is seen in Figure 19 which are mass chromatograms of m/e 246 and 235. The large peak at spectrum 126 is DDE, op'DDD appears at 130, DDT appears at 152, and pp'DDD and op'DDT elute as a partially resolved doublet near spectrum 139.

One of the major concerns in determining the quantity of DDE or DDT in the samples has been the possibility that the PCBs interfere with the analyses, giving high results. Although the routine checks of the silicic acid separation procedure indicated the DDT and DDD isomers were completely separated from the interfering PCBs, the composite of the methylene chloride fractions were also analyzed for molecular ions of the PCBs. In general, ions which arise from the 5, -6- and 7-Cl PCBs were low and insignificant. For example, Figures 20a and b are mass chromatograms which have been plotted as absolute intensities with no scale expansion. Figure 20c is the mass chromatogram for pentachlorobiphenyls which has been expanded tenfold. Even though the ion intensities cannot be compared in the strict sense to give relative masses of the two compounds in the mixture, the data indicates that PCB interference in the DDT analysis is less than 10 percent and likely insignificant after silicic acid separation in these composite samples.

Figure 17. Sum of Ion Intensity Mass Chromatogram for Green Bay Trout Extract

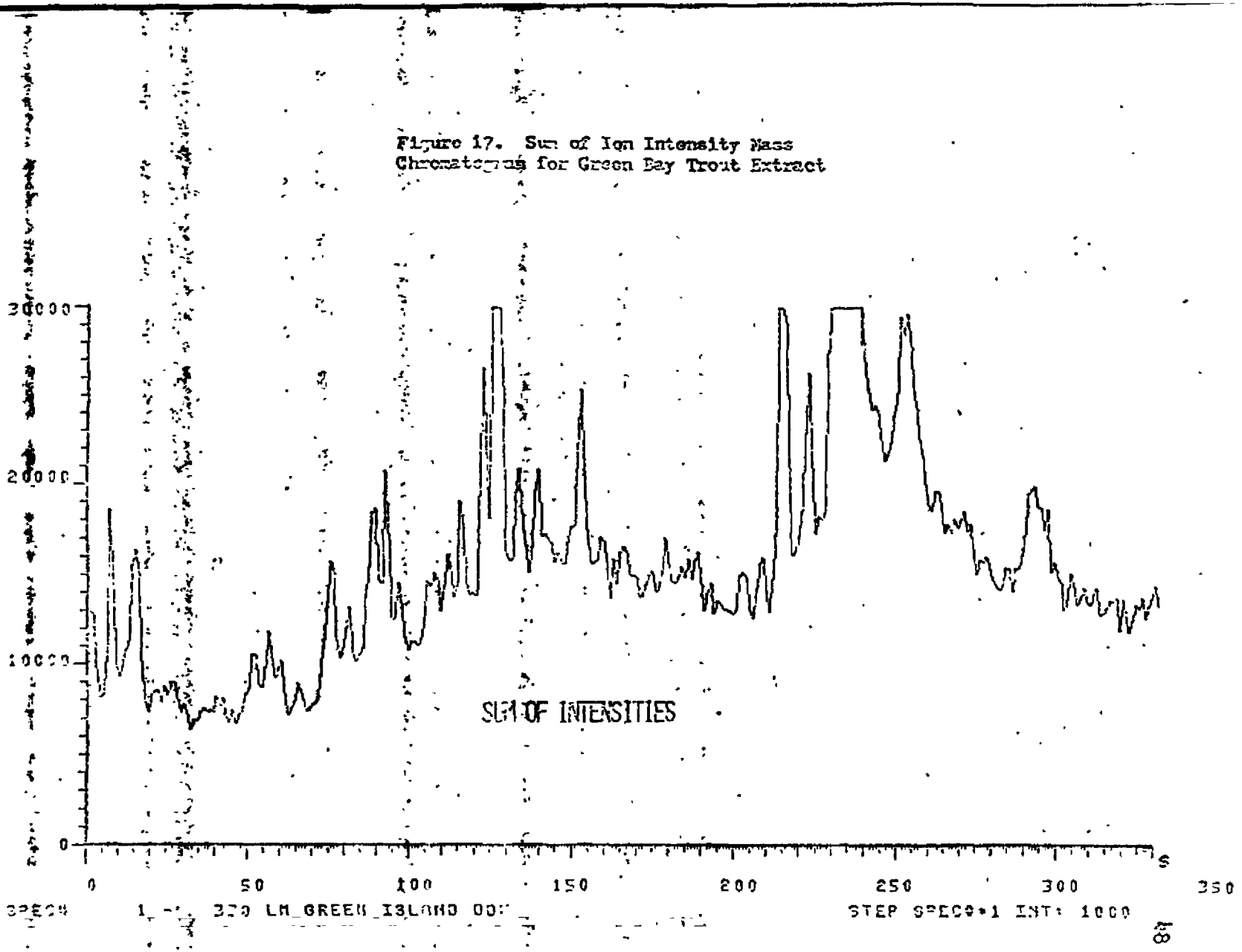


Figure 18. Mass Chromatogram for Masses 150-400
for the Green Island Trout Extract

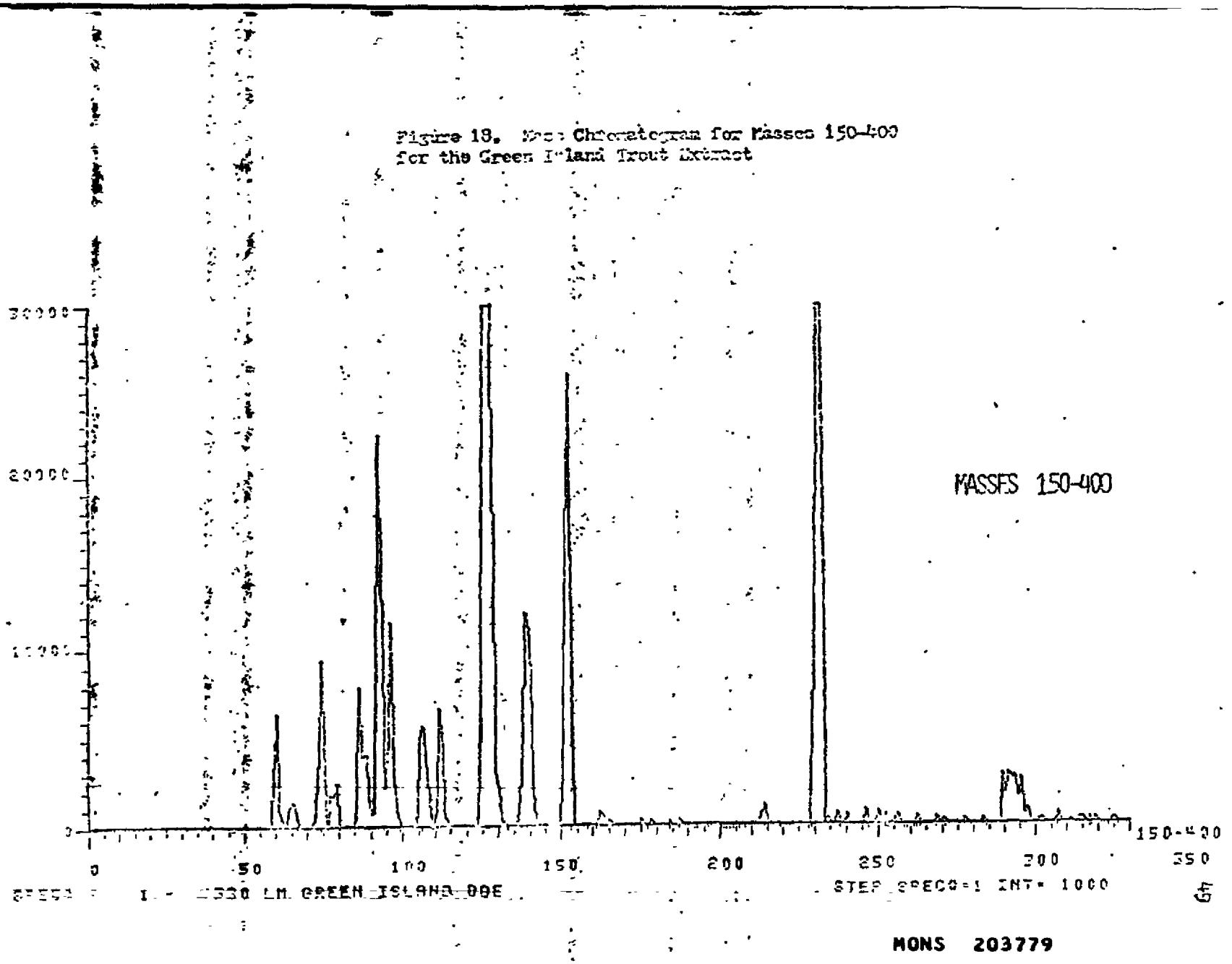
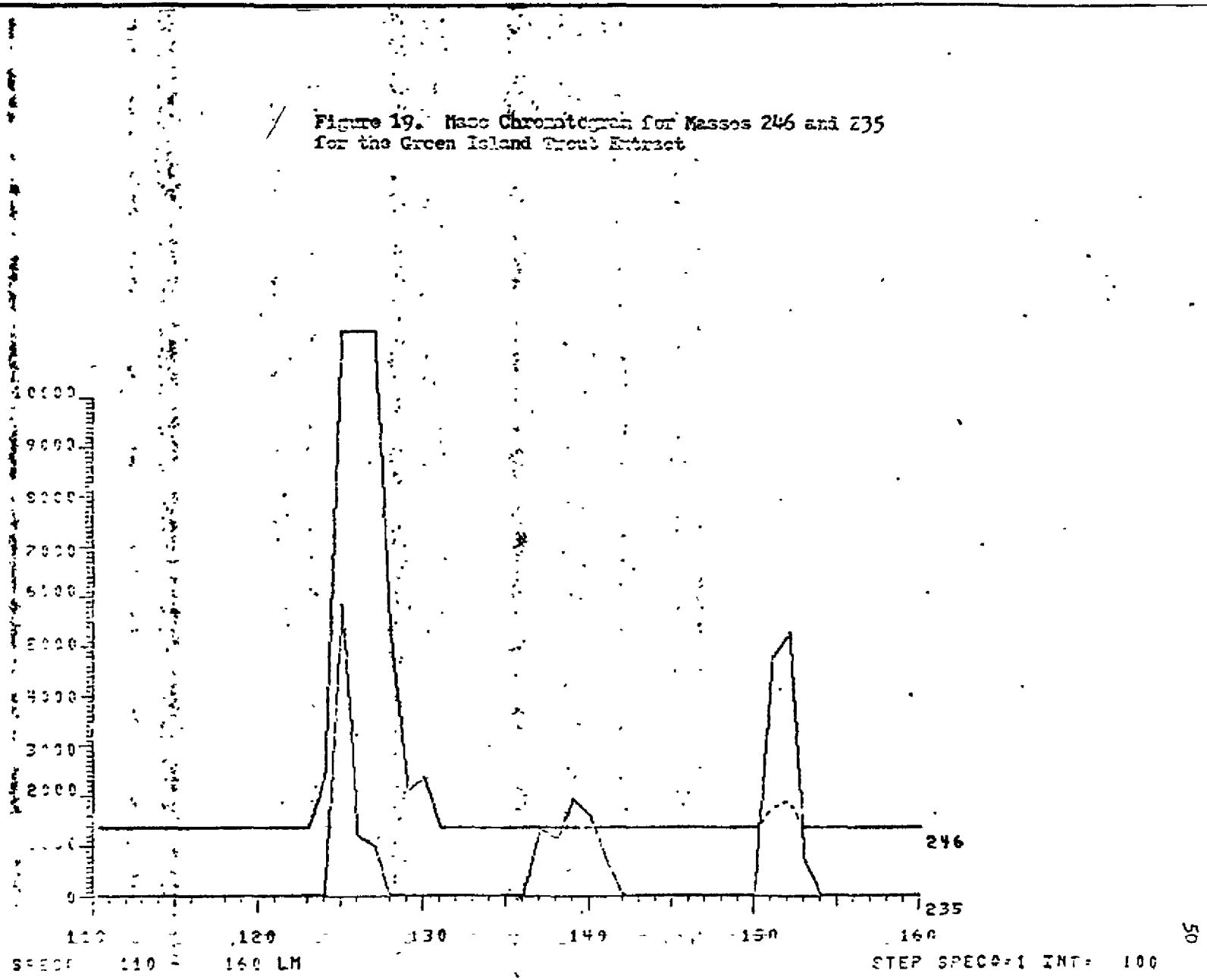
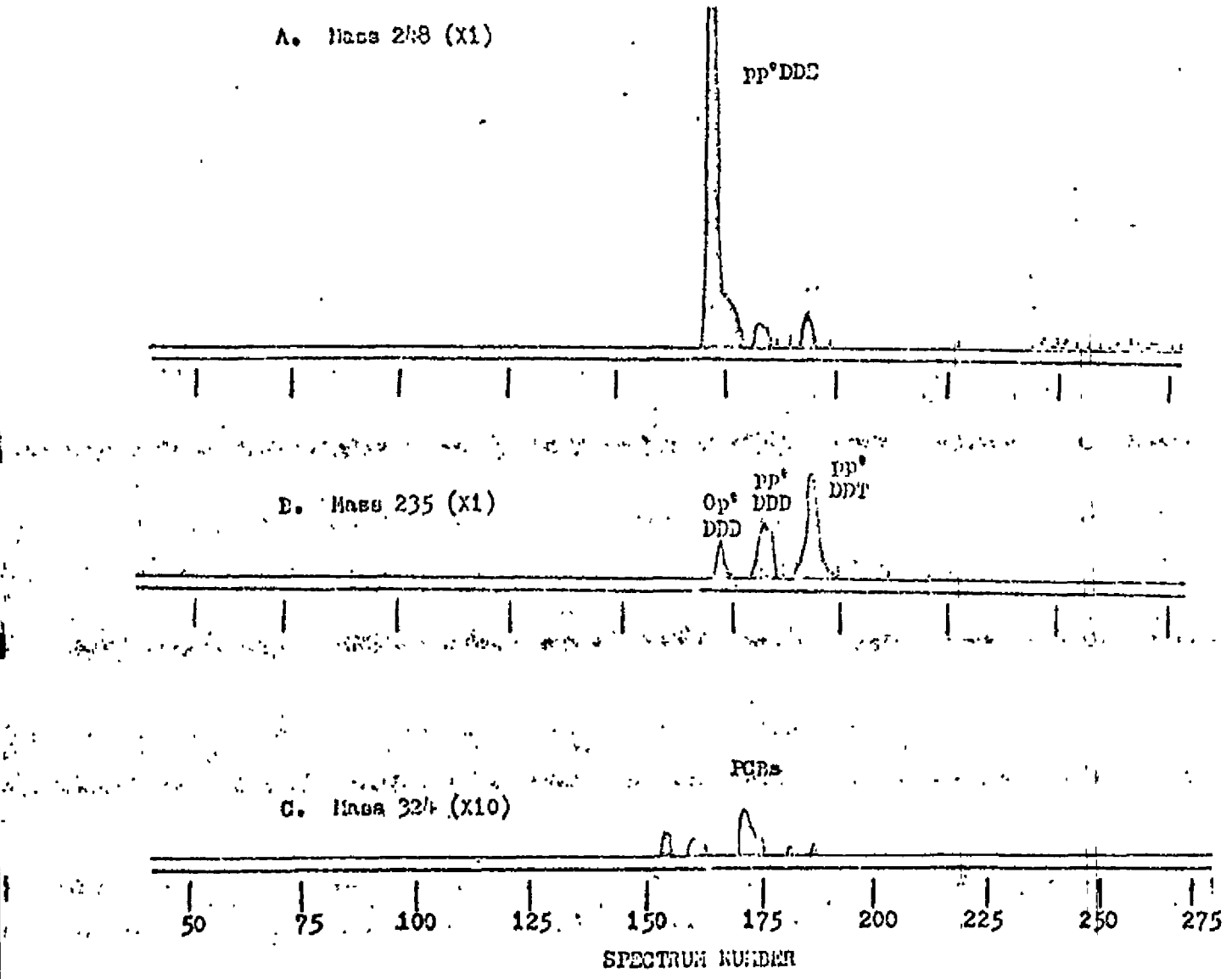


Figure 19. Mass Chromatogram for Masses 246 and 235 for the Green Island Trout Extract



MONS 203780

Figure 20 Selected Mass Chromatograms of the GC/MS Analysis of the DDE Fraction



MONS 203781

The major component eluting in spectrum 230 in Figure 18 has not been identified as yet. However, it contains no chlorine and appears to arise from the lipids. Figure 13 has indicated that a number of major components elute early in the chromatogram and that they have been identified as saturated, linear hydrocarbons. The predominant component eluting in spectrum 47 in Figure 13 has been identified as n-heptadecane with the substantiating data including mass spectra as in Figure 21, retention time, and bromination and related chemical reactivity studies. The presence of the C_{12}, C_{13}, and C_{17} hydrocarbons at comparatively high concentrations without the presence of C_{14}, C_{15}, and C_{16} hydrocarbons suggest that the hydrocarbons may be due to metabolic activities within the food chain rather than contamination of the waters by petroleum products. Considerable more research is needed to determine the source of these hydrocarbons.

The qualitative analysis also indicated the presence of several phthalate esters in the fish from Lake Michigan. In general, the di-n-butyl, a dihexyl, and a dioctyl (presumably di-2-ethylhexyl) phthalate were found, although some fish had lesser or undetectable quantities of the dihexylphthalate. The presence of these chemicals are readily detected by plotting the mass chromatogram for m/e 149, since m/e 149 is a predominant ion in the phthalate ester fragmentation. However, the low abundance of ions above 149 precludes explicit identification of the phthalate and GLC retention volume data were used to make the assignments presented here. The dihexyl-phthalate was not detected by electron capture GLC.

Also detected in some extracts was a dioctyl adipate. Figure 23a illustrates a m/e 147 mass chromatogram which is somewhat specific for the adipate esters. Although the adipate was found in the Green Bay trout extract and several others, no evidence was found for adipates in the trout from Michigan City and Saugatuck regions as evidenced by Figure 23b. A spectrum of the dioctyl-adipate from the Sheboygan trout extract is presented in Figure 24. The abundant m/e 147 ion is apparent; however, like the phthalates, the ion abundances in the parent ion region are low in electron impact spectra. The base peak at m/e 129 as well as the other major ions are often obscured by interference co-eluting from the column.

