MAY 28 1974

Exhibit 40

Measurements of PCB (Aroclor 1254) in Lake Superior Diluent Water During a Brook Trout Chronic Exposure

Frank A. Puglisi

U. S. Environmental Protection Agency
National Water Quality Laboratory
Duluth, Minnesota

MONS 204135

Concommitant with the need to investigate the chronic effects and bioconcentration factor of PCBs with fish is the need to measure the concentration of PCBs in the water. Brook trout are being exposed to Aroclor® 1254 at NWQL at nominal concentrations ranging from 0.01 µg/l to 1.0 µg/l. Water samples were taken daily from each tank and weekly composites were made using formalin to minimize microbial activity. Reagent blanks and recovery experiments using water from the control tanks spiked with 0.01 µg/l, 0.10ug/l, and 1.0 µg/l Aroclor® 1254 were conducted each week. In addition, estimates of PCBs in the Lake Superior water used in the exposure were made by perchlorinating the PCBs to decachlorobiphenyls.

Water samples (4 liters from each tank and 20 liters from Lake Superior water supply) were passed through glass columns packed with polyurethane foam after the extraction systems were exhaustively extracted with solvent to remove impurities which could interfere in the analysis. The PCBs were removed from the columns with acetone (50 ml) and hexane (225 ml). The extracts were dried over anhydrous Na_2SO_4 and concentrated to 5 ml in a Kuderna-Danish evaporative concentrator. The concentrates were transferred to one gram Florisil columns (prewashed with hexane) and the PCBs were eluted with 75 ml of hexane. The eluant was collected in a Kuderna-Danish concentrator and reduced to about 5 ml for GLC analysis.

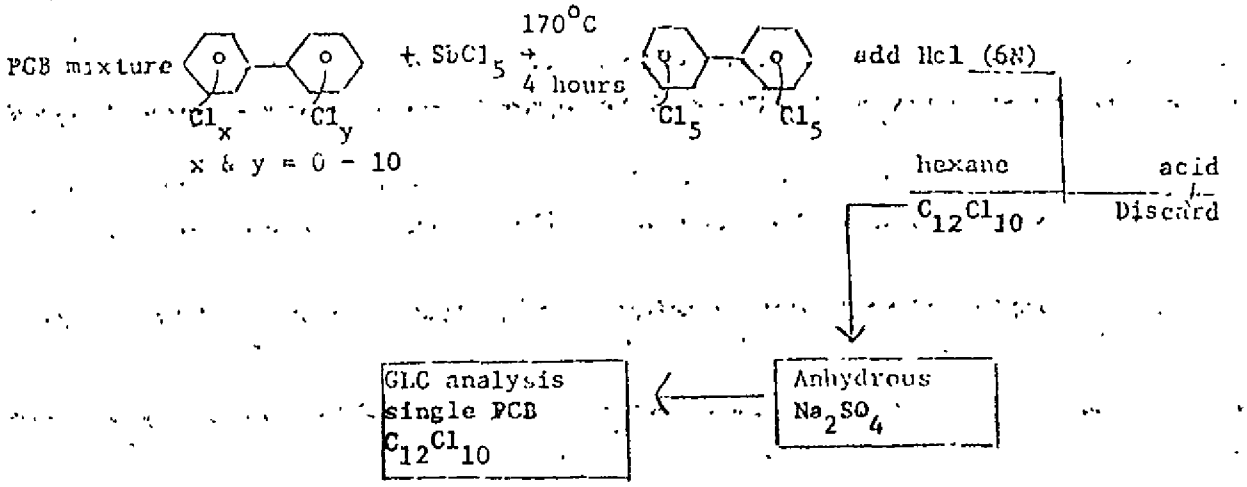
The results of the recovery experiments are presented in Table 1.

The data show that the mean recovery of PCBs from spiked Lake Superior water was greater than 60 percent over the range from 0.01 µg/l to 1.0 µg/l. The data also demonstrate that the standard deviation of the analyses using the four liter water samples increased from ± 14 percent at the 1 µg/l concentration to ± 59 percent at the 0.01 µg/l concentration.

Table 1. Recovery of Aroclor® 1254 Added to Water From NUQL Control Tanks

Quantity of PCBs added per liter	Number of recovery experiments	Recovery of PCBs (percent) Mean	Standard Deviation
1.00	59	62 ±	14
0.10	58	62 ±	21
0.01	57	79 ±	59

The extracts of the 20 liter water samples were analyzed for PCBs by perchlorinating the samples with $SbCl_5$. Briefly, the analytical scheme is as follows:



The increased sensitivity of this method is due to the conversion of all PCBs to a single compound and the greater sensitivity of the more highly chlorinated $C_{12}Cl_{10}$ to electron capture.

The GLC response is linear up to 0.2 ng $C_{12}Cl_{10}$ and the detection limit is about 0.005 to 0.01 ng $C_{12}Cl_{10}$. It is commonly observed that commercially available $SbCl_5$ produces a blank in this analysis, and both the $SbCl_5$ and reagent blanks generally limit the determinable limit in these analyses.

The experiment to measure the PCBs in Lake Superior water consisted of two perchlorination blanks, three reagent blanks which includes the extraction through the perchlorination steps, three replicate samples of Lake Superior water, and three replicate samples from the control tanks in the PCB bioassay system. The results of these analyses are summarized in Table 2. Since the perchlorination blanks were essentially the same as the overall reagent blank, the major source of PCBs is assumed to be the perchlorination step and all blanks were grouped together. The Lake Superior water samples contained approximately 0.8 ng/l PCBs as Aroclor 1254. However, the variability of the measurements (more than 50 percent) at this concentration does not permit precise estimates to be made. The estimated concentration of PCBs in the control tanks was approximately 3 ng/l.

Table 2. PCBs in Lake Superior Water and Control Bioassay Tanks

Sample Origin	Sample Number	Volume (liters)	Total ng $C_{12}Cl_{10}$ Mean ± S. E.	Estimated PCB concentration (ng/l as Aroclor 1254)
Reagent blanks	5	--	44 ± 12	--
Lake Superior	3	20.3 ± 0.02	62 ± 6	0.8 ± 0.4
Brook trout control tanks	3	21.1 ± 0.05	132 ± 11	3.2 ± 0.5

MONS 204139

Identification of Chlorinated Dibenzofurans in American Polychlorinated Biphenyls (Aroclor®)

MONS 204140

ABSTRACT

Chlorinated dibenzofurans, highly toxic compounds structurally related to the chlorinated dibenzodioxins, have been detected as contaminants in American preparations of polychlorinated biphenyls (PCB). They are assumed to account for some of the toxic properties of the crude PCB mixtures.

MONS 204141

Polychlorinated biphenyls (PCB) are now ubiquitous on Earth. These industrial chemicals have been detected in polar bears (Ursus maritimus) from remote areas of the Canadian Arctic and in snow petrels (Pagodroma nivea) from the Antarctic (1). They are widespread in both marine and fresh-water ecosystems, with highest concentrations recorded in fish-eating birds and mammals from waters adjacent to industrialized areas (2). The reproductive failures of many of these populations, particularly of the fish-eating birds, are among the examples of environmental degradation to which PCB may contribute; a role in eggshell thinning, however, has been discounted (3). Embryonic mortality accounts for a substantial portion of the reproductive failures; embryos in 20 per cent of the eggs of the herring gulls (Larus argentatus) in Lake Ontario colonies in 1973 were found to die during the first week of incubation (4). Experimental work has demonstrated the embryotoxic effects of PCB (5); the active components in German and French PCB, however, have been identified as chlorinated dibenzofurans, present in trace amounts as contaminants in the preparations (6). Like the structurally related chlorinated dibenzodioxins (Figure 1), the chlorinated dibenzofurans are toxic to embryos in sub-microgram amounts (6, 7). They are therefore among the most toxic compounds known. Since the chlorinated dibenzofurans and dibenzodioxins share chemical properties with the dominant chlorinated hydrocarbons in environmental samples, principally the DDT and PCB compounds, they are difficult to detect in the small amounts at which they exert biological effects (8). Techniques which detected the chlorinated dibenzofurans in the French and German PCBs failed to demonstrate their presence in the American PCB Aroclor® 1260 (6).

MONS 204142

In the present paper we report the presence of chlorinated dibenzofurans in the American PCBs Aroclor® 1248, 1254, and 1260, and their absence in detectable quantities in Aroclor® 1016.

Samples of PCB examined, obtained from the Monsanto Company in the years indicated, include: Aroclor® 1248, 1254, and 1260 (1969); Aroclor® 1254 (1970); and Aroclor® 1016 (1972). Amounts ranging from 1.0 to 2.0 g were dissolved in 400 ml hexane, placed on a Florisil column (180 g, column i.d. 31.5 mm), and eluted with: 1) an additional 1600 ml hexane, and successive 800 ml volumes each of 2) 5% ethyl-ether/hexane, 3) 25% ethyl-ether/hexane, and 4) acetone, at a rate of approximately 7 ml/min. The major portion of the PCB was eluted in the hexane fraction. This was discarded. Upon addition of the 5% mixture, the eluates were collected in 6 successive 400 ml volumes. To eliminate the polar solvents, each eluate was evaporated twice just to dryness and taken up each time in a minimal amount of hexane. Each fraction, in 1 ml hexane, was placed on a micro alumina column (9) and eluted with 10 ml each of 1% and 20% methylene chloride in hexane. These were also taken twice just to dryness and made up to a volume of 1 ml in hexane to eliminate the methylene chloride prior to gas chromatographic analysis. Aliquots of all fractions obtained before and after partitioning on alumina were injected into a 6 foot glass column containing 3% OV-1 on 100-120 mesh Supelcoport in a Tracor MT-220 gas chromatograph equipped with ^{63}Ni electron-capture detectors. PCB compounds were found to be present in each fraction eluted from the Florisil column in amounts sufficient to interfere with the detection of trace contaminants. Partitioning on the alumina columns separated most of the PCB interference into the 1% methylene chloride fractions.

Upon removal of this interference different peak patterns appeared in the chromatograms of the 20% methylene chloride fractions.

Compounds eluting in the 20% methylene chloride fraction were collected for mass spectrometric analysis with use of a 20:1 effluent splitter, and a trap consisting of a 1 mm i.d., 100 mm long capillary tube, bent to a U shape, immersed in a liquid nitrogen bath. Four microliters of 20% methylene chloride in hexane were injected into the capillary as a rinse, removed with a 1.0 µl micropipet, and placed directly upon the mass spectrometer probe. The probe was inserted into a GEC AEI MS-902 high resolution mass spectrometer and the solvent removed by the fore pump. The probe was rapidly inserted into the ion source and multiple scans were recorded in the on-line high resolution mode (10).

A gas chromatogram showing components derived from the Aroclor® 1254 obtained in 1969 is represented in Figure 2. The components were eluted in the second 400 ml Florisil fraction and recovered from the alumina column in 20% methylene chloride/hexane. Each of the numbered peaks was trapped as described above, and identified by mass spectrometric analysis. A nominal mass plot of the high resolution mass spectrum of peak 2 is shown in Figure 2. The plot includes all the ions with elemental compositions ranging to the maximum empirical formula $C_{13}H_6O^{35}Cl_5{}^{37}Cl_4{}^{13}C_2$. The molecular ion cluster at nominal m/e 304-310 fragments by successive losses of Cl to yield the ions at m/e 269-275 and CO to the ions at m/e 241-245. A minor loss of Cl from the peaks at m/e 269-275 also occurs to yield the ions at m/e 234-238, followed by CO elimination to m/e 206-210. The group of peaks at m/e 152-154 are the doubly charged molecular ions. An identical spectrum

MONS 204144

was obtained from an authentic standard of 2,3,7,8,-tetrachlorodibenzofuran. This latter compound has a retention time identical to that of peak 4. Peak 2 is therefore a steric isomer. The accurate mass measurements for the characteristic ions are within 2 ppm of the calculated exact masses. Peaks identified on this chromatogram and their retention times relative to dieldrin are as follows: (1) a mixture of tetra- and pentachlorobiphenyl (1.02); (2) tetrachlorodibenzofuran (1.30); (3) pentachlorobiphenyl (1.46); (4) tetrachlorodibenzofuran (1.57); (5) hexachloronaphthalene (1.75); (6) pentachlorobiphenyl (1.86); (7) hexachloronaphthalene (2.00); and (8) heptachloronaphthalene (3.46). In Aroclor® 1248, three tetrachloro- and four pentachlorodibenzofurans were found. Each of the Aroclor® 1254 preparations and Aroclor® 1260 contained eleven chlorinated dibenzofurans: two tetrachloro-, four pentachloro- and five hexachlorodibenzofurans.

Initially, because authentic standards were not available, the tetrachlorodibenzofurans were quantitated against 2,4,5,2',4',5'-hexachlorobiphenyl; pentachloro- and hexachlorodibenzofurans were quantitated against 2,3,5,6,2',3',5',6'-octachlorobiphenyl. Later an authentic standard of 2,3,7,8-tetrachlorodibenzofuran was obtained and its electron capture response was compared to that of the hexachlorobiphenyl. This comparison showed that use of the hexachlorobiphenyl had yielded estimates of the tetrachlorodibenzofurans that were high by a factor of approximately 2. In the absence of authentic standards of the penta- and hexachlorodibenzofurans it is assumed that the original estimates of their concentrations based upon the octachlorobiphenyl are also high by the same factor. Corrected estimates of the dibenzofuran concentrations in Aroclors® are presented in Table 1.

No chlorinated dibenzofurans were found in Aroclor® 1016, a PCB mixture containing approximately 42% chlorine whichhas replaced Aroclor® 1242 in many applications, principally as the dielectric fluid in capacitors (11).

Among the PCB preparations obtained in 1969, 1) the total dibenzofuran concentration decreases with increasing chlorine content of the PCB; 2) the total concentration of the tetrachlorodibenzofurans decreases by a factor of ten from Aroclor® 1248 to Aroclor® 1260; 3) hexachlorodibenzofurans could not be detected in the Aroclor® 1248 preparation, but constituted 17% and 57% respectively of Aroclor® 1254 and 1260. The relative proportions of the dibenzofuran components in the Aroclor® 1254 obtained in 1970 were similar to those of the Aroclor® 1254 obtained in 1969, but the total concentration was 74% lower.

PCB residues in the North American environment and in human food have been the subject of extensive scientific investigations, have prompted governmental action and have resulted in substantial changes in many industrial processes which formerly used PCB. Toxic and sublethal effects of crude PCB mixtures include, in addition to embryotoxicity, the production of subcutaneous and abdominal edema, hydropericardium, liver necrosis, porphyria, chloracne, immunosuppression, and the induction of hepatic enzymes that cause elevated levels of steroid metabolism (7). Edema production and embryotoxicity, and the major portion of the acnegenic and hepatotoxic effects of European PCB were attributed to contaminant dibenzofurans. Our results suggest that many of the toxic effects of PCB in North America, such as the low hatchability of eggs from chickens on PCB-contaminated diets (12) were caused by dibenzofurans in the PCB preparations. The possibility therefore exists that chlorinated dibenzofurans are present in

the environment and contribute to the reproductive failures and occasional birth defects observed in populations of fish-eating species of birds, including the herring gulls of Lake Ontario (4), and the ospreys (Pandion haliaetus) and common terns (Sterna hirundo) of Long Island Sound (13).

Gerald W. Bowes*

Michael J. Mulvihill

Canadian Wildlife Service
Toxic Chemicals Section
Ottawa, Canada K1A 0H3

Bernd R. T. Simoneit

A. L. Burlingame

Space Sciences Laboratory
University of California
Berkeley, California 94720

R. W. Risebrough

Bodega Marine Laboratory
University of California
Bodega Bay, California 94923

* Present address: State Water Resources Control Board
Division of Planning and Research
1416 Ninth Street
Sacramento, California 95814

MONS 204147

Table 1. Chlorinated dibenzofuran concentrations in Aroclor®*

PCB	4-Cl	5-Cl	6-Cl	Total
1248	0.24(20.9)	0.91(79.1)	nd	1.15
1254 (1969)	0.12(13.5)	0.62(69.7)	0.15(16.9)	0.89
1260	0.017(2.5)	0.27(40.3)	0.38(56.7)	0.67
1016	nd	nd	nd	----
1254 (1970)	0.012(5.2)	0.14(60.9)	0.08(34.8)	0.23

* Quantities are expressed as µg/g Aroclor®. Values in parentheses represent quantity as % of total dibenzofuran.

nd - not detected (< 0.001 µg/g)

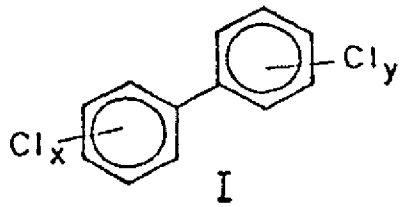
MONS 204148

Figure Captions

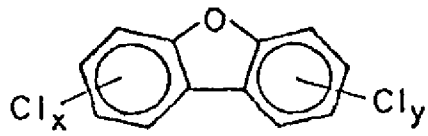
Figure 1. Skeletal structures of I: chlorinated biphenyl; x + y = 1 to 10; II: chlorinated dibenzofurans, x + y = 1 to 8; III: chlorinated dibenzodioxins, x + y = 1 to 8.

Figure 2. Top: Gas chromatogram of a fraction of Aroclor® 1254 containing a mixture of chlorinated biphenyls, dibenzofurans, and naphthalenes. Conditions and identity of peaks are given in the text. Bottom: Mass spectrum of peak 2, a tetrachlorodibenzofuran.

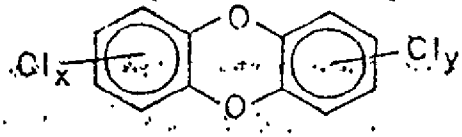
MONS 204149



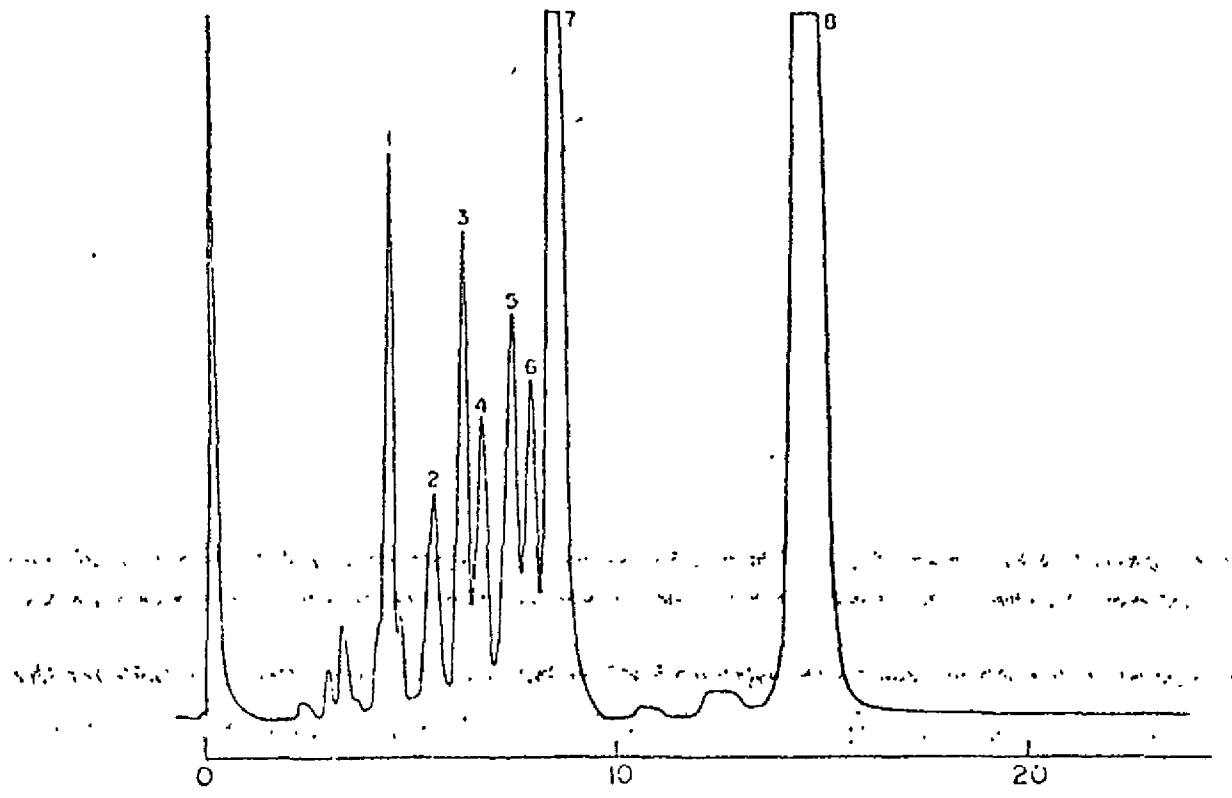
I

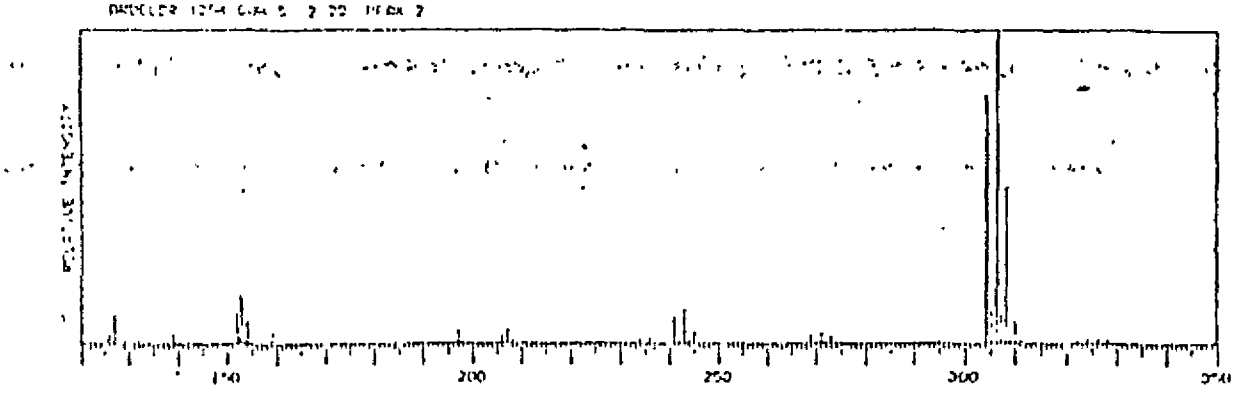


II



III





MONS 204151

References and Notes

1. G. W. Bowes and C. J. Jonkel, in preparation; R. W. Risebrough and G. M. Carmignani, in Conservation Problems in Antarctica, B. C. Parker, Ed. (Allen Press, Lawrence, Kansas, 1972), p. 63.
2. S. Jensen, A. G. Johnels, M. Olsson and G. Otterlind, Nature 224, 247 (1969); J. H. Koeman, M. C. Ten Noever de Brauw and R. H. de Vos, Nature 221, 1126 (1969); R. W. Risebrough, P. Reiche, D. B. Peakall, S. G. Herman and M. N. Kirven, Nature 220, 1098 (1968); R. W. Risebrough and B. W. de Lappe, Environ. Health Perspectives 1, 39 (1972); R. L. DeLong, W. G. Gilmartin and J. G. Simpson, Science 181, 1168 (1973).
3. D. B. Peakall, Bull. Environ. Contam. Toxicol. 6, 100 (1971); R. G. Heath, J. W. Spann, J. F. Kreitzer and C. Vance, Proc. XV International Ornithological Congress, 475 (1972); R. W. Risebrough, in Proceedings Sixth Berkeley Symposium Mathematical Statistics and Probability, L. Le Cam, J. Neyman and E. L. Scott, Eds. (Univ. of California Press, 1972), p. 443.
4. M. Gilbertson and R. Hale, Canadian Field-Naturalist, in press (1974).
5. D. C. Villeneuve, D. L. Grant, K. Khera, D. J. Clegg, H. Baer and W. E. J. Phillips, Environ. Physiol. 1, 67 (1971); J. G. Vos and J. H. Koeman, Toxicol Applied Pharmacol. 17, 656 (1970). C. F. Tumasonis, B. Bush and F. D. Baker, Archives Environ. Contam. 1, 312 (1973).
6. J. G. Vos, J. H. Koeman, H. L. Van der Maas, M. C. Ten Noever de Brauw and R. H. de Vos, Fd. Cosmet. Toxicol. 8, 625 (1970).
7. J. G. Vos, Environ. Health Perspectives 1, 105 (1972).