MONS 203782

Figure 21. Mass Spectrum of Heptadecane in Michigan City Trout Extract

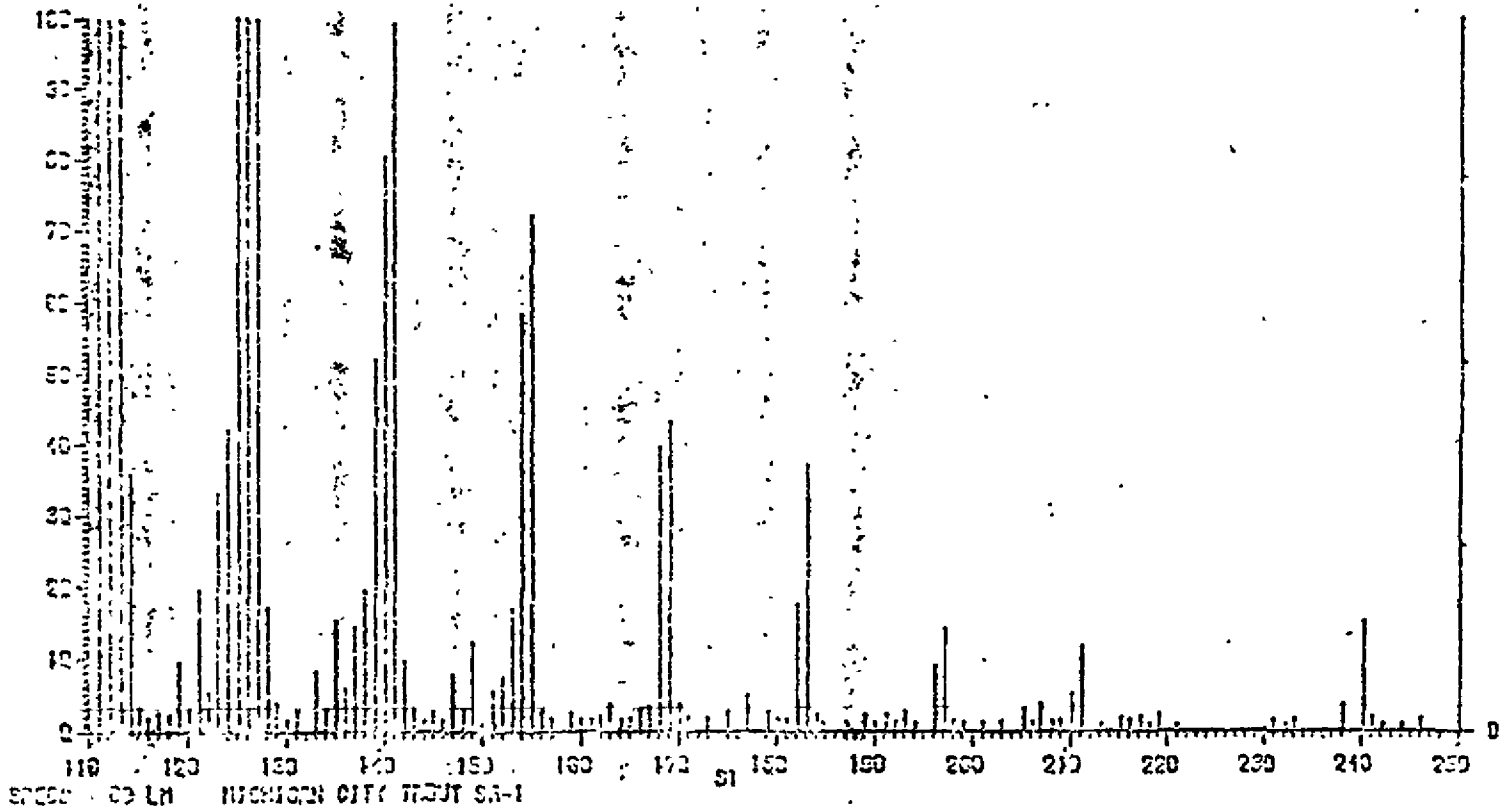
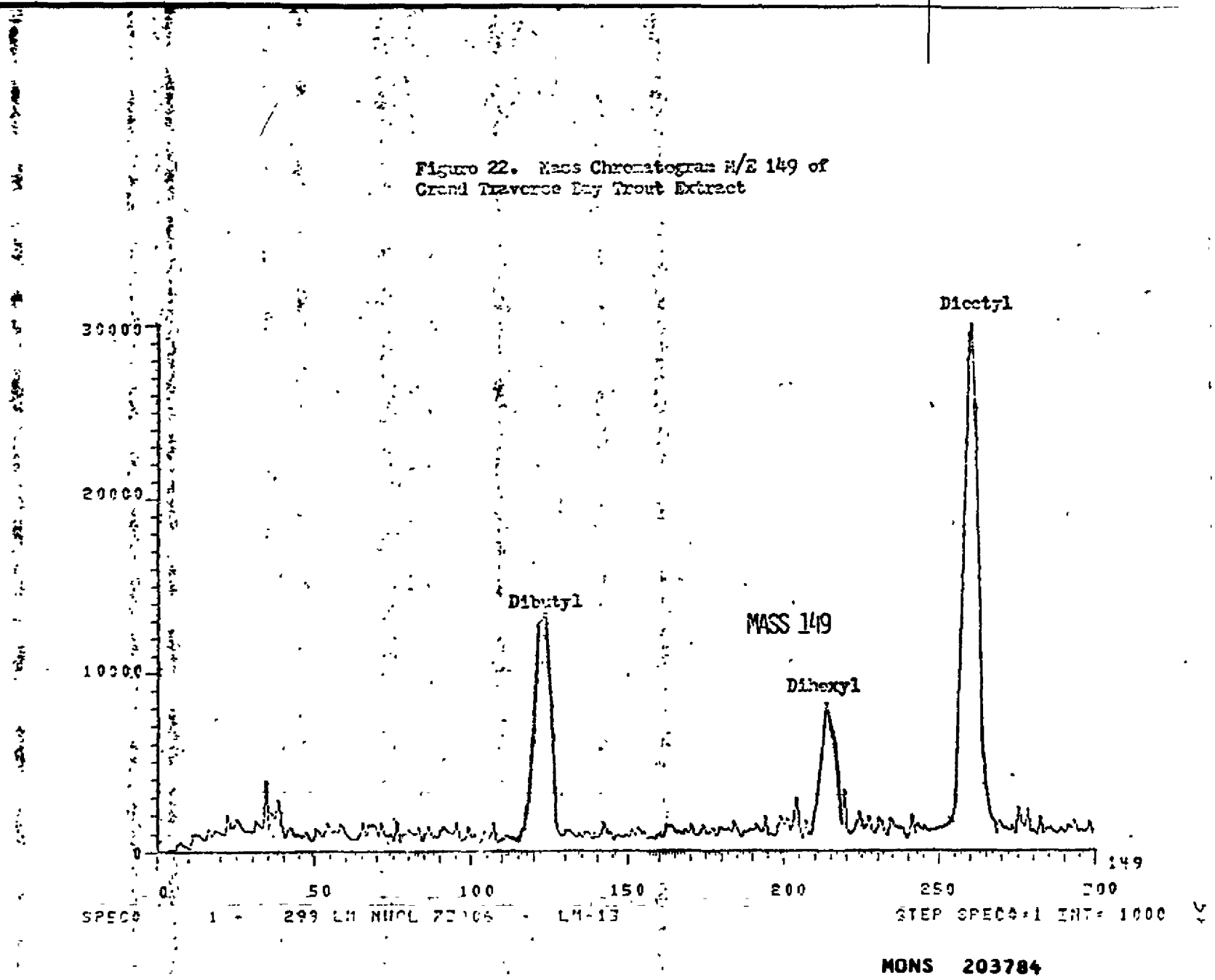
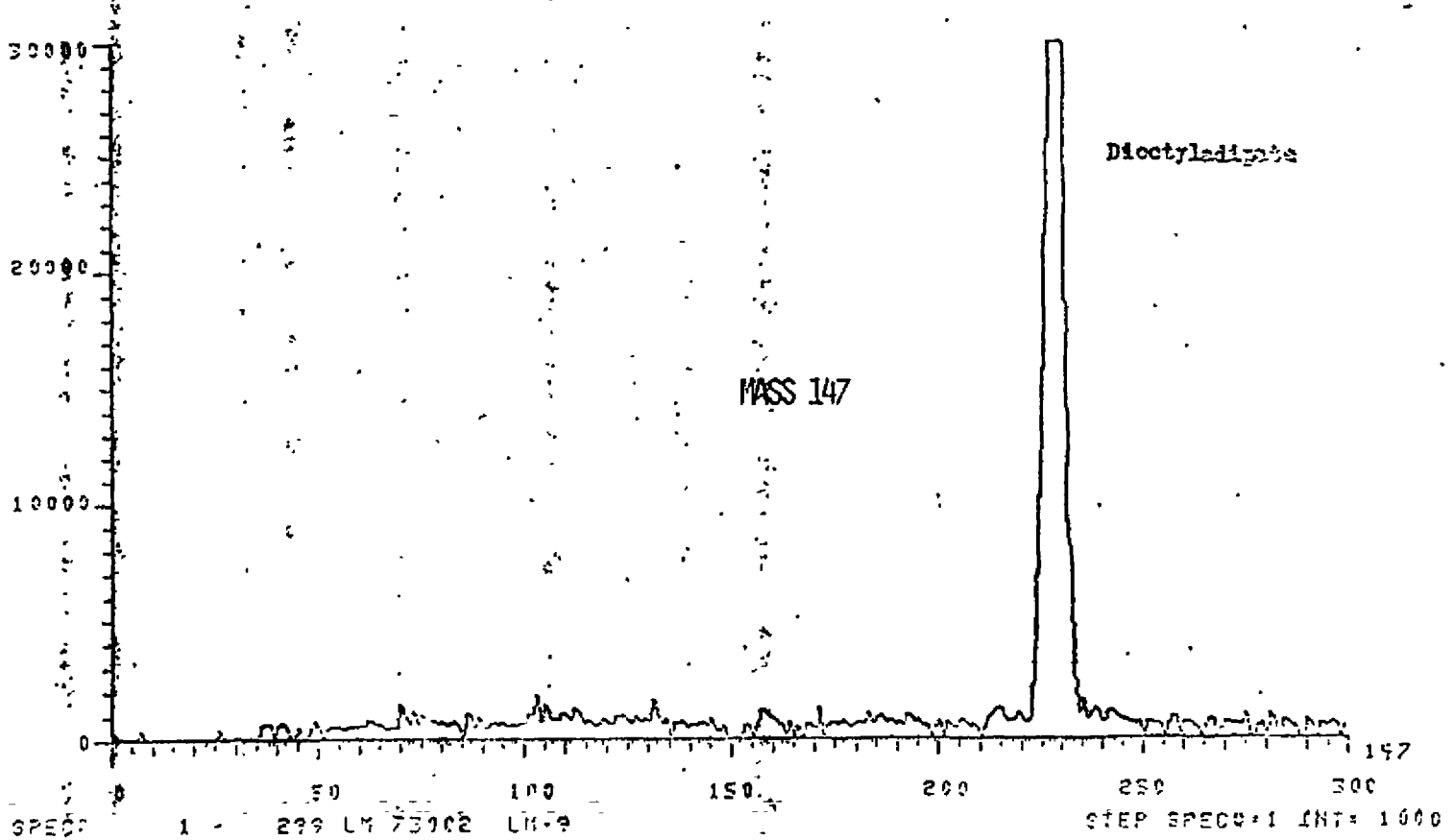


Figure 22. Mass Chromatogram M/E 149 of Grand Traverse Bay Trout Extract



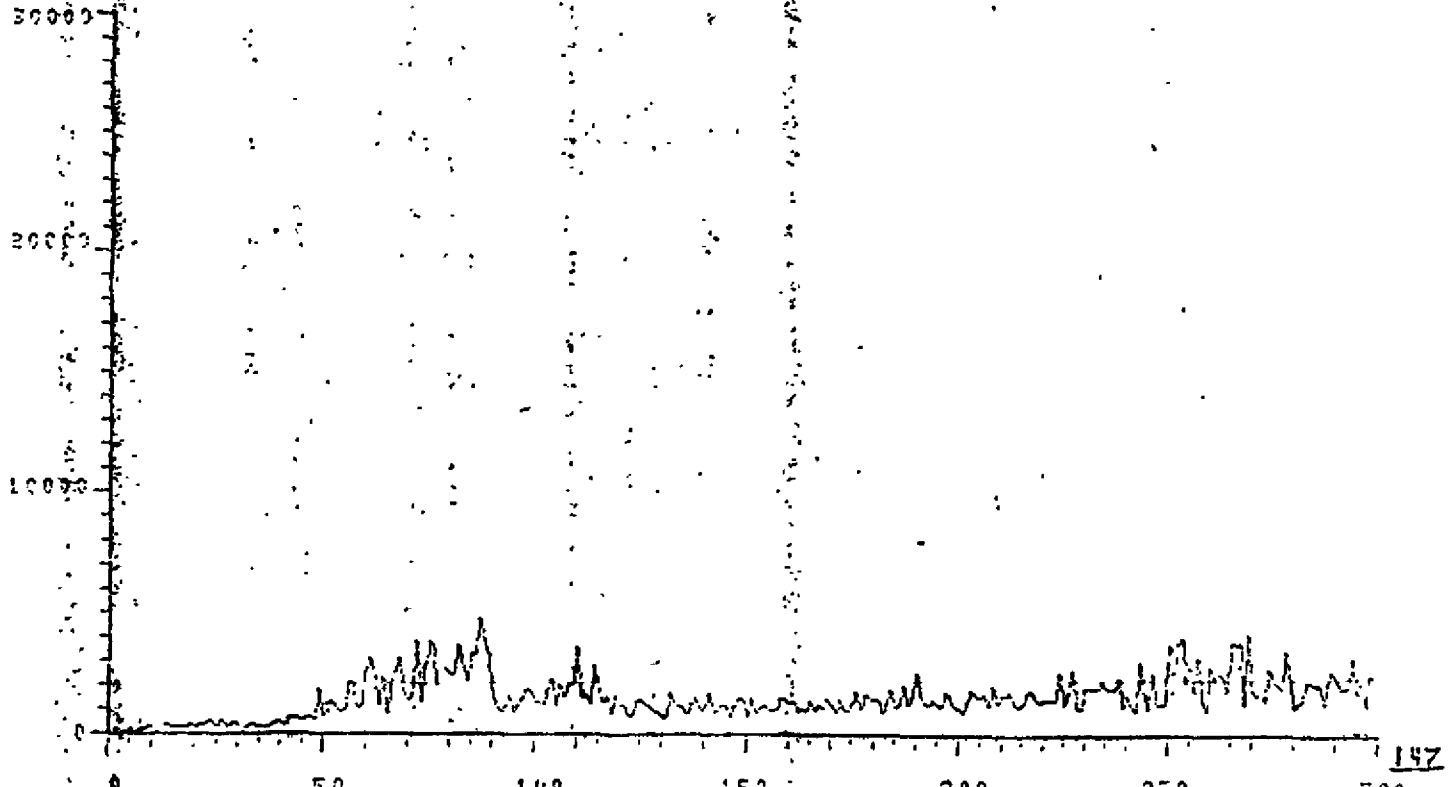
MONS 203784

Figure 23a. Mass Chromatogram M/E 147
for Adipates in Green Bay Trout



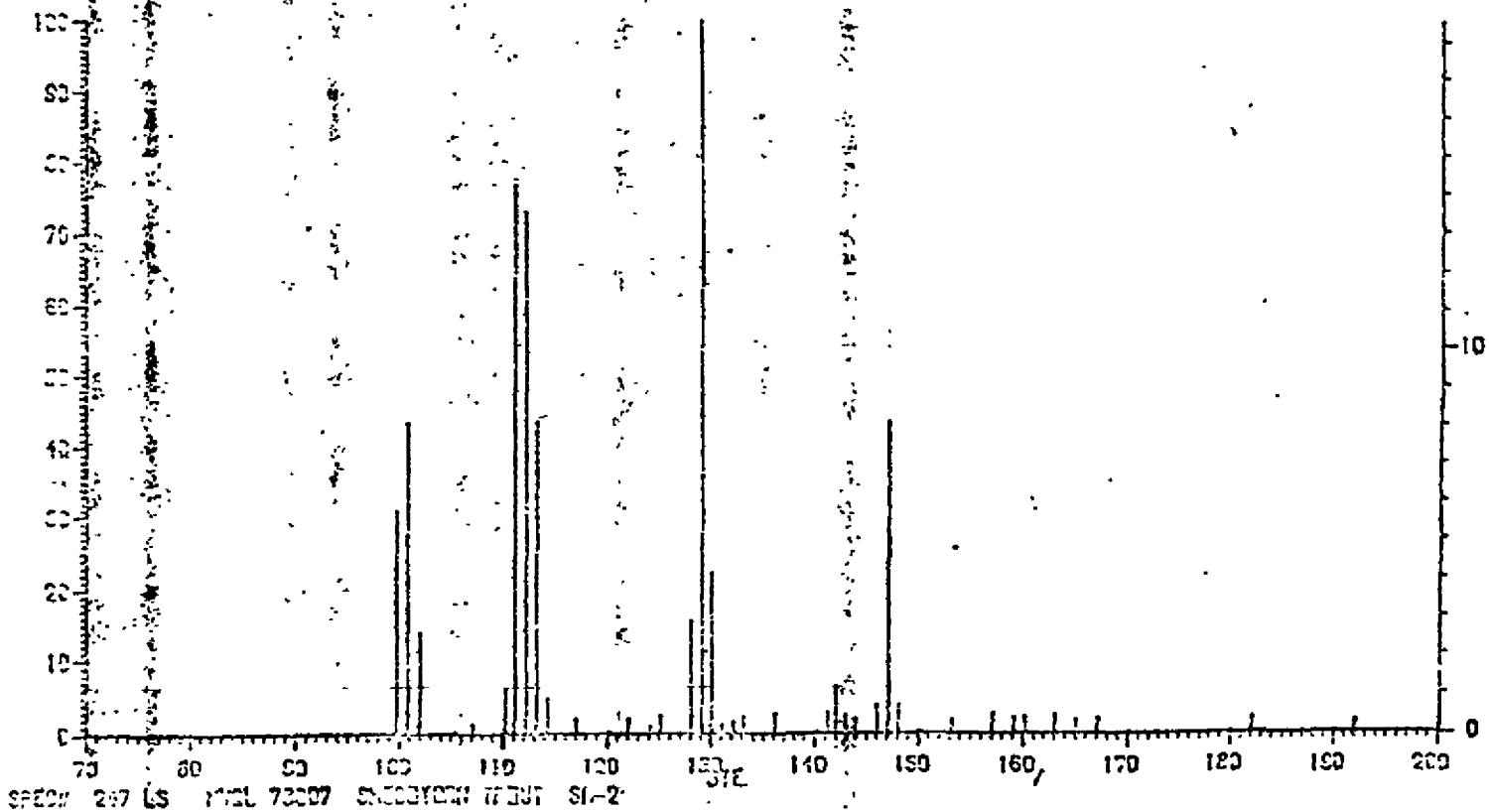
MONS 203785

Figure 23b. Mass Chromatogram M/S 147
for Adipates in Michigan City Trout



MONS 203786

Figure 24. Mass Spectrum of Dioctyladipate Isolated from Trout Captured Near Sheboygan

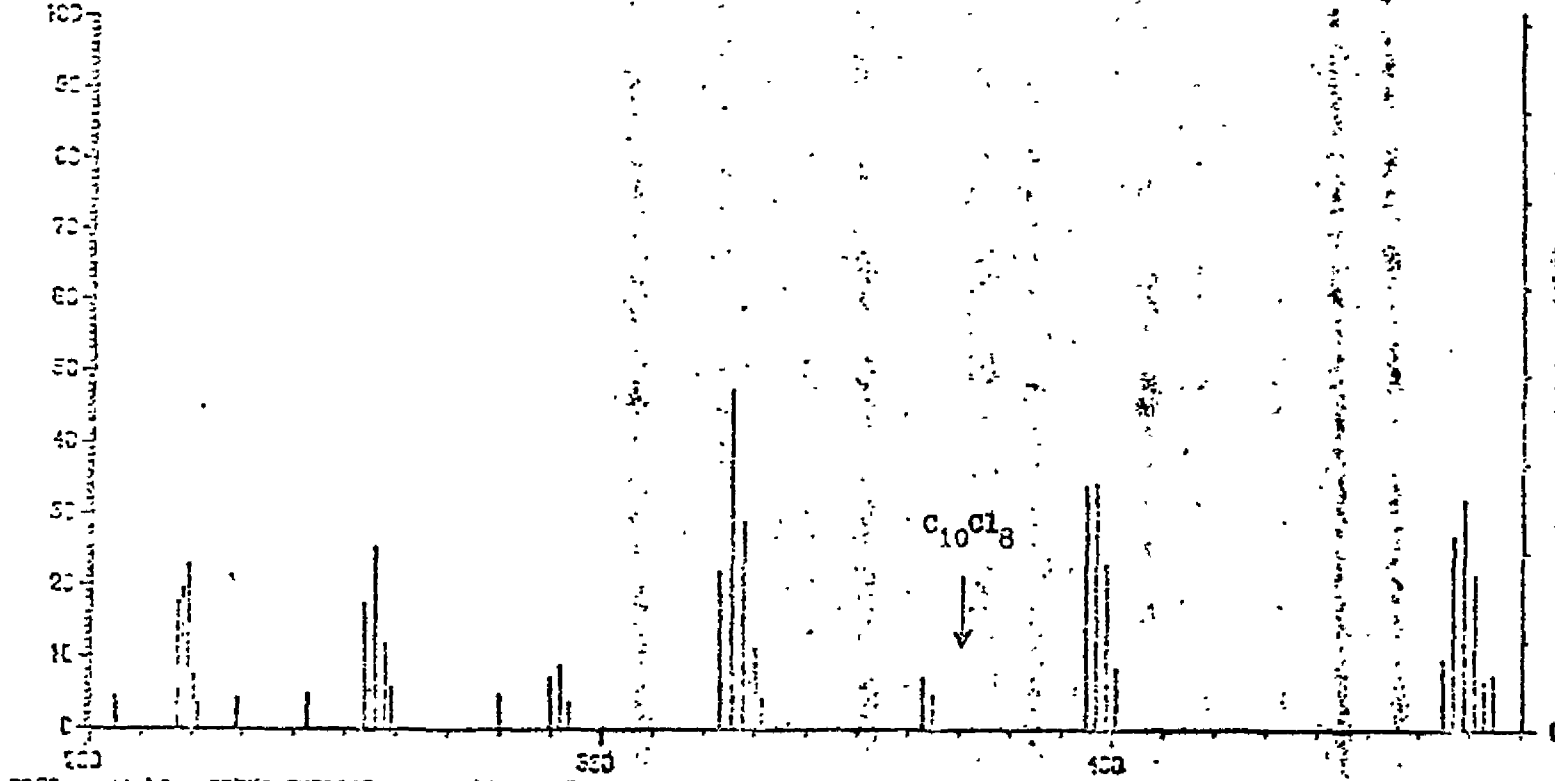


It must be emphasized that the elution behavior for phthalate esters and related esters do not behave as the pesticides on column chromatography and "quantitation" of these compounds must be considered estimates only.

The search for other potentially toxic chemicals in Lake Michigan fish is inherently open-ended. No evidence of lindane, hexachlorobenzene, chlordane, or other chlorinated pesticides has been found. However, the difficulty of obtaining explicit confirmation for dieldrin which is likely and often reported at concentrations of 0.3 ug/gm in the larger fish as well as spiked samples indicates that the GC/MS techniques employed may have to be refined for confirmations of minor components which may be present below approximately 0.1 ug/gm.

Of particular interest is the search for the chlorinated naphthalenes which may interfere with the PCB determination, if present. In all of the extracts analyzed by GLC and GC/MS, there was no evidence of the chlorinated naphthalenes. To further examine the extracts, the extracts were perchlorinated, and the products were analyzed by direct probe mass spectrometry. Figure 25 presents a spectrum in which the ions arising from decachlorobiphenyl are readily apparent. However, there is no evidence of ions at m/e 400 which is the parent ion region for octachloronaphthalene which results from the perchlorination. Sensitivities studies with this technique leads to an estimated detection limit of approximately 0.01 ug/gm.

Figure 25. Direct-Probe MS Analysis of the Perchlorination Products of a Lake Michigan Trout Extract



SECTION VI

RESULTS AND DISCUSSION

This section of the report presents data regarding the concentrations of PCBs and DDT (and metabolites) in the Lake Michigan fish collected in this study. Also presented are preliminary data regarding concentrations of two of the phthalate esters in the Lake Michigan fish.

A. Polychlorinated Biphenyl

Approximately 850 of the fish collected from Lake Michigan were analyzed for PCB mixtures resembling that of Aroclor 1254. A generalized summary of these results is presented in Table 6 in which the range and average concentrations of PCBs (as Aroclor 1254) is reported for 13 species of fish. The mean concentrations ranged from 2.7 µg/gm in smelt to 15 µg/gm in lake trout. The data show that the larger fish such as brown, lake and walleye trout and chinook and coho salmon contained PCBs at concentrations two to three times the 5 µg/gm action level established by the FDA. The mean PCB concentrations in suckers, smelt and whitefish were considerably less than 5 µg/gm and the mean concentrations in the alewife, carp, chub and perch were approximately 5 µg/gm, ranging from 4.2 to 6.0 µg/gm. As is expected, the larger fish with greater percentage fat contain greater concentrations of PCBs.

Table 6 also indicates that the mean concentration of DDT (total DDT defined as the sum of p,p'DDT, o,p'DDT, p,p'TDE, and p,p'DDE) in Lake Michigan fish ranged from 1.2 ug/gm in carp and smelt to 7.1 ug/gm in lake trout. As was found in the analyses of PCBs in these fish, the fish containing higher lipid concentrations contained greater concentrations of DDT.

Table 7 summarizes more detailed data collected for the 13 species of fish captured in Lake Michigan. The data show that the ratio of PCB/TotalDDT (a ratio often calculated and cited but not often applicable) ranged from 1.3 in red suckers which were caught primarily in the northern waters to 7.6 in carp. Of particular interest are the facts that the ratio of PCB/DDT in the majority of the fish was between 1.7

Table 6. Summary of PCB and Total DDT Concentrations In 1971 Lake Michigan Fish

Species	Numbers of Fish Analyzed	PCB's Mean	PCB's S.D.	Total Mean	Total S.D.
Alewife	85	4.6	2.1	2.2	1.1
Brown Trout	17	7.3	2.8	4.2	1.6
Carp	42	4.2	3.6	1.2	0.9
Chub	287	6.0	2.2	3.8	2.0
Chinook Salmon	21	11.4	4.0	6.8	2.5
Coho Salmon	56	11.5	5.7	6.3	2.8
Lake Trout	134	15.5	3.2	7.1	3.7
Perch	44	5.8	3.5	1.6	1.1
Rainbow Trout	11	9.3	4.1	4.2	1.8
Red Sucker	16	3.0	0.7	2.6	0.7
Smelt	38	2.7	1.3	1.2	0.6
White Sucker	51	3.9	3.6	1.6	1.2
Whitefish	43	3.0	1.9	1.4	0.6

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TABLE 7

GROSS MEAN CONCENTRATIONS OF HALOGENATED HYDROCARBONS FOR LAKE MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: Alewife

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	141	.100	.035
PERCENT FAT	82	4.5	3.9
PP'DDE	45	1.7	.8
PCB	76	9.6	4.1
OP'DDT	53	.03	.04
PP'DDT	53	.5	.2
PP'DDD	53	.20	.10
TOTAL DDT	65	2.2	1.1
PCB/TOTAL DDT	76	2.9	1.9
PP'DDE/PP'DDT	53	3.6	2.5
OP'DDT/PP'DDT	53	.2	.1
PP'DDE/TOTAL DDT	65	.8	.2
OP'DDT/TOTAL DDT	53	.0	.0
PP'DDT/TOTAL DDT	53	.2	.1
PP'DDD/TOTAL DDT	53	.1	.0

GROSS MEAN CONCENTRATIONS OF HALOGENATED HYDROCARBONS FOR LAKE MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: Brown Trout

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	13	3.494	1.066
PERCENT FAT	17	15.5	9.1
PP'DDE	17	2.7	1.0
PCB	17	2.3	2.8
OP'DDT	17	.16	.05
PP'DDT	17	1.1	.3
PP'DDD	17	.49	.11
TOTAL DDT	18	4.2	1.6
PCB/TOTAL DDT	17	1.8	.9
PP'DDE/PP'DDT	15	2.6	1.0
OP'DDT/PP'DDT	17	.1	.0
PP'DDE/TOTAL DDT	17	.6	.2
OP'DDT/TOTAL DDT	17	.0	.0
PP'DDT/TOTAL DDT	17	.3	.1
PP'DDD/TOTAL DDT	17	.1	.0

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TABLE 7

GROSS YEAR CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE-MICHIGAN FISH CAUGHT IN FALL,1971.

SPECIES: CARP

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	51	2.163	1.998
PERCENT FAT	51	10.0	7.0
PP'DDE	49	.7	.9
PCB	42	4.2	3.8
OP'DDT	15	.05	.08
PP'DDT	12	.2	.2
PP'DDD	30	.20	.27
TOTAL DDT	49	.9	1.2
PCB/TOTAL DDT	40	7.6	9.7
PP'DDE/PP'DDT	12	6.5	9.0
OP'DDT/PP'DDT	12	.3	.1
PP'DDE/TOTAL DDT	49	.6	.2
OP'DDT/TOTAL DDT	19	.0	.0
PP'DDT/TOTAL DDT	12	.1	.1
PP'DDD/TOTAL DDT	30	.3	.1

GROSS FILET CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE-MICHIGAN FISH CAUGHT IN FALL,1971.

SPECIES: Chinook Salmon

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	22	3.137	.876
PERCENT FAT	21	5.0	3.9
PP'DDE	22	5.2	1.5
PCB	21	11.4	7.0
OP'DDT	18	.21	.11
PP'DDT	18	1.3	.9
PP'DDD	18	.52	.32
TOTAL DDT	22	6.8	2.5
PCB/TOTAL DDT	21	1.7	.6
PP'DDE/PP'DDT	16	5.3	3.7
OP'DDT/PP'DDT	18	.2	.0
PP'DDE/TOTAL DDT	22	.8	.1
OP'DDT/TOTAL DDT	18	.0	.0
PP'DDT/TOTAL DDT	16	.2	.1
PP'DDD/TOTAL DDT	18	.1	.0

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TABLE 7

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: CHUB

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	175	.249	.270
PERCENT FAT	249	20.0	5.9
PP'DDE	277	2.5	1.1
PCB	231	6.3	2.2
OP'DDT	155	.22	.09
PP'DDT	154	1.6	.9
PP'DDD	156	.43	.20
TOTAL DDT	287	3.8	2.0
PCB/TOTAL DDT	224	2.2	2.2
PP'DDE/PP'DDT	145	1.9	3.3
OP'DDT/PP'DDT	155	.2	1.3
PP'DDE/TOTAL DDT	277	.7	.3
OP'DDT/TOTAL DDT	155	.0	.0
PP'DDT/TOTAL DDT	154	.4	.1
PP'DDD/TOTAL DDT	156	.1	.0

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: COHO Salmon

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	50	3.729	1.235
PERCENT FAT	62	6.5	2.1
PP'DDE	62	4.8	2.3
PCB	56	11.5	5.7
OP'DDT	51	.20	.08
PP'DDT	51	1.3	.7
PP'DDD	51	.49	.16
TOTAL DDT	63	6.3	2.8
PCB/TOTAL DDT	56	2.1	1.3
PP'DDE/PP'DDT	50	4.1	2.1
OP'DDT/PP'DDT	51	.2	.1
PP'DDE/TOTAL DDT	62	.7	.2
OP'DDT/TOTAL DDT	51	.0	.0
PP'DDT/TOTAL DDT	51	.2	.1
PP'DDD/TOTAL DDT	51	.1	.0

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TABLE 7

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: Fourhorn Sculpin

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	13	.020	.007
PERCENT FAT	13	5.9	.9
PP'DDE	13	1.9	.6
PCB	12	2.7	1.1
OP'DDT	9	.05	.04
PP'DDT	9	.2	1.0
PP'DDD	9	.15	.09
TOTAL DDT	13	2.9	3.7
PCB/TOTAL DDT	12	1.4	.6
PP'DDE/PP'DDT	9	1.5	.4
OP'DDT/PP'DDT	9	.1	.0
PP'DDE/TOTAL DDT	13	.7	.2
OP'DDT/TOTAL DDT	9	.0	.0
PP'DDT/TOTAL DDT	9	.4	.1
PP'DDD/TOTAL DDT	9	.1	.0

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: Lake Trout

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	58	1.617	1.3
PERCENT FAT	153	16.6	5.3
PP'DDE	142	5.0	2.8
PCB	139	15.5	12.3
OP'DDT	122	.26	.19
PP'DDT	122	2.0	1.0
PP'DDD	122	.63	.4
TOTAL DDT	149	7.1	3.7
PCB/TOTAL DDT	132	2.5	5.9
PP'DDE/PP'DDT	115	3.1	3.9
OP'DDT/PP'DDT	122	.2	.4
PP'DDE/TOTAL DDT	142	.7	.2
OP'DDT/TOTAL DDT	122	.0	.0
PP'DDT/TOTAL DDT	122	.3	.3
PP'DDD/TOTAL DDT	122	.1	.0

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TABLE 7

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: Perch

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	57	.146	.046
PERCENT FAT	46	6.1	1.7
PP'DDE	44	1.0	.6
PCB	44	5.6	3.5
OP'DDT	27	.08	.04
PP'DDT	27	.7	.7
PP'DDD	29	.24	.14
TOTAL DDT	47	1.6	1.1
PCB/TOTAL DDT	44	4.8	3.7
PP'DDE/PP'DDT	29	3.0	3.8
OP'DDT/PP'DDT	24	.1	.0
PP'DDE/TOTAL DDT	44	.8	.2
OP'DDT/TOTAL DDT	27	.0	.0
PP'DDT/TOTAL DDT	27	.3	.2
PP'DDD/TOTAL DDT	29	.1	.1

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: Rainbow Trout

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	11	4.199	1.394
PERCENT FAT	10	16.9	3.3
PP'DDE	11	3.4	1.3
PCB	11	9.3	4.1
OP'DDT	6	.16	.06
PP'DDT	6	1.2	.1
PP'DDD	6	.35	.11
TOTAL DDT	11	4.2	1.8
PCB/TOTAL DDT	11	2.1	1.0
PP'DDE/PP'DDT	6	3.8	1.4
OP'DDT/PP'DDT	6	.2	.0
PP'DDE/TOTAL DDT	11	.8	.2
OP'DDT/TOTAL DDT	6	.0	.0
PP'DDT/TOTAL DDT	6	.2	.1
PP'DDD/TOTAL DDT	6	.1	.0

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TABLE 7

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE-MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: Red Sucker

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	16	.902	.227
PERCENT FAT	16	4.6	1.2
PP'DDE	16	1.6	.5
PCB	16	3.0	.7
OP'DDT	14	.09	.03
PP'DDT	14	.7	.3
PP'DDD	14	.33	.10
TOTAL DDT	16	2.6	.7
PCB/TOTAL DDT	16	1.3	.5
PP'DDE/PP'DDT	14	3.1	2.9
OP'DDT/PP'DDT	14	.2	.1
PP'DDE/TOTAL DDT	16	.6	.2
OP'DDT/TOTAL DDT	14	.0	.0
PP'DDT/TOTAL DDT	14	.3	.1
PP'DDD/TOTAL DDT	14	.1	.0

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE-MICHIGAN FISH CAUGHT IN FALL, 1971.

SPECIES: Smelt

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	36	.061	.024
PERCENT FAT	39	5.8	1.8
PP'DDE	41	.8	.4
PCB	38	2.7	1.3
OP'DDT	39	.09	.02
PP'DDT	39	.3	.2
PP'DDD	39	.12	.04
TOTAL DDT	42	1.2	.6
PCB/TOTAL DDT	38	2.6	1.5
PP'DDE/PP'DDT	33	2.8	1.3
OP'DDT/PP'DDT	39	.1	.0
PP'DDE/TOTAL DDT	41	.7	.2
OP'DDT/TOTAL DDT	39	.0	.0
PP'DDT/TOTAL DDT	39	.3	.1
PP'DDD/TOTAL DDT	39	.1	.0

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TABLE 7

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE-MICHIGAN FISH CAUGHT IN FALL,1971.

SPECIES: WHITEFISH

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	77	1.100	.932
PERCENT FAT	47	12.4	9.9
PP'DDE	44	.8	.3
PCB	43	3.0	1.9
OP'DDT	34	.07	.04
PP'DDT	34	.5	.2
PP'DDD	34	.25	.1
TOTAL DDT	47	1.9	.4
PCB/TOTAL DDT	43	2.8	2.5
PP'DDE/PP'DDT	31	2.1	1.4
OP'DDT/PP'DDT	34	.2	.3
PP'DDE/TOTAL DDT	44	.7	.2
OP'DDT/TOTAL DDT	34	.0	.0
PP'DDT/TOTAL DDT	34	.3	.1
PP'DDD/TOTAL DDT	34	.2	.1

GROSS MEAN CONCENTRATIONS OF MAJOR CHLORINATED HYDROCARBONS FOR LAKE-MICHIGAN FISH CAUGHT IN FALL,1971.

SPECIES: WHITE SUCKER

VARIABLE	NUMBER PRESENT	MEAN	STANDARD DEVIATION
FISH WEIGHT (KG)	32	1.131	1.405
PERCENT FAT	53	5.9	2.8
PP'DDE	54	1.0	.5
PCB	51	3.9	3.4
OP'DDT	37	.05	.04
PP'DDT	42	.5	.4
PP'DDD	45	.24	.21
TOTAL DDT	54	1.6	1.2
PCB/TOTAL DDT	51	3.4	3.5
PP'DDE/PP'DDT	42	3.8	2.5
OP'DDT/PP'DDT	37	.1	.1
PP'DDE/TOTAL DDT	54	.7	.2
OP'DDT/TOTAL DDT	37	.0	.0
PP'DDT/TOTAL DDT	42	.2	.1
PP'DDD/TOTAL DDT	45	.2	.0

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and 2.8, and that only the carp, red suckers, and perch contained ratios outside this range. This ratio may become important in future studies to determine the relative rates of elimination of the chemicals from the Lake Michigan system now that the inputs to the Lake have been decreased.

The mean ratios of o,p'DDT/p,p'DDT ranged from 0.1 to 0.3 with the ratio in the majority of the fish being 0.2. Since technical DDT generally contains about 30 percent of the o,p'DDT isomer (ratio of 0.4), the data from Lake Michigan fish suggest that the degradation and/or other removal mechanisms in the lake are slightly greater for the o,p' isomer than for the p,p' isomer.

The data also show that greater than 80 percent of the "total DDT" residue is accounted for by p,p'DDE and p,p'DDT.

Not only does the PCB concentration vary considerably among the 13 species captured, but also the range of concentrations in a single species in the lake was generally greater than 100 percent (the range in red suckers was less; however, all were captured in the same region). Although some variation in concentration is expected because of the normal analytical precision, Table 7 reveals much larger ranges which are undoubtedly due to other factors which limit the usefulness of mean concentrations presented. Previous research has shown (Reinert, 1970) that the lipid content, size of fish, season of capture and concentration in the water may affect the observed concentration of chlorinated hydrocarbons in tissue considerably. Accordingly, the data in Table 7 must be considered in more detail.

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The above summary data for PCBs is presented in Table 8 where the regional variations and variations due to lipid content are expressed. The concentration of PCBs in alewives (on a fresh weight basis) were greater in southern Lake Michigan than in the northern regions, although anomolies are apparent. It is apparent that most of the alewives captured south of a line between Saugatuck and Sheboygan contained between 4.4 and 5.5 µg/gm PCBs whereas those caught north of the line contained between 3.5 and 4.4 µg/gm. An interesting exception is the high concentration, 8.9 µg/gm, in the alewives from Rock Island just off the Door County Peninsula in Wisconsin.

The analyses of brown trout suggested similar trends in that those from Michigan City at the southern end of the lake contained 11.9 µg/gm while those from Sheboygan and Gills Rock contained 7.9 and 6.7 µg/gm, respectively.

The carp from Michigan City also had higher levels of PCBs than did those from the northern region. In contrast to the 11.0 µg/gm found in the Michigan City carp, those from Saugatuck and Sheboygan contained 4.6 and 1.7 µg/gm, respectively.

The PCBs in chubs in southern Lake Michigan ranged from 4.9 µg/gm near Milwaukee to 8.1 µg/gm near Saugatuck. In general, the chubs from the northern regions were below 5 µg/gm. The concentration of PCBs in the five groups of chubs collected over three months near Sheboygan varied without trend between 3.7 and 6.1 µg/gm, although the mean was below 5 µg/gm. The variation is somewhat less when the data is expressed on a lipid basis. For example, the PCBs in the 8/27 and 10/15 chubs was 5.0 and 3.7 µg/gm--not weight, respectively. In contrast, the concentration of PCBs in the lipids was 23 µg/gm and 21 µg/gm, respectively. Thus, much of the observed variability is due to lipid content of the fish analyzed.

One hundred percent of the chinook salmon captured in Wisconsin contained greater than 5 µg/gm PCBs and mean concentrations ranged from 24 µg/gm in Milwaukee to 9.9 µg/gm in Strawberry Creek.

Little trend in the concentration of PCBs in coho salmon was observed with sampling region. With the exception of coho caught early in 1971

Table 8. PCB Concentrations
1971 Lake Michigan Fish

Number	Species	Location	Date (1971)	% Over 5 ppm	PCB (ppm as Aroclor 1254) Wet Weight	S.D.	Lipid Basis	S.D.
10	ALWF	MICHIGAN CITY	1015	40	4.4	2.0	169	172
10	ALWF	BENTON HARB	902	40	4.8	1.1	60	31
2	ALWF	SAUGATUCK	823		2.5	.1	47	11
12	ALWF	SAUGATUCK	911	41	5.3	2.2	51	21
4	ALWF	SHEBOYGAN	1015	50	5.5	1.4	79	50
14	ALWF	LUDDINGTON	703	18	4.4	1.4	207	154
10	ALWF	FRANKFORT	1005	20	3.7	1.2	62	35
3	ALWF	MANITOU IS	910	33	3.0	2.2	51	47
9	ALWF	ROCK IS	914	66	8.9	17.0	102	256
8	ALWF	ST MARTINS I	911	10	3.5	1.3	82	17
1	BRTR	MICHIGAN CITY	1015	100	11.9	.0	51	
5	BRTR	SHEBOYGAN	713	100	7.9	3.0	92	16
10	BRTR	GILLS ROCK	910	70	6.7	2.5	64	22
7	CARP	MICHIGAN CITY	1129	100	11.0	2.2	72	27
14	CARP	SAUGATUCK	1015	20	4.6	4.8	71	71
11	CARP	SHEBOYGAN	723		1.7	.8	30	14
9	CARP	PENSAUKEE HBR	1101	33	4.2	1.4	30	10
5	CARP	MANITOU IS	916	80	5.8	1.8	30	9
10	CHUB	BENTON HARB	902	60	5.0	1.2	29	5
8	CHUB	SAUGATUCK	810	88	8.1	1.9	96	33

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Table 8. PCB Concentrations
1971 Lake Michigan Fish

Number	Species	Location	Date (1971)	% Over 5 ug/g	PCB(ug/gm as Aroclor 1254) Wet Weight	S.D.	Lipid Basis	S.D.
24	CHUB	SAUGATUCK	618	79	7.0	2.2	43	16
15	CHUB	SAUGATUCK	619	86	6.9	1.8	34	12
11	CHUB	MILWAUKEE	719	36	4.6	1.2	20	6
13	CHUB	SHEBOYGAN	713	81	5.1	1.5	31	15
13	CHUB	SHEBOYGAN	722	76	6.1	1.8	21	5
6	CHUB	SHEBOYGAN	617		3.8	.6	15	2
7	CHUB	SHEBOYGAN	827	57	5.0	1.3	23	5
6	CHUB	SHEBOYGAN	1015		3.7	.8	21	5
43	CHUB	LUDDINGTON	703	93	7.4	1.8	41	12
8	CHUB	FRANKFORT	1005	12	4.7	.9	28	6
10	CHUB	MANITOU IS	910	30	4.6	1.1	24	6
6	CHUB	WASHINGTON I	807		4.1	.6	16	3
6	CHUB	ROCK IS	711	67	5.0	2.5	97	46
7	CHUB	ROCK IS	814	57	5.6	1.6	23	7
8	CHUB	ROCK IS	816	12	4.0	.7	16	3
9	CHUB	ST MARTINS I	719		3.4	.6	16	4
9	CHUB	MANITOULIN	527	33	4.8	.7	26	6
1	CISC	GREEN ISLAND	721		3.8	.0	24	
1	CRSH	MILWAUKEE	1015	100	24.0	.0	117	
8	CRSH	MANITOULIN	1021	100	11.3	3.1	209	73

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Table 8. PCB Concentrations
1971 Lake Michigan Fish

Number	Species	Location	Date (1971)	% Over 5 µg/gm	PCB(µg/gm as Aroclor 1254) Wet Weight	S.D.	Lipid Basis	S.D.
10	CKSH	STRAWBERRY CR	1021	100	9.9	2.8	373	154
2	CKSH	GILLS ROCK	916	100	12.7	.7	278	60
8	COHO	MICHIGAN CITY	917	12	3.6	1.7	51	24
8	COHO	MICHIGAN CITY	927	87	17.3	8.4	255	120
6	COHO	MICHIGAN CITY	1007	100	14.0	4.0	349	120
9	COHO	SHEBOYGAN	1021	100	12.1	2.8	270	36
4	COHO	LOUDINGTON	924	100	11.2	3.3	198	29
10	COHO	PLATTE RIVER	1007	100	12.9	1.3	226	49
11	COHO	GILLS ROCK	916	90	12.2	4.1	166	60
1	CREH	ROCK IS	916	--	7.6	4.0	31	--
4	LKTR	MICHIGAN CITY	1015	100	19.9	2.0	89	19
5	LKTR	MICHIGAN CITY	900	100	21.2	6.8	121	48
14	LKTR	SAUGATUCK	624	100	11.9	3.8	70	20
7	LKTR	SAUGATUCK	710	100	15.5	2.9	76	23
9	LKTR	SAUGATUCK	1004	100	19.7	4.7	113	43
1	LKTR	MILWAUKEE	919	100	21.2	.0	90	--
7	LKTR	MILWAUKEE	919	100	21.1	6.0	114	33
12	LKTR	MILWAUKEE	1015	100	19.9	3.6	127	74
1	LKTR	SHEBOYGAN	701	100	19.9	.0	58	--
36	LKTR	SHEBOYGAN	713	100	12.5	3.7	62	27

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Table 8. PCB Concentrations
1971 Lake Michigan Fish

Number	Species	Location	Date (1971)	% Over 5 µg/gm	PCB(µg/gm as Aroclor 1254) Wet Weight	S.D.	Lipid Basis	S.D.
10	LKTR	LUDDINGTON	701	100	6.1	1.8	51	14
8	LKTR	LUDDINGTON	709	100	6.5	1.7	58	11
9	LKTR	G TRAVERSE	1019	75	11.3	5.3	56	22
19	LKTR	GREEN ISLAND	721	100	9.0	1.7	48	12
10	LKTR	GILLS ROCK	916	100	14.7	6.4	63	30
1	LOUY	GREEN ISLAND	721		7.5	.0	40	
14	PRCH	MICHIGN CITY	1015	14	4.2	.7	72	21
9	PRCH	WAUKEGAN	828	88	6.1	1.5	70	27
10	PRCH	MIL-WAUKEE	1013	90	10.9	3.1	203	76
1	PRCH	LUDDINGTON	706	100	6.2	1.3	139	57
2	PRCH	FRANKFORT	1012	50	5.4	1.8	69	
10	PRCH	PENSAUKEE BR	625	10	2.7	2.1	49	28
1	RBTR	MICHIGN CITY	1015	100	12.0	.0	66	
9	RBTR	GILLS ROCK	916	77	6.8	4.3	47	22
7	ROSU	ROCK	911		2.8	.5	33	17
9	ROSU	ST MARTINS I	911		3.2	.5	37	6
12	SCUL	SAUGATUCK	1016		2.7	1.1	46	25
15	SHLT	MICHIGN CITY	1015		3.2	.9	45	15
13	SHLT	SHEBOYGAN	1015		1.2	.2	18	14
5	SHLT	GREEN ISLAND	721		7.6	1.1	57	27

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Table 8. PCB Concentrations
1971 Lake Michigan Fish

PCB(ug/g as Aroclor 1254)

Number	Species	Location	Date (1971)	% Over 5 ug/gm	Wet Weight	S.D.	Lipid Basis	S.D.
8	SHLT	ROCK -15	911	25	2.9	1.7	49	28
4	SHLT	ST MARTINS I	719	-	1.3	.3	31	8
6	WHSU	MICHIGAN CITY	1015	100	10.6	4.0	180	44
3	WHSU	SAUGATUCK	1015	60	6.0	2.9	149	49
5	WHSU	SAUGATUCK	1016	-	2.3	.4	40	11
7	WHSU	PENSAUKEE BR	804	14	3.2	1.1	97	35
14	WHSU	G TRAVERSE W	1014	-	2.3	.7	60	26
1	WHSU	GREEN ISLAND	721	-	2.1	.0	48	-
10	WHSU	ROCK -15	911	-	2.5	.9	46	25
4	WHFH	MICHIGAN CITY	1015	100	6.1	.9	25	1
7	WHFH	SAUGATUCK	619	-	2.7	.5	16	3
2	WHFH	SAUGATUCK	621	-	3.1	1.3	70	6
7	WHFH	GRAND HAVEN	908	71	4.8	.9	29	5
13	WHFH	G TRAVERSE B	1014	-	1.8	.3	13	4
10	WHFH	ROCK -15	911	-	1.5	.2	9	1

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near Michigan City, the mean concentration of PCBs ranged from 11 μg/gm in those near Ludington to 14 μg/gm in those near Michigan City.