MONS 204152

8. R. Baughman and H. Heselson, Environ. Health Perspectives 5, 27 (1973); G. W. Bowes, B. R. Simoneit, A. L. Burlingame, B. W. de Lappe, and R. W. Risebrough, Environ. Health Perspectives 5, 191 (1973).

9. H. L. Porter and J. A. Burke, J. Assoc. Offic. Anal. Chem. 54, 1426 (1971).

10. A. L. Burlingame, R. W. Olsen and R. McPherron, Adv. Mass Spectrometry 6, 1053 (1974).

11. I. C. T. Nisbet and A. F. Sarofim, Environ. Health Perspectives 1, 21 (1972).

12. A. C. Kolbye, Environ. Health Perspectives 1, 85 (1972).

13. P. R. Spitzer, R. W. Risebrough, J. W. Grier, and C. R. Sindelar, in Proceedings North American Osprey Symposium, J. Ogden, Ed., in press; H. Hays and R. W. Risebrough, Auk. 89, 19 (1972).

14. We thank J. A. Burke and H. L. Porter, Food and Drug Administration, Washington, D. C., and J. G. Vos, Institute of Veterinary Pathology, Utrecht, for helpful discussions of analytical methodology; A. S. Kende, Department of Chemistry, University of Rochester, for providing an authentic standard of 2,3,7,8-tetrachlorodibenzofuran; and F. C. Walls for assistance with the mass spectrometry. Research was supported by the Canadian Wildlife Service, the National Aeronautics and Space Administration (Grant NGL 05-003-003) and the National Science Foundation (Grants GX-32885 and GB-36593).

MONS 204153

MA 1,

Environmental Protection Agency

Washington, DC

Office Of The
General Counsel

May 15, 1974

NOTE

Enclosed are Exhibits 1, 2, 6, 7, 8, 9 and 12 from Dr. Alan V. Nebeker's statement. Exhibits 4, 11, 13 and 14 will be mailed directly to you by Dr. Nebeker. All remaining exhibits are available in open literature.

MONS 204154

Exhibit 1

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

Alan V. Nebeker[1] and Frank A. Puglisi

U. S. Environmental Protection Agency

National Water Quality Laboratory

Duluth, Minnesota 55804

[1]Present Address: Western Fisheries Ecology Station

NWQL, 1350 S. E. Goodnight Ave.

Corvallis, Oregon 97330

Trans. Amer. Fish. Soc. 103(4): Oct., 1974

MONS 204155

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 0.43 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.

MONS 204156

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 DeVoe 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

MONS 204157

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.

MONS 204158

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 (Paratanytarsus) 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 Nebeker, 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.

MONS 204163

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).

MONS 204164

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. Arbclor-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.

MONS 204167

Acknowledgements

We thank Arlene Shelhon and Marjorie Starr for aid in conducting the static bioassays, Dr. Kenneth Biesinger for helpful suggestions concerning Daphnia rearing and testing, Kenneth Campbell for assistance with chemical analyses and preliminary testing, David DeFoe for help with chemical analysis and construction of the continuous-flow test facility, and Dr. David L. Stalling for residue analysis of scud tissues.

We also thank Monsanto Chemical Company for their cooperation and for making Aroclor samples available for testing.

MONS 204168

Literature Cited

American Public Health Association. 1965. Standard methods for the examination of water and wastewater 12th ed. New York. 769 p.

Biesinger, K. E., and G. M. Christensen. 1972. Effects of various metals on survival; growth, reproduction, and metabolism of Daphnia magna. J. Fish. Res. Board Can. 29: 1691-1700.

Bioassay Committee. MS 1971. Recommended bioassay procedure for fathead minnows Pimephales promelas Rafinesque. National Water Quality Laboratory, Duluth, Minn. 13 p.

Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor 1254) in the water, sediment, and biota of Escambia Bay, Florida. Bull. Env. Cont. Toxicol. 5: 171-180.

Litchfield, J. T., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicity studies. Water Res. 1: 21-29.

Nebeker, A. V., F. A. Puglisi, and D. L. DeFoe. 1972. Toxicity of Polychlorinated Biphenyls to Fish and other Aquatic Life. Final report. USEPA, National Water Quality Laboratory, Duluth, MN 55804.

Nebeker, A. V., F. A. Puglisi, and D. L. DeFoe. 1974. Effect of polychlorinated biphenyl compounds on survival and reproduction of fathead minnow and flagfish. Trans. Amer. Fish. Soc. (In press). July, 1974

Peakall, D. B., and J. L. Lincer. 1970. Polychlorinated biphenyls. Another long-life widespread chemical in the environment. Bio-Science 20: 958-964.

MONS 204169

Risebrough, R., and V. Dodine. 1970. More letters in the wind. Environment 12: 16-27

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793-821.

Stalling, D. L. 1970. Summary of PCB research at the Fish-Pesticide Research Laboratory. Report of the Annual Meeting of the Great Lakes Fishery Comm., June 16-18, 1970.

Stalling, D. L., and J. L. Johnson. 1971. Residues of Aroclor 1254 in Scud. Sp. Rep. on PCB's. Fish-Pesticide Laboratory, Columbia, Missouri.

Veith, G. D., and G. F. Lee. 1970. A review of chlorinated biphenyl contamination in natural waters. Water Res. 4: 265-269.

Zitco, V. 1970. Polychlorinated biphenyls (PCB) solubilized in water by nonionic surfactants for studies of toxicity to aquatic animals. Bull. Env. Contam. Tox. 5: 279-285.

MONS 204170

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

MONS 204171

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 (µg/liter)	Confidence limits (0.05)	Reproductive impairment (µg/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

MONS 204172

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.

MONS 204173

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.

MONS 204174

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

MONS 204175

3/

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

MONS 204176

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) A	B	Mean	Concentration factor		Nominal Column
A-1242						
2.8	71(14)[a]	80(17)	76	22,000	27,000	3.5
8.7	246(9)	387(10)	316	29,000	36,000	11.0
26	409(1)	—	409	14,000	16,000	33.0
A-1248						
0.18	9(33)	—	9	24,000	50,000	0.37
0.54	15(32)	—	15	14,000	28,000	1.10
2.2	127(33)	—	127	38,000	58,000	3.3
5.1	552(24)	—	552	55,000	108,000	10.0

[a]Mean of (N) individual samples.

MONS 204177

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.

MONS 204178

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	-			-		

MONS 204179

Exhibit 6 Nebcrol 4/1/79

(6)

Q-984-7

Dietary Effects of Polychlorinated Biphenyls on Mink

(1) (2)
N. S. Platonow and L. H. Karstad*

ABSTRACT

Poisoning occurred in 32 mink fed diets containing meat from cows which had been fed a polychlorinated biphenyl (PCB), Aroclor 1254. No live kits were produced and all adult mink died during a 105 day period of feeding a ration containing 3.57 ppm of PCB. At a level of 0.64 ppm of PCB in ration one of 12 mink produced three kits, all of which died during the first day after birth. Clinical signs were limited to weight loss and passage of black tarry feces. The gross lesions seen were yellowish discoloration of the liver and hemorrhage into the abdominal cavity or gastrointestinal tract. Microscopic lesions were nephrosis, fatty degeneration and necrosis of the liver, brain edema, disseminated intravascular coagulation, and fibrosis of coronary arteries. It is concluded that mink are highly sensitive to small quantities of PCB fed for an extended period of time.

RÉSUMÉ

On a empoisonné 32 visons en leur servant une ration qui contenait de la viande de vaches auxquelles on avait donné un diphényle polychloré (PCB), l'Aroclor 1254. On n'obtint aucun petit et tous les visons adultes moururent au cours d'une période de 105 jours durant laquelle on leur servit une ration contenant 3.57 ppm de PCB. A la concentration de 0.64 ppm de PCB dans la ration, une femelle, sur un total de 12, mit bas trois petits qui moururent en moins de 24 heures après leur naissance. Les signes cliniques se traduisirent par une perte de poids et le passage de fèces d'apparence goudronnée. Les lésions macroscopiques consistaient en une décoloration jaunâtre du foie et en des hémorragies intra-abdominales ou gastro-intestinales. Les lésions microscopiques étaient les suivants: néphrose, dégénérescence graisseuse et nécrose hépatiques, oedème cérébral, coagulation intra-vasculaire disséminée et fibrose des artères coronaires.

Les auteurs en viennent à la conclusion que le vison est très vulnérable à l'ingestion prolongée de petites quantités de PCB.

INTRODUCTION

Polychlorinated biphenyl (PCB) compounds are now recognized as widespread environmental contaminants of aquatic or terrestrial ecosystems. Since fish and various biproducts made from domestic animals are generally included in the diet of ranch-raised mink, it seemed to be important to know the effects of PCB compounds on mink, when included in the diet for an extended period of time.

MATERIALS AND METHODS

Thirty-two ranch mink approximately one year old were divided into two groups with four standard dark males, four pastel females, and eight standard dark females in each group. Each group of 16 mink was fed a separate ration. Rations were prepared from two Jersey cows which had been given orally ten consecutive daily doses of 1 and 10 mgm per kgm, respectively of a PCB,

(1) Departments of Biomedical Sciences and Pathology, University of Guelph, Guelph, Ontario, CANADA.

This paper was presented in part at the Fifteenth Annual Meeting of the Canadian Federation of Biological Societies, Quebec, Quebec, June 1972.

This research was financially supported by the Canada Mink Breeders' Association and the Ontario Ministry of Agriculture and Food.

Submitted August 8, 1972.

(1) Univ Guelph, Dept Pathol / Guelph / Ontario / Canada

MONS 204180

namely Aroclor 1254[1], dissolved in a small amount of olive oil and mixed with dairy concentrate. Twenty-four hours after the last doses were given, the cows were killed; fat, musculature, liver and kidneys were removed, thoroughly ground and mixed with commercial mink food cereal at a level of 21% cereal. The resulting rations contained 0.61 ppm and 3.57 ppm of total PCB, respectively.

The cows did not have any clinical, gross or histopathological signs of intoxication. If these animals had been slaughtered at an inspected meat plant, they would without doubt have passed as fit for human consumption.

Feeding *ad libitum* was begun two months before the breeding season, i.e. on January 7 and continued until June 17, 1971 (160 days).

Beginning in early March the 12 females in each test diet group were presented daily for breeding to one of the four males in the group, until the female was found to be receptive and mating took place. The semen of each male, collected by vaginal aspiration from a just-bred female, was examined microscopically for motile sperm.

The remaining 101 breeding female mink plus males and mink designated for other experiments in the institutional herd from which the test mink were taken were maintained on a commercial mixed ration, purchased locally. They were managed during the breeding and whelping seasons just as were the experimental PCB-fed animals. For practical purposes, they were regarded as no-treatment controls. During the course of the PCB experiments 50 to 60 mink from this herd were removed for aleutian disease research, either killed at once for tissue cultures or maintained on other premises for infection experiments.

All of these mink were examined for gross and histopathological lesions.

Necropsies were performed on experimental mink which died and those which were killed after PCB feeding was discontinued. Blood from the heart and samples of tissues from the major body organs and psoas muscle were taken for PCB analysis. Portions of brain, lungs, heart, spleen, liver, kidney, intestine, adrenal and reproductive organs were fixed in 10% formalin, processed for histology by paraffin embedding and sectioned at approximately 6 microns. Hematoxylin-eosin stain was used routinely. Where necessary for study of lesions of blood vessels, selected tissue sections were stained by the allochrome procedure (5).

TABLE I. Lesions Seen in Mink Fed PCB

PCB (ppm)	3.57	3.57	0.61	0.61
No of mink	12	4	12	4
Sex	F	M	F	M
No of deaths	12/12	4/4	2/12	0/4
Mean survival (days)	74	96	151	160*
Gross Lesions				
Weight loss	8/12	2/4	0/12	0/4
Hemorrhages	9/12	3/4	0/12	0/4
Yellow liver	6/12	2/4	1/12	0/4
Microscopic Lesions				
Nephrosis	5/12	1/4	1/12	0/4
Liver necrosis	2/12	2/4	0/12	0/4
Disseminated intravascular coagulation	2/12	0/4	1/12	0/4
Brain edema	3/12	0/4	0/12	0/4
Coronary arteriosclerosis	0/12	1/4	1/12	1/4

*All mink in this group survived to the end of the feeding period, 160 days

The PCB were extracted from various tissues as well as from samples of rations by the method of Grant *et al* (3) and subsequently analyzed by the method of Luchenbrecker and Ecobichon (12) using a Micro-Tech, Model MT-220[2], gas chromatograph, equipped with a ^{63}Ni high temperature electron capture detector. The quantitation of the PCB was performed by measuring total peak area as the detector response, using an Infotronics, Model 208[3], automatic digital integrator equipped with a baseline tracking and drift corrector. The PCB were separated from DDT and its analogs by the method described by Armour and Burke (2).

RESULTS

Reproduction

Fifteen of the 24 females were bred normally. Of the remaining nine, five on the higher PCB diet which were not bred, died

[1]Aroclor 1254 (mixture of chlorinated biphenyls containing 54% chlorine) was generously supplied by Monsanto Canada Ltd., Toronto, Ontario.

[2]Tracer Inc., Austin, Texas, U.S.A.

[3]Infotronics Ltd., Shannon, Ireland.

MONS 204181

either before or during the breeding period. Two of the four on the low PCB diet died during May. In all other cases, failure to breed was caused either by the female rejecting the male or because the female was too weak to participate.

All males produced motile sperm, yet no litters were produced by the females fed the ration containing 3.57 ppm of PCB and only one of the females fed 0.64 ppm PCB produced a litter. Three kits were born to this female but they died during their first day of life.

Clinical Signs and Mortality

The first mink to die was an adult female fed the ration with the higher level PCB which was dead on day 43 of the experiment. Deaths occurred sporadically thereafter. All of the mink fed the 3.57 ppm PCB ration were dead by day 105. Two of the mink fed 0.64 ppm PCB died on days 122 and 129, respectively. The feeding of this ration was terminated on the 160th day, at which time four of the surviving mink, three males and one female, were killed with an overdose of ether so that specimens could be taken for PCB analysis and histopathology. From day 161 to day 277 the surviving mink which had been fed the 0.64 ppm PCB ration were fed the regular commercial ration which was fed to the no-treatment controls. Three female and one male mink were killed for examination on each of days 40 and 82 after the cessation of PCB feeding. The remaining two female mink were killed on day 277.

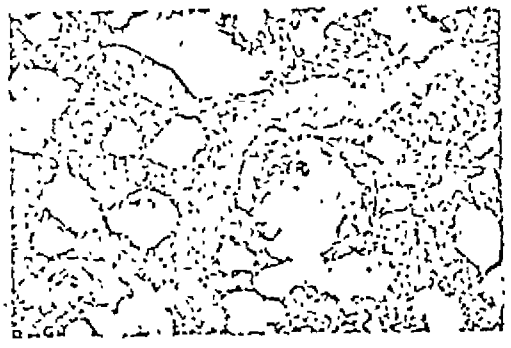


Fig. 1. Lung of a female standard dark mink which died suddenly with disseminated thrombosis on day 129 of 0.64 ppm PCB feeding. Note the vein occluded by a large platelet thrombus. H & E. X45.

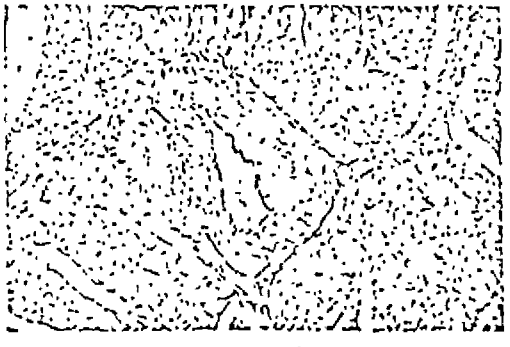


Fig. 2. Coronary artery of the mink described in Fig. 1. Most of the smooth muscle of the media has been replaced by fibrous connective tissue. Alleichrome. X288.

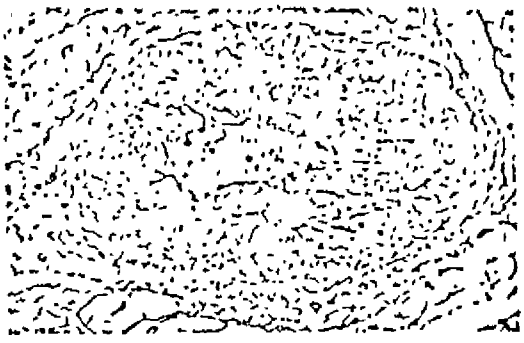


Fig. 3. Lesions similar to those of Fig. 2 in the coronary artery of a standard dark male mink killed by ether inhalation after 140 days of 0.64 ppm PCB feeding. Alleichrome. X130.

At both treatment levels, males survived longer than females. There was a marked difference in survival between treatment levels (Table I).

Clinical signs were either absent or nonspecific. A few mink died suddenly while they were still in good flesh, but many of the mink became emaciated on the PCB diets; they had poor appetites and in some cases became lethargic and very weak before they died. Some passed tarry feces, indicating gastrointestinal hemorrhage.

Gross Lesions

The gross lesions seen at necropsy were emaciation, blood in the gastrointestinal tract or intra-abdominal hemorrhage, and yellowish discoloration of the livers. These changes were not consistent in all cases. Their frequencies are given in Table I. Hemorrhages seemed to be more often chronic rather than massive and acute. It was not possible to relate the blood present in the abdominal cavity to lesions in the blood vessels or visceral organs. Bleeding

MONS 204182

TABLE II. Mean Concentrations (± stand. dev.) in ppm of Polychlorinated Biphenyls (PCB) in Various Tissues of Mink Fed Rations Containing Ingredient(s) Contaminated with PCB

PCB level in ration (ppm)	Number of Animals	Period[a]	Blood	Brain	Kidney	Liver	Muscle	Heart
3.57	16	—	1.80 ± 1.42	4.52 ± 3.31	7.12 ± 4.59	11.99 ± 11.0	3.31 ± 0.93	8.31 ± 7.21
0.64	2[b]	•	0.71 ± 0.01	0.52 ± 0.01	1.29 ± 0.28	1.10 ± 0.03	0.52 ± 0.12	1.10 ± 0.23
0.64	4	0	0.12 ± 0.02	1.53 ± 0.45	1.74 ± 0.66	1.23 ± 0.19	0.57 ± 0.51	1.12 ± 0.45
0.64	4	1	0.10 ± 0.05	0.60 ± 0.26	1.12 ± 0.87	0.87 ± 0.15	0.43 ± 0.43	1.60 ± 0.71
0.64	4	2	0.24 ± 0.03	0.90 ± 0.13	1.86 ± 0.43	1.21 ± 0.05	0.77 ± 0.19	1.25 ± 0.25
0.64	2	3	0.05 ± 0.07	0.32 ± 0.01	1.09 ± 0.61	1.33 ± 0.16	0.61 ± 0.09	1.11 ± 0.03
Control (0.30 ± 0.03)	6	—	0.12 ± 0.03	0.32 ± 0.09	0.29 ± 0.07	0.39 ± 0.14	0.23 ± 0.15	0.35 ± 0.11

[a]Period (in months) after withdrawal of PCB ration
[b]Died during feeding the ration containing 0.64 ppm of PCB

seemed to have occurred by diapedesis. Similarly, the blood which was present in the gastrointestinal tracts of eight of the 18 mink which died was not traceable to ulcers or other gross lesions. Usually the blood was partially digested.

Slight splenic enlargement was found in about half the dead mink. Hydropericardium and hydroperitoneum, seen in birds poisoned with PCB, were never found.

Microscopic Lesions

The most frequently observed microscopic lesions are listed in Table I, with their frequencies of occurrence. Some degree of nephrosis was noted in about half of the mink which died. This consisted of vacuolar degenerative changes in tubular epithelial cells, flattening of the epithelium in some of the convoluted tubules, and dilatation of lumens containing fibrin casts. Foamy or flocculent eosinophilic exudates were sometimes present in Bowman's capsules.

Minimal to moderate focal liver necrosis was seen in four mink on the high PCB diet. The yellow color seen on gross inspection was due to the presence of large fat vacuoles in many hepatic cells. Brain edema, characterized by mild or moderate spongiosis and diffusely distributed, was seen in three of the dead mink.

Disseminated intravascular coagulation was a prominent lesion in three mink. These animals appeared to have died of extensive disseminated thrombosis followed by multiple hemorrhages in organs such as the lung (Fig. 1), and brain and either hemorrhage or irregular pooling of blood in sinusoids of the spleen and liver. Some of the spleens had populations of megakaryocytes which seemed to be greater than normal.

Fibrosis of the media of coronary arteries (Figs 2 and 3) was a lesion which apparently developed late in the course of the experiment. It was found in two standard dark mink which died on days 105 and 129 of PCB feeding and in another which was killed for examination on day 160. The mink which died on day 105 was on the high level PCB diet; the other two were on the lower level diet. Healing and recent infarcts were present in the left ventricular myocardium of the mink which died on day 105.

No lesions were noted in the male and female reproductive organs. Many of the females had uterine hypertrophy, evidence of recent pregnancy, and the female mink which died during late March and April all

MONS 204183

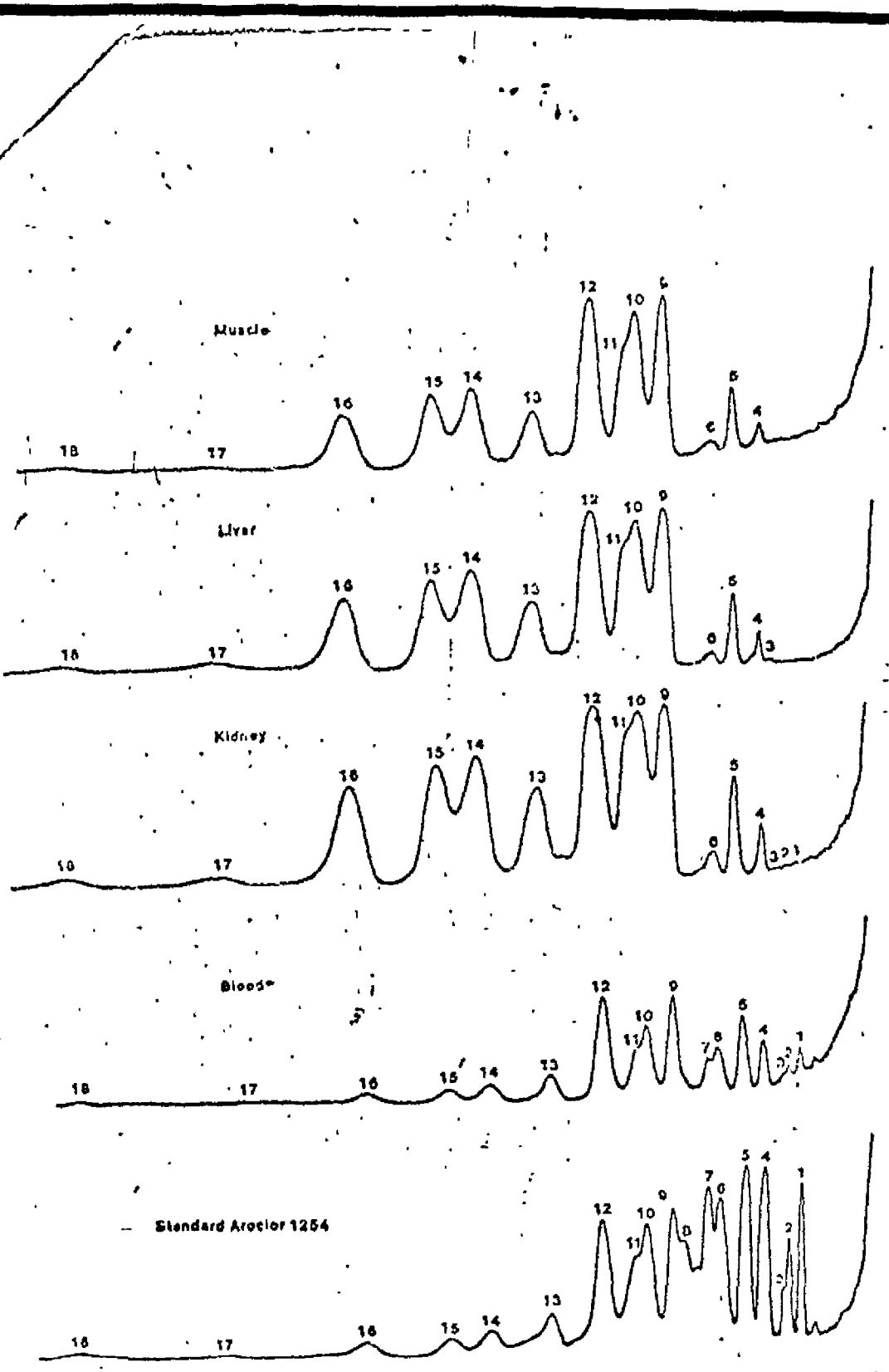


Fig. 4. Chromatograms of tissue extracts from mink given a ration containing 3.47 ppm PCB as well as a fraction of a standard Aroclor 1254.

MONS 204184

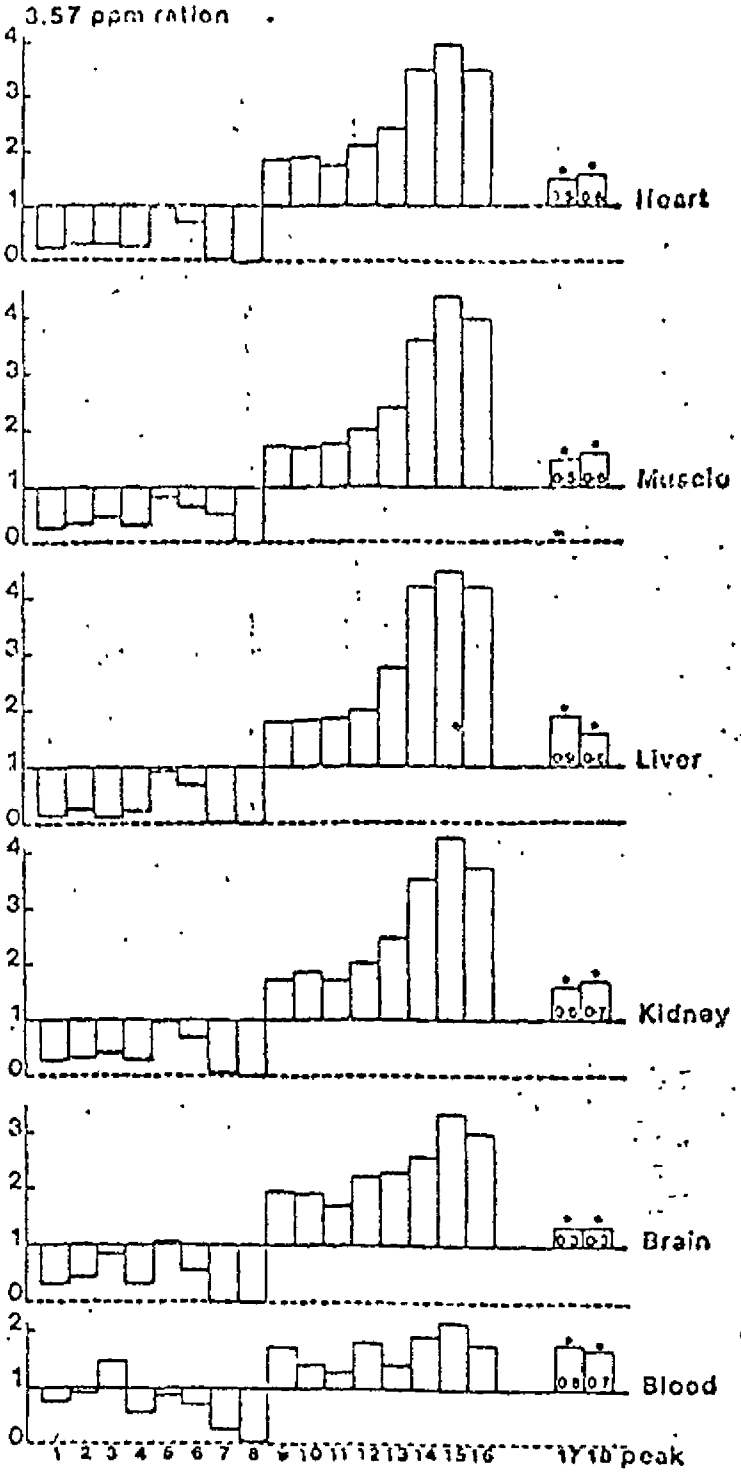


Fig. 1. Ratios between the mean percentages of chromatographic peaks of various tissue extracts of the group given the ration containing 3.57 ppm PCB, to the percentage of the same peak in the Aroclor 1254 standard.

MONS 204185

and corpora lutea present, in various degrees of regression. Ovarian follicles seemed to be present in adequate numbers during the breeding period, although many of the follicles were undergoing regressive changes in mink which died. Females which died before or at the beginning of the breeding period had a few uteri which were juvenile in appearance. Male mink which died during March and April had histological evidence of spermatogenesis. Those which died or were killed later had testicular changes typical of post-breeding regression.

No lesions were observed in the adrenal glands.

Tissue Distribution of PCB

The concentrations of PCB in various tissues of principal and control mink are given in Table II.

In the group of mink fed the higher level of PCB, the concentrations of these compounds were lowest in the blood and highest in the liver. In the same group, the PCB concentrations in the brain were relatively high, especially when one considers that they were higher than in the skeletal muscle. The concentrations of PCB in kidneys were higher than in the brain, but lower than in the hearts and livers.

In mink fed the ration containing 0.64 ppm of PCB, the PCB concentrations in tissues were similar in all organs analyzed, except in blood, where the lowest levels were detected. Similarly, in mink fed the commercial control ration, the PCB concentrations were nearly identical in the analyzed tissues, except in blood.

The resolution by gas-chromatography of Aroclor 1254 standard as well as extracts of various feed or tissue samples (under the operating conditions used in the present study) resulted in up to 18 distinct peaks. Figure 4 depicts the chromatographic tracing of tissue extracts from mink fed the ration containing 3.57 ppm PCB as well as the tracing of a standard Aroclor 1254. Note the decrease of early emerging peaks of tissue extracts, disappearance of peak 8 and increase of late emerging peaks.

The data depicted in Fig. 5 and Fig. 6 show the ratios between the mean percentages of peaks from each tissue extract of groups given 3.57 ppm and 0.64 ppm PCB to the percentages of the corresponding peak of the standard Aroclor 1254. The base line in each case is unity. The zero value indicates the disappearance of the peak; the values between unity and zero indicate the percentage decrease of the peak; the values higher than unity indicate the increases in percentage above the value of the standard.

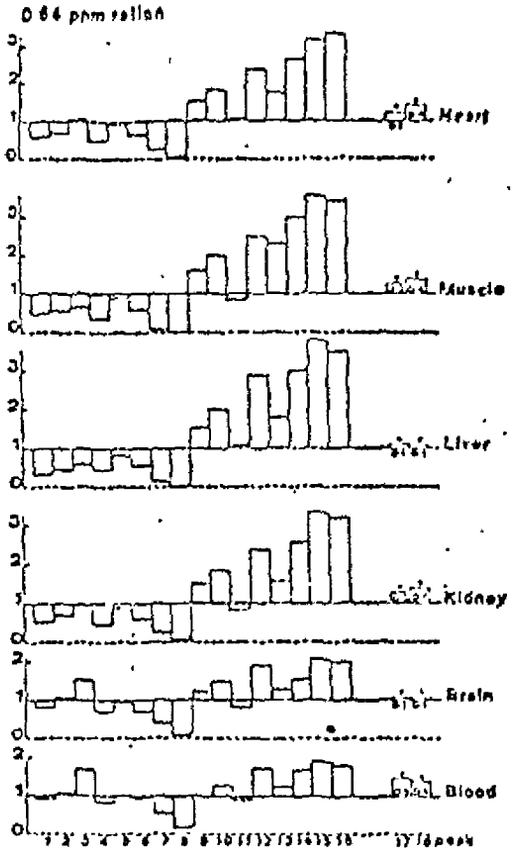


Fig. 6. Ratios between the mean percentages of chromatographic peaks of various tissue extracts of the group given ration containing 0.64 ppm PCB, to the percentage of the same peak in the Aroclor 1254 standard.

The percentage reductions of the first eight peaks and the percentage increases in the subsequent peaks in these two figures are evident. The first eight emerging peaks were significantly reduced in all tissues, except in blood, where the reduction was not always significant. The second eight peaks were always significantly increased proportionally to the Aroclor 1254 standard. These changes were most pronounced in the liver and least so in the blood. Peak 8 was reduced to zero in all tissues, except in blood; peak 7 was the next most strongly reduced. In peaks emerging after peak 8, the proportional percentage increase was nearly directly related to the retention time, i.e. peak 16 increased more than peak 9.

MONS 204186

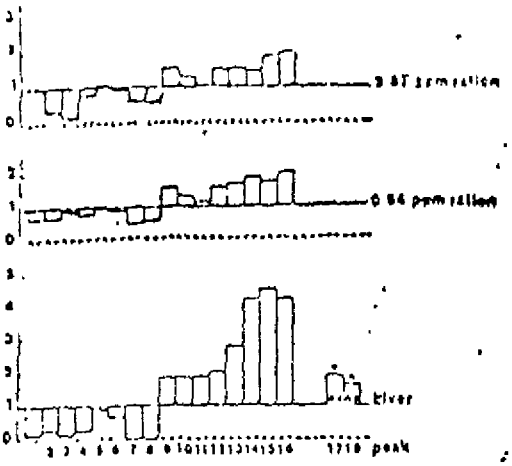


Fig. 7. Ratios between the percentages of chromatographic peaks of ration containing 3.57 ppm (upper figure) and ration containing 0.64 ppm PCB (middle figure). The lower figure, given for comparison, are the ratios of the liver of the group of mink fed 3.57 ppm PCB.

This tendency was not observed in blood. On all chromatograms a substantial percentage increase was noted in peaks 17 and 18. These two peaks are below 0.1% in the Aroclor 1254 mixture.

Figure 7 shows the ratios expressed similarly to Fig. 5 and Fig. 6 but give the ratios for both PCB rations fed, and for comparison the values of livers of the group fed 3.57 ppm.

Reproduction and Health of Control Mink

The 101 mink which received the commercial ration and which were regarded as no-treatment controls, produced only an average of 1.81 kits per female bred (satisfactory production is considered to be four kits per female).

When the commercial ration was analyzed the following results were obtained. The data represent means of six samples taken at intervals during the experiment.

Percent fat	— 8.2
p,p′ — DDE	— 0.012 ppm
p,p′ — DDD	— 0.010 ppm
o,p′ — DDT	— 0.004 ppm
p,p′ — DDT	— 0.008 ppm
Total DDT	— 0.033 ppm
Dieldrin	— 0.006 ppm
PCB	— 0.30 ppm

No outbreak of infectious disease was diagnosed in the herd during these experiments. Lesions similar to those of the principal PCB feeding groups were found in several dozen control mink killed for tissue culture purposes and used in aleutian disease research.

DISCUSSION

From the impaired reproduction and mortality observed, and the tissue concentrations of PCB found, it can be concluded that the mink were very susceptible to the effects of PCB compounds as dietary contaminants. The clinical and gross and microscopic tissue abnormalities were rather nonspecific, so that diagnosis in cases of naturally occurring PCB poisoning would be very difficult. Reproductive failure, weight loss and tarry droppings could suggest either starvation or aleutian disease, a common disease of mink. The PCB-fed mink may have been on an inadequate nutritional plane, but if so, it was not because the diet lacked essential nutrients but rather that the mink lost appetite and did not eat enough of the feed that was put before them. Aleutian disease would be ruled out on histopathology, however, since the severe hepatitis and nephritis of aleutian disease, marked by proliferation of plasma cells, was not seen in the PCB-poisoned mink.

The occurrence of liver damage in mink which also developed hemorrhagic tendencies suggests a breakdown in hemostasis in which prothrombin deficiency may be a causative factor. Blood coagulation studies should be made in future research on PCB poisoning in mink. Splenic enlargement and increased numbers of megakaryocytes in the spleen may represent compensatory responses in attempts to maintain hemostasis. The cause of the disseminated intravascular coagulation seen in the three mink is unknown. Hormonal effects of PCB may have been responsible for the reproductive failure. The presence of corpora lutea in the ovaries of the mink which died during or immediately after the normal gestation period indicates that ovulation did occur but that gestation did not continue to term.

Fatty degeneration of the liver and liver necrosis has been described in other species treated with PCB compounds. Degenerative kidney lesions also have been

MONS 204187

scribed in other species by some workers. Platonow *et al* (9) have described perivascular hemorrhages around coronary arteries in the hearts of chickens fed Aroclor. The coronary arterial lesions seen in three of the mink in these experiments are unexplained but thought to be related to the PCB feeding, since lesions of this type have not been seen by the authors previously. Their appearance in two of three mink which died between days 105 and 129 of PCB feeding and in one of four mink killed on day 160 may mean that lesions in these vital arteries develop as a late response to low-level PCB ingestion. This should be investigated further in mink and also in other species.

The poor reproduction in the no-treatment controls may be referable to the low (0.3 ppm) but significant amounts of PCB found in the control commercial ration. The controls, therefore, actually were an unplanned lower PCB treatment group of mink. Lesions referable to the PCB content of the control ration were not seen in the mink from this herd which were used for research on aleutian disease during the course of these PCB experiments. The low concentrations of dieldrin as well as DDT and its analogs and metabolites in the control ration are for all practical purposes of no toxicological significance.

It has been suggested that PCB affect enzyme systems that metabolize several sex hormones, which ultimately leads to reproductive failure. Thus, Risebrough *et al* (11) reported that the PCB induce the activity of hydroxylating enzyme which metabolizes estradiol. The estrogenic activity of various PCB mixtures upon the rat uterus was described by Bitman and Cecil (2). Platonow and Funnel (7, 8) reported that chronic feeding of PCB in cockerels resulted in anti-androgenic effects, as assessed by decreased development of testicles and comb. These signs appeared as early as one week after the start of continuous feeding of a PCB-contaminated diet.

It was recently demonstrated by Nowicki and Norman (6) that the post-mitochondrial hepatic fraction (microsomes + cytosol) from PCB-treated cockerels and pullets metabolized testosterone, estradiol-17β and 4-androstene-3, 17-dione at increased rates. The metabolic rates of these three natural steroid hormones were increased approximately three-fold over untreated birds. Platonow, Liptrap and Geissinger (8) reported on the effect of oral administration of PCB (Aroclor 1241) in the adult boar. The doses of PCB were such that no gross or histological lesions could be seen. However, the presence of significant biochemical alterations were recorded. These changes consisted in drastic reduction of urinary levels of two gonadal hormones: dehydroepiandrosterone and estrogen, indicating that PCB have a deleterious effect upon reproductive activity. The primary site of o,p'-DDD inhibition of ACTH-induced steroidogenesis in the adrenal cortex appears to be on the ACTH-regulated intramitochondrial conversion of cholesterol to pregnenolone (4). Since the chemical formulae of PCB's are similar to that of DDT, it is possible that the hormonal disturbances due to PCB are located at the same site. At least this is a probable site of PCB effect in the boar, because pregnenolone is a precursor of dehydroepiandrosterone in steroidogenesis.

In comparison to domestic animals (8, 9, 10, 13) mink seem to extensively metabolize the PCB, Aroclor 1254 used in this experiment. The changes occurring on the chromatograms of various tissue extracts indicated significant reduction of the first eight peaks, with simultaneous increases of the peaks emerging thereafter. The order of appearance of peaks on the PCB chromatogram is related to the extent of chlorination of biphenyls. Thus, monochlorbiphenyls appear before dichlorobiphenyls, trichlorobiphenyls before tetrachlorobiophenyls, etc., and decachlorobiphenyl (the completely chlorinated biphenyl) emerges as the last peak. The reduction of peaks of lower retention indicates that mink can metabolize PCB of lower chlorination numbers. Since this reduction in faster emerging peaks is much less pronounced in the cow (13), pig (10), or chicken (8), one might suggest that the extent of PCB metabolism is related to the extent of susceptibility to the toxic effect of PCB. Mink appear to be more susceptible to PCB than the domestic animals studied. However, further studies are required to confirm or reject the above hypothesis.

ACKNOWLEDGMENTS

The authors are grateful to Drs. Joan Budd and Peter Lusis for assistance in necropsies, to Mrs. N. Y. Chen for performing

MONS 204188

...analyses of PCB, and to Mr. Hugh Bel... for care of the experimental milk. The histopathology was done while the junior author was a visiting professor at Utah State University.

REFERENCES

1. ABBOTT, D. A. and J. A. BURKE. Method for extraction polychlorinated biphenyls from DDT and its analogs. J. Ass. off. analyt. Chem. 53: 761-765, 1970.
2. BITMAN, J. and H. C. CECIL. Estrogenic activity of DDT analogues and polychlorinated biphenyls. J. agr. Fd Chem. 18: 1108-1112, 1970.
3. GRANT, D. L., W. J. J. PHILLIPS and D. C. VILLENEUVE. Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. Bull. environ. Contam. Toxic. 5: 193-197, 1971.
4. HAHN, H. M. and J. A. STEAN. Studies on the site of action of o,p'-DDD in the dog adrenal cortex. I. Inhibition of ACTH-mediated progesterone synthesis. Steroids 17: 669-679, 1971.
5. LILLIE, R. D. Allocatory stain. Am. J. clin. Path. 31: 494, 1959.
6. [illegible] and A. W. [illegible]. [illegible] Aroclor 1254 [illegible] Fd Cosmet. Toxicol. 9: 487-494, 1971.
7. PLATONOW, N. S. and H. S. FUNNELL. Acute and chronic effect of polychlorinated biphenyls in rainbow trout. Vet. Rec. 88: [illegible], 1971.
8. PLATONOW, N. S. and H. S. FUNNELL. The distribution and some effects of polychlorinated biphenyls (Aroclor 1254) in rainbow trout (Salmo gairdneri). Can. J. comp. Med. 36: 1-5, 1972.
9. PLATONOW, N. S., I. H. KARLSAR and H. W. KANSUN. Tissue distribution of polychlorinated biphenyls (Aroclor 1254) in rainbow trout in relation to the duration of exposure and clearance from epithelioma. Can. J. comp. Med. 37: 29-32, 1973.
10. PLATONOW, N. S., H. M. KARLSAR and H. H. FUNNELL. The distribution and excretion of polychlorinated biphenyls (Aroclor 1254) and their effect on urinary steroid levels in the horse. Fd Cosmet. Toxic. 11: 745-754, 1973.
11. RISEBROUGH, R. W., P. REICHE, D. B. PEAKALL, S. G. HERMAN and M. N. KIRVEN. Polychlorinated biphenyls in the global ecosystem. Nature, Lond. 220: 1098-1102, 1968.
12. SASCHENBRECKER, P. W. and D. J. ECOBICHON. Extraction and gas chromatographic analysis of chlorinated insecticides from animal tissue. J. agric. Fd Chem. 19: 168-170, 1971.
13. SASCHENBRECKER, P. W., N. S. PLATONOW and H. E. FUNNELL. Histochemical study of polychlorinated biphenyls in lactating cows. Proc. Can. Fed. Biol. Soc. 14: 65, 1971.