The PCBs in lake trout were most concentrated in the trout from Michigan City, Saugatuck and Milwaukee where mean concentrations were generally between 15 and 22 μg/gm. The trout from the northern areas such as Sheboygan, Ludington, Grand Traverse Bay and Gill's Rock were considerably lower, and the mean concentrations ranged between 8 and 15 μg/gm.

The concentration of PCBs were unexpectedly high in the perch caught near Milwaukee in that the mean of the 10 fish was 11 μg/gm. In contrast, perch from other regions appear to be less by a factor of two, and those from lower Green Bay contained only 2.7 μg/gm.

Rainbow trout were only caught near Michigan City and Gill's Rock. The Michigan City trout had 12 ug/gm PCBs while those from Gill's Rock averaged only 8.8 μg/gm PCBs.

The concentration of PCBs in suckers and smelt were generally between 2 and 4 μg/gm, although the average concentration in the three white suckers from Saugatuck was 6.0 μg/gm. With the exception of whitefish caught at Grand Haven and Michigan City, the PCB concentration in whitefish was less than 3 μg/gm.

B. DDT and Analogs

The concentrations of pp'DDT, op'DDT, pp'TDE and pp'DDE are summarized in Table 9 along with the total DDT and percentage of fish which contained over the 5 μg/gm action level established by the FDA. The total DDT in alewives ranged from 1.6 μg/gm near Waukegan to 5.0 near the Manitou Islands. There was no trend in the variations with region. A brown trout from Michigan City contained 6.4 μg/gm total DDT whereas those from Sheboygan and Gill's Rock averaged 4.4 μg/gm. With the exception of the carp from Michigan City which averaged 4.6 μg/gm total DDT, the average concentrations in Lake Michigan carp were 1.0 μg/gm or less.

The total DDT in chubs ranged from 3.8 μg/gm at Frankfort to 6.2 μg/gm at Sheboygan. There are no trends for DDT in chubs observed in

Table 9. Concentration of DDT and Metabolites in 1971 Lake Michigan Fish (Wet-Weight Basis)

Number	Species	Location	Date	Concentration (ug/gm) pp DDE	pp TDE	pp DDT	op DDT	Total DDT	% Over 5 ug/gm
10	ALWF	MICHIGN CITY	1015	1.4	.19	.5	.08	2.2	10
10	ALWF	BENTON HRBR	902	1.8	.23	.5	.08	2.7	
2	ALWF	WAUKEGAN	823	1.0	.10	.5	.07	1.7	
9	ALWF	SAUGATUCK	911	1.9	.22	.0	.10	2.7	11
1	ALWF	SHEBOYGAN	1015	2.2	.20	.7	.07	3.2	
9	ALWF	FRANKFORT	1005	1.2	.10	.6	.00	1.9	
1	ALWF	MANITOU IS	910	3.2	.32	1.3	.16	5.0	
4	ALWF	ROCK IS	911	1.1	.11	.7	.04	1.9	
5	ALWF	ST HARRIS	911	1.0	.10	.3	.05	1.5	
1	BRTR	MICHIGN CITY	1015	3.7	.70	1.7	.22	6.4	100
6	BRTR	SHEBOYGAN	713	2.6	.40	1.7	.18	4.9	25
10	BRTR	GILLS ROCK	810	2.2	.48	1.0	.14	4.4	40
2	CARP	MICHIGN CITY	1129	3.3	.31	1.3	.14	4.8	50
10	CARP	SAUGATUCK	1015	.8	.20	.1	.02	.9	0
6	CARP	SHEBOYGAN	723	.2	.07	.0	.01	.3	
6	CARP	PENSAUKEE BR	1101	.8	.19	.0	.01	1.0	
10	CHUB	BENTON HRBR	902	2.5	.31	1.5	.19	4.5	30
8	CHUB	SAUGATUCK	911	1.9	.47	1.6	.19	4.2	37
10	CHUB	SAUGATUCK	918	2.4	.63	2.4	.25	5.7	64
2	CHUB	SAUGATUCK	919	1.8	.47	2.4	.21	5.1	60

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Table 9. Concentration of DDT and Metabolites in 1971 Lake Michigan Fish (Wet-Weight Basis)

Number	Species	Location	Date	Concentration (µg/gm) pp DDE	pp TDE	pp DDT	op DDT	Total DDT	% Over 5 µg/gm
9	CHUB	MILWAUKEE	919	3.4	.30	2.0	.20	6.0	80
2	CHUB	SHEBOYGAN	7-13	1.3	.40	2.2	.29	4.1	50
11	CHUB	SHEBOYGAN	722	3.4	.34	2.0	.24	5.9	81
5	CHUB	SHEBOYGAN	810	2.6	.35	1.3	.16	4.4	20
5	CHUB	SHEBOYGAN	827	3.2	.47	2.3	.28	6.2	80
7	CHUB	SHEBOYGAN	1015	2.4	.28	1.7	.17	4.5	42
20	CHUB	LUDDINGTON	703	2.0	.58	2.2	.28	5.0	46
6	CHUB	FRANKFORT	1005	2.2	.33	1.2	.10	3.8	
2	CHUB	MANITOU IS	910	3.1	.50	1.3	.20	5.1	50
5	CHUB	WASHINGTON I	909	3.2	.34	1.9	.18	5.6	60
5	CHUB	ROCK IS	911	2.8	.20	1.1	.08	4.4	20
10	CHUB	ROCK IS	914	2.7	.42	2.0	.22	5.4	50
5	CHUB	ROCK IS	916	2.8	.29	1.3	.14	4.5	40
10	CHUB	ST MARTINS I	913	2.2	.38	1.1	.17	5.0	
5	CHUB	MANISTIQUE	507	2.9	.42	1.8	.14	5.2	80
1	CISC	ROCK IS	911	.8	.32	.5	.10	1.7	
1	CKSH	MILWAUKEE	1015	6.0	1.67	4.7	.53	13.0	100
7	CKSH	MANITOWOC	1021	6.3	.52	1.2	.21	8.3	100
9	CKSH	STRAWBERRY CO	1021	4.2	.25	1.0	.17	5.4	88
1	CKSH	GILLS ROCK	910	5.0	.49	1.2	.20	7.3	100

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Table 9. Concentration of DDT and Metabolites in 1971 Lake Michigan Fish (Wet-Weight Basis)

Number	Species	Location	Date	Concentration (µg/gm) pp DDE	pp TDE	pp DDT	op DDT	Total DDT	% Over 5 µg/gm
5	CHUB	MILWAUKEE	919	3.4	.36	2.0	.20	6.0	80
2	CHUB	SHEBOYGAN	7-13	1.3	.40	2.2	.24	4.1	50
11	CHUB	SHEBOYGAN	722	3.4	.34	2.0	.24	5.8	81
5	CHUB	SHEBOYGAN	810	2.6	.35	1.2	.16	4.4	20
5	CHUB	SHEBOYGAN	827	3.2	.47	2.3	.28	6.2	80
7	CHUB	SHEBOYGAN	1015	2.4	.28	1.7	.17	4.5	42
20	CHUB	LUDDINGTON	705	2.0	.58	2.2	.28	5.0	45
6	CHUB	FRANKFORT	1005	2.2	.33	1.2	.16	3.8	0
2	CHUB	MANITOU IS	810	3.1	.50	1.8	.20	5.8	50
5	CHUB	WASHINGTON I	809	3.2	.34	1.3	.18	5.5	60
5	CHUB	ROCK IS	911	2.6	.41	1.1	.08	4.0	20
6	CHUB	ROCK IS	914	2.7	.42	2.0	.22	5.4	50
5	CHUB	ROCK IS	910	2.6	.29	1.7	.14	4.5	40
10	CHUB	ST MARTINS I	718	2.2	.34	1.1	.17	3.8	0
5	CHUB	MANISTIQUE	527	2.9	.42	1.8	.14	5.2	80
1	CISC	ROCK IS	911	.7	.32	.5	.10	1.7	0
1	CKSH	MILWAUKEE	1015	8.1	1.67	4.7	.53	13.0	100
7	CKSH	MANITOWOC	1021	6.3	.52	1.2	.21	8.3	100
5	CKSH	STRAWBERRY PT	1021	4.6	.25	1.0	.17	6.0	80
1	CKSH	GILLS ROCK	915	5.2	.49	1.2	.20	7.3	100

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Table 9 . Concentration of DDT and Metabolites in 1971 Lake Michigan Fish (Wet-Weight Basis)

Number	Species	Location	Date	Concentration (µg/gm) pp DDE	pp TDE	pp DDT	op DDT	Total DDT	% Over 5 µg/gm
5	COHO	MICHIGAN CITY	417	.8	.17	.5	.09	1.6	
7	COHO	MICHIGAN CITY	927	5.3	.40	1.2	.20	7.2	85
5	COHO	MICHIGAN CITY	1007	4.5	.41	1.4	.18	6.5	60
9	COHO	SHEBOYGAN	1021	6.6	.57	1.4	.27	8.6	100
3	COHO	LUDDINGTON	828	6.8	.39	.9	.19	8.2	100
9	COHO	PLATTE RIVER	1007	5.5	.40	1.4	.24	7.6	100
8	COHO	GILLS ROCK	916	4.9	.45	1.2	.20	6.7	87
3	LKTR	MICHIGAN CITY	1015	2.9	.68	3.1	.35	7.3	100
2	LKTR	MICHIGAN CITY	908	9.5	1.12	3.9	.45	14.9	100
9	LKTR	SAUGATUCK	621	4.7	.65	2.1	.27	7.6	88
6	LKTR	SAUGATUCK	710	6.9	.70	2.0	.24	9.8	100
8	LKTR	SAUGATUCK	1001	6.7	.84	2.8	.27	10.6	100
2	LKTR	MILWAUKEE	914	10.1	.36	3.0	.47	14.5	100
5	LKTR	MILWAUKEE	919	10.6	1.05	3.3	.45	15.4	100
10	LKTR	MILWAUKEE	1015	2.3	.44	1.3	.15	4.2	[illegible]
28	LKTR	SHEBOYGAN	713	4.7	.51	1.7	.24	6.8	64
10	LKTR	LUDDINGTON	701	3.3	.52	1.6	.22	5.6	60
6	LKTR	LUDDINGTON	704	3.2	.63	2.0	.30	6.1	66
9	LKTR	G TRAVERSE B	1019	6.0	.73	2.0	.30	9.0	77
11	LKTR	GREEN ISLAND	721	3.6	.50	1.5	.21	6.0	72

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Table 9. Concentration of DDT and Metabolites in 1971 Lake Michigan Fish (Wet-Weight Basis)

Number	Species	Location	Date	Concentration (ug/gm) pp DDE	pp TDE	pp DDT	op DDT	Total DDT	% Over 5 ug/gm
8	LKTR	GILLS ROCK	916	5.1	.62	1.9	.28	8.3	62.
1	LOUY	GREEN ISLAND	721	.5	.14	.3	.03	.9	
10	PRCH	MICHIGN CITY	1015	.8	.15	.5	.04	1.3	
9	PRCH	MILWAUKEE	1017	1.1	.30	.7	.00	2.1	
2	PRCH	LUDDINGTON	70C	2.2	.30	.9	.13	3.6	
2	PRCH	FRANKFORT	1013	1.7	.43	2.2	.14	4.5	
1	PRCH	PENSAUKEE PR	625	.4	.10	.0	.01	.5	
8	RPTR	GILLS ROCK	916	3.1	.34	.2	.16	4.5	40
6	ROSU	ROCK IS	911	1.7	.30	.8	.09	2.7	
8	ROSU	ST MARTINS I	911	1.5	.35	.8	.09	2.7	
2	COHO	GILLS ROCK	916	6.4	.52	1.0	.24	8.7	100
9	SCUL	SAUGATUCK	1010	1.4	.19	1.2	.06	2.8	11
11	SMLT	MICHIGN CITY	1015	.9	.11	.4	.05	1.4	
3	SMLT	SHEBOYGAN	1015	.6	.12	.3	.03	1.0	
4	SMLT	GREEN ISLAND	721	.7	.12	.2	.02	1.0	
9	SMLT	ROCK IS	911	1.8	.14	.5	.05	1.0	
3	SMLT	ST MARTINS I	719	.7	.09	.2	.04	1.0	
2	WHFH	MICHIGN CITY	1015	.5	.32	.5	.05	1.4	
3	WHFH	SAUGATUCK	619	.6	.21	.4	.06	1.2	
6	WHFH	GRAND HAVEN	906	.7	.38	.8	.10	1.9	

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Table 9. Concentration of DDT and Metabolites in 1971 Lake Michigan Fish (Wet-Weight Basis)

Number	Species	Location	Date	Concentration (µg/gm) pp DDE	pp TDE	pp DDT	op DDT	Total DDT	% Over 5 µg/gm
12	WHFH	G TRAVERSE B	1019	1.2	.25	.4	.05	2.0	
6	WHFH	ROCK IS	511	.7	.16	.4	.05	1.4	
3	WHSU	MICHIGN CITY	1015	1.8	.02	1.0	.05	4.0	[illegible]
7	WHSU	SAUGATUCK	1015	.7	.14	.2	.03	1.0	
7	WHSU	PERSAUKEE BR	306	.3	.15	.2	.04	.7	
14	WHSU	G TRAVERSE B	1019	1.4	.21	.4	.05	2.1	
1	WHSU	GREEN ISLAND	721	.7	.19	.4	.03	1.3	
12	WHSU	ROCK IS	911	.8	.220	.3	.04	1.4	

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Table 9 . Similarly, the total DDT in coho was somewhat constant throughout the lake, and the mean concentration ranged between 6.5 µg/gm to 8.8 µg/gm for the coho caught in the fall of 1971. The five coho caught near Michigan City in the spring averaged only 1.6 µg/gm total DDT.

As was previously discussed, the concentration of chlorinated materials is dependent on the size of the fish. Thus, lake trout from Michigan City averaged 14.9 µg/gm total DDT on 9/8/71 while those caught on 10/15/71 averaged only 7.3 µg/gm. The difference is likely due to the size differences. The lake trout from Milwaukee also contained approximately 15 µg/gm in October, but the smaller trout caught near Milwaukee averaged only 4.2 µg/gm total DDT. The DDT in the northern areas of the lake were less than 8 µg/gm, and approximately 60 to 70 percent of the lake trout contained over 5 µg/gm total DDT.

The perch from lower Green Bay had the lowest DDT content (0.5 µg/gm) while perch from other areas contained between 1.3 and 4.5 µg/gm total DDT. None of the perch contained greater than 5 µg/gm total DDT.

The concentration of DDT in smelt, whitefish and suckers averaged approximately 1 to 2 µg/gm, although the white suckers from Michigan City averaged 4.6 µg/gm total DDT.

C. Phthalate Esters

A group of Lake Michigan fish were selected for analysis of n,n dibutyl phthalate (DBP) and 2-ethylhexyl phthalate (DEHP) by Analytical Bio-Chemist Laboratories, Inc. of Columbia, Missouri. Although the presence of DBP was indicated in most of the fish, DEHP was indicated in only five percent of the samples analyzed. The data presented in this section must be regarded as preliminary--subject to confirmatory mass spectral data and further evaluation of the procedures used for the phthalate ester analysis in fish.

Table 10 presents the data concerning the concentration of DBP in the selected fish. The mean DBP concentration in chubs ranged from 0.8 µg/gm near Saugatuck to 3.7 µg/gm at Benton Harbor. Although sufficient data were not obtained to determine trends of DBP in the lake, the concentrations in the fish from the northern regions were all

Table 10. Concentration of n,n Dibutyl phthalate in Selected Lake Michigan Fish (GLC Data Only)

Species	Region	No. of Fish in Sample	Concentration[a] (µg/gm)
Chubs	Sheboygan	3	1.5
Chubs	Rock Is.	2	1.1
Chubs	Milwaukee	4	1.1
Chubs	Benton Harbor	2	3.7
Chubs	Saugatuck	3	0.8
Chubs	Manitou Is.	1	1.5
Lake Trout	Green Bay	1	1.4
Lake Trout	Sheboygan	1	1.4
Lake Trout	Milwaukee	2	1.8
Lake Trout	Saugatuck	2	2.3
Lake Trout	Ludington	2	0.8
Lake Trout	West Arm Grand Traverse Bay	1	1.1
Perch	Green Bay	2	1.9

[a] means for more than one sample

less than 1.5 µg/gm whereas the two chubs from Benton Harbor in southern Lake Michigan contained 2.1 and 3.7 µg/gm DBP.

The levels of DBP in lake trout and perch were similar in magnitude in that the range observed was from 0.8 µg/gm in the trout from Ludington to 2.3 µg/gm near Saugatuck. The perch from lower Green Bay were found to contain 1.8 and 2.0 µg/gm DBP.

The concentration of DEHP in five fish from Lake Michigan are presented in Table 11. The levels ranged from 1.0 µg/gm in a lake trout from Ludington to 65 µg/gm in the whitefish captured in the west arm of Grand Traverse Bay. In the limited data available, it is noted that the fish from Milwaukee and Sheboygan contained 22 and 14 µg/gm, respectively, which is considerably more than the 1.0 and 2.0 µg/gm in the fish from Ludington and Saugatuck, respectively.

D. Summary

There are insufficient data on PCBs in Great Lakes fish to adequately relate the results of this work on Lake Michigan fish with those from other Great Lakes. The concentration of PCBs ranged from less than 2 µg/gm in the small fish of low lipid content to over 20 µg/gm in the larger fish. Based on unpublished data provided by the Ontario Pesticide Residue Laboratory, the concentration of PCBs in Lake Michigan coho salmon appears to be two to three times greater than coho from Lake Huron, approximately 1.5 times greater than those in Lake Ontario and approximately ten times greater than those from Lake Erie and Lake Superior. Approximately 50 to 80 percent of the chubs from Lake Michigan contain greater than 5 µg/gm PCBs and essentially 100 percent of large salmon and trout contain greater than 5 µg/gm PCBs.

Table 11. Concentration of 2-Ethylhexylphthalate in Selected Fish from Lake Michigan (GLC Data Only).

Species	Region	Concentration (µg/gm)
Chub	Sheboygan	14
Chub	Milwaukee	22
Chub	Saugatuck	2.0
Lake Trout	Ludington	1.0
Whitefish	West Arm Grand Traverse Bay	65

SECTION VII

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SECTION VIII

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Samples collected from the Grand Traverse Bay and Beaver Island areas were obtained with the assistance of Merrill Keller and Lee Moffit of the Michigan Department of Natural Resources, Great Lakes Fishery Station at Charlevoix, Michigan.

Robert A. Koch of the Michigan City office of the Indiana Department of Natural Resources collected some of the lake trout from the Michigan City area. The remaining samples from Michigan City were captured with the assistance of William Ritter of Ritter Brother's Fish Company in Michigan City.

Although no samples were obtained from the Chicago area of Lake Michigan, the assistance of Robert Schacht of the Illinois Environmental Protection Agency and A. C. Lopinot and Paul Vidal of the Illinois Department of Conservation is appreciated.

Samples collected from Waukegan, Illinois, were provided by a fish population study of Industrial Bio-Test Laboratories, Incorporated of Northbrook, Illinois. Permission for the sampling was granted by Steven

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Hastings of Commonwealth Edison Company of Chicago with the cooperation of William Johnson of Industrial Bio-Test Laboratories. Bruce Muench and Jay Smith captured the fish for this study.

The Milwaukee area was sampled by the Wisconsin Department of Natural Resources and by Leland La Fond of Milwaukee. Ronald Poff of the Department of Natural Resources transported the fresh fish to the Water Chemistry Laboratory in Madison, Wisconsin. In addition, James Moore and Russell Daley of the Department of Natural Resources collected the trout from the Sheboygan and Green Island areas. Herman Schwarz of Schwarz Fish Company in Sheboygan provided fish from Sheboygan and assisted in coordinating the collection of fish from area fishermen. Donald Euers, Lake Michigan Enforcement Coordinator for the State of Wisconsin, collected and transported trout from pond nets which are operated by Marvin Weberg near Gills Rock to a Green Bay freezer plant for pickup. Steve Ellefson of Washington Island assisted immeasurably in the collection of samples for the Washington Island area as well as northern Lake Michigan. All of the fish collected from lower Green Bay were captured by Norbert, Francis and Arthur Swaer of Pensaukee, Wisconsin.