(1) Univ Guelph, Dept Pathol / Guelph / Ontario / Canada 391-400

MONS 204189

Exhibit 7

Nebeker

(7)

Biological Magnification of a Polychlorinated Biphenyl (Aroclor® 1254) from Water by Aquatic Invertebrates

by Herman O. Sanders and Jack H. Chandler

Fish-Pesticide Research Laboratory
Bureau of Sport Fisheries and Wildlife
United States Department of the Interior
Columbia, Mo.

Polychlorinated biphenyls (PCBs) are chemical mixtures of chlorinated biphenyl isomers. These mixtures have a wide range of industrial application and are marketed in the United States as the Aroclor® 1200 series. A recent report describes the widespread occurrence of PCBs in the environment and their potential detrimental effects on organisms in aquatic ecosystems (1). Their insecticidal properties are well documented in literature (2,3). Only a few studies report the toxicity of PCBs to aquatic invertebrates (4,5). Some excellent reviews by Penkall and Linder (6); Gustafson (7); and Veith and Lee (8) summarize and evaluate our current knowledge concerning contamination of the environment by PCBs.

Results of previous bioassay experiments with aquatic invertebrates indicated that crustacea and immature insects rapidly accumulate total body concentrations of DDT or aldrin many thousands of times that of surrounding water (9). Although concentrations of PCBs entering the aquatic environment are generally below levels acutely toxic to aquatic invertebrates, these pollutants can be accumulated to appreciable concentrations by aquatic invertebrates. Thus, organisms at higher trophic levels may be exposed to significant amounts of PCBs via the food chain.

The purpose of this study was to determine the rate of accumulation and biological magnification of ^{36}Cl-labeled Aroclor® 1254 from water by eight species of aquatic invertebrates. The invertebrates were exposed to Aroclor® 1254 at concentrations less than 3 ppb, which is similar to levels detected in the water of Escambia Bay, Florida (4). In addition to measurements of accumulation, scud exposed to Aroclor® 1254 were analyzed by gas-liquid chromatography to investigate potential shifts in residue composition which may have occurred as a result of metabolism.

Materials and Methods

The organisms tested were four species of crustacea; scud, Gammarus pseudolimnaeus Bousfield; glass shrimp, Palaemonetes kadiakensis Rathbun; crayfish, Orconectes nais Faxon; a daphnid, Daphnia magna Strauss, and four species of immature aquatic insects, stonefly (naiad), Pteronarcys dorsata Say; dobsonfly (larvae) Corydalus cornutus Linnaeus; mosquito (larvae), Culex tarsalis Coquillett; and the phantom midge, Chaoborus punctipennis (Say). Daphnids were obtained from laboratory-reared cultures and all other

Bulletin of Environmental Contamination & Toxicology, Vol. 5, No. 3, 1970 by Springer-Verlag New York Inc.

MONS 204190

TABLE I

Biological magnification of ^{36}Cl-labeled Aroclor® 1254 by aquatic invertebrates

Organism	Organisms per sample 1/	Water concentration ppb $\bar{x}$ ± SE 2/	Organism concentration (4 day exposure) ppm $\bar{x}$ ± SE	Magnification factor 3/ 1-day	4-day	7-day	14-day	21-day
Daphnid Daphnia magna	60	1.1 ± 0.2	52 ± 2.0	24,700	47,000	-	-	-
Phantom midge Chaoborus punctipennis	5	1.3 ± 0.1	30 ± 1.6	22,000	23,000	23,800	24,800	-
Scud Gammarus pseudolimnaeus	6	1.6 ± 0.1	39 ± 3.0	17,000	24,000	26,000	27,500	27,000
Mosquito larvae Culex tarsalis	10	1.5 ± 0.3	27 ± 2.0	12,600	18,000	20,000	-	-
Glass shrimp Palaemonetes kadiakensis	3	1.3 ± 0.1	16 ± 2.6	10,300	12,300	13,700	14,200	16,600
Stonefly Pteronarcys dorsata	3	2.8 ± 0.8	7.0 ± 0.30	2,100	2,500	2,800	2,900	2,800
Dobsonfly Corydalus cornutus	3	1.1 ± 0.1	5.1 ± 0.23	1,400	4,600	5,700	6,600	6,800
Crayfish Orconectes nais	2	1.2 ± 0.1	0.2 ± 0.20	570	1,700	3,400	4,500	5,100

1/ Samples were taken in triplicate.

2/ Samples were taken in triplicate and expressed as mean value ± standard error (P=.05).

3/ Concentration in organism/concentration in water.

MONS 204191

organisms were collected from streams and ponds near the Fish-Pesticide Research Laboratory, Columbia, Missouri.

The ^{36}Cl-labeled Aroclor® 1254 used in these experiments was prepared by neutron irradiation in a nuclear reactor (10). This irradiated material was purified by silicic acid chromatography (10,11). The specific activity of this compound was 90.2 cpm/µg (disintegrations/min per microgram).

The experiments were conducted in a continuous-flow bioassay system designed for exposing small invertebrates to constant concentrations of a toxicant over extended periods. Test water came from a deep well and had a pH of 7.4; alkalinity and hardness were 260 and 270 mg/l as $CaCO_3$, respectively. The desired concentration of ^{36}Cl-labeled Aroclor 1254 was prepared in a reservoir and pumped through glass tubing into exposure vessels by a metering unit, at approximately 5 ml/min. Exposure vessels were 2-l glass aquaria containing one liter of water maintained at 21 ± 1 C°. To allow for concentration equilibrium, the bioassay system was operated for at least 24 hr prior to addition of organisms. All invertebrates were allowed to adjust to the test environment for 48 hr prior to exposure. The organisms were not fed during the experiments and mortality in the test population and controls were less than 10%. Previous studies (12,13) have shown that aquatic insect controls can live for several weeks without feeding.

Invertebrate samples for radiometric analysis were taken in triplicate. Sample size ranged from three crayfish to sixty daphnids. Individual samples were prepared directly for analysis by homogenizing the whole organism in a tissue grinder. The homogenate was obtained by adding 6 ml of Triton X-100®: toluene (2:3 v/v) emulsifier to each sample during grinding (9). The homogenate was then transferred to a glass vial for counting and a toluene-fluor mixture was used to bring the total volume of the scintillation vial up to 15 ml. The radioactivity of the sample was measured in a Beckman 200-L liquid scintillation counter. The concentration of Aroclor® 1254 in water was monitored radiometrically by extracting triplicate 100-ml water samples with redistilled hexane and by preparing the sample as previously described for the liquid scintillation method.

Concentrations of PCBs in the samples were obtained by converting cpm (counts/min) to dpm (disintegrations/min) after determining the efficiency of liquid scintillation counting. All samples were corrected for quench by use of external and internal standards. Residue concentrations presented in the text and tables were computed on whole-body dry-weight basis. Dry-weight conversion factors were obtained by drying the organisms to a constant weight at 50° C. Magnification was expressed as the ratio of concentration in organism to concentration in water.

Results and Discussion

PCB uptake kinetics

The rate of uptake and biological magnification of Aroclor® 1254 by some aquatic invertebrates was very rapid (Table 1). For example, when *D. magna* were exposed for 4 days to water containing 1.1 ± 0.2 ppb of 1254, they accumulated total body concentrations

MONS 204192

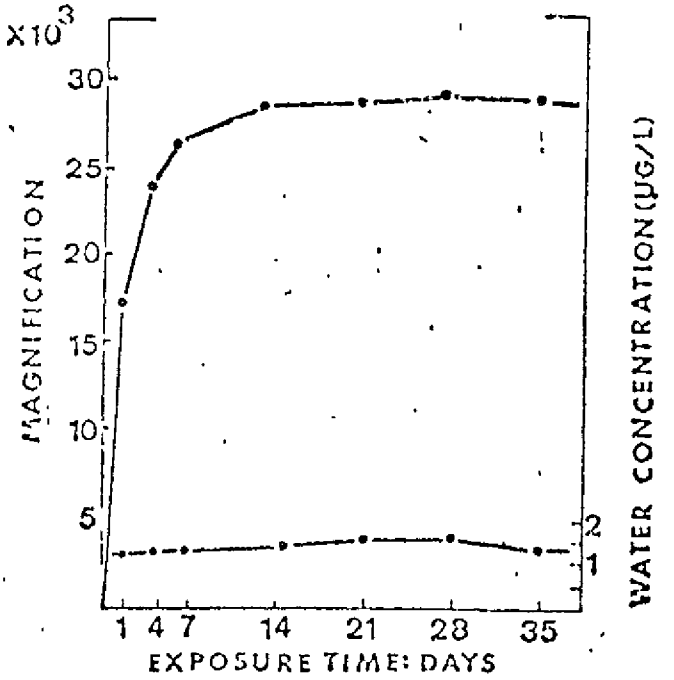


Figure 1. Biological magnification of ^{36}Cl-labeled Aroclor® 1254 from water by scud Gammarus pseudolimnaeus during 35 days of exposure. -•- = concentration in water; -o- = magnification in organism (total body residue/water concentration).

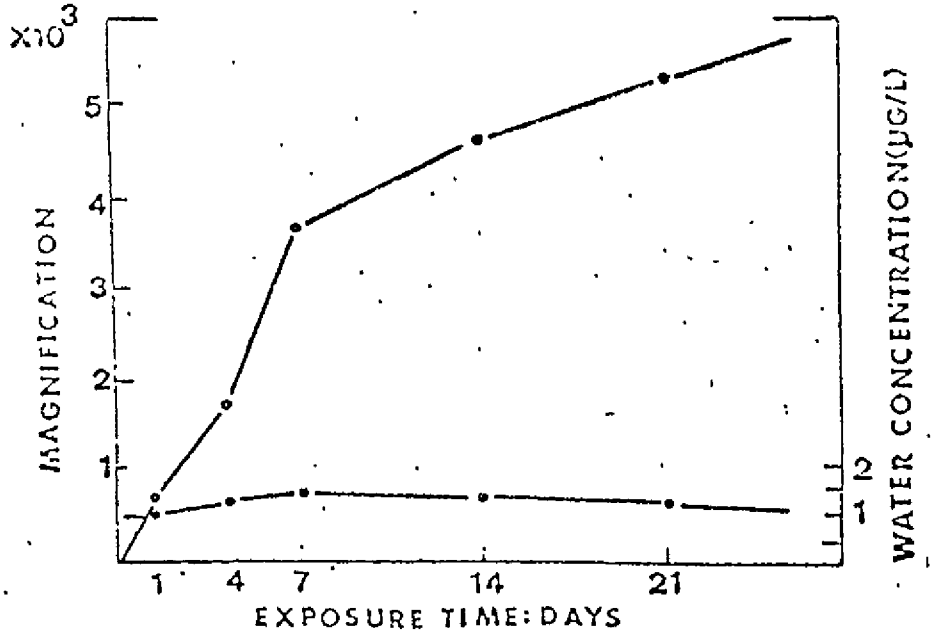


Figure 2. Biological magnification of ^{36}Cl-labeled Aroclor® 1254 from water by crayfish Orconectes nais during 21 days of exposure. -•- = concentration in water; -o- = magnification in organism (total body residue/water concentration).

MONS 204193

40,000 times greater than those in water.

In similar experiments, late-instar mosquito larvae exposed to water containing 1.5 ± 0.3 ppb of 1254 accumulated 19 ppm of this compound within 24 hr, which represents a 12,600-fold magnification. This accumulation continued until the seventh day of exposure when the mosquito larvae metamorphosed into the pupal stage. The pupae showed no apparent symptoms of toxicosis after this 7-day exposure. However, many of the pupae were unable to metamorphose into the adult form. Control organisms pupated with success. Moriarty (3) reported that the acute toxicity of Aroclor® 1254 to grasshoppers is not a significant factor. However, he suggested that a possible latent toxicity of this compound to grasshopper nymphs occurred at ecdysis, resulting in higher mortality for moulted individuals.

In some experiments, we were able to continue the exposure until an equilibrium concentration was reached within the organism. For example, scud exposed continuously to 1.6 ± 0.1 ppb of Aroclor® 1254 in water reached an equilibrium concentration in 14 days. Total body residues at this time were 44 ppm, a concentration 27,500 times that in water (Figure 1). Once this equilibrium was reached within the organism, no further magnification of residue was detected after an additional 21 days of exposure. Stonefly naiads, exposed to water containing 2.8 ppb of 1254, accumulated 8 ppm after a 7-day exposure; residues remained at 8-9 ppm after an additional 14 days of exposure. Early instar crayfish apparently reached an equilibrium concentration somewhat more slowly than the other invertebrates investigated. Crayfish accumulated 1254 at a linear rate until the experiment was terminated after 21 days of exposure (Figure 2).

Isomer changes of PCB residues in scud

To further elucidate the uptake of PCBs by scud, component analysis of the PCB residues were made by gas-liquid chromatography (GLC). Scud were exposed to 1.6 ppb of Aroclor® 1254 for 14 days. This exposure was previously shown to result in maximal residue accumulation in the organism. After sample extraction and cleanup of the extracts, the extracts were analyzed by GLC (14). The GLC peak area of each PCB component was measured and each component area expressed as a relative peak area (RPA). The PCB component RPA was determined by dividing the individual component area by the sum of all PCB component areas. The component relative peak areas were also calculated for Aroclor® 1254. To express the change in PCB residue composition in scud a concentration factor for each sample component was calculated by determining the ratio of the sample components RPA to the Aroclor® component RPA (Table 2).

The isomer uptake of Aroclor® 1254 by scud was characterized by progressively greater uptake of the lower chlorinated PCB isomers. Significant shifts in the ratios of the individual components occurred. Concentration factors ranged from 2.00 for a trichloro biphenyl isomer; 1.00-1.31 for pentachloro isomers; 0.49-0.80 for hexachloro isomers; and 0.25-0.50 for heptachloro isomers. No lower chlorinated components were observed in the chromatograms of the PCB residues in scud indicating that in these exposures reductive dechlorination of Aroclor® 1254 did not occur.

MONS 204194

We believe that ratios of the various components should be determined in examining the residues of PCBs from environmental samples. Knowledge of changes in the relative ratios of the PCB isomers may be important in evaluating the toxicological effects of PCBs.

Acknowledgements

The authors gratefully acknowledge the contributions of Dr. David Stalling and Mr. James Johnson of our staff for GLC residue analysis of scud samples.

TABLE 2

Changes in isomer ratios of Aroclor® 1254 residues in scud

Peak No.	Rel. Ret. Time 1/	No. Cl's 2/	Concentration Factor 3/ Sample 1	Sample 2	Average	Aroclor® 1254 R.P.A.
1	0.27	3	2.00	2.00	2.00	0.03
2	0.37	4	1.69	1.72	1.71	0.29
3	0.45	4	1.81	1.81	1.81	0.11
4	0.50	4	1.80	2.00	1.90	0.05
5	0.63	5	1.31	1.31	1.31	0.51
6	0.77	5	1.00	1.00	1.00	1.00
7	0.98	5	1.13	1.16	1.15	0.38
8	1.07	5	1.07	1.16	1.12	0.57
9	1.19	6	0.33	0.66	0.49	0.03
10	1.31	6	0.84	0.85	0.85	0.65
11	1.59	6	0.55	0.55	0.55	0.33
12	1.67	6	0.80	0.80	0.80	0.30
13	1.79	7	0.43	0.57	0.50	0.07
14	2.04	6	0.56	0.60	0.58	0.45
15	2.61	6	0.50	0.62	0.56	0.08
16	3.22	7	0.25	0.50	0.37	0.04
17	3.68	7	0.25	0.25	0.25	0.04

1/ Retention time relative to p,p' DDE on GLC column of 2 mm i.d. x 1.8 m 0.3% w/w OV-7 on 80-100 mesh Corning® 110 glass beads; 15 ml/min. flow of nitrogen carrier gas; column operated at 155° C.

2/ Number of chlorine atoms substituted on biphenyl ring determined by gas chromatography-mass spectrometry (9).

3/ Concentration factor = Relative Peak area of sample ÷ Relative Peak areas as Aroclor® 1254.

MONS 204195

Literature Cited

1. Risebrough, R. W., Reiche, P. and Olcott, H. S., Bull. Environ. Contam. and Toxicol., 4, 192 (1970).
2. Wildish, D. J., Bull. Environ. Contam. and Toxicol., 5, 202 (1970).
3. Moriarty, F., Entomol. Exptl. and Appl., 12, 206 (1969).
4. Duke, T. W., Lowe, J. I. and Wilson, Jr., A. J., Bull. Environ. Contam. and Toxicol., 5, 171 (1970).
5. Zitko, V., Bull. Environ. Contam. and Toxicol., 5, 279 (1970).
6. Peakall, D. B. and Lincer, J. L., BioScience, 20, (1970).
7. Gustafson, C. G., Environ. Sci. and Technol., 4, 814 (1970).
8. Veith, G. D. and Lee, G. F., Water Res., 4, 265 (1970).
9. Johnson, B. T., Saunders, C. R. and Sanders, H. O., Jour. Fish. Res. Bd. of Can., 4, 705 (1971).
10. Stalling, D. L. and Huckins, J. N., J. Assoc. Official Agr. Chemists, 54, 801 (1971).
11. Armour, J. and Burke, J., J. Assoc. Official Agr. Chemists, 53, 761 (1970).
12. Nebeker, A. V. and Lemke, A. E., J. Kansas Entomol. Soc., 41, 413 (1968).
13. Bell, H. L. and Nebeker, A. V., J. Kansas Entomol. Soc., 42, 230 (1969).
14. Stalling, D. L., Proceedings of 2nd International Congress of Pesticide Chemistry, February 22-26, 1971, Tel Aviv, Israel.

MONS 204196

Exhibit 8

(8)

Pete Benville, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Toxicity of Aroclors® to Aquatic Organisms

Fish: Tests to determine the chronic toxicity of Aroclors® to fish are continuing. Aroclors® 1242, 1248, and 1254 were about equally toxic to yellow perch (30-day TL50 = 72 to 92 µg/l), while Aroclor® 1260 was less than half as toxic.

Herman O. Sanders, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Invertebrates

Preliminary static bioassays with individual PCB isomers were begun using scud (Gammarus pseudolimnaeus). These organisms were selected as the amounts of pure isomers available did not permit bioassays using larger organisms.

No. Cl	PCB Component	TL50 (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
4	mixture of 3, decachlorobiphenyls	0.30	0.11

MONS 204197

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND MONITORING
NATIONAL WATER QUALITY LABORATORY

REPLY TO:
6201 Congdon Boulevard
Duluth, Minnesota 55804

FIELD STATIONS
Newtown, Cincinnati, Ohio
Corvallis, Oregon
Monticello, Minnesota

30 March 1972

(Hebert Field Address)

Dear Newsletter Subscribers:

Attached is the fourth issue of the PCB Newsletter. I have again tried to put your contributions in with as little change as possible. I have placed the news items into rather arbitrary categories, as Analytical Methods, Environmental Occurrence and Fate, Etc., to make finding the subject areas you are interested in more convenient.

Sincerely,

Virginia M. Snarski
Research Aquatic Biologist

Attachment:
PCB Newsletter (fourth issue)

MONS 204198

Exhibit 9

(9)

Toxicities of PCBs to Fish and Environmental Residues

by David L. Stalling and Foster Lee Mayer, Jr.*

Polychlorinated biphenyls (PCBs) have been found in fish and wildlife from many parts of the world at levels which may adversely affect aquatic organisms and interfere with pesticide residue analyses (1, 2, 3, 4). The environmental occurrence, uses, and present toxicological aspects of PCBs were recently reviewed by Peakall and Lincer (5), Gustaffson (6), and Risebrough (7). PCBs have gas chromatographic characteristics similar to many organochlorine insecticides. Jensen (8) first identified unknown gas chromatographic peaks as PCBs in extracts of pike and an eagle which were analyzed for organochlorine insecticides.

The Monsanto Company is the sole manufacturer of PCBs in the United States (6) and markets eight formulations of chlorinated biphenyls under the trademarks Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. The latter two digits designate the percent chlorine of each formulation. Aroclor 1248 and 1254 are the materials produced in greatest quantities and are used as a dielectric fluid in capacitors and in closed-system heat exchangers (6). Aroclor 1242 is used as a hydraulic fluid, and Aroclor 1260, as a plasticizer. Chlorinated terphenyls are marketed under the trademark Aroclor 5442 and 5460. A mixture of bi- and terphenyls is designated Aroclor 4465. The Monsanto Company has restricted the sale of PCBs for uses in which disposal of the end products cannot be controlled, such as the use of PCBs as plasticizers (6).

* Chemist and Fishery Biologist, respectively, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Columbia, Missouri 65201.

Chemical Composition and Analysis

PCBs occur in the aquatic environment as mixtures of chlorinated biphenyl isomers (10, 11). The biphenyl structure may be substituted with one to ten chlorine atoms, and over 200 compounds are possible (6). The isomer composition and chromatographic characteristics of each formulation have been described by Stalling and Huckins (12) and Bagley (13). The PCBs can cause serious interference in gas chromatographic (GC) determination of chlorinated insecticides (4). Analysis of PCBs is best accomplished by GC after separation of PCBs and pesticides during sample cleanup using silicic acid column cleanup (14). No standardized GLC method has been proposed for the analysis of mixtures of PCB formulations in environmental samples. The solubility of these formulations in water have not been precisely determined but is reported in the range of 100–1000 μg/l (9). Considerable research remains to be done on the structure of the isomers of each technical material and how environmental residues of various Aroclors are to be reported.

Environmental PCB Residues

Analyses of 40 fish (cross-check sample) from the 1970 National Pesticide Monitoring Program were made using sample preparation techniques which separated PCBs from pesticides and other industrial residues (Table 1). These analyses employed GC and gas chromatography-mass spectrometry (GC-MS) for confirmation. The samples were prepared using gel permeation chromatography (23), and PCBs were separated from the pesticides with silicic acid (14). Only

MONS 204199

Table 1. Composition of PCB residues in selected fish samples from the 1970 National Pesticide Residue Monitoring Program.

River	Location	Species	PCB residue as Aroclor type (μg/g whole body)				
			1232	1248	1254	1260	total
Ohio	Cincinnati, O.	Carp	10.2	75	42	6.0	131
Ohio	Cincinnati, O.	White crappie	15.9	17	27	5.6	66
Ohio	Marietta, O.	Channel catfish	38	23	11	4.9	77
Ohio	Marietta, O.	Channel catfish	15.6	6.2	12.6	4.6	39
Yazoo	Redwood, Miss.	Smallmouth buffalo	72	—	1.4	—	73
Hudson	Poughkeepsie, N.Y.	Goldfish	8.6	173	32	—	213
Allegheny	Natrona, Pa.	Walleye	—	5.2	25	4.6	35
Delaware	Camden, N.J.	White perch	—	8.0	6.8	3.9	19
Cape Fear	Elizabeth Town, N.C.	Gizzard shad	18.9	—	2.6	1.1	23
Lake Ontario	Port Ontario, N.Y.	White perch	12.9	—	4.6	1.2	19
Mississippi	Memphis, Tenn.	Drum	11.2	—	4.5	3.4	19
Merrimac	Lowell, Mass.		13.8	75	6.1	3.2	98

one of the forty samples contained ess than 1 μg/g PCB residue. The composition of PCB residues in a lake trout and coho salmon were examined by GC-MS and found to be a mixture of Aroclor 1248 and 1254 (Figure 1), and residues of PCBs were in excess of the "DDT" residue levels (Table 2).

Widespread pollution of the major waterways has occurred, and appreciable PCB residues exist in fish. In view of the PCB bioconcentration in fish, PCB levels as high as 5 μg/l may exist in the waters of the Hudson River. These levels are certain to have adverse effects on aquatic organisms.

Toxicity and Bioaccumulation

Fish

Chronic and acute toxicity studies on aquatic organisms are quite limited. Due to the low solubility of PCBs, tests to obtain 96-hr LC_{50} values do not adequately reflect their toxicities to fish (Table 3). For Aroclor 1221-1268, 96-hr LC_{50} values ranged from 1,170 to 50,000 μg/l for cutthroat trout (15). 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 bluegills resulted in 15-day

Table 2. Pesticide and PCB Residues in a Coho Salmon and a Lake Trout from Lake Michigan.

	Residues in μg/g whole fish									
	Dieldrin	p,p' isomers			o,p' isomers		Total "DDT"	PCB (Aroclor)[1]		
		DDE	DDD	DDT	DDE	DDT		1248	1254	Total
Lake trout[2]	0.10	13.0	1.8	5.2	0.9	1.8	22.7	12.0	16.0	28.0
Coho salmon[3]	0.10	4.3	0.8	2.3	0.4	0.5	8.3	6.7	6.7	13.4

[1] Calculated from ratios of characteristic 1248 and 1254 peaks.

[2] Lake trout (33 L)—taken 10-14-69. 600 mm, 2500 gm, immature M. Taken in 2.5 fathoms in Little Bay de Noc, Michigan.

[3] Coho salmon (7Ca)—taken 8-3-69. 675 mm, 5715 gm F. Taken in 17–19 fathoms 4 miles NW of Manistee, Michigan.

95

MONS 204200

LC_{50} values of 51, 76 and 204 μg/l, respectively (Table 4). Exposures to channel catfish gave 20-day LC_{50} values of 107, 127, and 741 μg/l for the same Aroclors. All LC_{50} values decreased significantly with longer exposures. The 20-day LC_{50} values for Aroclors 1248, 1254, and 1260 were 10, 135, and 245 μg/l for bluegills and 300, and 206 μg/l for Aroclors 1254 and 1260 with channel catfish at 20°C. The toxicity of Aroclor 1248 to bluegills and channel catfish increased two-fold at 27°C. The toxicity of the PCBs to fish is inversely related to percent chlorine (higher Aroclor number).

In chronic tests to determine the effects of Aroclor 1242 and 1254 on fathead minnow reproduction, all fish exposed to greater than 8.3 μg/l

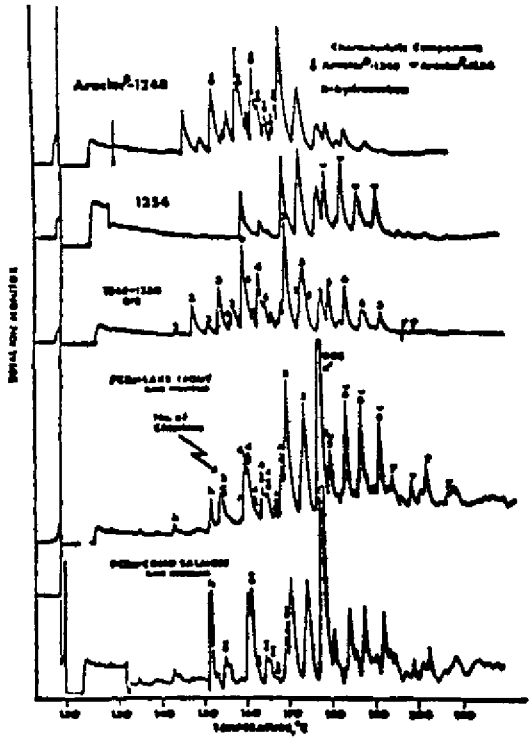


FIGURE 1. Composition of PCB residues in fish. Arrows and triangles designate the most characteristic peaks of the Aroclor. All numbered peaks in chromatograms of fish extracts are PCBs. These numbers designate the number of chlorine atoms in the compounds as determined by mass spectrometry. Temperature program rate, 4°C/min, He 9 psig, 60"×0.020" I.d. SE-30 SCOT column, PE-2708 GC-MS.

Table 3. Acute toxicity[1] of several Aroclors to selected fishes.

Aroclor	Species	Temperature (C)	96-hr LC_{50} (μg/l)
1221	Cutthroat trout	8.0[2]	1,170
1232	Cutthroat trout	8.9	2,000
1242	Cutthroat trout	8.9	5,430
1248	Cutthroat trout	8.9	5,750
1254	Cutthroat trout	8.9	42,000
1260	Cutthroat trout	8.9	60,000
1262	Cutthroat trout	8.9	60,000
1268	Cutthroat trout	8.9	60,000
1248	Channel catfish	19.3[3]	6,000
1254	Channel catfish	18.3	12,000
1248	Bluegills	18.3[3]	273
1254	Bluegills	18.3	2,740

[1] Static bioassay.
[2] Alkalinity, 150 ppm; pH 7.6.
[3] Alkalinity, 35 ppm; pH 7.1.

of each Aroclor died. Reproduction occurred at and below 8.3 μg/l Aroclor 1242 and at and below 2.8 μg/l Aroclor 1254 (17).

Accumulation of Aroclor 1248 and 1254 by bluegills chronically exposed to 2–10 μg/l was from 25,300 to 71,400 times the exposure levels for both PCBs. Concentration factors were not strongly dependent upon water exposure levels, but a direct correlation of water concentrations and whole-body residues may exist. No major modification of the PCB isomer ratios was observed in the tissue residues, and no new components were identified (18).

Coho salmon fed Aroclor 1254 for 240 days at concentrations of 14.5 to 14,500 μg/kg body weight per day (dietary concentrations of 0.4 to 580 μg/g) accumulated whole-body residues which were 0.9 to 0.5 times the exposure levels (300 mg/kg highest residue value). Growth rates were not affected; however, all fish exposed to the highest treatment died after 240 days' exposure, and thyroid activity was stimulated in all but the lowest treated group (19).

Direct water exposures appear to represent a greater hazard to fish than dietary exposures, although both contribute to tissue residues. However, in the environment the kinetics of residue uptake from dietary sources could be more important, since PCBs have a high affinity for

MONS 204201

Table 4. Intermittent-flow bioassays of Aroclor against three fishes.[1]

Aroclor	Species	LC_{50} (μg/l) 5 days	10 days	15 days	20 days	25 days	30 days
1254	Rainbow trout	150	8	—	—	—	—
1260		—	240	94	21	—	—
DDT		2.28	0.87	0.28	—	—	—
1242	Bluegills	154	72	54	—	—	—
1248		307	160	70	10	—	—
1254		—	443	201	135	54	—
1260		—	—	—	245	212	151
1242	Channel catfish	—	174	107	—	—	—
1248		—	225	127	—	—	—
1254		—	—	741	300	113	—
1260		—	—	—	206	166	137
1248[2]	Bluegills	137	76	—	—	—	—
1248[2]	Channel catfish	—	94	57	—	—	—

[1] Temperature, 20°C; alkalinity, 260; pH, 7.4.
[2] Temperature, 27°C.

sediments and PCBs can readily enter food chains (20, 21).

Aquatic Invertebrates

Daphnia magna: Aroclor 1248 is the most toxic of the Aroclor series to *Daphnia*, and preliminary studies indicate that levels above 5 μg/l are not safe for reproduction (17). After 48 hours' exposure to 300 μg/l, *Daphnia* concentrated Aroclor 1254 by 48,000 times (22).

Gammarus pseudolimnaeus: The level of Aroclor 1248 that did not affect reproduction was comparable to that for daphnids, ca 5 μg/l (17). The 96-hr LC_{50} values for Aroclor 1248 and 1254 were 52 μg/l and 2,400 μg/l, respectively (Table 5). Scud were more sensitive to Aroclor 1242 (96-hr LC_{50}=10 μg/l). After exposing another species of scud (*Gammarus fasciatus*) to 1.6 μg/l Aroclor 1254 for 14 days, the PCBs were concentrated 27,000 times the exposure level. No further increase in PCB residue resulted after an additional 21 days of exposure (22).

Orconectes nais: Crayfish were more susceptible to Aroclor 1242 (7-day LC_{50}=30 μg/l) than they were to Aroclor 1254 (7 day LC_{50}=80 μg/l). PCB residues in crayfish did not reach equilibrium after a 28-day exposure to Aroclor 1254, and the uptake was linear during this period (22).

Palaemonetes kadiakensis: Glass shrimp were very sensitive to Aroclor 1254, having a 7-day LC_{50} value of 3 μg/l compared to a 5-day LC_{50} of 1 μg/l for DDT (22).

Marine Organisms

Immature pink shrimp were extremely sensitive to Aroclor 1254 exposures. Fifty-one percent of the shrimp died within 15 days when continuously exposed to 0.04 μg/l Aroclor 1254 (25). Mortalities in two estuarine fishes (*Lagodon rhomboides* and *Leiostomus xanthurus*) exposed for 14 to 45 days to Aroclor 1254 were observed at 5 μg/l. Fish mortalities were not observed at 1 μg/l during the same exposure time (24). PCB concentration factors in these fish were similar to uptake by freshwater fish ($1–5\times10^6$ times the exposure levels).

Correlation of Reproduction and PCB Residues

In preliminary investigations, Jensen et al. (25) reported a possible relationship between PCB residues in salmon eggs and egg mortality in Sweden. On a fat basis, PCB residues in eggs ranged from 7.7 to 34 μg/g and mortality ranged from 16 to 100 percent. A regression analysis of their data resulted in a coefficient of correlation of 0.85, and the correlation was significant at P .001.

47

MONS 204202

Table 5. Aroclor and DDT toxicity to invertebrates.

Compound	Organism[1]	Bioassay type[2]	Exposure (days)	LC50 (μg/l)
Aroclor 1242	Crayfish	static	7	30
Aroclor 1254	Crayfish	static	7	100
Aroclor 1254	Crayfish	continuous-flow	7	60
DDT	Crayfish	static	4	100
Aroclor 1242	Scud	continuous-flow	4	10
Aroclor 1242	Scud	continuous-flow	10	5.0
Aroclor 1248	Scud	static	4	52
Aroclor 1254	Scud	static	4	2,400
DDT	Scud	static	4	3.2
DDT	Scud	continuous-flow	5	0.6
Aroclor 1254	Glass shrimp	continuous-flow	7	3.0
DDT	Glass shrimp	static	5	1.0
DDT	Glass shrimp	continuous-flow	5	1.3
Aroclor 1242	Dragonfly	static	7	800
Aroclor 1254	Dragonfly	static	7	1,000
Aroclor 1242	Damselfly	continuous-flow	4	400
Aroclor 1254	Damselfly	continuous-flow	4	200
DDT	Damselfly	static	4	56

[1] Crayfish (*Orconectes nais*), Scud (*Gammarus fasciatus*), Glass shrimp (*Palaemonetes kadiakensis*), Dragonfly (*Macromia sp.*), Damselfly (*Ischnura verticalis*).

[2] Temperature, 15.6°C; alkalinity, 35 ppm; pH, 7.1.

Summary

The occurrence of high levels of PCB residues in aquatic organisms (fish and invertebrates) as complex mixtures of Aroclor formulations has been determined by GC analysis and confirmed by GC-MS. Suitable methods for sample cleanup include gel permeation and silicic acid column chromatography. Agreement on methods for reporting PCB residues is lacking.

The highest concentrations of PCB residues in freshwater fish occur in rivers which are associated with industrialized areas, i.e. Hudson River (ca 200 μg/g whole body residue) and the Ohio and Allegheny Rivers (ca 100 μg/g). Residue levels associated with these rivers approach PCB residues which were associated with fish mortalities in chronic continuous-flow exposures (500–600 μg/g).

Concentration of PCBs by fish is greater than 40,000 times the exposure levels. Similar concentration factors have been observed for invertebrates. Adverse effects on reproduction of aquatic organisms may occur at PCB concentrations of 5 μg/l or less. Further studies are required to determine more definitely the exposure levels which do not adversely effect aquatic organisms.

The problem of PCB residues in industrialized areas can be greatly improved by close monitoring of effluents and by identification of those PCB uses which contribute to effluent contamination. Elimination of widespread PCB residues in fish at concentrations of 1–20 μg/g will require greater efforts, such as the control of PCBs from sewage treatment plants and other as yet unidentified sources of PCBs into the aquatic environment.

Related Materials

Chlorinated napthalenes are recommended for uses similar to those of the PCBs (27). These materials are recovered by the same analytical procedures as the PCBs (28) and may be recognized by their gas chromatographic elution patterns if PCBs are not present. The materials are designated Halowax 1000, 1031, 1099, 1013, 1014, and 1051 and are listed in an order of increasing chlorine content (27). Aquatic toxicity information is not presently available, nor has

MONS 204203

the persistence and fate of these materials been examined in the aquatic environment.

REFERENCES

1. Jensen, S. et al. 1969. DDT and PCB in marine animals from Swedish waters. Nature 224: 247.
2. Holmes, D. C., Simmons, J. H. and Tatton, J. O'G. 1967. Chlorinated hydrocarbons in British wildlife. Nature 216: 227.
3. Koeman, J. H., Ten Noever de Brauw, M. C. and Vos, R. H. de 1969. Chlorinated biphenyls in fish, mussels, and birds from the River Rhine and the Netherlands coastal area. Nature 221: 1126.
4. Risebrough, R. W. et al. 1968. Polychlorinated biphenyls in the global ecosystem. Nature 220: 1098.
5. Penkall, D. B. and Lincer, J. L. 1970. Polychlorinated biphenyls: another long-life widespread chemical in the environment. BioScience 20: 933.
6. Gustafson, C. G. 1970. PCBs—prevalent and persistent: intensified research is needed to minimize their dangers. Environ. Sci. Tech. 4: 814.
7. Risebrough, R. 1970. More letters in the world. Environ. 12: 16.
8. Jensen, S. 1966. Report of a new chemical hazard. New Scientist 32: 612.
9. Papageorge, W. B. 1970. PCBs in the Environment, present in part at a meeting sponsored by the National Water Quality Laboratory, FWQA, Duluth, Minnesota, March 17, 1970. (Monsanto Company).
10. Stalling, D. L. and Johnson, J. L. 1971. Unpublished data from laboratory cross-check analyses from the 1970 National Pesticide Monitoring Program. Fish-Pesticide Research Laboratory.
11. Stalling, D. L. (In press). Analysis of Organochlorine Residues in Fish—Current Research at the Fish-Pesticide Research Laboratory, Conference on Pesticide Chemistry, February, 1971, Tel Aviv, Israel.
12. Stalling, D. L. and Huckins, J. N. 1971. GC-MS characterization of PCBs (Aroclors) and ^{36}Cl labeling of Aroclor 1248 and 1254. J. Assoc. Off. Anal. Chem. 54: 801.
13. Bagley, G. E., Reichel, W. L. and Cromartie, E. 1970. Identification of polychlorinated biphenyls in two bald eagles by combined gas-liquid chromatography mass spectrometry. J. Assoc. Off. Anal. Chem. 53: 251.
14. Armour, J. and Burke, J. 1970. Method for separating polychlorinated biphenyls from DDT in its analysis. J. Assoc. Off. Anal. Chem. 53: 761.
15. Swellberg, D. 1970. Special report on PCBs, In: Bureau of Sport Fisheries and Wildlife, Progress in Sport Fishery Research, Resource Pub., U.S. Govt. Print. Off. (In press).
16. Mayer, F. L. Ibid.
17. Nebeker, A. 1971. Unpublished data, National Water Quality Laboratory, EPA, Duluth, Minnesota
18. Stalling, D. L. and Huckins, J. N. Unpublished data. Fish-Pesticide Research Laboratory, Columbia, Missouri.
19. Mehrle, P. M. and Grant, B. F. Unpublished data. Fish-Pesticide Research Laboratory, Columbia, Missouri.
20. Duke, T. W., Lowe, J. I. and Wilson, A. J. 1970. Vertebrate insecticide resistance: the in vitro enzyme effect on succinic dehydrogenase activity on endrin-resistant and susceptible mosquitofish. Bull. Environ. Contamin. Toxicol. 5: 171.
21. Nimmo, D. R. et al. 1971. Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. Nature 231: 50.
22. Sanders, H. O. 1970. Special Report on PCBs. In: Progress in Sport Fishery Research, Bureau of Sport Fisheries and Wildlife, Resource Publication, USGPO, (In press).
23. Stalling, D. L., Tindle, R. C. and Johnson, J. L. (In press). Pesticide and PCB cleanup by gel permeation chromatography. J. Assoc. Off. Anal. Chem.
24. Hansen, D. J. et al. 1971. Chronic toxicity, uptake, and retention of Aroclor 1254 in two estuarine fishes. Bull. Environ. Contamin. Toxicol. 6: 113.
25. Duke, T. W. 1971. Unpublished data. Gulf Breeze Laboratory, Environmental Protection Agency, Gulf Breeze, Florida.
26. Jensen, S., Johansson, N. and Olsson, M. 1970. PCB-Indications of Effects on Salmon. PCB Conference, Stockholm, September 29, 1970. Swedish Salmon Research Institute-Report LF1 MEDD 7/1970.
27. Halowax Chlorinated Naphthalenes, Technical Bulletin, Koppers Co., Inc., Tar Products Division, Pittsburgh, Pennsylvania.
28. Armour, J. A. and Burke, J. A. 1971. Behavior of chlorinated naphthalenes in analytical methods for organochlorine pesticides and polychlorinated biphenyls. J. Assoc. Off. Anal. Chem. 54: 178.

MONS 204204

Exhibit 12

N-6269

12

Effects of Aroclor 1242[1] (a Polychlorinated Biphenyl) and DDT on Cultures of an Alga, Protozoan, Daphnid, Ostracod, and Guppy[1]

By Janet R. Morgan
College of Fisheries, University of Washington, Seattle, Wash. WA 98105

The polychlorinated biphenyls (PCB's) are a group of chemicals having physical and chemical properties sufficiently similar to those of DDT to warrant investigation (10). The Monsanto Company, the sole producer of PCB's in the United States, markets them under the trade name of Aroclor. Aroclor 1242 is a mixture of PCB compounds containing approximately 42% chlorine by weight. Diverse in their commercial applications, but most commonly employed as plasticizers, PCB's have increased in usage dramatically since the forties (6). Jensen first reported finding PCB's in pike, other fish, eagle feathers and human hair from Sweden in 1966 (1). Subsequently, scientists from around the world began analyzing wildlife tissues for the presence of PCB's. Chlorinated biphenyls were detected in fish, mussels, and birds from the Rhine River and the coast of the Netherlands (7), in sparrowhawks, kestrels and kittiwakes from Great Britain (4) and peregrine falcons from the United States (5). Cultures of the marine diatom Cylindrotheca closterium have been shown capable of absorbing Aroclor 1242 from sea water containing 0.1 ppm and concentrating it 900 to 1000 times (6). This concentration caused, however, a marked reduction in growth, chlorophyll index, and RNA synthesis of the marine diatom, but no noticeable effect on DNA synthesis.

The aim of the present study was to investigate some of the effects of Aroclor 1242 and DDT on a variety of aquatic organisms. For a more detailed report, please refer to the Master's thesis (9).

R Registered Trademark, Monsanto Co., St. Louis Mo.

[1] Fisheries contribution No.350

Bulletin of Environmental Contamination & Toxicology, Vol. 4, No. 3, 1972, © by Springer Verlag New York Inc.