A special thanks goes to the technical assistants who conducted the analyses on this project. Joan Karnauskas, Judy Capelli, William Rasmus, Gaylord Plummer, Ronald Kawalczek and Richard Pyter assisted in coding the samples and conducted the tissue preparation, extraction and cleanup of the fish. Robert Philbin assisted in much of the analytical work and wrote the necessary computer programs for the compilation and analysis of data. Donald Mazei conducted the majority of GLC analyses. Kathryn Benkert and Wally Gunthier prepared solvents, sorbents and glassware for this study and assisted in preparing the samples for GLC analysis.

A special thanks is extended to Dr. Gary Glass of the EPA National Water Quality Laboratory in Duluth, Minnesota, who as Project Officer for this study, provided continual guidance to the study and assisted immensely in establishing priorities in sample collection and analysis.

In addition, the National Water Quality Laboratory provided the mass spectrometry facilities for the qualitative analyses, and the assistance of Mr. Douglas Kuehl in obtaining the data is appreciated.

MONS 203821

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MONS 203822

SECTION IX
APPENDICES

A. Summary of Data on Fish Analyzed for PCBs and DDT

MONS 203823

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

(µg/gm)

Sample Number	Species	1971 Date	Location	Dpth	No.*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
[illegible]														

*Number of fish, if composite sample

MONS 203824

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Drh	No*	Lgth	Wt	% Fat	(ug/gm) PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
[illegible]														

*Number of fish, if composite sample

MONS 203825

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(ug/gm)					
0864	ALWF	1005	4440 [illegible]	[illegible]	2	07.4	00.093	6.8	4.8	.6	.11	1.6	.17	2.5
0872	ALWF	1005	4440 [illegible]	[illegible]	2	07.5	00.051	11.9	3.3	.6	.08	1.3	.21	2.1
0869	ALWF	0910	4510 [illegible]	[illegible]	1	[illegible]	[illegible]	6.9	5.0	1.3	.16	3.2	.33	5.0
0532	ALWF	0911	4520 [illegible]	[illegible]	2	[illegible]	00.032	2.1	7.3	.3	.05	1.3	.13	1.6
0531	ALWF	0911	4520 [illegible]	[illegible]	2	[illegible]	00.030	6.2	7.6	.4	.06	.8	.10	1.3
0533	ALWF	0911	4520 [illegible]	[illegible]	2	05.3	00.040	5.3	15.8	.1	.02	1.4	.05	1.6
0534	ALWF	0911	4520 [illegible]	[illegible]	2	[illegible]	[illegible]	6.3	3.7	.3	.04	.7	.11	1.2
0536	ALWF	0911	4520 [illegible]	[illegible]	2	05.5	00.030	3.6	3.6	.3	.04	1.1	.14	1.5
0539	ALWF	0911	4520 [illegible]	[illegible]	2	[illegible]	00.032	2.7	3.7	.4	.05	1.2	.17	2.1
0540	ALWF	0911	4520 [illegible]	[illegible]	2	05.9	00.035	3.7	2.4	.2	.04	1.0	.13	1.4
[illegible]	ALWF	0911	4520 [illegible]	[illegible]	2	[illegible]	[illegible]	[illegible]	[illegible]	.2	.02	[illegible]	[illegible]	[illegible]
1052	BRTR	1015	4343 [illegible]	[illegible]	1	25.0	05.130	23.4	11.9	1.7	.22	3.7	.70	6.4
0224	BRTR	0717	4345 [illegible]	[illegible]	1	27.2	[illegible]	19.0	6.7	1.5	.24	2.[illegible]	.57	4.6
0227	BRTR	0713	4345 [illegible]	[illegible]	1	21.5	[illegible]	20.1	6.9	[illegible]	.12	2.5	.36	4.2
0221	BRTR	0713	4345 [illegible]	[illegible]	1	24.7	[illegible]	20.4	7.0	1.2	.10	2.5	.41	5.4
0228	BRTR	0713	4345 [illegible]	[illegible]	1	19.5	[illegible]	18.6	13.2	.9	.18	1.3	.40	2.5
0947	BRTR	0916	4517 [illegible]		1	21.0	02.324	12.2	4.8	.9	.12	2.2	.24	3.6
0945	BRTR	0916	4517 [illegible]		1	21.0	02.724	10.4	5.3	1.0	.14	2.0	.44	3.7
0950	BRTR	0916	4517 [illegible]		1	23.5	02.859	9.9	7.4	1.2	.13	2.9	.45	4.7
0943	BRTR	0916	4517 [illegible]		1	24.0	02.951	14.5	4.2	1.1	.22	3.3	.52	5.2

*Number of fish, if composite sample

MONS 203826

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Drh	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(ug/gm)					
0948	BRTR	0916	4517 8700		1	26.0	05.675	17.5	9.0	1.3	.18	4.2	.66	6.3
0940	BRTR	0916	4517 8700		1	[illegible]	[illegible]	[illegible]	6.1	1.0	.13	.2	.35	1.7
0953	BRTR	0915	4517 8700		1	21.0	02.351	11.9	8.0	1.1	.14	3.8	.47	5.4
0943	BRTR	0916	4517 8700		1	25.0	[illegible]	[illegible]	6.7	.6	.13	2.7	.45	3.9
0949	BRTR	0915	4517 8700		1	22.0	[illegible]	11.9	11.7	1.3	.13	4.2	.68	1.4
0944	BRTR	0916	4517 8700		1	19.0	02.457	10.2	2.3	.6	.08	2.0	.28	3.0
1054	CARP	1129	4143 8695	12	1	29.0	07.700	15.0	11.1	.5	.13	4.0	1.18	5.8
1052	CARP	1129	4143 8695	12	1	22.0	05.413	12.7	10.5	.2	.10	2.7	.44	3.4
1272	CARP	1015	4240 8620	03	1	07.0	00.102	6.0	2.0	.0	.00	.3	.05	.3
1273	CARP	1015	4240 8620	03	2	[illegible]	[illegible]	4.2	2.4	.0	.00	.3	.06	.3
1271	CARP	1015	4240 8620	03	2	05.0	00.091	[illegible]	[illegible]	.0	.00	.4	.07	.3
1265	CARP	1015	4240 8620	03	1	06.0	00.089	2.2	1.8	.0	.00	.1	.05	.1
1268	CARP	1015	4240 8620	03	1	06.0	00.087	3.4	1.7	.0	.00	.3	.03	.3
1167	CARP	1015	4240 8620	03	1	[illegible]	[illegible]	[illegible]	[illegible]	.5	.12	[illegible]	.22	[illegible]
1277	CARP	1015	4240 8620	03	2	05.0	00.042	3.4	2.5	.0	.00	.1	.10	.2
1178	CARP	1015	4240 8620	03	1	23.0	04.430	22.4	12.5	.1	.00	1.8	.00	2.0
1187	CARP	1015	4240 8620	03	1	22.0	03.020	21.5	6.2	.3	.05	.6	.33	1.3
1184	CARP	1015	4240 8620	03	1	17.0	01.050	12.7	5.2	.0	.00	.4	.27	.7
1276	CARP	1015	4240 8620	03	2	05.5	00.049	1.9	2.1	.0	.00	.1	.06	.2
0291	CARP	0723	4345 8720	03	1	20.0	[illegible]	[illegible]	1.5	.0	.00	.1	.04	.1

*Number of fish, if composite sample

MONS 203827

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dpth	No*	Lgth	Wt	% Fat	(µg/gm) PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	11.2	2.1	.1	.01	.2	.11	.4
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4.3	1.3	.0	.00	.1	.05	.1
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.0	.01	.1	.07	.2
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.1	.02	.2	.10	.4
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	6.2	2.8	.0	.01	.2	.12	.1
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4.0	1.1	.0	.00	.1	.01	.1
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2.8	1.1	.0	.00	.1	.04	.2
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.0	.00	.0	.10	.1
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.0	.00	.1	.12	.1
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.1	.02	.2	.15	.7
[illegible]	CARP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.0	.00	1.0	.22	.4
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*Number of fish, if composite sample

MONS 203828

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No.*	Lgth	Wt	% Fat	(ug/gm) PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	10.8	7.1	2.8	.31	2.4	.57	6.1
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	20.7	[illegible]	1.5	.20	[illegible]	.26	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.19	.7	.72	2.1
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	12.6	7.1	2.1	.29	2.2	.24	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	10.7	[illegible]	[illegible]	.26	.3	.52	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	10.5	[illegible]	1.6	.21	1.0	.37	2.4
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.15	2.9	.23	4.1
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.10	[illegible]	.32	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.14	2.1	.24	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.20	[illegible]	.32	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.14	[illegible]	.33	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.41	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*Number of fish, if composite sample

MONS 203829

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Drh	No*	Lgth	Wt	% Fat	(µg/gm) PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
[illegible]														

*Number of fish, if composite sample

MONS 203830

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Drh	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
0175	CHUB	0722	4345 8730	35	1	12.0	[illegible]	26.6	5.1	3.4	.39	3.9	.47	7.2
0180	CHUB	0722	4345 8730	35	1	11.4	[illegible]	25.8	5.0	2.2	.24	3.7	.43	7.0
0187	CHUB	0722	4345 8730	35	1	11.0	[illegible]	24.8	5.0	1.9	.20	3.3	.41	5.3
0189	CHUB	0722	4345 8730	35	1	11.8	[illegible]	25.8	3.8	1.5	.16	2.6	.19	4.5
0188	CHUB	0722	4345 8730	35	1	10.3	[illegible]	27.9	6.5	1.6	.24	3.3	.26	5.9
0594	CHUB	0819	4345 8730	47	1	09.5	00.224	23.3	3.7	.6	.17	3.4	.20	4.1
0595	CHUB	0819	4345 8730	47	1	09.5	00.224	30.9	3.7	.9	.13	2.6	.25	4.6
0597	CHUB	0819	4345 8730	47	1	09.3	00.152	21.5	7.5	1.4	.01	2.6	.30	4.3
0600	CHUB	0819	4345 8730	47	1	09.3	00.133	21.7	4.2	2.1	.30	2.5	.59	5.1
0599	CHUB	0819	4345 8730	47	1	09.3	00.177	34.9	4.8	1.2	.15	2.1	.35	3.2
0607	CHUB	0827	4345 8730	47	1	10.0	00.133	16.4	5.0	2.9	.26	3.3	.52	6.5
0606	CHUB	0827	4345 8730	47	1	09.2	00.111	24.6	4.7	1.3	.21	2.2	.35	4.1
0608	CHUB	0827	4345 8730	47	1	09.3	00.170	21.0	7.7	2.7	.34	4.7	.59	8.3
0605	CHUB	0827	4345 8730	47	1	10.0	00.171	17.5	3.2	2.4	.27	2.5	.47	5.5
0604	CHUB	0827	4345 8730	47	1	09.0	00.181	22.9	5.9	2.0	.30	3.4	.40	6.2
0830	CHUB	1015	4345 8730	[illegible]	1	09.5	00.122	16.0	3.3	2.3	.28	2.7	.40	5.7
0837	CHUB	1015	4345 8730	[illegible]	1	10.5	00.177	17.4	2.8	1.0	.11	1.4	.21	2.7
0840	CHUB	1015	4345 8730	[illegible]	1	09.5	00.132	14.2	3.1	1.6	.16	2.7	.24	4.2
0832	CHUB	1015	4345 8730	[illegible]	1	09.0	00.159	15.3	4.8	2.3	.17	3.3	.37	6.6
0835	CHUB	1015	4345 8730	[illegible]	1	10.0	00.175	15.5	4.1	1.7	.15	2.7	.20	4.3

*Number of fish, if composite sample

MONS 203831

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dft	No*	Length	Wt	% Fat	(µg/gm) PCB	pp' DDE	op' DDT	pp' DDD	pp' DDT	Total DDT
[illegible]														

*Number of fish, if composite sample

MONS 203832

Appendix A. Summary of Data on Fish Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dth	No.*	Length	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	17.3	[illegible]	1.2	.10	1.8	.27	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0160	CHUB	0615	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	13.4	[illegible]	.7	.10	1.4	.20	2.0
0167	CHUB	0616	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	22.4	[illegible]	[illegible]	.15	.9	.40	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0158	CHUB	0616	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	20.5	[illegible]	2.1	.27	2.2	.33	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	0616	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.07	[illegible]	.22	[illegible]
0112	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0109	CHUB	0618	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0103	CHUB	0618	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.24	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0102	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	22.2	[illegible]	[illegible]	.20	[illegible]	.58	[illegible]
0110	CHUB	0618	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	19.1	[illegible]	[illegible]	.14	[illegible]	.39	[illegible]
[illegible]	CHUB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*Number of fish, if composite sample

MONS 203833

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
1161	CHUB	0911	4520 8645	19	1	16.0	00.700	5.0	3.1	.7	.07	1.9	.23	2.9
1164	CHUB	0911	4520 8645	19	1	25.5	01.760	5.2	9.1	2.9	.13	4.4	.79	8.2
1159	CHUB	0911	4520 8645	19	1	23.0	01.300	5.4	6.3	1.2	.09	3.1	.29	4.7
1163	CHUB	0911	4520 8645	19	1	23.0	01.470	6.6	7.9	.2	.03	2.4	.19	2.8
1165	CHUB	0911	4520 8645	19	1	21.0	01.570	9.7	6.6	.8	.00	2.4	.31	3.6
0550	CHUB	0914	4520 8635	43	1	07.5	00.199	26.1	5.8	2.1	.27	2.5	.40	5.4
0546	CHUB	0914	4520 8635	43	1	08.8	00.178	22.2	6.8	3.6	.35	3.3	.76	8.0
0545	CHUB	0914	4520 8635	43	1	08.3	00.173	26.7	4.3	1.5	.20	2.6	.32	4.6
0503	CHUB	0916	4520 8645	17	1	10.5	00.243	20.1	4.0	1.7	.19	2.8	.35	5.0
0511	CHUB	0916	4520 8645	17	1	10.5	00.266	24.3	3.9	.9	.13	1.9	.19	3.1
0508	CHUB	0916	4520 8645	17	1	09.5	00.187	16.6	3.3	1.0	.07	2.5	.27	3.9
0509	CHUB	0916	4520 8645	17	1	11.0	00.231	26.8	3.9	1.4	.13	4.0	.31	5.8
0812	CHUB	0719	4530 8525	30	1	11.5	00.223	21.4	4.0	1.3	.21	2.3	.44	4.2
0809	CHUB	0719	4530 8525	30	1	11.5	00.259	23.[illegible]	2.5	.5	.20	1.2	[illegible]	3.2
0813	CHUB	0719	4530 8525	30	1	10.0	00.318	26.6	2.9	1.1	.17	2.3	.27	3.0
0814	CHUB	0719	4530 8525	30	1	10.5	00.293	21.6	3.1	.8	.14	1.5	.48	3.0
0810	CHUB	0719	4530 8525	30	1	10.0	00.250	24.6	4.3	1.1	.16	2.3	.27	3.8
0807	CHUB	0719	4530 8525	30	1	11.5	00.324	16.7	3.1	1.3	.17	2.1	.33	3.0
0806	CHUB	0719	4530 8525	30	1	12.0	00.274	21.9	8.9	1.2	.17	2.9	.29	4.6
0808	CHUB	0719	4530 8525	30	1	10.5	00.262	18.9	4.1	.6	.17	2.0	.41	3.2

*Number of Fish, if composite sample

MONS 203834

Appendix A. Summary of Data on Fish Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dsh	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(ug/gm)					
0815	CHUB	0719	4530	6625	30	1	09.5	00.137	13.9	3.0	1.0	.11	2.1	.19	3.4
0622	CHUB	0527	4550	0615	40	1	10.6	00.165	13.0	5.0	1.8	.21	2.4	.56	5.0
0641	CHUB	0527	4550	0615	40	1	10.1	00.163	17.1	5.3	1.5	.00	3.6	.39	5.4
0638	CHUB	0527	4550	0615	40	1	09.0	00.176	15.3	4.6	1.9	.19	2.6	.42	5.1
0636	CHUB	0527	4550	0615	40	1	10.6	00.226	23.7	4.6	1.5	.10	2.7	.27	4.6
0889	CKSH	1015	4305	8745	10	3	22.5	01.936	20.9	24.0	0.7	.53	4.6	1.67	33.6
0899	CKSH	1021	4410	8740		1	24.0	03.178	5.0	14.4	2.0	.31	8.3	.65	11.3
0893	CKSH	1021	4410	8740		1	22.5	02.497	6.0	12.0	1.2	.19	4.8	.66	6.9
0905	CKSH	1021	4410	8740		1	24.0	02.997	7.7	14.0	1.2	.22	5.7	.63	7.7
0898	CKSH	1021	4410	8740		1	24.0	03.059	5.4	7.1	1.0	.16	4.9	.40	6.5
0897	CKSH	1021	4410	8740		1	22.0	04.313	3.9	10.9	1.0	.16	6.0	.00	7.6
0894	CKSH	1021	4410	8740		1	22.0	02.961	4.9	14.7	1.5	.29	9.5	.50	11.9
0896	CKSH	1021	4410	8740		1	23.0	02.729	5.9	7.8	.7	.12	5.1	.34	6.3
0919	CKSH	1021	4446	8720		1	25.0	03.170	5.5	12.3	1.2	.19	5.0	.46	6.8
0907	CKSH	1021	4446	8720		1	23.0	02.770	5.0	8.4	.9	.19	3.9	.31	5.3
0908	CKSH	1021	4446	8720		1	25.0	02.728	3.3	14.2	1.3	.06	6.2	.19	7.7
0913	CKSH	1021	4446	8720		1	22.0	03.178	8.4	7.3	1.3	.23	4.1	.47	6.9
0913	CKSH	1021	4446	8720		1	25.0	03.178	7.4	6.7	1.3	.20	5.4	.52	7.5
0917	CKSH	1021	4446	8720		1	23.0	02.497	7.4	11.9	.9	.10	4.2	.35	5.5
0910	CKSH	1021	4446	8720		1	25.0	03.059	4.6	8.4	1.0	.20	3.0	.49	5.5

*Number of fish, if composite sample

MONS 203835

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dch No*	Length	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
								(µg/gm)					
0916	CKSH	1021	9496 8720	1	21.0	02.093	3.6	8.3	1.1	.25	4.9	.51	6.8
0918	CKSH	1021	9496 8720	1	21.0	02.093	1.0	7.6	.8	.11	2.0	.30	4.0
0966	CKSH	0916	9617 8700	1	20.0	04.767	5.7	32.4	1.2	.20	5.5	.44	7.3
0642	COHO	0917	9193 8655	03	1	10.4	00.850	7.7	2.6	.5	.06	.6	.16	1.4
0646	COHO	0917	9193 8655	03	1	20.5	00.990	8.2	2.6	.4	.00	.9	.20	1.6
0644	COHO	0917	9193 8655	03	1	20.0	00.980	7.2	3.2	.5	.07	.9	.17	1.6
0649	COHO	0917	9193 8655	03	1	19.1	01.183	7.6	2.7	.5	.09	.8	.17	1.5
0642	COHO	0917	9193 8655	03	1	19.2	01.200	3.0	3.0	.6	.10	.8	.17	1.7
0989	COHO	0927	9193 8655	1	21.0	01.350	7.2	32.2	1.3	.21	5.0	.67	7.1
1000	COHO	0927	9193 8655	1	20.0	02.790	5.4	19.4	1.2	.24	4.7	.42	6.6
1002	COHO	0927	9193 8655	1	24.5	04.070	7.3	19.4	1.0	.16	5.0	.40	6.6
1007	COHO	0927	9193 8655	1	24.0	03.520	6.9	15.6	1.9	.25	6.2	.50	8.3
1004	COHO	0927	9193 8655	1	21.0	02.315	7.5	16.3	1.6	.23	4.5	.69	7.1
1010	COHO	0927	9193 8655	1	23.0	02.825	9.9	1.6	.3	.05	.7	.15	1.2
1023	COHO	1007	9193 8655	1	19.0	01.655	4.1	19.0	2.0	.22	6.3	.51	9.0
1025	COHO	1007	9193 8655	1	20.0	01.640	3.2	14.5	.9	.13	4.5	.28	5.8
1020	COHO	1007	9193 8655	1	20.[illegible]	02.[illegible]	5.2	18.3	2.2	.29	6.5	.50	9.6
1021	COHO	1007	9193 8655	1	19.0	01.970	3.5	11.2	1.0	.15	3.3	.37	4.8
1024	COHO	1007	9193 8655	1	13.5	00.540	0.2	10.1	1.0	.09	1.9	.34	3.3
0926	COHO	1021	9395 8730	1	22.0	03.069	4.0	11.6	1.9	.28	3.8	.55	6.0

*Number of fish, if composite sample

MONS 203836

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dpth	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(ug/gm)					
0936	COHO	1021	4345 8730		1	28.0	04.086	4.3	12.0	1.2	.17	5.9	.40	7.7
0923	COHO	1021	4345 8730		1	27.0	03.405	4.9	10.9	1.9	.33	5.7	.70	8.7
0929	COHO	1021	4345 8730		1	24.0	03.637	3.5	10.0	1.3	.35	7.0	.69	9.2
0928	COHO	1021	4345 8730		1	28.0	03.659	4.7	15.1	1.3	.27	8.3	.62	10.5
0930	COHO	1021	4345 8730		1	27.0	04.313	3.7	12.3	1.3	.22	6.8	.62	8.9
0931	COHO	1021	4345 8730		1	23.0	03.642	3.7	8.1	1.7	.31	6.6	.70	9.4
0933	COHO	1021	4345 8730		1	23.0	02.749	6.9	17.3	1.1	.29	7.5	.39	9.2
1318	COHO	0828	4355 8640		1	27.8	"	9.1	11.3	1.7	.20	5.9	.40	8.2
1316	COHO	0828	4355 8640		1	25.2	"	9.6	6.5	.6	.22	5.8	.37	7.0
1331	COHO	0829	4355 8640		1	26.8	"	9.6	12.8	.9	.16	9.3	.32	10.7
0776	COHO	1007	4443 8610		1	27.5	"	6.0	12.9	1.6	.24	6.9	.47	9.1
0781	COHO	1007	4443 8610		1	29.0	"	5.1	11.0	1.2	.19	5.1	.40	6.9
0773	COHO	1007	4443 8610		1	29.5	"	6.3	15.4	1.1	.21	6.9	.32	8.4
0783	COHO	1007	4443 8610		1	27.0	"	6.4	13.3	1.5	.23	6.0	.40	8.1
0774	COHO	1007	4443 8610		1	26.5	"	4.5	12.5	1.0	.18	5.4	.40	7.2
0779	COHO	1007	4443 8610		1	26.0	"	3.6	12.9	1.0	.24	5.9	.49	8.1
0782	COHO	1007	4443 8610		1	29.5	"	5.7	12.9	1.2	.17	5.9	.37	7.7
0771	COHO	1007	4443 8610		1	25.0	"	5.9	11.6	1.4	.34	5.9	.56	8.2
0774	COHO	1007	4443 8610		1	26.5	"	6.9	12.6	2.1	.34	6.1	.86	9.3
0950	COHO	0914	4517 8700		1	26.0	03.859	7.0	10.9	1.4	.22	6.0	.47	8.1