MONS 204205

Methods

Five different organisms were used in the present investigation: the alga Chlamydomonas reinhardtii, the ciliate protozoan Tetrahymena vorax, the cladoceran Daphnia pulex, the ostracod Cypridopsis vidua, and the guppy Poecilia reticulata. Media 63 (12) was the experimental medium in which the effects of the toxicants upon algae, cladocerans, and ostracods were studied. Bioassay media, modified from Pernell and Rosen (3) to contain 1/100 of the organic components and 1/10 of the organic salts, was used for the Tetrahymena studies. Coarsely-filtered pond water was used in the guppy toxicity tests. Since both DDT and PCB have a tendency to adhere to surfaces (2, 14), disposable glassware was used to prevent residues of the chlorinated hydrocarbons from affecting the results of subsequent experiments. The culture vessels were either quart or half-gallon mason jars with half of a disposable plastic Petri plate for a lid or disposable glass test tubes for the protozoan studies. At the start of an experiment all culture vessels and media were autoclaved at 250° C. for 25 minutes to insure sterility (except the guppy experiments in which pond water was used). One hundred percent p,p'-DDT, designated as the "ESA pesticide reference standard" was used in all DDT experiments and the Aroclor 1242 used was obtained directly from Monsanto in St. Louis, Missouri. The test chemical was generally added to the growth media prior to the introduction of the organisms. In order to distribute the test chemical throughout the media, and to ease in making dilutions, acetone was used as a vehicle for DDT, and 95% ethyl alcohol was used for the PCB. A concentration of 0.2 ml solvent/liter media was used, which did not appear to adversely affect the tested organisms as shown by preliminary experiments and usually demonstrated by the solvent controls. Once algal density was determined, a known volume of inoculum was added to each culture so that the initial concentration in the culture vessel would be 0.1×10^6 cells/ml. Adult Daphnia, ostracods, and fish were removed from their stock cultures, randomized, and counted before placing them in the test cultures. Experimental cultures containing algae and/or Daphnia were grown at 28.5° C. ± 1°C. under a bank of fluorescent lights which delivered 900 foot-candles

MONS 204206

(as measured by a GE model 213 lightmeter with a blue filter). Tetrahymena vorax cultures, grown in test tubes, were placed on a roller tube apparatus which rotated the tubes at 1/5 rpm. Protozoan, ostracod, and guppy bioassays were conducted under the conditions of 23.5° C±1°C. and 75 footcandles of light.

During the course of an experiment, population counts of the organisms present in each culture vessel were recorded. The Chlamydomonas cells were killed and

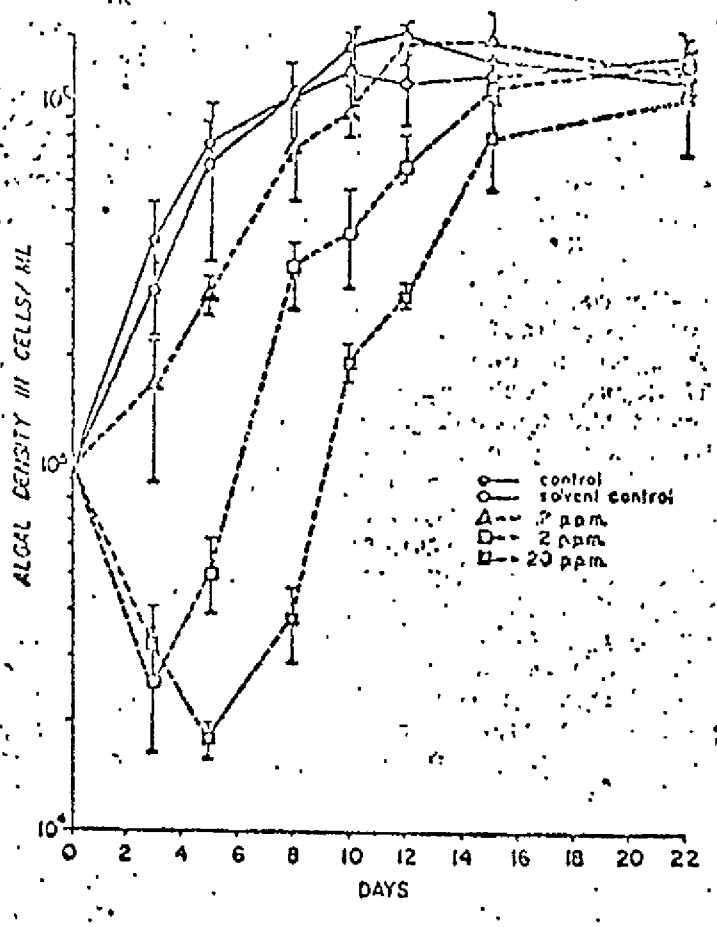


Fig. 1. Effect of the addition of 0.2, 2, and 20 ppm Aroclor 1242 on the growth of Chlamydomonas reinhardtii. Each point represents the mean of two observations whose individual values are indicated as the range.

MONS 204207

stained by adding a drop of I_2-KI solution, then counted using a haemacytometer at 100X. The Tetrahymena were stained in the same manner, then ten 0.01 ml drops of the culture were distributed on a Falcon counting plate and the cells in each counted at 50X. Carbon-14 uptake by algal cells subjected to various concentrations of both DDT and PCB was determined by incubating 5 ml samples with $NaH^{14}CO_3$ and using a standard filtration-planchet method. Gas chromatography analysis was performed by Dr. James Saddler of the Fisheries Research Institute on five algal cultures--a control, a solvent control, and three cultures to which 20, 2, and 0.2 ppm Aroclor had been added. The algal pellets and supernatant samples were extracted with acetonitrile-petroleum ether; purified by elution on a Florisil column; and injected into a gas chromatograph equipped with an electron capture detector.

Observations--Discussion

Figure 1 illustrates the effect of three different doses (0.2, 2, and 20 ppm) of Aroclor 1242-alcohol on the density of Chlamydomonas over a period of 22 days. Both the control and the solvent control followed the typical parabolic growth curve with no initial lag period. There was a period of rapid growth (the log phase) followed by a leveling off of the population size as the culture approached the stationary phase. Aroclor-containing cultures did not follow this pattern, however, but took a much longer time to approach the cell density of the controls. In the Chlamydomonas cultures to which the two highest dosages of Aroclor were added there appeared to be not only a delay before exponential growth began, but an initial drop in the algal density below the original inoculum size. The severity of the response was dose related. After day 5, the growth rate of these inhibited cultures increased so that by day 22 they had cell densities similar to the controls. By day 22, the standing crop of Chlamydomonas in all of the cultures appeared to be about the same. This experiment was repeated several times with similar results, although the degree of inhibition from equal additions of PCB varied somewhat. This variation may be due to heterogeneity of the PCB globular suspensions (14), or to differences in other physical factors from experiment to experiment.

MONS 204208

In order to determine whether ^{14}C uptake by Chlamydomonas cells was also affected by the addition of Aroclor 1242, the experiment was repeated using five different doses of PCB (0.002, 0.02, 0.2, 2, and 20 ppm), incubating $NaH^{14}CO_3$ with samples taken from the cultures at three different points in the growth period. On day 4, the control had the greatest ^{14}C uptake and the PCB containing cultures had uptakes per cell and per ml in inverse relationship to their PCB concentration. By days 11 and 15, the ^{14}C uptake per cell were all markedly reduced and showed no dose-related response. These results were undoubtedly influenced by the varying amounts of nutrients still available. The ^{14}C uptake per ml tended to increase during the duration of the experiment, the values generally in inverse relation to the dose.

A possible reason for the apparent recovery of Chlamydomonas from the initial effects of PCB-containing media is that the PCB was degraded or otherwise made less effective during the course of the experiment. Since an experiment conducted by Risebrough (11) indicated that PCB compounds could be broken down by ultraviolet light, the possibility that the fluorescent lights used in growing the algae cultures were causing degradation of the added PCB was investigated. Mason jars of Media 63 containing 2 ppm Aroclor 1242 were placed under the bank of fluorescent lights for 7, 5, 2 or 0 days prior to their inoculation with Chlamydomonas. All of the PCB-containing cultures exhibited delayed algal growth and there did not appear to be any correlation between degree of inhibition and the length of time the media was under the lights after the PCB was added. This seemed to indicate that neither degradation by light nor adsorption of PCB on the glass were significant factors in the delayed growth and subsequent recovery of the Chlamydomonas cultures. Another possibility is that the PCB may have been metabolized either by the algae or by contaminating bacteria. No difference, however, was noted in the chromatograms between contaminated and axenic cultures, nor in degree of algal growth inhibition, hence bacterial degradation of Aroclor 1242 was probably not a significant factor.

Data from the gas chromatography analysis seem to indicate that the Chlamydomonas was concentrating the PCB, for a much higher concentration was found in the

MONS 204209

algal pellet (which took up about 0.05 ml in volume) than in the one liter of supernatant. For example, in cultures to which 20 ppm (mg/l) PCB had been added, 0.52 mg were detected in the algae, and only 0.04 mg in the supernatant. A large percentage of PCB that was added was not recovered from either the media or the algae. This may have been lost because of adherence to the glass sides of the mason jars, sinking of small PCB globules to the bottom, vaporization, codistillation with water, metabolism by the algae, or loss in the extraction, Florisil clean-up, or injection processes.

The effect of DDT on the growth and ^{14}C uptake of Chlamydomonas cultures was studied. In the tested concentration range of 0.2 ppm to 20 ppm DDT, the algal growth curves did not differ appreciably from those of the controls. While both Wurster (13) and Menzel et al. (8) were able to demonstrate that DDT reduced ^{14}C uptake in various species of marine phytoplankton, in the present study no significant effect of DDT on ^{14}C uptake by Chlamydomonas was noted. Mensel et al. (8) did observe that Dunaliella tertiolecta, a green flagellate, did not appear to be affected by concentrations of DDT as high as 1 ppm. It is thus possible that Chlamydomonas, like Dunaliella, is relatively DDT resistant, and yet may be effected by polychlorinated biphenyls which may restrict ^{14}C uptake by a different mechanism.

The toxicity of PCB and DDT to organisms other than algae was then investigated. The growth curves of the protozoan Tetrahymena vorax grown in the presence of Aroclor 1242 (0.02 to 20 ppm) and in the presence of DDT (0.01 to 100 ppm) seem to indicate that neither chemical has any effect upon the growth rate or population size. Aroclor 1242 appeared to be fairly toxic to Daphnia pulex, however. Young Daphnia died at levels as low as 0.02 ppm. Mortality may be linked with other stresses as well, however, for when the Daphnia were supplied with algal culture, the death rate was cut to one-third. A comparison of the relative toxicity of PCB and DDT to ostracods revealed a greater mortality of Cypridopsis vidua at 20 ppm PCB than at the same concentration of DDT. At 20 ppm there were no survivors out of 20 ostracods in the Aroclor-containing cultures while there were 13 survivors in the DDT cultures. At lower concentrations, however, DDT appeared to have more of an effect than

MONS 204210

the PCB. The guppy Poecilia reticulata had 100% mortality at 20 and 2 ppm, but only 25% mortality at 0.2 ppm. The behavior of the fish just prior to death in PCB-containing cultures was quite different from the normal symptoms of hydrocarbon pesticide poisoning. In some cases behavior included rapid pivoting of the body with the nose pressed close to the bottom of the jar; fungus-like growths were also often associated with dying or recently-dead fish.

To explore the passage of PCB through the food chain, Daphnia in PCB-free media were supplied with algal cells that had been grown in Aroclor-containing media, centrifuged, and resuspended in PCB-free media. All of the three-day old Daphnia fed algae cells grown in media containing 20 ppm were dead by the fourth day, and there were only three survivors from among the thirty fed algae from the culture containing 2 ppm. The mortality rate was higher among control Daphnia that were starved than among those that were given PCB-free algae.

Summary

Aroclor 1242 appeared to temporarily inhibit the growth rate of Chlamydomonas as evidenced by cell counts and ^{14}C uptake, while DDT did not appear to have any effect. A greater concentration of PCB was found in the algal cells than in the media, indicating that concentration and passage of the biphenyl compounds through the food chain may take place. Neither Aroclor 1242 nor DDT in the amounts tested appeared to have any effect on the growth or cell density of Tetrahymena vorax cultures. A summary of the results of the toxicity tests on Daphnia, ostracods, and guppies is presented in Table 1. According to the table, Daphnia pulex were quite sensitive to additions of Aroclor 1242 as low as 0.02 ppm. The toxicity of PCB and DDT to the ostracod Cypridopsis vidua appeared to be approximately the same. Young guppies died in concentrations of 2 ppm Aroclor 1242. Aroclor 1242 appears to have a much lower toxicity to cladocerans and guppies than p,p'-DDT. Although the effect of PCB on algal growth appeared to be temporary, in view of its evident capability to be passed through the food chain and its selective

MONS 204211

TABLE 1

Summary of PCB toxicity studies. +++ = many survivors; ++ = most survivors; + few survivors; 0 = no survivors.

Test Situation	Table #	age	dose of PCB (ppm) 0.0002	0.002	0.02	0.2	2	20	observation day
Daphnia									
fed PCB-containing cells*	2	young	---	---	---	+++	+	0	4
direct addition of PCB-alcohol									
non-fed	3	young	+++	+++	++	0	0	0	4
fed	3	young	+++	+++	++	0	0	0	4
PCB-containing algal cultures	4	adult	---	---	---	---	0	0	3
	5	adult	---	+++	+++	++	0	0	2
	5	adult	---	+++	+++	0	0	0	9
Ostracods									
PCB-containing algal cultures	6	adult	---	+++	+++	+++	++	0	3
Guppies									
direct addition of PCB-alcohol	7	young	---	---	---	---	0	0	7
	8	young	---	+++	+++	++	0	0	1

* Daphnia in PCB-free media were fed washed Chlamydomonas cells grown in PCB suspensions of the indicated doses.

What does a dash dash death mean?

MONS 204212

toxicity to certain zooplankton, it would be unwise to attempt to predict its effect on the ecosystem without further study.

Acknowledgements

For assistance with the research and constructive criticism of the manuscript, I wish to thank Dr. Frieda Taub and the members of her laboratory staff. This research was supported by the Environmental Protection Agency, Grant 16050 DXN.

References

1. ANONYMOUS, New Sci., 32, 612 (1966).
2. DUSTMAN, E. and L. STICKEL, Ann. N. Y. Acad. Sci., 160, 162 (1969)
3. FERNELL, W. and G. ROSEN, Brit. J. Nutr., 10, 143 (1956).
4. HOLMES, D., J. SIMMONS, and J. TATTON, Nature, 216, 227 (1967).
5. JENSEN, S., A. JOHNELS, M. OLSSON, and G. OTTERLIND, Nature, 224, 247 (1969)
6. KEIL, J., L. PRIESTER, and S. SANDIFER, Bull. Environ. Contam. Tox., 6, 156 (1971).
7. KOEMAN, J.,M. TEN NOEVER DE BRAUW, and R. DE VOS, Nature, 221, 1126 (1969)
8. MENZEL, D., J. ANDERSON, and A. RANDTKE, Science, 167, 1724 (1970).
9. MORGAN, J. Unpub. Master's thesis, Fish-Ocean. Lib., U. of Wash., Seattle (1971)
10. REYNOLDS, L., Bull. Environ. Contam. Tox., 4, 128 (1969)
11. RISEBROUGH, R., P. REICHE, and H. OLCOTT, Bull. Environ. Contam. Tox., 4, 192 (1969)
12. TAUB, F. and A. DOLLAR, Limnol. Oceanogr., 13, 607 (1968)
13. WURSTER, C., Science, 159. 1474 (1968).
14. ZITKO, V., Bull. Environ. Contam. Tox., 5, 279 (1970).

MONS 204213

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.

MONS 204214

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.

Bad - 8 mo. lab study results in 2×10^5 bio-conc. factor

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.

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 3.5 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 (0.7 ppm) A-1248 in control fish to 1036 µg/gram (ppm) 1254 in fish held for 8 months in water containing 4.6 µg/liter (ppb) A-1254.

(2)

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, Gu 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

MONS 204223

Foster L. Mayer, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Chronic Toxicology of PCBs to Fish

TL50 values (5-30 days) from continuous flow exposures of Aroclors® 1242, 1248, 1254, and 1260 to three species of fish have been determined. Comparative toxicity data for DDT was also determined.

Compound	Species	TL50[1] (ug/l) for 5-30 days					
		5	10	15	20	25	30
DDT	RBT[2]	4.22	1.39	0.51	0.41	0.22	-
	BG[3]	1.18	1.43	1.63	1.27	0.59	0.35
	CCF[4]	>1.0	1.54	2.33	1.32	0.87	0.57
1242	RBT	67	48	18	10	12	-
	BG	125	125	164	125	120	84
	CCF	>100	125	219	150	132	87
1248	RBT	54	38	16	6.4	3.4	-
	BG	136	115	111	106	100	78
	CCF	>100	121	121	115	104	75
1254	RBT	142	160	64	39	27	-
	BG	>200	>200	303	260	239	177
	CCF	>200	303	286	293	181	139
1260	RBT	232	326	143	78	49	51
	BG	>400	>400	>400	>400	>400	>400
	CCF	>400	535	482	512	465	433

[1] temperature, 17°C

[2] Rainbow trout

[3] Bluegills

[4] Channel Catfish

MONS 204224

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND MONITORING
NATIONAL WATER QUALITY LABORATORY

REPLY TO:
6201 Congdon Boulevard
Duluth, Minnesota 55804

30 March 1972

FIELD STATIONS
Newtown, Cincinnati, Ohio
Corvallis, Oregon
Monticello, Minnesota

(Hold for Final Abstracts due)

Dear Newsletter Subscribers:

Attached is the fourth issue of the PCB Newsletter. I have again tried to put your contributions in with as little change as possible. I have placed the news items into rather arbitrary categories, as Analytical Methods, Environmental Occurrence and Fate, Etc., to make finding the subject areas you are interested in more convenient.

Sincerely,

Virginia M. Snarski
Research Aquatic Biologist

Attachment:
PCB Newsletter (fourth issue)

MONS 204225

Exhibit 12

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.

MONS 204226

19a

Exhibit 13

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 204227

195

Exhibit 11

United States Department of the Interior

FISH AND WILDLIFE SERVICE
BUREAU 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 204228

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.

Lennon, R. E., and C. R. Walker. (1964). Laboratories and methods for screening fish-control chemicals. U. S. Bur. Sport Fish. & Wildl. Invest. in Fish Control No. 1 and Circ. 185:1-15.

Litchfield, J. T. and F. Wilcoxon. (1949). A simplified method of evaluating dose-effect experiments. J. Phatmacol. Exper. Therap., 96:99.

Mount, D. I. and W. A. Brungs. (1967). A simplified dosing apparatus for fish toxicology studies. Water Research, I:21-29.

MONS 204229

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 204230

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				
Species	Size (g)	Temp (°C)	pH	Alkal (mg/l)	Hard. (mg/l)	96 hr. LC50	Terminal Value Day	Terminal Value LC50	TI LC10	TI 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 204231

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 t PCBs are bioconcentrated in fish by factors greater than 10^5, that the residu resulting by bioconcentration from less than 0.01 ug/l in water have decrease 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 encounter in numerous other analyses such as chlordane and toxaphene. The need to mea PCBs in large numbers of fish from a bioassay or the Great Lakes where biolo variation is subtracted dictates that the methodology permit the necessary numbers of samples to be analyzed, and the interpretation of the residue dat is based on more detailed analyses of a small number of representative sampl

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-liqu 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 chemica 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 chromatogra (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 incomple 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 = 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 methodol 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 componen 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 estimate 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. Exhib 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 concep

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 2 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.

(3)

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 x 10^4 and 5 x 10^4 respectively (Exhibit 28). Exhibit 27 also reports bioconcentration factors ranging from 2 x 10^4 to 6 x 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 x 10^5 to 2.4 x 10^5. Exhibit 29 and 30 report that goldfish in the lower Milwaukee River accumulated Aroclor 1248 approximately 0.7 x 10^5 to 2 x 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 x 10^5 to 1.5 x 10^5; in good agreement with other measurements.

In summary, for river systems and in laboratory studies many of the aqua organisms studied appear to bioconcentrate PCB mixtures containing 3, 4, 5, a 6 chlorine atoms per molecule approximately 3 x 10^3 to 2 x 10^5 times the conc tration 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 de study presented in Exhibit 15 shows that the PCBs resembling Aroclor 1254 we 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 co

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 1.8 ug/l in 1971 and from 0.01 ug/l to 0.7 ug/l in 1972. Aroclor 1254 was f in the same tributaries at concentrations up to 0.8 ug/l. In an extensive s 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 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 int water of municipalities along the Michigan shore are, with one exception, al 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 approxim 15×10^5.

The PCB residues present in the Lake Michigan biota have adversely affec the value of the biological resources of the lake as a food supply for animal feeds. Exhibit 34 (DDT only) is an early indication that Great Lakes fish were suspecte 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 oxida 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 re duced reproduction and that 1 ug/gm of Aroclor 1254 in the diet caused a slig 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 concentr 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, lo lipid content fish contain up to approximately 1.5 ug/gm (on a wet tissue bas whereas the larter trout contained up to 5.6 ug/gm. Exhibit 39 presents corr borating data for the PCB concentrations in Lake Superior trout obtained by t 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 cont 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 Lak Superior fish is approximately 9×10^5.

In summary, the data from the Great Lakes demonstrate that PCBs are pres 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.

(12)

Chlorodibenzofurans in Aroclor[R] PCB Mixtures

Exhibit 41 presents confirmations tetra-, penta- and hexachlorodibenzofuran 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.

REFERENCE LIST FOR GILMAN D. VEITH

Ex. 1. Mackay, D. and A.W. Wolkoff. Rate of Evaporation of Low-Solubility Contaminants from Water Bodies to Atmosphere. Environmental Science & Technology 7:611-614(1973)

Ex. 2. Ahling, B. and S. Jensen. Reversed Liquid-Liquid Partition in Determination of Polychlorinated Biphenyl (PCB) and Chlorinated Pesticides in Water. Analytical Chemistry 42:1483-1486(1970)

Ex. 3. Gesser, H.D., A. Chow, F.C. Davis, J.F. Uthe and J. Reinke. The Extraction and Recovery of Polychlorinated Biphenyls (PCB) Using Porous Polyurethane Foam. Analytical Letters 4:883-886(1971)

Ex. 4. Jensen, S., L. Renberg, and R. Vaz. Problems in the Quantitation of PCB in Biological Material. PCB Conference II Stockholm 1972

Ex. 5. Armour, J. and J.A. Burke. Method for Separating Polychlorinated Biphenyls from DDT and Its Analogs. Journal of the AOAC 53:761-768 (1970)

Ex. 6. Masumoto, H.T. Study of the Silicic Acid Procedure of Armour and Burke for the Separation of Polychlorinated Biphenyls from DDT and Its Analogs. Journal of the AOAC 55:1092-1100(1972)

Ex. 7. Armour, J.A. and J.A. Burke. Behavior of Chlorinated Naphthalenes in Analytical Methods for Organochlorine Pesticides and Polychlorinated Biphenyls. Journal of the AOAC 54:175-177(1971)

Ex. 8. Stalling, D.L. and J.N. Huckins. Reverse Phase Thin Layer Chromatography of Some Aroclors, Halowaxes, and Pesticides. Journal of the AOAC 56:367-372(1973)

Ex. 9. Armour, J.A. Gas Chromatographic Data for Polychlorinated Biphenyl Components in Six Aroclors. Journal of Chromatography 72:275-282 (1972)

Ex. 10. Sissons, D. and D. Welti. Structural Identification of Polychlorinated Biphenyls in Commercial Mixtures by Gas-Liquid Chromatography, Nuclear Magnetic Resonance and Mass Spectrometry. Journal of Chromatography 60:15-32(1971)

Ex. 11. Webb, R.G. and A.C. McCall. Quantitative PCB Standards for Electron Capture Gas Chromatography. Journal of Chromatographic Science 11:366-373(1973)

Ex. 12. Rote, J.W., and W.J. Morris. Use of Isotopic Abundance Ratios in Identification of Polychlorinated Biphenyls by Mass Spectrometry. Journal of the AOAC 56:188-198(1973)

MONS 204244

Ex. 13. Gregory, N.L. Electron Capture by Chlorinated Biphenyls. Journal of The Chemical Society, Section B:295-300(1968)

Ex. 14. Zitko, V., et al. Retention Times and Electron-Capture Detector Responses of Some Individual Chlorobiphenyls, Bul. Env. Cont. Toxicology 6:160-163(1971).

Ex. 15. Veith, G.D. Chlorinated Hydrocarbons in Fish from Lake Michigan. EPA Project 16020 PBE

Ex. 16. Rote, J.W. and P.G. Murphy. A Method for the Quantitation of Polychlorinated Biphenyl (PCB) Isomers. Bulletin of Environmental Contamination & Toxicology 6:377-384(1971)

Ex. 17. Beezhold, F.L. and V.F. Stout. The Use and Effect of Mixed Standards on the Quantitation of Polychlorinated Biphenyls. Bulletin of Environmental Contamination & Toxicology 10:10-14:23:(1973)

Ex. 18. Armour, J.A. Quantitative Perchlorination of Polychlorinated Biphenyls as a Method for Confirmatory Residue Measurement and Identification. Journal of the AOAC 56:987-993(1973)

Ex. 19. Hutzinger, O, W.D.J. Jamieson, S.S. Safe, and V.Z. Zitko. Exhaustive Chlorination as a Technique in the Analysis of Aromatic Hydrocarbons. Journal of the AOAC 56:982-986(1973)

Ex. 20. Holden, A.V. Monitoring PCB in Water and Wildlife. PCB Conference II Stockholm 1972

Ex. 21. Hamelink, J.L., R.C. Waybrant, and R.C. Ball. A Proposal: Exchange Equilibria Control the Degree Chlorinated Hydrocarbons are Biologically Magnified in Lentic Environments. Transactions of the American Fisheries Society 100:207-214(1971)

Ex. 22. Nebeker, A.V., F.A. Puglisi, and D.L. DeFoe. Effect of Polychlorinsted Biphenyl Compounds on Survival and Reproduction of the Fathead Minnow and Flagfish. Transactions of the American Fisheries Society 103: (3) (July, 1974)

Ex. 23. Snarski, V.M., and F.A. Puglisi. Exposure of Brook Trout to Aroclor 1254. A Summary of NWQL Research Concerning the Effects of Polychlorinated Biphenyl on Water Quality and Aquatic Life. EPA May 197

Ex. 24. Hansen, D.J., P.R. Parrish, J.I. Lowe, A.J. Wilson, Jr., and P.D. Wilson. Chronic Toxicity, Uptake, and Retention of Aroclor 1254 in Two Estuarine Fishes. Bulletin of Environmental Contamination & Toxicology 6:113-119(1971)

Ex. 25. Murphy, P.G. Effects of Salinity on Uptake of DDT, DDE and DDD by Fish. Bulletin of Environmental Contamination & Toxicology 5:404-407(1970)

Ex. 26. Sodergren, A. and Bj. Svensson. Uptake and Accumulation of DDT and PCB by Ephemera Danica (Ephemeroptera) in Continuos-Flow Systems.

Ex. 27. Annual Report Progress in Sport Fishery Research (1970)

Ex. 28. Nebeker, A.V. and F.A. Puglisi. Effect of Polychlorinated Biphenyls (PCB's) on Survival and Reproduction of Daphnia, Gammarus, and Tanytarsus. Transactions of the American Fisheries Society 103: (4) (October, 1974)

Ex. 29. Veith, G.D. and G.F. Lee. Chlorobiphenyls (PCBs) in the Milwaukee River. Water Research 5: 1107-1115(1971)

Ex. 30. Veith, G.D. and G.F. Lee. PCBs in Fish from the Milwaukee Region. Proc. 14th Conf. Great Lakes Res. 1971:157-169 Internat. Assoc. Great Lakes Res.

Ex. 31. Report to: Lake Michigan Toxic Substances Committee May, 1973. Monitoring for Polychlorinated Biphenyls in the Aquatic Environment. Report done by: Michigan Water Resources Commission, Bureau of Water Management, Department of Natural Resources, Environmental Protection Branch. Unpublished.

Ex. 32. PCB's in Lake Michigan. Unpublished.

Ex. 33. Dube, J.D. Polychlorinated Biphenyls in Effluents from Sewage Treatment Plants in Southeastern Wisconsin. Unpublished.

Ex. 34. Aulerich, R.J., R.K. Ringer, H.L. Seagran, and W.G. Youatt. Effects of feeding Coho Salmon and other Great Lakes Fish on Mink Reproduction. Canadian Journal of Zoology 49:611-616(1971)

Ex. 35. Hartsough, G.R. Great Lakes Rish Now Suspect as Mink Food. News of GLMA(Great Lakes Mink Association) pages 25 and 27.

Ex. 36. Ringer, R.K., R.J. Aulerich and M. Zabik. Effect of Dietary Polychlorinated Biphenyls on Growth and Reproduction of Mink. Papers Presented at 164th Nat'l. Meeting American Chemical Society 12:149-154(1972)

Ex. 37. Platonow, N.S. and L.H. Karstad. Dietary Effects of Polychlorinated Biphenyls on Mink.

Ex. 38. Veith, G.D., and G.E. Glass. PCBs and DDT in Fish from Western Lake Superior. Unpublished.

Ex. 39. _____ _____ on Polychlorinated Biphenyls in Michigan Waters. June 1973. Unpublished.

Ex. 40. Puglisi, F.A. Measurements of PCB (Aroclor 1254) in Lake Superior Diluent Water During a Brook Trout Chronic Exposure. Unpublished.

Ex. 41. Bowes. Identification of Chlorinated Dibenzofurans in American Polychlorinated Biphenyls (Aroclor). Unpublished.

MAY 3 19

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

1 "A Polychlorinated Biphenyl (Aroclor 1254) in the Water,
Sumner D-1 Sediment and Biota of Escambia Bay, Florida." T. W. Duke,

MONS 204248

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

Same as D-3 3 " 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

Same as D-26 4 "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.

Same as D-5 5 "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

Same as D-21 6 "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.

(1) Armour, J. A. and Burke, J. A., J. Assoc. Official Anal Chemists, 53, 761 (1970)

Alfred J. Hilton Jr.

MAY 3 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

MONS 204254

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 biologic 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 chromatograp (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 incomplet isolation is observed. Exhibit 6 discusses some of the commonly observed problems in using this group separation technique and specifically points out that DDT 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 mixtures. For example, Exhibit 15 summarizes the concentration of PCB mixtures resembling Aroclor 1254 in Lake Michigan fish. It was observed that [illegible] DDE 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. Exhibit 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 collaborative 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 foun in the same tributaries at concentrations up to 0.8 ug/l. In an extensive stud 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 fo 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 approximat 15×10^5.

The PCB residues present in the Lake Michigan biota have adversely affect 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 oxidat 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 re- duced 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 larger 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® PCB Mixtures

Exhibit 41 presents confirmations tetra-, penta- and hexachlorodibenzofuran in the Aroclor® 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.

STATEMENT OF THOMAS W. DUKE

My name is Thomas W. Duke. I am the director of the Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory at Gulf Breeze, Florida. I have directed research activities at this laboratory since 1968. The mission of the Gulf Breeze Laboratory is to study the effects of toxic organics on marine organisms.

My educational background is as follows: I received my B.S. degree in Zoology from Texas A & M University in 1953, my M.S. degree in Oceanography from Texas A & M University in 1960 and my Ph.D. in Oceanography from Texas A & M University in 1962. A list of my scientific publications is appended to this testimony.

My testimony will be taken from completed and ongoing research, as well as pertinent results of related studies in the literature. My testimony is concerned with the routes, rates and reservoirs of Aroclor® 1254 in Escambia Bay, Florida and with the effects of various Aroclors on marine organisms as determined in the laboratory.

The following exhibits are included in my testimony:

1. Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970. A Polychlorinated Biphenyl (Aroclor® 1254) in the Water, Sediment, and Biota of Escambia Bay, Florida. Bulletin of Environmental Contamination and Toxicology, Volume 5, No. 2, pages 171-180.

2. Nimmo, D. R., D. J. Hansen, J. A. Couch, N. R. Cooley, P. R. Parrish, and J. I. Lowe. 1974. Toxicity of Aroclor® 1254 and Its Physiological Activity in Several Estuarine Organisms. Archives Environmental Contamination and Toxicology. (In press)

3. Nimmo, D. R., P. D. Wilson, R. R. Blackman and A. J. Wilson, Jr. 1971. Polychlorinated Biphenyl Absorbed from Sediments by Fiddler Crabs and Pink Shrimp. Nature, Volume 231, No. 5297, pages 50-52.

4. Wilson, A. J., Jr. 1972. Escambia Bay PCB Survey.- Unpublished data.

5. Nimmo, D. R., R. R. Blackman, A. J. Wilson, Jr. and J. Forester. 1971. Toxicity and Distribution of Aroclor® 1254 in the Pink Shrimp Penaeus duorarum. Marine Biology, Volume 11, No. 3, pages 191-197.

6. Nimmo, D. R., J. Forester, P. T. Heitmuller and G. H. Cook. 1974. Accumulation of Aroclor® 1254 in Grass Shrimp (Palaemonetes pugio) in Laboratory and Field Exposures. Bulletin of Environmental Contamination and Toxicology. (In press)

7. Staff, Gulf Breeze Environmental Research Laboratory. 1969-1974. Aroclor ® 1254 in Escambia Bay System. Unpublished data.

8. Proceedings. 1971. Second Session of Conference in the Matter of Pollution of the Interstate Waters of Escambia River Basin (Alabama-Florida) and the Intrastate Portions of the Escambia Basin and Bay within the State of Florida. Second Session.

MONS 204267

9. Proceedings. 1972. Third Session of Conference in the Matter of Pollution of the Interstate Waters of Escambia River Basin and the Intrastate Portions of the Escambia Basin and Bay within the State of Florida. Third Session.

10. Staff, Gulf Breeze Environmental Research Laboratory. 1969-1974. Aroclor® 1254 in Selected Oysters from Escambia Bay, Florida. Unpublished data.

11. Cooley, N. R., J. M. Keltner, Jr. and J. Forester. 1973. The Polychlorinated Biphenyls, Aroclors® 1248 and 1260: Effect on and Accumulation by Tetrahymena pyriformis. Journal of Protozoology, Volume 20, No. 3, pages 443-445.

12. Lowe, Jack I. 1969. Gulf Breeze Environmental Research Laboratory - Bioassay Screening Test on Aroclor® 1248 and 1260. Unpublished data.

13. Cooley, N. R., Keltner, J. M., Jr. and J. Forester. 1972. Mirex and Aroclor® 1254: Effect on and Accumulation by Tetrahymena pyriformis Strain W. Journal of Protozoology, Volume 19, No. 4, pages 636-638.

14. Wildish, D. J. 1972. Polychlorinated Biphenyls (PCB) in Sea Water and Their Effect on Reproduction of Gammarus oceanicus. Bulletin of Environmental Contamination and Toxicology, Volume 7, No. 2/3, pages 182-187.

15. Wildish, D. J. and V. Zitko. 1971. Uptake of Polychlorinated Biphenyls from Sea Water by Gammarus oceanicus. Marine Biology, Volume 9, No. 3, pages 213-218.

16. Parrish, P. R. 1972. Gulf Breeze Environmental Research Laboratory - Bioassay Screening Test on Aroclors® 1242 and 1254. Unpublished data.

17. Bourquin, A. W. 1974. Growth Responses of Estuarine Bacteria to Mirex, Heptachlor and Polychlorinated Biphenyls. Unpublished manuscript.

18. Kinter, W. B., L. S. Merkens, R. H. Janicki and A. M. Guarino. 1972. Studies on the Mechanism of Toxicity of DDT and Polychlorinated Biphenyls (PCB's): Disruption of Osmoregulation in Marine Fish. Environmental Health Perspectives, Volume 1, pages 169-173.

19. Hansen, D. J., P. R. Parrish and J. Forester. 1974. Aroclor® 1016: Toxicity to and Uptake by Estuarine Animals. Environmental Research. (In press)

20. Staff, Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory. 1974. Toxicity of Aroclors 1260, 1254, 1248, 1242 and 1016 to Salt Water Organisms in 48- and 96-hour Bioassays. Unpublished summary.

21. Lowe, J. I., P. R. Parrish, J. M. Patrick, Jr. and J. Forester. 1972. Effects of the Polychlorinated Biphenyl Aroclor® 1254 on the American Oyster Crassostrea virginica. Marine Biology, Volume 17, No. 3, pages 208-214.

22. Parrish, Patrick R. 1974. Aroclor® 1254, DDT and DDD, and Dieldrin: Accumulation and Loss by American Oysters (Crassostrea virginica) Exposed Continuously for 56 Weeks. Proceedings of National Shellfisheries. (In press)

MONS 204269

23. Couch, John A. 1974. Free and Occluded Virus, Similar to Baculovirus, in Hepatopancreas of Pink Shrimp. Nature, Volume 147, No. 5438, pages 229-231.

24. Nimmo, D. R. and L. H. Bahner. 1973. Physiological Consequences of Polychlorinated Biphenyl and Salinity-stress in Penaeid Shrimp. Symposium on Pollution and the Physiological Ecology of Estuarine and Coastal Water Organisms, November 14-17, 1973, Hobcaw Barony, South Carolina.

25. Wildish, D. J. 1970. The Toxicity of Polychlorinated Biphenyls (PCB) in Sea Water to Grammarus Oceanicus. Bulletin of Environmental Contamination and Toxicology, Volume 5, No. 3, pages 202-204.

26. Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and P. D. Wilson. 1971. Chronic Toxicity, Uptake, and Retention of Aroclor® 1254 in Two Estuarine Fishes. Bulletin of Environmental Contamination and Toxicology, Volume 6, No. 2, pages 113-119.

27. Schimmel, Steven C., David J. Hansen, and Jerrold Forester. 1974. Effects of Aroclor® 1254 on Laboratory-reared Embryos and Fry of Sheepshead Minnows (Cyprinodon variegatus). Transactions of the American Fisheries Society. (In press)

28. Hansen, D. J., S. C. Schimmel and J. Forester. 1974. Aroclor® 1254 in Eggs of Sheepshead Minnows: Effect on Fertilization Success and Survival of Embryos and Fry. Proceedings of the Southeastern Game and Fish Commission. (In press)

29. Johansson, N., S. Jensen and M. Olsson. 1970. PCB - Indications of Effects on Fish. In "PCB Conference, Wenner-Gren Center," September 29, 1970, pages 59-68. National Environmental Protection Board, Stockholm.

30. Hansen, David J. 1974. Aroclor® 1254: Effect on Developing Estuarine Animal Communities in the Laboratory. Contributions to Marine Science. (In press)

31. Hansen, D. J., S. C. Schimmel and J. Forester. 1974. Effects of Aroclor® 1016 on Embryo, Fry, Juvenile and Adult Sheepshead Minnows (Cyprinodon variegatus). Manuscript approved by Gulf Breeze Environmental Research Laboratory.

32. Staff, Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory. 1974. Toxicity of Aroclors 1254 and 1016 to Salt Water Organisms in Flow-through Bioassays Exceeding 96-hour Duration. Unpublished summary.

MONS 204271

ROUTES, RATES AND RESERVOIRS OF AROCLOR® 1254 IN ESCAMBIA BAY, FLORIDA

We detected the polychlorinated biphenyl, Aroclor® 1254, in the water, sediment and biota of Escambia Bay in 1969 (Exhibit 1) and continue to follow the movement and accumulation of this chemical within the bay system. Only one source of the chemical, the Monsanto chemical plant on the Escambia River, has been located at this time. The Aroclor reportedly entered the plant's effluent through accidental leakage of a heat exchange fluid. The leakage was stopped after the company was notified of the appearance of PCBs in the bay system. However, a small amount of this chemical continues to enter the system, possibly from leaching from contaminated sediments into the plant's effluent ditch. Several conclusions have been reached from this study:

(1) Much of the chemical entering the water of Escambia River appeared in particulate form in the water samples

(2) Aroclor® 1254 was distributed from the point of entry in the Escambia River to sediment and biota in the Escambia Bay system by physical or biological transport

(3) Some of the organisms containing residues of Aroclor® 1254 are used as seafood by man

(4) Although the quantity of the Aroclor in the Escambia River was reduced drastically, residues continue

to appear in oysters approximately 8 miles downstream.

Exhibit 1 summarizes the history of the discovery of the chemical in the bay. Exhibit 2, Table 1, gives Aroclor® 1254 concentrations in water, sediment and biota from September 1969 to December 1971 after the leakage was stopped. By comparison, samples of sediments had high concentrations ranging from non-detectable to 30.0 mg/kg (dry weight). In the initial survey (1970) of sediments from the river and bay, concentrations were high at the source of the pollutant (Exhibit 3, Fig. 1; see below the industry) and also at the mouth of the river (Exhibit 3, Fig. 1). Other surveys (1970-1972) show that concentrations of Aroclor® 1254 in sediments from three locations in the river and bay appear to decrease with time (Exhibit 2, Fig. 3). Also, the concentrations in sediment strata appear to decrease with depth. While the number of samples was inadequate for absolute comparison, these data indicate a trend. Data from the latest survey (1972) taken in the channel (Exhibit 4, page 4, see Transect A-middle) indicate that in November, 1972, the sediments above the trestle still had considerable amounts of Aroclor® 1254. Concentrations ranged from 0.16 to 9.0 mg/kg (ppm) (dry weight).

The sediment reservoir of Aroclor® 1254 is probably a source of this chemical to the biota of the bay because residues continued to appear after the amount in the water was reduced drastically. For example, the initial survey of biota

(Exhibit 1, Table 1) showed biota with levels as high as 12.0 mg/kg. In surveys conducted in 1969-70, shrimp collected far from the original source had whole body concentrations as high as 14 mg/kg wet weight (Exhibit 2, Fig. 4). Concentrations of Aroclor found in species collected in Escambia and East Bays are shown in Exhibit 2, Fig. 5. The biota in East Bay are about 35 kilometers from the original source of the chemical, and yet have detectable levels of the chemical. There is also a higher concentration of Aroclor® 1254 in higher trophic levels in both East and Escambia Bays' biota which could implicate a food chain transfer from sediment to large animals.

Three separate studies have been conducted to study the capacity of benthic animals to accumulate Aroclor® 1254 from contaminated sediments. The first of these (Exhibit 3) showed that shrimp placed on various naturally-occurring substrates containing this PCB accumulated the chemical in proportion to the concentration of the chemical in the sediment. The second study showed that penaeid shrimp (Penaeus duorarum), placed on Aroclor® 1254-contaminated sediments, in upper Escambia Bay, accumulated the material (Exhibit 5, page 197). Grass shrimp, Palaemonetes pugio, exposed for 3 months to sediments in the bay accumulated Aroclor® 1254 to about 0.4 mg/kg whole body (Exhibit 6) in the third study.

Additional data on the levels of Aroclor® 1254 in the water of the Escambia Bay system are shown in Exhibit 7. We analyzed both filtered and unfiltered samples of surface and bottom water and monitored water from the new outfall that the industry constructed in 1971. Duplicate samples were also analyzed periodically, and in general, there was good agreement. The water column was fairly homogeneous judging from the consistency of surface to bottom samples. But in filtered samples of water, little Aroclor® 1254 was recoverable. This observation probably means that most of the chemical was attached to suspended particulates in the river water. Other evidence of this is shown in Exhibit 8, Fig. 1; Exhibit 9, Fig. 1, where waters in Escambia Bay show only trace levels (~~0.5~~ 0.03 to 0.05 µg/l), whereas the levels in the sediments are relatively high (Exhibit 8, Fig. 2; Exhibit 9, Fig. 2).

The Aroclor® 1254 concentrations from a new outfall constructed by the industrial plant in 1971 are listed in Exhibit 7. Levels of this PCB continue to be above 0.03 µg/l but well below those found in the initial surveys (1969). Some Aroclor® 1254 is now found in the river above the industry and we assume this is due to the effects of high tides and winds moving surface waters slightly up the river.

We originally discovered Aroclor® 1254 in the bay in oysters from Devil's Point Light and we have continued to

monitor these mollusks (Exhibit 10). The increases in Aroclor® 1254 levels in May to July each year are probably due to the increase in egg mass which could have a high affinity to these chemicals because of a high lipid content. The levels drop shortly after the oysters spawn. This phenomenon also is observed in the laboratory. If the data from Devil's Point Light are analyzed by linear regression analysis it is projected that these mollusks would reach the <0.03 µg/g level by 1985. This prediction assumes that no more chemical is put into the system and that climate, hydrologic or other factors remain the same.

EFFECTS OF AROCLORS ON MARINE ORGANISMS

At the time Aroclor® 1254 was discovered in Escambia Bay, essentially no data existed on the effects of this chemical or other PCBs on marine organisms. Since it is difficult and sometimes impossible to determine toxic effects of specific chemicals in the natural environment because of the presence of other chemicals and fluctuating environmental conditions, we conducted specific toxicity test with Aroclor® 1254 and other Aroclors under controlled conditions in the laboratory. In order to make our bioassay information as meaningful as possible, we conducted most of our tests with indigenous organisms from Escambia Bay or similar areas, and utilized flowing seawater when possible. The toxicity of the test chemicals was determined for specific marine organisms for a given period of time. Detailed test conditions are given in the exhibits.

Residue analyses were conducted where possible to determine if the animals concentrated the chemical from the water in which they were tested. This is an important aspect of these bioassays because Aroclor® 1254 occurred in animals from Escambia Bay that are used by man for food. Obviously, these animals could serve as a vector for the movement of Aroclor® 1254 from site of application to humans. If residues in marine organisms were to exceed levels deemed harmful for human consumption, the fishery resource could not be harvested and would be adversely affected. Consequently, the well-being of commercial fishermen and those with related occupations would be adversely affected.