*Number of fish, if composite sample

MONS 203837

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Drb	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDS	pp' DDE	Total DDT
									(ug/gm)					
0962	COHO	0916	9517	8700	1	27.0	04.767	7.0	11.9	1.6	.22	4.9	.54	7.2
1030	COHO	0916	9517	8700	1	26.0	03.510	7.0	18.9	1.7	.26	3.0	.58	9.6
0972	COHO	0916	9517	8700	1	28.0	04.994	7.1	16.9	1.2	.27	4.7	.41	8.6
0961	COHO	0916	9517	8700	1	16.0	01.135	8.2	3.2	.6	.00	1.4	.22	2.2
1029	COHO	0916	9517	8700	1	24.0	03.150	7.3	16.1	.9	.14	3.6	.38	5.0
1020	COHO	0916	9517	8700	1	25.0	03.150	7.5	15.2	.6	.20	5.3	.51	6.6
0969	COHO	0916	9517	8700	1	25.5	03.859	7.5	12.2	1.6	.25	6.8	.61	9.3
0959	COHO	0916	9517	8700	1	23.5	02.941	9.6	9.9	1.5	.20	4.3	.42	6.4
0955	COHO	0916	9517	8700	1	25.5	01.086	9.6	12.2	1.3	.24	5.5	.49	7.5
1013	LKTR	1015	9193	8455	12	1	20.5	01.415	14.1	15.9	2.6	.24	5.1	.59	8.9
1014	LKTR	1015	9193	8455	12	1	21.0	02.005	10.7	13.0	3.6	.50	7.0	1.27	12.4
1011	LKTR	1015	9193	8455	12	1	22.0	02.039	17.9	18.9	3.6	.34	5.9	.83	10.6
1018	LKTR	0909	9195	8650	10	1	24.6	02.445	15.0	21.9	3.7	.60	10.1	1.13	15.6
1019	LKTR	0909	9195	8650	10	1	22.0	01.865	20.4	24.3	3.9	.39	6.7	1.19	14.1
0236	LKTR	0421	9240	8620	10	1	22.2		16.1	12.0	1.8	.20	4.9	.56	7.4
0252	LKTR	0421	9240	8620	10	1	21.0		19.1	7.4	1.6	.29	2.5	.50	4.9
0250	LKTR	0421	9240	8620	10	1	23.8		16.3	11.3	1.3	.18	3.4	.41	5.0
0249	LKTR	0421	9240	8620	10	1	22.3		17.4	15.9	2.4	.34	4.8	.91	8.5
0236	LKTR	0421	9240	8620	10	1	23.2		16.5	6.0	.9	.16	4.5	.30	5.9
0246	LKTR	0421	9240	8620	10	1	25.3		16.5	18.7	2.8	.39	6.0	.80	10.0

*Number of fish, if composite sample

MONS 203838

Appendix A. Summary of Data on Fish Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
0295	LKTR	0621	9240 8620	13	1	20.6	.	17.6	8.2	2.5	.10	4.4	.50	7.7
0292	LKTR	0621	9240 8620	13	1	20.8	.	19.9	14.6	2.4	.30	5.0	.72	8.4
0298	LKTR	0621	9240 8620	10	1	24.5	.	15.9	13.9	2.6	.34	5.4	.95	9.3
1319	LKTR	0710	9240 8620	10	1	24.4	.	19.0	18.2	2.8	.35	8.0	.81	12.0
1317	LKTR	0710	9240 8620	10	1	25.8	.	15.0	18.3	.3	.73	9.5	.80	10.9
1327	LKTR	0710	9240 8620	10	1	21.4	.	21.4	17.3	2.1	.19	6.9	.69	9.8
1321	LKTR	0710	9240 8620	10	1	22.6	.	20.7	14.4	3.5	.29	4.4	.93	9.0
1315	LKTR	0710	9240 8620	10	1	23.6	.	22.0	13.0	2.5	.24	6.3	.69	9.7
1320	LKTR	1001	9240 8620	15	1	20.7	.	18.1	11.4	2.6	.20	2.6	.59	6.1
1326	LKTR	1001	9240 8620	15	1	22.5	.	16.1	12.3	1.9	.19	4.0	.53	6.8
1330	LKTR	1001	9240 8620	15	1	22.8	.	15.3	17.7	.3	.33	6.9	1.02	8.6
1324	LKTR	1001	9240 8620	15	1	24.8	.	24.3	19.1	4.0	.45	8.3	1.09	13.7
1333	LKTR	1001	9240 8620	15	1	23.0	.	13.6	21.1	1.9	.24	4.6	.62	7.4
1314	LKTR	1001	9240 8620	15	1	22.5	.	17.9	13.1	3.3	.26	4.6	.90	9.1
1332	LKTR	1001	9240 8620	15	1	23.0	.	18.9	20.9	4.3	.43	10.5	1.06	16.3
1310	LKTR	1001	9240 8620	15	1	22.6	.	17.9	25.6	3.7	.03	11.7	.90	16.3
1090	LKTR	0919	9305 8745	10	1	26.8	04.740	23.4	21.2	3.2	.46	9.2	1.00	13.8
1056	LKTR	0919	9305 8745	10	1	28.0	04.980	19.0	25.5	3.9	.59	15.0	1.22	20.7
1055	LKTR	0919	9305 8745	10	1	25.5	02.070	18.4	31.7	3.7	.53	13.4	1.12	18.7
1083	LKTR	0919	9305 8745		1	24.5	02.945	14.5	17.7	5.5	.49	7.5	1.15	14.6

*Number of fish, if composite sample

MONS 203839

Appendix A. Summary of Data on Fish Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Drh	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
1004	LKTR	0719	9305	0745	1	24.5	02.945	19.7	17.8	2.3	.31	7.2	.06	10.7
1005	LKTR	0719	9305	0745	1	25.0	03.225	14.9	21.1	2.9	.91	10.1	.93	14.9
0887	LKTR	1015	9305	0745	10	19.0	01.076	12.1	11.3	1.2	.16	2.8	.99	4.3
0895	LKTR	1015	9305	0745	10	19.5	01.012	12.2	6.9	1.0	.14	2.7	.35	4.2
0891	LKTR	1015	9305	0745	10	13.5	00.209	5.2	0.3	.08	.08	1.0	.27	2.2
0888	LKTR	1015	9305	0745	10	19.9	01.112	14.9	8.6	.9	.13	2.8	.32	4.1
0888	LKTR	1015	9305	0745	10	13.4	00.2[illegible]	7.5	13.5	1.0	.10	1.5	.32	2.9
0894	LKTR	1015	9305	0745	10	19.5	01.305	14.6	13.9	1.5	.26	2.3	.70	4.7
0886	LKTR	1015	9305	0745	10	23.5	02.220	20.7	13.7	2.3	.29	3.9	.61	7.0
0892	LKTR	1015	9305	0745	10	13.0	00.292	5.8	17.8	.9	.06	1.6	.36	2.9
0896	LKTR	1015	9305	0745	10	13.8	00.329	5.9	6.1	.6	.02	1.2	.21	2.1
0673	LKTR	0713	9345	0730	15	23.5	00.245	9.0	12.1	.9	.12	3.9	.30	5.3
0313	LKTR	0713	9345	0730	15	24.4	[illegible]	19.0	15.7	3.1	.98	7.3	.83	11.5
0269	LKTR	0713	9345	0730	15	23.5	[illegible]	23.0	20.2	1.2	.25	8.2	.42	10.1
0270	LKTR	0713	9345	0730	15	17.5	[illegible]	17.0	9.9	1.6	.25	2.7	.48	5.1
0254	LKTR	0713	9345	0730	05	24.0	[illegible]	16.0	13.7	2.3	.37	7.4	.65	10.7
0253	LKTR	0713	9345	0730	05	19.5	[illegible]	16.0	9.9	1.2	.20	3.7	.39	5.4
0231	LKTR	0713	9345	0730	05	23.5	[illegible]	16.0	12.1	2.5	.32	5.3	.72	8.9
0263	LKTR	0713	9345	0730	15	21.5	[illegible]	18.1	12.9	2.6	.34	3.7	.80	7.4
0319	LKTR	0713	9345	0730	15	26.0	[illegible]	18.1	14.4	3.9	.33	7.0	.93	12.2

*Number of fish, if composite sample

MONS 203840

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(ug/gm)					
0671	LKTR	0713	4345 8730				00.177	8.1	12.8	.2	.12	2.7	.33	3.8
0674	LKTR	0713	4345 8730				00.169	8.2	7.1	.6	.07	1.8	.17	2.6
0271	LKTR	0713	4345 8730	15	1	18.5		10.2	9.9	1.9	.19	2.1	.37	4.1
0675	LKTR	0713	4345 8730				00.254	13.2	10.6	1.1	.09	3.9	.32	5.4
0672	LKTR	0713	4345 8730				00.179	8.3	12.0	.7	.09	3.2	.29	4.4
0271	LKTR	0713	4345 8730	15	1	20.0		14.3	12.6	1.1	.14	2.6	.30	4.1
0265	LKTR	0713	4345 8730	15	1	23.6		21.3	17.3	2.8	.37	3.9	1.01	8.2
0311	LKTR	0713	4345 8730	15	1	25.5		19.0	17.9	2.3	.26	5.6	.69	8.8
0262	LKTR	0713	4345 8730	15	1	19.0		15.4	9.8	1.1	.14	2.6	.31	4.2
0733	LKTR	0713	4345 8730	05	1	25.2		16.5	16.9	2.5	.30	6.6	.78	10.3
0670	LKTR	0713	4345 8730				00.223	8.6	6.1	.7	.09	2.0	.24	3.1
0230	LKTR	0713	4345 8730	05	1	22.8		17.4	17.9	2.7	.38	5.3	.68	9.1
0269	LKTR	0713	4345 8730	15	1	25.6		23.7	13.3	2.0	.33	4.6	.58	7.5
0316	LKTR	0713	4345 8730	15	1	20.0		15.2	8.7	1.6	.20	3.0	.52	5.3
0318	LKTR	0713	4345 8730	15	1	21.5		16.2	12.1	1.5	.25	5.3	.55	7.6
0315	LKTR	0713	4345 8730	15	1	20.0		21.0	12.0	2.7	.31	6.2	.73	9.9
0229	LKTR	0713	4345 8730	15	1	22.0		17.8	8.4	1.3	.27	2.9	.49	5.0
0676	LKTR	0713	4345 8730				00.210	14.0	12.0	1.1	.15	3.7	.32	5.2
0669	LKTR	0713	4345 8730				00.198	10.9	14.0	.9	.09	3.4	.12	4.1
0310	LKTR	0711	4345 8690	13	1	20.5		20.0	6.3	1.5	.21	2.4	.55	4.6

*Number of fish, if composite sample

MONS 203841

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(ug/gm)					
0214	LKTR	0701	9355 8640	13	1	22.6		19.1	8.7	2.2	.40	3.7	.67	7.0
0213	LKTR	0701	9355 8640	13	1	20.2		18.3	5.6	1.6	.20	1.9	.54	4.3
0215	LKTR	0701	9355 8640	13	1	20.3		18.3	6.1	1.5	.12	2.2	.46	4.3
0304	LKTR	0701	9355 8640	13	1	21.0		18.3	9.7	1.7	.27	3.9	.52	6.3
0223	LKTR	0701	9355 8640	13	1	21.2		17.9	9.3	1.8	.35	4.9	.64	7.7
0309	LKTR	0701	9355 8640	13	1	20.0		16.9	8.2	1.6	.21	3.3	.54	5.7
0308	LKTR	0701	9355 8640	13	1	22.0		17.7	11.3	2.1	.23	3.0	.60	6.7
0306	LKTR	0701	9355 8640	13	1	21.7		13.7	9.0	1.7	.20	2.0	.66	5.3
0216	LKTR	0704	9355 8640	10	1	21.0		19.2	9.5	2.4	.33	4.4	.73	7.9
0219	LKTR	0704	9355 8640	10	1	20.4		11.3	8.2	2.0	.39	4.0	.79	7.9
0256	LKTR	0704	9355 8640	10	1	20.5		13.3	6.7	1.5	.24	2.0	.48	4.2
0210	LKTR	0704	9355 8640	10	1	21.3		19.5	7.0	1.9	.29	2.5	.67	5.4
0305	LKTR	0704	9355 8640	10	1	21.6		14.6	9.4	2.1	.27	3.6	.52	6.5
0221	LKTR	0704	9355 8640	13	1	20.0		15.2	6.0	1.9	.31	1.9	.61	4.7
0751	LKTR	1019	9455 8535		1	24.0		16.0	12.2	2.6	.30	4.7	.78	8.3
0766	LKTR	1019	9455 8535		1	24.0		25.0	20.2	2.5	.49	12.0	.89	16.0
0763	LKTR	1019	9455 8535		1	20.5		24.2	12.5	2.9	.39	10.0	.53	13.8
0755	LKTR	1019	9455 8535		1	20.6		24.3	14.1	2.6	.30	5.6	.76	9.3
0754	LKTR	1019	9455 8535		1	25.0		19.3	10.6	2.0	.24	5.7	.56	8.5
0767	LKTR	1019	9455 8535		1	25.0		15.4	3.9	1.1	.17	2.5	.46	4.2

*Number of fish, if composite sample

MONS 203842

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
0750	LKTR	1019	4455 8535		1	26.0	.	15.6	12.6	1.8	.28	4.3	.69	7.0
0752	LKTR	1019	4455 8535		1	18.8	.	15.7	4.2	.7	.19	2.3	.48	3.7
0282	LKTR	0721	4505 8730	20	1	22.0	.	23.0	5.6	.8	.10	3.3	.22	4.8
0320	LKTR	0721	4505 8730	20	1	24.0	.	18.1	8.0	1.1	.12	3.3	.45	4.9
0279	LKTR	0721	4505 8730	20	1	23.0	.	19.1	10.9	1.1	.19	3.5	.42	5.2
0209	LKTR	0721	4505 8730	20	1	22.5	.	21.2	9.9	1.8	.22	4.5	.53	7.0
0276	LKTR	0721	4505 8730	20	1	23.0	.	14.3	10.7	1.7	.26	4.3	.54	6.8
0324	LKTR	0721	4505 8730	20	1	24.8	.	16.5	11.3	1.4	.22	5.0	.57	7.2
0211	LKTR	0721	4505 8730	20	1	22.0	.	20.6	7.3	2.3	.17	3.4	.43	6.3
0321	LKTR	0721	4505 8730	20	1	23.5	.	18.7	11.3	1.4	.19	2.2	.49	4.3
0281	LKTR	0721	4505 8730	20	1	23.5	.	21.7	9.2	1.4	.22	3.3	.50	5.3
0208	LKTR	0721	4505 8730	20	1	23.0	.	20.8	9.5	1.9	.41	5.1	.79	8.2
0212	LKTR	0721	4505 8730	20	1	23.5	.	20.9	9.8	1.5	.25	4.5	.64	6.9
1046	LKTR	0916	4517 8700		1	19.0	01.340	14.1	8.4	.9	.12	2.[illegible]	.33	[illegible]
1049	LKTR	0916	4517 8700		1	19.0	01.080	17.1	7.0	.9	.10	2.3	.35	3.6
1048	LKTR	0916	4517 8700		1	21.0	01.570	17.1	12.5	1.5	.23	5.0	.48	7.1
1035	LKTR	0916	4517 8700		1	25.0	03.550	15.1	18.7	1.8	.28	6.6	.56	9.2
1043	LKTR	0916	4517 8700		1	27.0	03.365	18.3	24.7	2.4	.34	9.4	.69	12.8
1045	LKTR	0916	4517 8700		1	19.5	01.165	17.3	7.5	.7	.10	2.8	.35	3.9
0967	LKTR	0916	4517 8700		1	24.0	03.170	18.6	14.2	3.5	.33	7.4	1.13	12.4

*Number of fish, if composite sample

MONS 203843

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
1050	LKTR	0916	4517 8700		1	26.0	03.640	22.7	22.1	3.5	.45	7.5	1.06	12.6
0705	LOUY	0721	4505 8730	20	1	14.0	[illegible]	6.1	2.5	.3	.03	.5	.13	.9
1298	PRCH	1015	4143 8655	12	1	06.0	00.072	5.0	3.8	.4	.04	.9	.15	1.5
1287	PRCH	1015	4143 8655	12	1	08.0	00.095	7.0	2.5	.2	.02	.8	.09	1.1
1297	PRCH	1015	4143 8655	12	1	08.0	00.079	5.1	5.2	.3	.05	1.1	.20	1.7
1305	PRCH	1015	4143 8655	12	1	08.0	00.086	6.3	5.0	.4	.04	.9	.15	1.5
1309	PRCH	1015	4143 8655	12	1	07.5	00.097	4.3	4.9	.5	.03	.7	.19	1.4
1303	PRCH	1015	4143 8655	12	1	07.5	00.083	5.5	4.3	.1	.02	.7	.05	.9
1300	PRCH	1015	4143 8655	12	1	07.5	00.061	6.5	4.9	.4	.04	.7	.16	1.2
1292	PRCH	1015	4143 8655	12	1	07.5	00.086	9.6	4.3	.3	.04	.6	.15	1.1
1308	PRCH	1015	4143 8655	12	1	08.0	00.106	7.7	4.4	.5	.06	.7	.20	1.5
1290	PRCH	1015	4143 8655	12	1	08.0	00.099	6.9	3.8	.4	.05	.8	.17	1.4
0790	PRCH	1013	4305 8745	10	1	09.5	00.146	5.0	14.3	.5	.05	1.0	.25	1.8
0792	PRCH	1013	4305 8745	10	1	09.0	00.196	5.1	8.0	.7	.11	.8	.25	1.4
0793	PRCH	1013	4305 8745	10	1	10.5	00.253	8.1	12.6	1.2	.16	1.4	.63	3.4
0799	PRCH	1013	4305 8745	10	1	09.5	00.166	6.2	8.9	.4	.06	.8	.21	1.5
0794	PRCH	1013	4305 8745	10	1	09.5	00.187	5.5	12.2	.6	.06	1.3	.27	2.3
0791	PRCH	1013	4305 8745	10	1	09.5	00.145	3.8	12.5	.6	.02	1.1	.27	2.1
0795	PRCH	1013	4305 8745	10	1	08.5	00.111	4.6	8.4	.5	.06	1.3	.29	2.1
0796	PRCH	1013	4305 8745	10	1	09.5	00.221	6.2	13.9	.6	.11	1.0	.41	2.4

*Number of fish, if composite sample.

MONS 203844

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
0798	PRCH	1013	4305 8795	10	1	08.5	00.134	5.8	4.8	.4	.00	.7	.12	1.3
0337	PRCH	0706	4355 8640	11	3	10.5	.	4.0	6.5	.1	.03	2.4	.12	2.7
0335	PRCH	0706	4355 8640	11	4	11.0	.	5.2	5.4	1.7	.22	2.1	.47	4.5
0719	PRCH	1012	4440 8625	11	1	11.3	.	9.0	6.2	1.7	.19	2.2	.39	4.4
0795	PRCH	1012	4440 8625	11	1	12.0	.	6.4	4.5	2.8	.09	1.3	.48	4.7
0343	PRCH	0825	4445 8750	07	1	08.5	00.132	4.4	1.5	.1	.02	.4	.12	.6
0347	PRCH	0825	4445 8750	07	1	08.8	00.165	7.9	8.1	.0	.00	.3	.06	.3
0339	PRCH	0825	4445 8750	07	1	08.6	00.190	4.9	1.5	.0	.00	.4	.11	.5
1033	RBTR	0916	4517 8700		1	24.0	04.180	22.0	7.3	1.0	.15	1.9	.32	3.3
0960	RBTR	0916	4517 8700		1	20.0	02.497	14.5	1.6	.3	.07	1.6	.13	2.1
0973	RBTR	0916	4517 8700		1	28.0	05.676	16.6	14.0	1.0	.18	5.3	.37	6.0
1036	RBTR	0916	4517 8700		1	26.0	04.035	21.8	7.3	1.3	.19	3.4	.52	5.4
1031	RBTR	0916	4517 8700		1	23.0	02.540	14.9	8.2	1.1	.16	3.1	.34	4.7
1134	RDSU	0911	4520 8645	14	1	15.0	00.590	5.1	2.3	.6	.07	1.0	.33	2.0
1133	RDSU	0911	4520 8645	14	1	15.0	00.770	8.2	3.3	.7	.09	1.5	.37	2.6
1130	RDSU	0911	4520 8645	14	1	19.5	01.440	9.4	1.5	.2	.09	2.2	.16	2.6
1137	RDSU	0911	4520 8645	14	1	17.0	00.765	9.4	2.3	.5	.07	1.5	.27	2.3
1129	RDSU	0911	4520 8645	14	1	18.0	01.145	8.5	4.1	1.0	.11	2.6	.42	4.1
1140	RDSU	0911	4520 8645	14	1	18.0	00.930	7.8	3.3	.4	.02	1.5	.25	2.3
1125	RDSU	0911	4530 8650	14	1	17.0	01.000	10.2	3.2	1.1	.15	1.9	.59	3.7

*Number of fish, if composite sample

MONS 203845

Appendix A. Summary of Data on Fish Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dph	No*	Length	Wt	% Fat	PCB	pp' EDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
1118	RDSU	0911	9530 8650	19	1	16.5	00.863	6.4	3.9	.7	.08	.2	.29	1.3
1124	RDSU	0911	9530 8650	19	1	16.5	01.005	8.5	2.8	.8	.06	1.5	.21	2.2
1119	RDSU	0911	9530 8650	19	1	17.5	00.800	8.5	3.3	1.1	.10	1.7	.42	3.3
1120	RDSU	0911	9530 8650	19	1	16.5	00.730	6.6	2.6	.7	.08	1.8	.31	2.9
1121	RDSU	0911	9530 8650	19	1	17.0	00.960	10.7	2.5	.7	.10	1.6	.39	2.6
1128	RDSU	0911	9530 8650	19	1	15.4	00.700	8.9	3.2	.7	.08	1.9	.29	3.0
1116	RDSU	0911	9530 8650	19	1	17.0	01.195	9.9	3.5	.7	.08	1.6	.31	2.6
1252	SCUL	1014	9290 8620	50	3	05.4	00.029	4.1	4.8	3.1	.09	2.6	.31	6.0
1260	SCUL	1014	9290 8620	50	4	04.6	00.022	6.1	2.9	.8	.05	1.4	.12	2.4
1261	SCUL	1014	9290 8620	50	5	04.6	00.017	6.5	2.3	.5	.05	.9	.10	1.5
1248	SCUL	1014	9290 8620	50	4	05.0	00.022	5.5	3.7	2.1	.07	2.2	.27	4.6
1253	SCUL	1014	9290 8620-50	4	03.8	00.012	5.5	1.8	.4	.03	.6	.09	1.1
1256	SCUL	1014	9290 8620	50	4	04.2	00.019	5.8	1.8	.4	.04	.8	.09	1.3
1250	SCUL	1014	9290 8620	50	2	05.4	00.036	6.9	4.0	2.0	.12	2.5	.23	4.8
1249	SCUL	1014	9290 8620	50	3	05.0	00.023	7.9	2.1	.6	.07	1.0	.10	2.0
1239	SMLT	1015	9193 8655	12	3	07.6	00.050	9.0	3.6	.9	.03	1.0	.12	1.5
1229	SMLT	1015	9193 8655	12	1	09.5	00.011	6.1	4.6	.7	.07	1.0	.22	2.0
1233	SMLT	1015	9193 8655	12	1	08.5	00.070	7.2	3.6	.4	.06	1.0	.12	1.5
1236	SMLT	1015	9193 8655	12	2	07.5	00.047	9.2	3.2	.8	.09	1.9	.11	1.9
1241	SMLT	1015	9193 8655	12	3	06.5	00.030	7.2	2.3	.2	.03	.8	.10	1.1

*Number of fish, if composite sample

MONS 203846

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dth	No*	Lgth	Wt	% Fat	(ug/gm) PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
1249	SMLT	1015	4143	8655	12	4	05.5	00.021	5.7	1.5	.2	.02	.9	.05	.17
1240	SMLT	1015	4143	8655	12	3	06.5	00.030	5.7	1.9	.2	.03	.9	.06	1.2
1246	SMLT	1015	4143	8655	12	4	05.6	00.021	7.8	3.1	.3	.03	.7	.06	1.0
1234	SMLT	1015	4143	8655	12	1	06.5	00.072	7.0	4.3	.9	.04	1.0	.15	1.6
1243	SMLT	1015	4143	8655	12	2	06.5	00.036	7.8	3.8	.3	.03	.9	.07	1.3
1231	SMLT	1015	4143	8655	12	1	08.5	00.040	4.0	4.3	.6	.06	1.1	.17	1.9
0842	SMLT	101	4345	8730		4	05.5	.	5.1	.7	.2	.03	.8	.15	1.2
0843	SMLT	1015	4345	8730		2	07.0	.	2.7	.8	.8	.04	.5	.12	1.0
0841	SMLT	1015	4345	8730		9	04.5	.	1.9	.5	.2	.02	.3	.10	.7
0171	SMLT	0721	4505	8730	20	2	05.5	.	5.3	2.7	.1	.01	.3	.11	.6
0172	SMLT	0721	4505	8730	20	2	05.6	.	3.5	1.0	.1	.01	.3	.06	.4
0170	SMLT	0721	4505	8730	20	2	06.5	.	3.6	3.6	.1	.01	.4	.09	.6
0168	SMLT	0721	4505	8730	20	2	09.5	.	9.9	2.7	.4	.06	2.0	.23	2.7
0527	SMLT	0911	4520	8645	19	2	07.7	00.043	8.1	5.1	.3	.05	1.0	.15	2.2
0519	SMLT	0911	4520	8645	19	1	08.0	00.061	5.3	9.4	.5	.04	.7	.16	1.4
0522	SMLT	0911	4520	8645	19	1	08.3	00.072	9.1	9.8	.5	.04	.8	.11	1.3
0517	SMLT	0911	4520	8645	19	2	07.2	00.046	5.6	5.8	.3	.03	.8	.10	1.3
0520	SMLT	0911	4520	8645	19	2	07.0	00.039	4.4	2.8	.3	.03	.8	.11	1.2
0523	SMLT	0911	4520	8645	19	1	08.0	00.066	4.6	1.4	.4	.04	.5	.10	1.1
0524	SMLT	0911	4520	8645	19	2	07.0	00.042	5.0	1.8	.3	.06	.6	.13	1.1

*Number of fish, if composite sample

MONS 203847

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

(ug/gm)

Sample Number	Species	1971 Date	Location	Drh	No*	Length	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
0005	SALT	0719	9530	8525	30	2	09.0	.3.2	1.3	.9	.05	.7	.09	1.2
0004	SALT	0719	9530	8525	30	3	06.5	4.3	1.0	.1	.05	.0	.10	1.1
0002	SALT	0719	9530	8525	30	9	05.5	4.6	1.7	.1	.03	.5	.07	1.7
0994	WHFH	1015	9143	8655	12	1	19.0	01.360	27.1	6.9	.9	.06	.6	.32	1.9
0992	WHFH	1015	9143	8655	12	1	18.0	01.350	24.2	5.6	.5	.06	.9	.31	1.3
0325	WHFH	0619	9240	8620	07	1	13.3	17.1	1.9	.6	.09	.6	.24	1.5
0326	WHFH	0619	9240	8620	07	1	14.8	22.3	2.8	.5	.10	.7	.21	1.5
0331	WHFH	0621	9240	8620	10	1	14.8	14.3	2.5	.3	.04	.9	.19	1.0
1090	WHFH	0906	9304	8625	19	1	16.0	01.135	19.0	5.5	.3	.03	.6	.21	1.1
1092	WHFH	0906	9304	8625	19	1	21.0	01.950	21.2	6.5	1.0	.09	.7	.41	2.2
1089	WHFH	0906	9304	8625	19	1	22.5	01.540	23.3	5.0	.4	.07	.6	.29	1.5
1093	WHFH	0906	9304	8625	19	1	17.0	01.390	22.7	4.6	.5	.06	.5	.23	1.3
1094	WHFH	0906	9304	8625	19	1	16.0	01.110	22.7	9.3	1.2	.21	.9	.62	2.9
1091	WHFH	0906	9304	8625	19	1	17.5	01.230	26.9	5.5	1.2	.13	.8	.53	2.5
0709	WHFH	1019	9455	8535		1	19.0	20.0	1.5	.1	.21	.9	.06	1.3
0701	WHFH	1019	9455	8535		1	18.5	12.0	1.9	.5	.06	1.3	.30	2.2
0698	WHFH	1019	9455	8535		1	18.0	14.0	1.0	.3	.06	1.2	.26	1.9
0702	WHFH	1019	9455	8535		1	19.0	13.7	2.3	.9	.07	1.6	.23	2.3
0695	WHFH	1019	9455	8535		1	19.5	14.2	1.8	.5	.06	1.1	.24	1.9
0696	WHFH	1019	9455	8535		1	18.0	15.3	1.8	.9	.06	1.2	.29	2.0

*Number of fish, if composite sample

MONS 203848

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dch	No*	Lth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(mg/gm)					
0692	WHFH	1019	4455 8535		1	18.0	.	12.3	1.7	.4	.06	1.3	.24	2.1
0697	WHFH	1019	4455 8535		1	19.0	.	13.4	2.0	.4	.07	1.7	.29	2.5
0694	WHFH	1019	4455 8535		1	21.5	.	9.4	1.0	.3	.04	.0	.27	1.5
0705	WHFH	1019	4455 8535		1	18.0	.	18.5	1.1	.7	.09	1.2	.21	2.2
0699	WHFH	1019	4455 8535		1	19.0	.	10.7	1.6	.4	.04	1.2	.26	1.9
0693	WHFH	1019	4455 8535		1	19.0	.	12.8	2.1	.5	.08	1.2	.32	2.0
1119	WHFH	0911	4520 8645	14	1	18.5	01.270	14.0	1.5	.5	.05	.7	.10	1.4
1111	WHFH	0911	4520 8645	14	1	16.0	00.865	14.4	1.3	.4	.04	.7	.15	1.3
1107	WHFH	0911	4520 8645	14	1	16.0	00.925	13.5	1.5	.4	.04	.7	.12	1.3
1101	WHFH	0911	4520 8645	14	1	18.0	01.270	17.5	1.5	.3	.02	.7	.17	1.3
1105	WHFH	0911	4520 8645	14	1	17.0	01.210	17.6	1.5	.6	.06	.0	.22	1.6
1103	WHFH	0911	4520 8645	14	1	18.0	01.260	13.6	1.3	.2	.04	.8	.12	1.2
1108	WHFH	0911	4520 8645	14	1	18.0	01.255	17.6	1.8	.5	.06	.6	.17	1.4
1106	WHFH	0911	4520 8645	14	1	17.0	00.291	12.9	1.3	.5	.05	.6	.22	1.5
0985	WHSU	1015	4143 8655	12	1	16.0	01.310	9.4	7.1	.8	.00	1.5	.55	2.8
0984	WHSU	1015	4143 8655	12	1	18.0	01.370	13.8	14.2	2.2	.00	2.0	.99	4.2
1166	WHSU	1015	4240 8620	03	1	19.5	02.030	5.8	7.9	.5	.06	.8	.34	1.8
1169	WHSU	1015	4240 8620	05	1	16.0	00.700	3.8	5.0	.2	.02	.6	.15	1.0
1173	WHSU	1018	4240 8620	09	1	13.5	00.460	7.0	1.9	.1	.02	.4	.09	.5
1172	WHSU	1018	4240 8620	09	1	14.0	00.510	6.1	2.6	.2	.04	.5	.15	.6

*Number of Fish, if composite sample

MONS 203849

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dsh	No*	Lgth	Wt	% Fat	PCB	pp' DDT	op' DDT	pp' DDE	pp' TDE	Total DDT
									(µg/gm)					
1170	WHSU	1018	4240	8420	09	1	11.0	00.930	5.2	1.6	.2	.03	1.7	.12	2.0
1171	WHSU	1018	4240	8420	09	1	11.0	00.960	5.4	2.5	.0	.00	.9	.05	.9
1174	WHSU	1018	4240	8420	09	1	12.0	00.950	4.6	2.6	.1	.02	.9	.12	.6
0302	WHSU	0806	9995	8750	06	1	11.5	00.324	3.3	1.7	.0	.01	.2	.05	.3
0297	WHSU	0806	9995	8750	06	1	12.0	00.348	6.3	2.5	.2	.02	.5	.11	.8
0290	WHSU	0806	9995	8750	06	1	13.5	00.396	9.5	3.1	.0	.00	.3	.05	.4
0300	WHSU	0806	9995	8750	06	1	12.5	00.312	5.6	2.1	.0	.01	.3	.09	.4
0299	WHSU	0806	9995	8750	06	1	13.0	00.303	7.8	10.0	1.5	.22	.3	.59	2.6
0296	WHSU	0806	9995	8750	06	1	12.0	00.309	9.9	2.6	.0	.00	.3	.05	.4
0685	WHSU	1019	9955	8535		1	16.5		5.0	3.1	.6	.00	2.1	.97	3.1
0686	WHSU	1019	9955	8535		1	17.0		9.1	3.2	.6	.07	1.7	.39	2.7
0683	WHSU	1019	9955	8535		1	17.0		2.1	1.9	.2	.02	1.0	.20	1.4
0689	WHSU	1019	9955	8535		1	15.0		3.1	1.8	.3	.03	.9	.21	1.4
0677	WHSU	1019	9955	8535		1	12.5		3.3	2.5	.3	.01	1.0	.27	1.9
0681	WHSU	1019	9955	8535		1	17.0		2.4	1.6	.4	.05	1.5	.29	2.2
0682	WHSU	1019	9955	8535		1	18.0		3.8	2.7	.3	.02	1.2	.32	1.9
0687	WHSU	1019	9955	8535		1	17.5		1.8	1.8	.1	.00	.9	.22	1.2
0684	WHSU	1019	9955	8535		1	16.5		4.8	2.0	.3	.04	1.9	.30	2.1
0680	WHSU	1019	9955	8535		1	18.5		4.9	2.5	.2	.03	1.2	.39	1.8
0678	WHSU	1019	9955	8535		1	18.0		4.9	3.5	.5	.10	2.9	.67	4.7

*Number of fish, if composite sample

MONS 203850

Appendix A. Summary of Data on Fish
Analyzed for PCBs and DDT.

Sample Number	Species	1971 Date	Location	Dpth	No*	Length	Wt	% Fat	(µg/gm) PCB	pp' DDT	op' DDT	pp' DDE	pp' DDD	Total DDT
0691	WHSU	1019	9455 8535		1	16.0		3.9	2.9	.06	.05	1.4	.37	2.3
0679	WHSU	1019	9455 8535		1	19.0		.9	1.3	.02	.01	.9	.15	1.2
0204	WHSU	0721	9505 8730	20	1	14.0		10.0	2.1	.04	.08	.7	.19	1.3
1148	WHSU	0911	9520 8645	19	1	16.5	01.045	7.7	4.7	.06	.04	1.5	.35	2.5
1152	WHSU	0911	9520 8645	19	1	18.0	01.230	6.3	1.5	.05	.03	.7	.17	1.4
1142	WHSU	0911	9520 8645	19	1	18.0	01.330	6.4	1.3	.01	.01	.6	.09	.8
1145	WHSU	0911	9520 8645	19	1	18.0	01.065	7.4	1.7	.02	.03	.7	.17	1.1
1151	WHSU	0911	9520 8645	19	1	18.5	01.260	3.5	1.7	.02	.02	.9	.11	1.3
1149	WHSU	0911	9520 8645	19	1	18.0	00.615	9.4	2.5	.04	.04	1.0	.21	1.7
1153	WHSU	0911	9520 8645	19	1	18.0	01.220	9.6	3.9	.05	.05	.9	.30	1.0
1144	WHSU	0911	9520 8645	19	1	17.5	01.090	4.7	1.8	.03	.04	.8	.17	1.3
1154	WHSU	0911	9520 8645	19	1	17.0	00.910	5.0	2.6	.04	.05	1.2	.17	1.9
1143	WHSU	0911	9520 8645	19	1	17.0	01.115	2.8	2.3	.02	.03	.8	.12	1.2
1155	WHSU	0911	9520 8645	19	1	16.0	00.950	4.0	2.7	.03	.04	.8	.27	1.4
1150	WHSU	0911	9520 8645	19	1	20.0	01.920	6.9	2.9	.03	.03	.7	.22	1.2

*Number of fish, if composite sample

MONS 203851

Appendix B. Selected Mass Spectra of Composite Samples of Lake Michigan Fish

MONS 203852

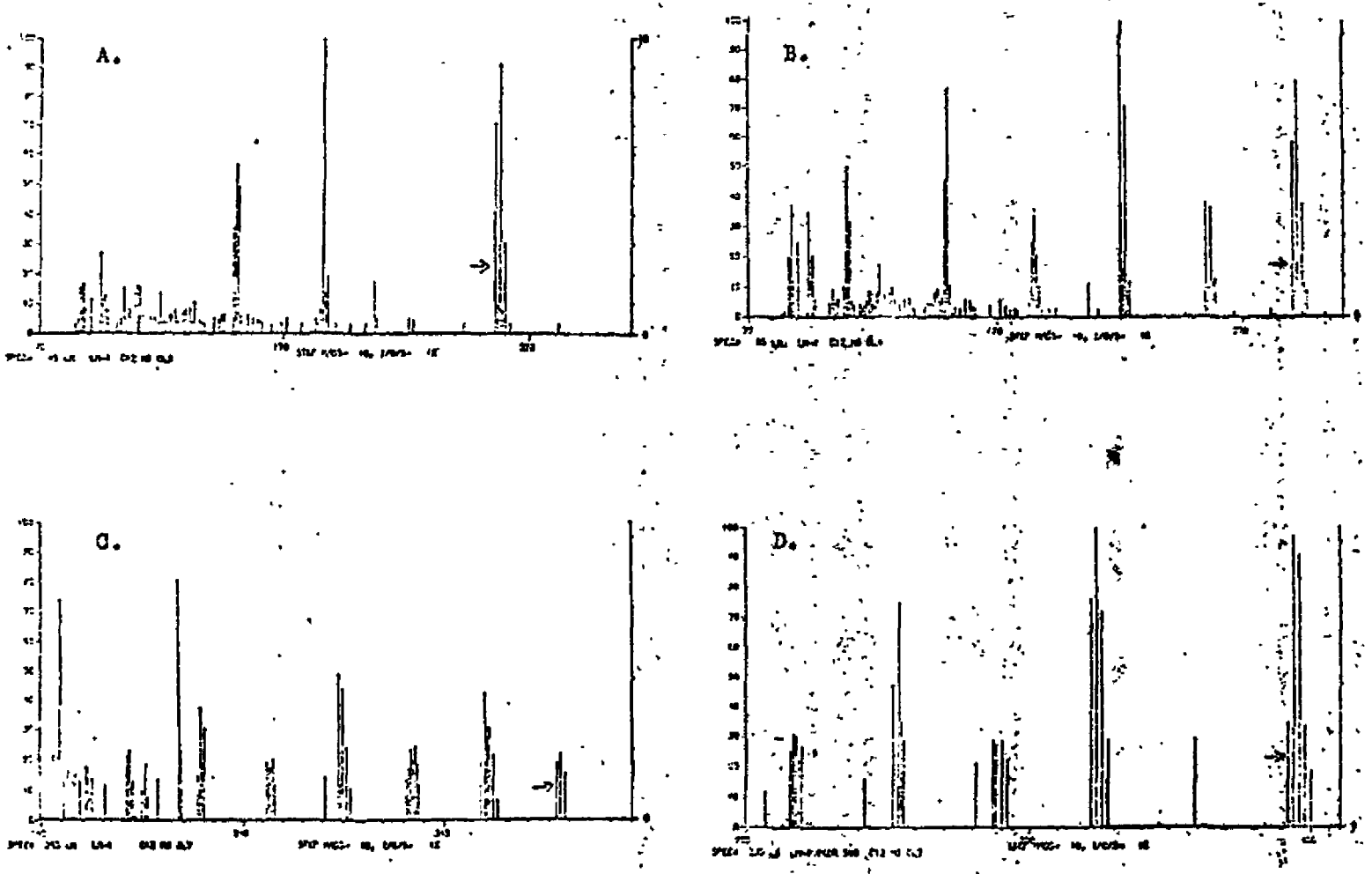


Figure I. Mass Spectra of Major PCBs in Lake Trout from the Milwaukee Area

MONS 203853

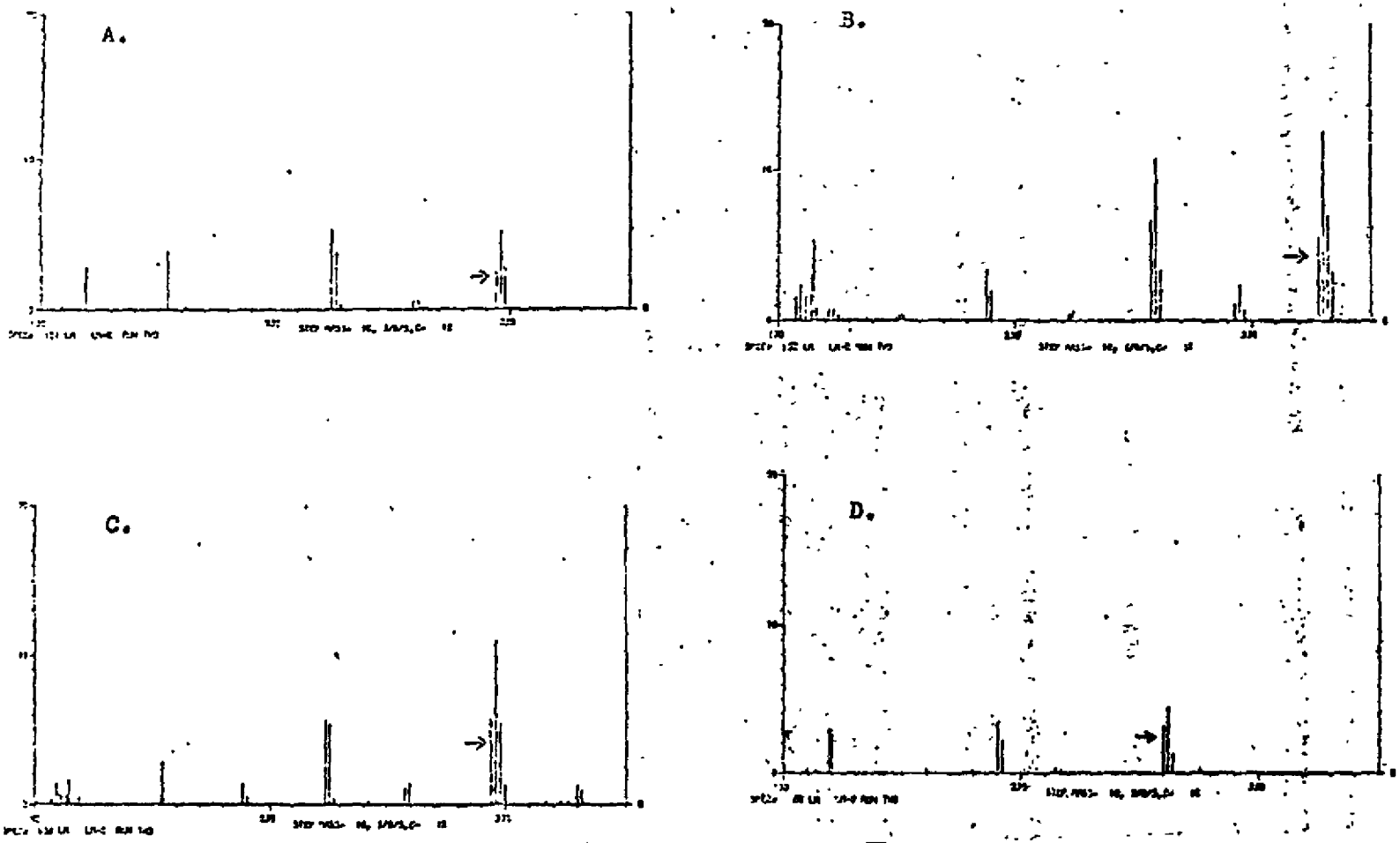


Figure II. Mass Spectra of Major PCBs in Lake Trout from the Saugatuck Area

MONS 203854

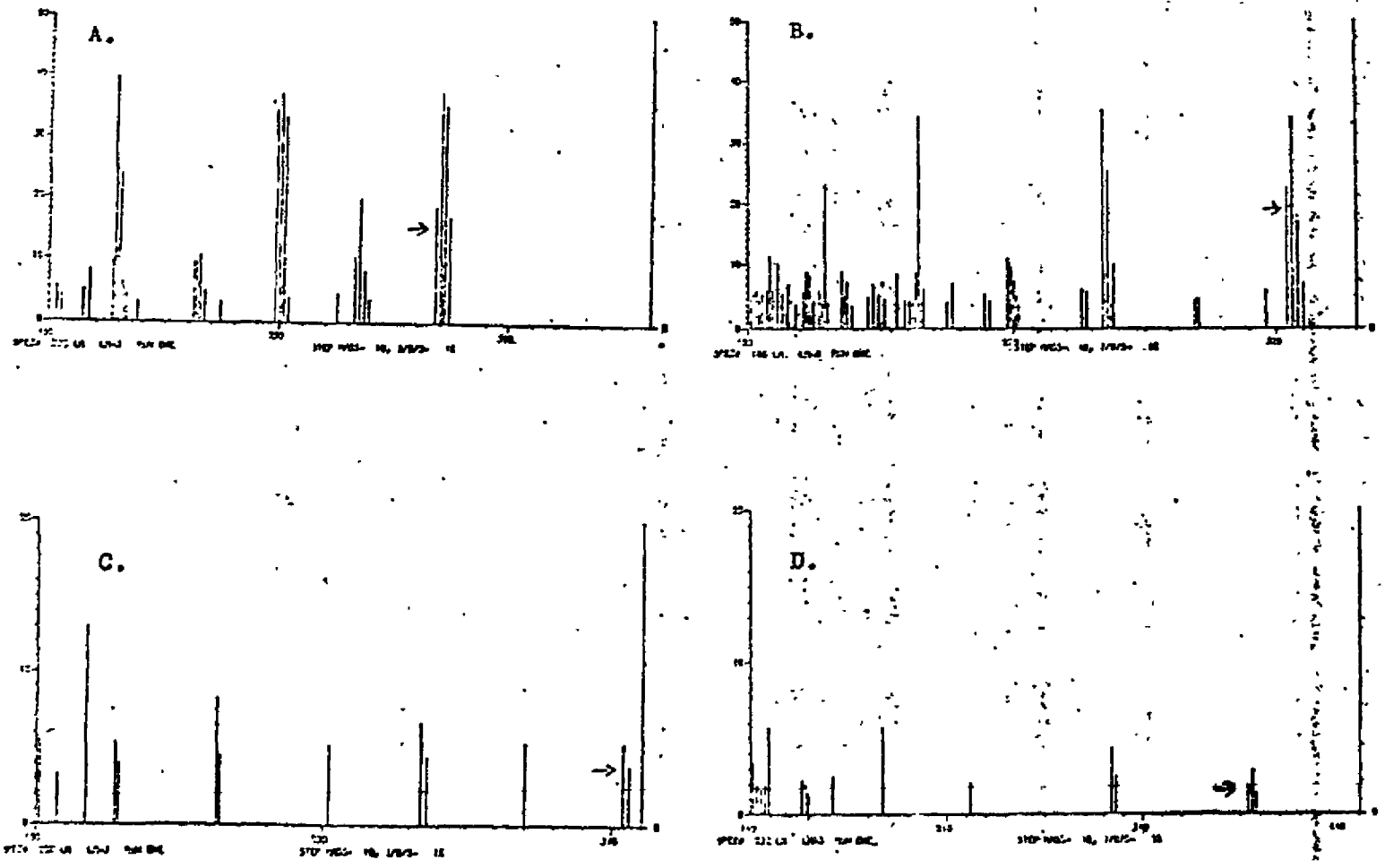


Figure XII. Mass Spectra of Major PCBs in Lake Trout from the Ludington Area.

MONS 203855

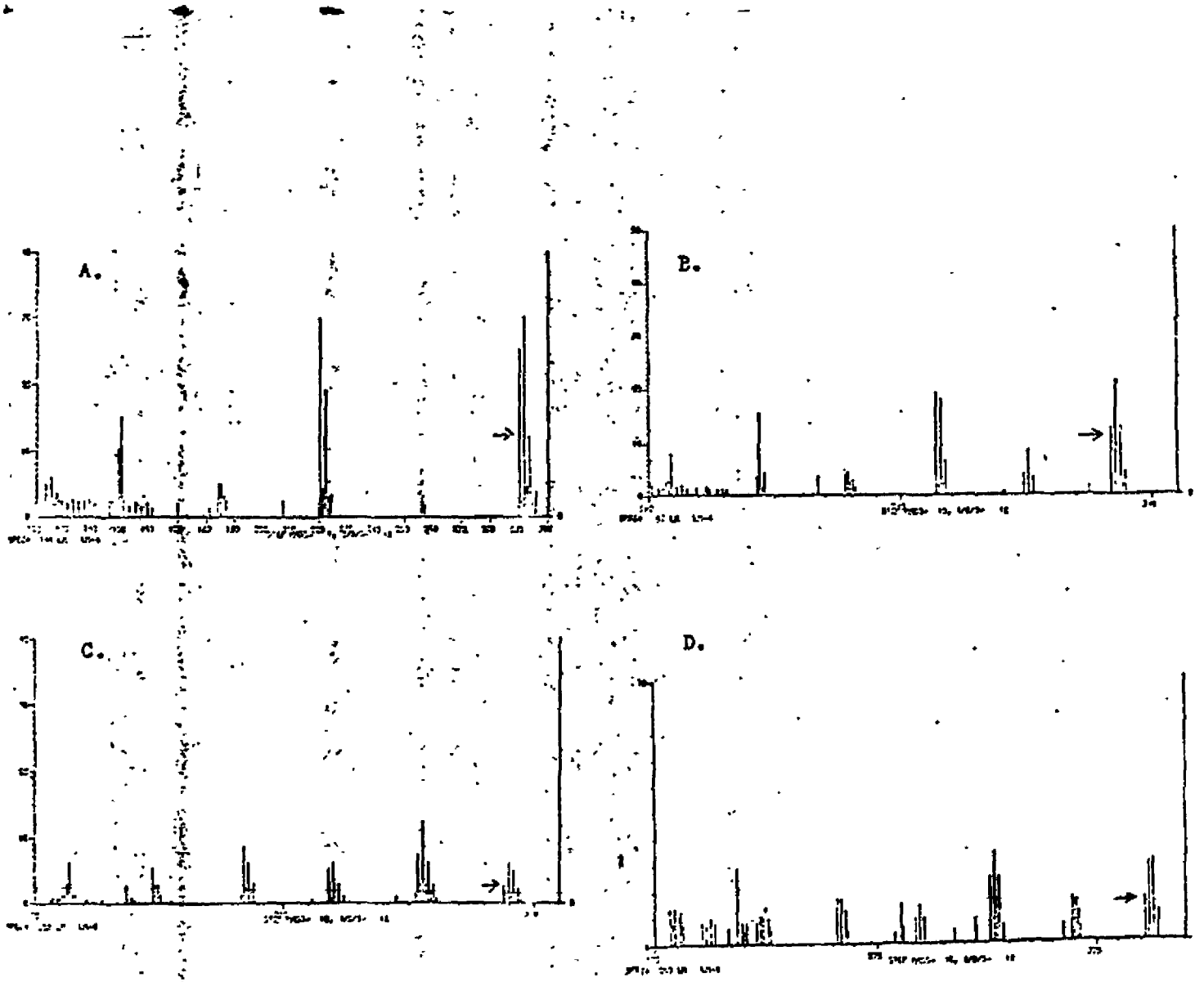


Figure I/. Mass Spectra of Major PCBs in Lake Trout from the Manitowoc Area

MONS 203856

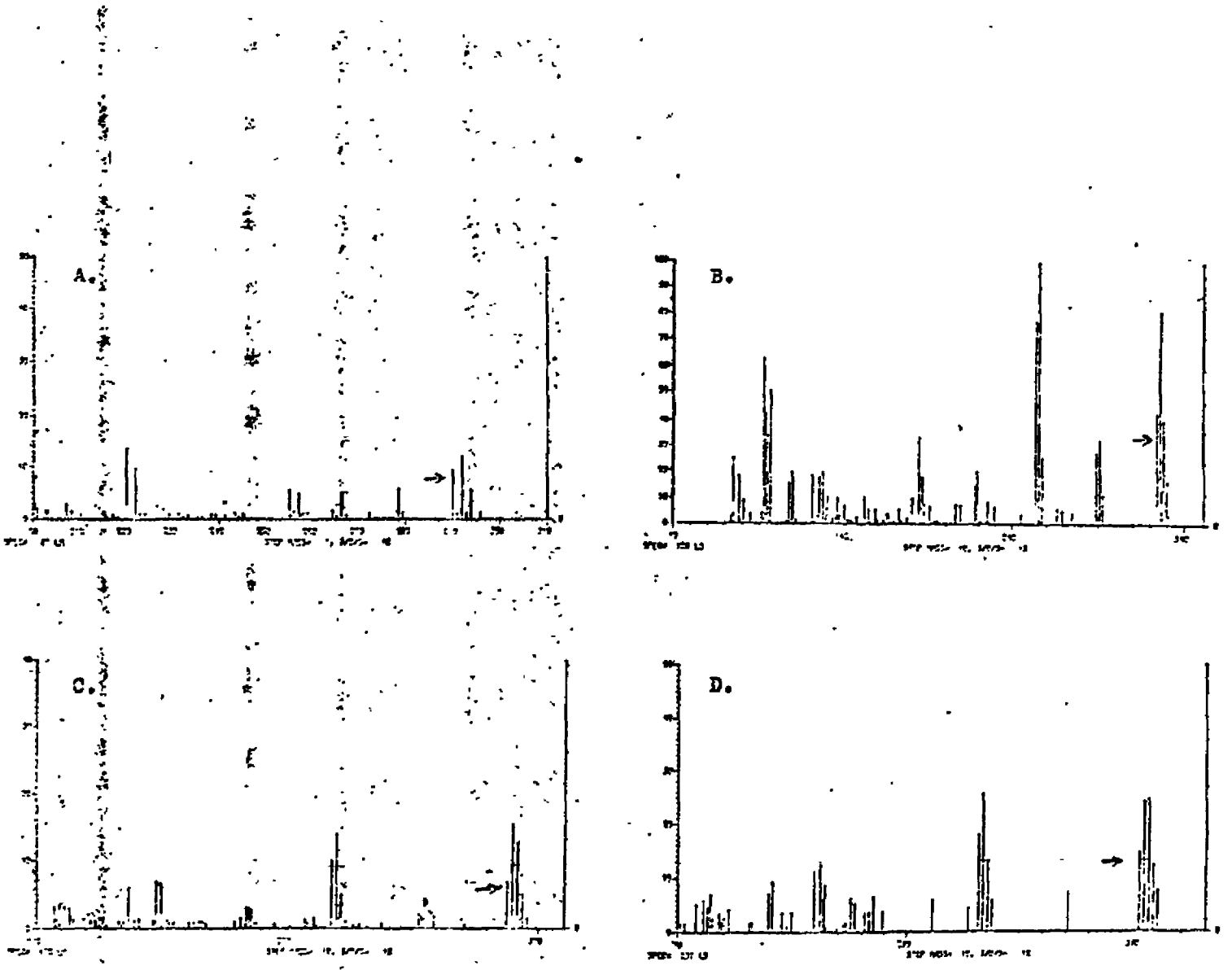


Figure V . Mass Spectra of Major PCBs
in Lake Trout from Traverse Bay

MONS 203857

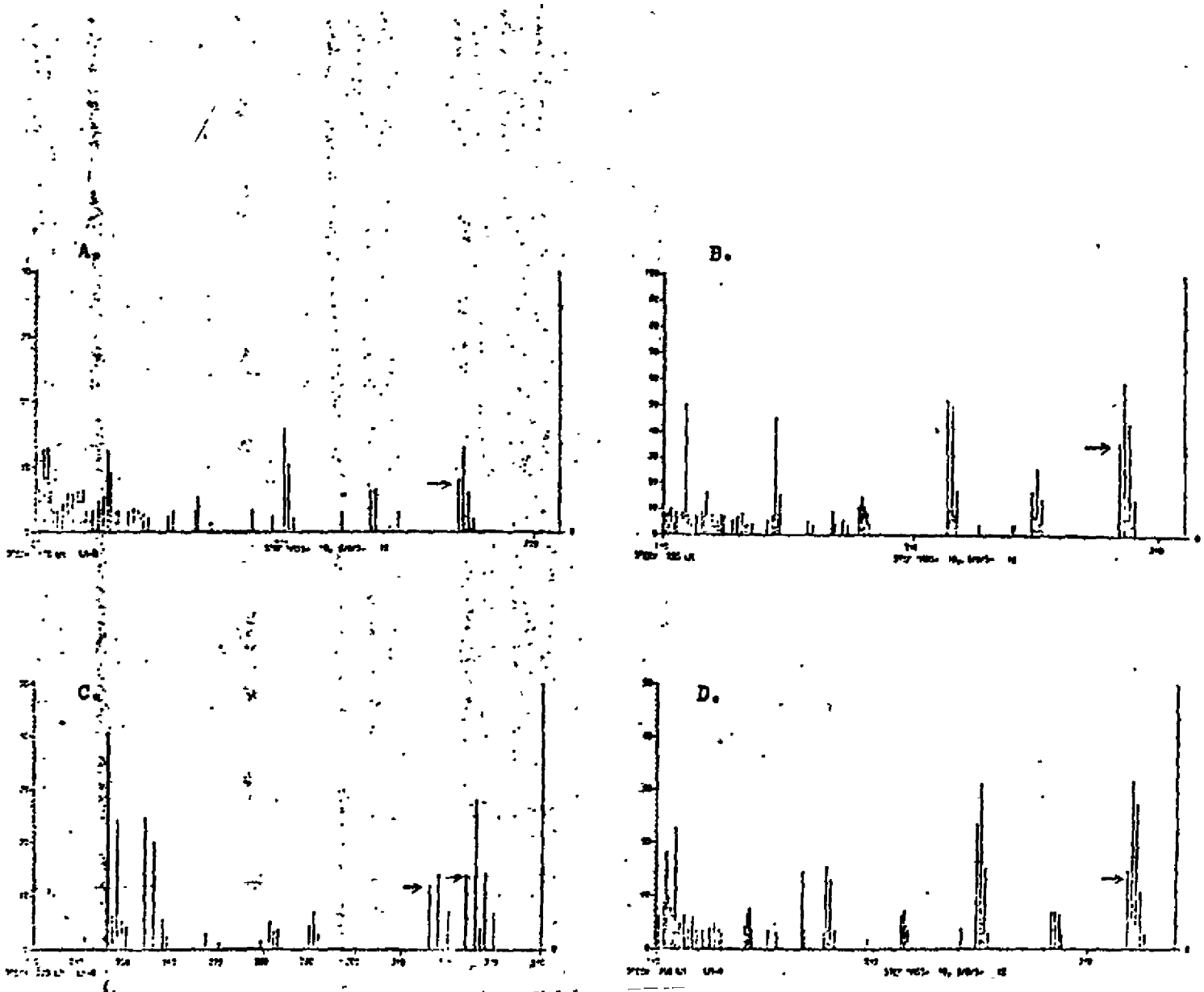


Figure VI. Mass Spectra of Major PCBs
In Lake Trout from the Michigan City Area MONS 203858

Appendix C. Other Field Studies
Supported by Grant 16020 FDS

Due to conflicts arising from changes in the funding period of this grant, the scheduled sampling of major rivers around the lake was not possible. Ship time for the University of Wisconsin Sea Grant Program vessel was obtained for one week in May, 1972. The cruise consisted of transects normal to the shore at Sturgeon Bay, Manitowoc, Sheboygan, Milwaukee, Waukegan, Indiana Harbor, and Michigan City in which plankton, water, sediments, and bottom organisms were sampled for PCB and pesticide analysis. The results will be presented and discussed in the forthcoming Ph.D. thesis of Mr. Richard Flotard of the Water Chemistry Program. The cruise also provided samples for two other graduate studies on trace metals in Lake Michigan by the Water Chemistry Program as well as a study of the food chain transport of PCBs into bird populations on Lake Michigan by the Department of Wildlife Ecology.

Although the data is only partially analyzed at this time, a brief examination of the available data as presented in the following table shows that the PCB levels in Pontiporeia are approximately five times greater off Waukegan than those from Sturgeon Bay. The trend of decreasing levels is also observed in samples from increasing depth (and distance from shore), suggesting that gradients of PCB levels in the water exit along the shore and with depth or distance from the shore.

MONS 203859

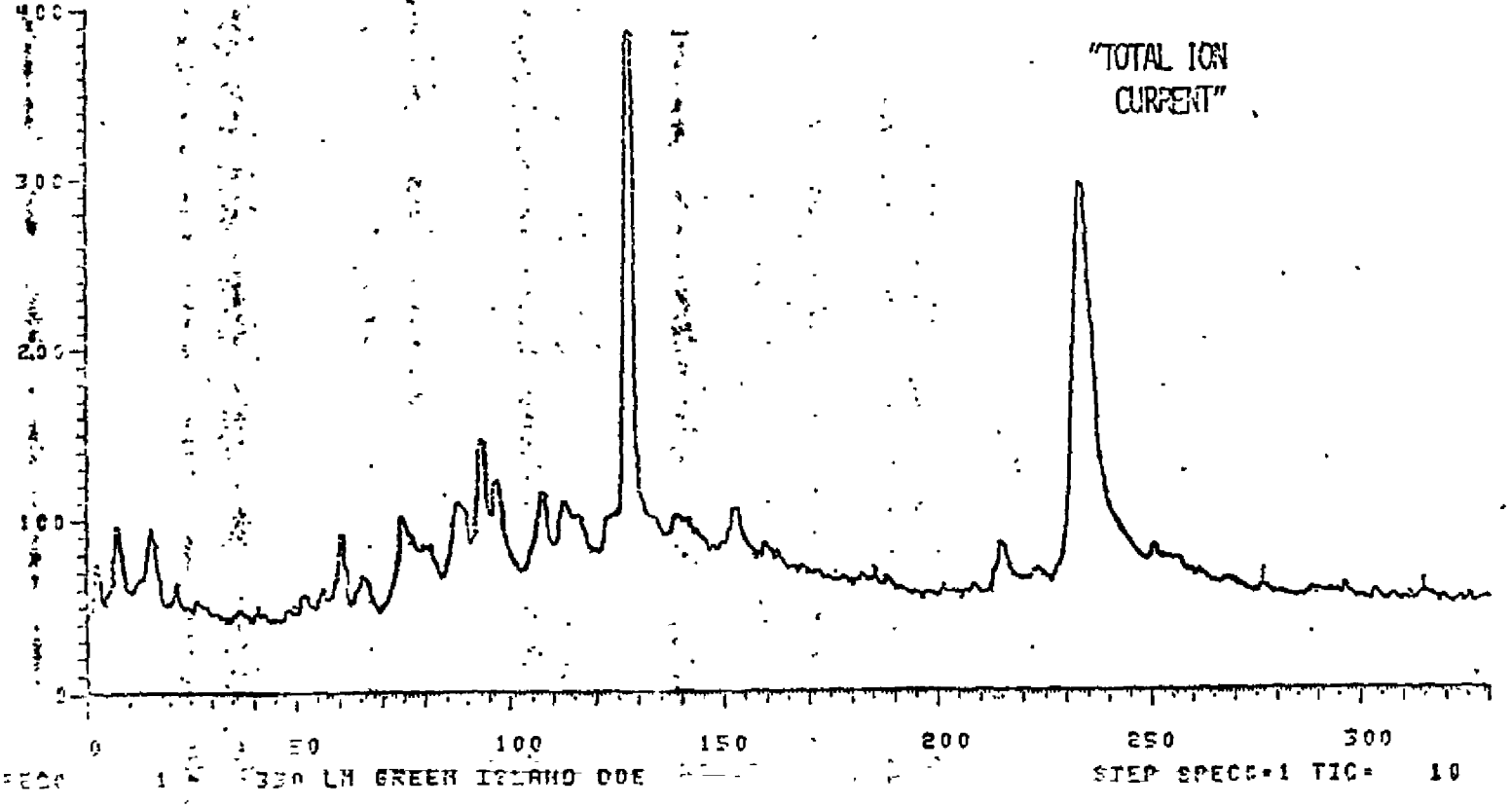
Appendix C. Concentration of PCBs and DDT in *Pontoporeia* from the Western Shore of Lake Michigan[a] (Spring, 1972).

Sampling Region	Depth (ft)	Concentration (μg/gm-wet) PCBs[b]	DDT	DDD	DDE	"Total" DDT
Sturgeon Bay	100	0.06	0.03	0.08	0.03	0.14
Manitowoc	50	0.21	0.03	0.01	0.04	0.08
	100	0.11	0.04	0.01	0.05	0.10
	150	0.17				
Sheboygan	100	0.32	0.04	0.02	0.05	0.11
	150	0.23				
Waukegan	27	0.69				
	100	0.45	0.08	0.03	0.09	0.20
	150	0.34				

[a] Data from Ph.D. thesis (Water Chemistry) of Richard Plotard at the University of Wisconsin, partially supported by this study.

MONS 203860

Figure 16. Total Ion Current Chromatogram of the Polar Eluant Fraction (Silicic Acid) of a Green Bay Trout Extract



MONS 203777