The following conclusions were reached from laboratory bioassay experiments:

(1) All of the Aroclors tested are acutely toxic for certain estuarine organisms.

(2) Bioassays lasting longer than 96 hours demonstrate that short-term or acute tests underestimate toxicities of some of the Aroclors.

(3) Bioassays lasting over 96 hours indicate that Aroclor® 1254 is toxic to commercially valuable shrimps at concentrations less than 1 µg/l and its toxicity is greatest to young shrimp and to shrimp that are subjected to stress.

(4) Fishes, particularly sheepshead minnows, are extremely sensitive to Aroclor® 1254. Concentrations

of about 0.1 µg/l in water are lethal to fry and affect reproduction of this fish. About 7 µg/g in eggs of the fish is lethal to fry hatching in water free of this chemical.

(5) The acute or short-term toxicity of Aroclor® 1016 to estuarine organisms is similar to other Aroclors but it appears less toxic to fishes in long exposures than does Aroclor® 1254.

BIOASSAYS OF 96 HOURS OR LESS DURATION

Aroclor 1260: Acute bioassays lasting as long as 96 hours show that Aroclor® 1260 is toxic to a wide variety of animals. Growth of the ciliate, Tetrahymena pyriformis, was reduced after 96 hours in growth media containing 1,000 µg/l of Aroclor® 1260 (Exhibit 11). Pinfish were apparently unaffected and 10% of the pink shrimp died in 100 µg/l of the PCB (Exhibit 12) during a 48-hour test. Oysters, however, were sensitive to Aroclor® 1260 with growth diminished by 44% in 10 µg/l and 52% in 100 µg/l in a 96-hour test.

Aroclor 1254: Acute bioassays indicate that Aroclor® 1254 may be more toxic to some estuarine organisms than Aroclor® 1260. Growth rates of the ciliate protozoan, T. pyriformis, were significantly reduced by 96 hours exposure to 1 µg/l (part per billion) of Aroclor® 1254 in 10 ml of growth medium (Exhibit 13). A concentration of 100 µg/l of Aroclor® 1254 in 48-hour flowing water bioassays appeared to have no effect on

juvenile pinfish, Lagodon rhomboides, but killed 100% of the juvenile pink shrimp, Penaeus duorarum. Shell growth of oysters, Crassostrea virginica, exposed for 96 hours was completely inhibited by 100 µg/l and decreased 41% over that of unexposed controls by 10 µg/l of Aroclor® 1254 (Exhibit 13). All four species accumulated Aroclor® 1254 with quantities in oysters, pinfish, and pink shrimp exposed to 10 µg/l in excess of 1 µg/g (part per million). The Aroclor® 1254 is toxic to the amphipod, Gammarus oceanicus, (Exhibit 14). The amphipod also accumulates Aroclor® 1254 from water (Exhibit 15).

Aroclor 1248: The toxicity of Aroclor® 1248 to T. pyriformis (Exhibit 11) is about 1,000 times less than that of Aroclor® 1254. Its toxicity to shrimp, oysters, and pinfish was similar to that of Aroclor® 1254 (Exhibit 12).

Aroclor 1242: Bioassays lasting 96 hours demonstrate that this PCB is toxic to oysters and shrimps at concentrations of 100 µg/l or less (Exhibit 16). This PCB, like the others tested, had no apparent effect on pinfish. Aroclor® 1242 inhibits the growth of certain strains of estuarine bacteria (Exhibit 17).

Aroclor 1221: In static bioassays lasting 96 hours, 80% of the killifish, Fundulus heteroclitus, exposed to 25,000 µg/l of Aroclor® 1221 died (Exhibit 18). All control and 7,500 µg/l exposed fish survived the 96-hour experiment. Lethal concentrations of Aroclor® 1221 did decrease the ability of this killifish to osmoregulate. The osmolarity and Na concentration of the serum was significantly increased in fish exposed

to lethal levels of Aroclor®1221.

Aroclor 1016: Bioassays lasting 96 hours show that Aroclor®1016, like other PCBs, is acutely toxic to many estuarine organisms at concentrations of 100 µg/l or less (Exhibit 19). About 10 µg/l was estimated to be lethal to 50% of the oysters, brown shrimp (Penaeus aztecus) and grass shrimp (Palaemonetes pugio) in flowing water bioassays. Only 18% of the pinfish exposed to 100 µg/l died in the 96-hour exposure. All four of these species accumulated the PCB with the quantities accumulated being related to concentrations in the test water and apparently not to species of animal. Aroclor®1016 inhibits the growth of certain strains of estuarine bacteria (Exhibit 17).

Data on the acute toxicity PCBs tested is summarized in Exhibit 20.

BIOASSAYS EXCEEDING 96 HOURS DURATION

Aroclor 1254:

Mollusca - Young oysters were continuously exposed to Aroclor®1254 for 24 or 30 weeks in flowing, unfiltered sea water (Exhibit 21). Growth rates (height and in water weights) were significantly reduced in oysters exposed for 24 weeks to 5 µg/l (3.9 µg/l measured), but apparently were not affected by 30 weeks exposure to 1 µg/l (0.64 µg/l measured). Survival of oysters was not affected by these concentrations of the chemical. Oysters accumulated as much as 101,000 X the concentration in the test water. Concentration was <0.3 µg/g in 5 µg/l exposed oysters after 28 weeks of depuration in PCB-free

water and $<0.2\mu g/g$ in $1\mu g/l$ exposed oysters after 12 weeks of depuration. Oysters exposed to $5\mu g/l$ for 24 weeks displayed general tissue alterations in the vesicular connective tissue around the digestive diverticula of the hepatopancreas.

In another experiment, oysters were exposed continuously, with no significant mortality, for 56 weeks to $0.01\mu g/l$ of Aroclor® 1254 and showed a concentration factor of 165,000 (Exhibit 22). Maximum concentration of $1.65\mu g/g$ occurred in oysters exposed for eight weeks. Patterns of accumulation and loss were seasonal. Residues decreased 45% to 81% in early July and late October, apparently as a result of spawning, and increased following these periods. This data indicates that life-history of oysters must be known when evaluating residue data from monitoring programs.

Crustacea - blue crabs. Juvenile blue crabs, Callinectes sapidus, exposed for 20 days to $5\mu g/l$ (3.5 to $4.2\mu g/l$ measured) of Aroclor® 1254 were apparently not affected (Exhibit 1). Average concentration in five crabs was $23\mu g/g$. The Aroclor® 1254 was slow to flush from crabs. After one week in PCB-free water concentrations in a second group of five crabs averaged $22\mu g/g$ and after 4 weeks six crabs contained $11\mu g/g$.

Fiddler crabs. Fiddler crabs, Uca pugilator, were exposed to Aroclor® 1254 contaminated sediment from Escambia Bay, Florida to determine if the crabs would accumulate the Aroclor®

1254 from the sediment (Exhibit 3). Fiddler crabs accumulated Aroclor in quantities directly related to the amounts in sediments. The chemical was accumulated by ingestion or by its leaching into the water.

Pink shrimp. Pink shrimp were exposed for 20 days to 5 µg/l of Aroclor® 1254 (3.5 to 4.2 µg/l measured) in flowing unfiltered water (Exhibit 1). Seventy-two percent (18 of 25) died during the exposure, no controls died. The first shrimp died on the tenth day of exposure, and a few died each day during the next 10 days. Several died during molting. Symptoms of insecticide poisoning were not exhibited by dying shrimp.

A series of additional bioassays using pink shrimp showed that Aroclor® 1254 is differentially toxic to juvenile and adult shrimp (Exhibit 5). In flowing water bioassays, a measured concentration of Aroclor of 0.94 µg/l killed 51% of the juvenile shrimp (2.5 to 3.8 cm) within 15 days. Exposure to 3.5 µg/l (measured concentration) for 35 days resulted in a mortality of 50% of a group of (9.5 to 12.5 cm) adult shrimp. Dying shrimp exhibited no apparent outward symptoms of poisoning. Mortality was delayed, they died at the rate of one or two per day and seemed most susceptible during molting. Shrimp obtained the PCB from water and food and concentrated it in the greatest amounts in the hepatopancreas. Shrimp placed in PCB-free water lost about 40% of their total body burden of PCB within 5 weeks. If Aroclor® 1254 caused shrimp mortality in the environment, it would be unlikely that it could be observed because:

(1) shrimp would die over an extended period of time, (2) dying shrimp would decompose rapidly or be eaten by predators, and (3) dead shrimp would not be visible, because unlike fish, they do not float on the surface of the water.

Pathological examinations of exposed pink shrimp demonstrated that, even if there were no outward symptoms of poisoning, there were alterations at the cellular and sub-cellular level. The hepatopancreas of shrimp exposed to Aroclor® 1254 did exhibit dramatic tissue changes which includes the presence of pyramidal crystalloids in the nuclei of epithelial cells (Exhibit 2). Along with the presence of this crystalloid were rod-shaped, free (non-occluded) and occluded virons which appear similar to the Baculovirus group of invertebrate viruses (Exhibit 23). Aroclor® 1254 may facilitate the transmission or enhance the expression of latent viral infections.

Brown shrimp - adult brown shrimp (Penaeus aztecus) were as sensitive to Aroclor® 1254 as adult pink shrimp (Exhibit 24). Three µg/l was lethal to both shrimps within 30 days. Brown shrimp exposed to a sublethal quantity of the Aroclor® 1254 for seven days died if the salinity of the test water decreased gradually in 8 hours from 30‰ to 10‰ and 7‰. Salinity changes such as this occur during tidal cycles. Osmoregulation seemed impaired because concentrations of most major ions in sera of PCB-exposed shrimp became significantly less as the

ambient salinity decreased. This was reflected as a decrease in the sum of the major ions (e.g. Na, Ca, Mg, K, Cu, and Cl) and particularly in the sodium, chloride, and calcium ions.

Grass shrimp - grass shrimp, Palaemonetes pugio, were exposed to measured concentrations of Aroclor® 1254 ranging from 0.17 to 12.5 µg/l for 7 or 16 days (Exhibit 6). A concentration of 4.0 µg/l produced significant mortality but mortality of 1.3 µg/l exposed shrimp was not significantly different from that of control shrimp. Shrimp exposed for seven days to from 0.17 to 9.1 µg/l contained Aroclor in amounts ranging from 3,200 to 11,000 X the measured concentration in the test water. Grass shrimp accumulated the Aroclor® 1254 in ever increasing amounts in exposures lasting as long as 35 days.

Amphipod - the amphipod, Gammarus oceanicus, was exposed to nominal concentrations of Aroclor® 1254 of from 1 to 10,000 µg/l for up to 30 days (Exhibit 25). Aroclor® 1254 was lethal at concentrations as low as 10 µg/l. Some Gammarus had severely necrosed branchiae. Branchial necrosis was found in some animals exposed to 1 µg/l of the Aroclor® 1254.

Fishes - Spot. In three separate flow-through bioassays, 5 µg/l of Aroclor® 1254 (measured concentration with ± 20% of nominal) was lethal to juvenile spot (Leiostomus xanthurus) exposed in flowing water for 20, 26 or 45 days (Exhibit 26). Spot exposed to 1 µg/l showed no apparent effects. Affected

spot usually ceased feeding, became emaciated, and developed ragged fins and lesions on the body. In some instances, fish continued to die even though they were place in PCB-free water. Aroclor® 1254 was accumulated by exposed spot with amounts related to duration and extent of exposure and not apparently to mortality. Spot exposed to 1 µg/l of the Aroclor® 1254 for 56 days rapidly stored this chemical, maximum levels were attained in 14 to 28 days. Maximum amount stored in whole fish was 37,000 times that in the test water. After 84 days in PCB-free water, 39% of the Aroclor® 1254 remained in the fish.

Pinfish - in two separate bioassays lasting 14 or 35 days, 5 µg/l of Aroclor® 1254 was lethal to juvenile pinfish (Exhibit 26). Affected fish usually developed fungus-like lesions on the body, especially around the mouth. Concentration of the Aroclor® 1254 in whole pinfish exposed for 35 days to 5 µg/l was 109 µg/g (21,800 X the amount in the test water).

Sheepshead minnows. Sheepshead minnows are the most sensitive estuarine organism to Aroclor® 1254 that we have tested (Exhibit 27). Intermittent flow bioassays lasting three weeks were conducted with embryo, fry, juvenile and adult fish. Newly hatched fry were the most sensitive life-stage tested. Significant fry mortality occurred in 0.3 µg/l (0.16 µg/l measured) and 0.1 µg/l (0.06 µg/l measured) had no apparent effect. Fry did not die immediately upon hatching but were killed over most of the two weeks following hatching. Embryos

and juveniles of this fish were affected by 10 µg/l (3.5 µg/l measured) but this concentration was not lethal to adults. Many fry, juvenile and adult fish developed fin-rot.

Aroclor® 1254 in eggs from adult sheepshead minnows exposed in bioassays to as little as 0.3 µg/l (0.14 µg/l measured) of the Aroclor® 1254 can decrease survival of embryos and fry that are maintained in water free of this chemical (Exhibit 28). Fertilization success was unimpaired by concentrations in eggs as high as 201 µg/g but survival of embryos and fry was significantly reduced. Usually, fry from eggs containing 7.0 µg/g or more began dying 24-48 hours after hatching. Some preliminary research indicates that PCBs in eggs may decrease fertility and survival in early stages of embryonic development in Atlantic salmon, Salmo salar (Exhibit 29).

Communities of Estuarine Organisms:

Aroclor® 1254 affected the composition of communities of estuarine animals that developed from planktonic larvae in salt water that flowed through 10 control aquaria and 10 aquaria contaminated with 0.1, 1, or 10 µg/l of the Aroclor® 1254 (Exhibit 30). Communities that developed in control aquaria and aquaria that received 0.1 µg/l of PCB in water for four months were dominated (>75%) by arthropods, primarily the amphipod Corophium volutator. In aquaria receiving 1 and 10 µg/l, the number of arthropods decreased and the number of chordates, primarily the tunicate Molgula manhattensis, increased; over 75% of the animals in 10 µg/l aquaria were tunicates. Numbers of phyla, species, and individuals (particularly

amphipods, bryozoans, crabs, and mollusks) were decreased, but there was no apparent effect on the abundance of annelids, brachipods, coelenterates, echinoderms or nemerteans. The Shannon-Weaver index of species diversity was not altered by Aroclor®1254.

Aroclor 1016 - Pinfish. The chronic toxicity and uptake of Aroclor®1016 by pinfish was tested in three separate flow-through bioassays lasting 42 days (Exhibit 19). Mortality of pinfish exposed to 32 µg/l (13 µg/l measured in one experiment and 21 µg/l in the second) or 100 µg/l (59 µg/l measured) was significantly greater than mortalities of unexposed pinfish. Mortality of 10 µg/l (about 7 µg/l measured) exposed fish was negligible. Color of most dying fish darkened, they stopped feeding and they swam erratically with their head inclined downward. Difficulty in swimming progressed until the fish swam upside down just prior to death. Pathological examination of 32 µg/l exposed fish revealed several liver and pancreatic alterations that distinguished them from control fish. Concentrations of Aroclor®1016 stored by the pinfish exposed for 42 days ranged from 11,000 to 24,000 times the nominal concentration in the test water. Concentrations in pinfish exposed to 1 µg/l for 56 days increased for three or four weeks and then stablized. When placed in PCB-free water for 56 days, concentrations (µg/g) in these fish decreased by 51%.

Sheepshead minnows: The chronic toxicity of Aroclor® 1016 to fry, juvenile and adult sheepshead minnows was investigated in intermittent flow bioassays lasting 28 days (Exhibit 31). Fry, juvenile, and adult fish were apparently not affected by nominal concentrations of 0.1, 0.32, 1.0, 3.2 or 10 µg/l but died in 32 and 100 µg/l of this chemical. Chemical analyses of the test water showed about 4 µg/l in the 10 µg/l aquaria, 15 µg/l in 32 µg/l aquaria and 42 µg/l in the aquaria contaminated with 100 µg/l of Aroclor® 1016. Responses to poisoning included darkened body coloration, uncoordinated swimming, cessation of feeding, lesions on the body of many juveniles and adults, and death. Sheepshead minnows accumulated the PCB in proportion to its concentration in the test water. Fry contained 2,500 to 8,100 X the nominal concentration in the test water, adults 4,700 to 14,000 X and juveniles 10,000 to 34,000 X. Eggs from exposed adults were fertilized and placed in PCB-free water. As much as 77 µg/g of the PCB in these eggs apparently did not affect survival of embryos and fry for two weeks.

Information on flow-through bioassays lasting longer than 96 hours is summarized in Exhibit 32.

Published Works:

Duke, Thomas W. 1961. Availability of sediment-sorbed radionuclides to the biota. Ph.D. Dissertation. Texas A & M University.

With Don Hood and Bernadette Stevenson. 1961. Measurement of toxicity of organic wastes to marine organisms. J. Water Pollution Control Federation 9: 982-996.

Duke, Thomas W. 1962. Observations on the movement of radionuclides in selected marine environments. Bull. Ecological Soc. of America 43: 120 (Abstract).

Duke, Thomas W., E. R. Ibert, and K. M. Rae. 1963. Availability of sediment-sorbed material to the biota. In First National Symposium on Radioecology, V. Schultz and W. Klement (eds.), Reinhold Publishing Company, pp. 171-175.

Duke, Thomas W. 1964. Use of radioisotopes in marine biological research. Proceedings of the Second Oak Ridge Radioisotope Conference. U. S. Atomic Energy Commission TID-7689, pp. 69-72.

Duke, Thomas W. 1965. Biogeochemcial cycling of radionuclides in the estuarine environment. Proceedings of the 17th Annual Conference Southeastern Association of Game and Fish Commission, pp. 315-323.

Duke, Thomas W., James N. Willis, and Thomas J. Price, 1965. Cycling of zinc in experimental marine environments. Association of Southeastern Biologists Bulletin 12: 45 (Abstract).

Duke, Thomas W., J. N. Willis, and T. J. Price. 1966. Cycling of trace elements in the estuarine environment. I. Movement and distribution of zinc 65 and stable zinc in experimental ponds. Chesapeake Science 7: 1-10.

Duke, Thomas W., J. P. Baptist, and D. E. Hoss. 1966. Bioaccumulation of radioactive gold as a sediment tracer in the estuarine environment. U.S. Fish and Wildlife Service, Fishery Bulletin 65(2): 427-436.

Duke, Thomas W. 1967. Possible routes of zinc 65 from an estuarine environment to man. J. Water Pollution Control Federation 39(4): 536-542.

Duke, Thomas W., James N. Willis, and Douglas A. Wolfe. 1967. Studies of the exchange of trace elements between estuarine sediments and water. Association of Southeastern Biologists Bulletin 14(2): 27 (Abstract).

MONS 204289

Published works: (continued)

Duke, T. W., J. N. Willis, T. J. Price, and K. Fischler. 1967. Influence of environmental factors on the concentration of Zn^{65} by an experimental community. Proceedings of the Second National Symposium on Radioecology held in Ann Arbor, Michigan, May 15-17, 1967.

Duke, T.W., and T.R. Rice. 1967. Cycling of nutrients in estuaries. Proceedings of the Gulf and Caribbean Fisheries Institute, 19th Annual Session, pp. 59-67.

Duke, Thomas W. 1968. Salt Water Radioecology. Wildlife in North Carolina 32(5): 4-6.

Duke, Thomas W., James N. Willis, and Douglas A. Wolfe. 1968. A technique for studying the exchange of trace elements between estuarine sediments and water. Limnology and Oceanography 13(3): 541-545.

With Kenneth R. Tenore, and Donald B. Horton. 1968. Effects of bottom substrate on the brackish water bivalve Rangia cuneata. Chesapeake Science 9(4): 238-248.

With T.R. Rice. 1969. Radioactivity in the Sea. In Encyclopedia of Marine Resources, Frank Firth (ed.), Van Nostrand Reinhold, pp. 566-569.

With D.W. Hayne, and T.J. Sheets. 1969. Pesticides in Estuaries. In, H.T. Odum, B.J. Copeland and Elizabeth McMahan. Coastal ecological systems of the United States. (A Report to the Federal Water Pollution Control Administration. Institute of Marine Science, University of North Carolina, Morehead City, N.C.).

Duke, Thomas W. 1970. Estuarine Pesticide Research--Bureau of Commercial Fisheries. Proceedings of the Gulf and Caribbean Fisheries Institute, Twnety-second Annual Session, November, 1969, pp. 146-153.

Duke, T.W., J.I. Lowe, and A.J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor® 1254) in the water, sediment, and biota of Escambia Bay, Florida. Bulletin of Environmental Contamination & Toxicology 5(2): 171-180, 1970.

Duke, Thomas W. 1970. Effects of pesticides on estuarine organisms. Marine Pollution Bulletin 1(8): 126, August, 1970.

MONS 204290

Published works: (continued)

With T.R. Rice, J.P. Baptist, and F.A. Cross. 1970. Potential hazards from radioactive pollution of the estuary. Proceedings of the FAO Technical Conference on Marine Pollution and its Effects on the Living Resources and Fishing, Rome, Italy, 9-18 December 1970.

With Ford A. Cross and James N. Willis. 1970. Biogeochemistry of trace elements in a coastal plain estuary: Distribution of manganese, iron, and zinc in sediments, water, and polychaetous worms. Chesapeake Science, 11(4): 221-234.

With J. I. Lowe, P. R. Parrish. A. J. Wilson, Jr., and P. W. Wilson. 1971. Effects of mirex on selected estuarine organisms. Transactions of the 36th North American Wildlife and Natural Resources Conference, March 7-10, 1971, Portland, Oregon, pp. 171-186.

Duke, Thomas W. and A. J. Wilson, Jr. 1971. Chlorinated hydrocarbons in livers of fishes from the Northeastern Pacific Ocean. Pesticides Monitoring Journal 5(2): 228-232.

With D. L. Coppage. 1971. Effects of pesticides in estuaries along the Gulf and Southeast Atlantic Coasts. Proceedings of the 2nd Gulf Coast Conference on Mosquito Suppression and Wildlife Management, New Orleans, Louisiana, October 20-22, 1971, pp. 24-31.

With C. G. Bookhout, A. J. Wilson, Jr., and J. I. Lowe. 1972. Effects of mirex on the larval development of two crabs. Water, Air, and Soil Pollution 1: 165-180.

With Borthwick, P. W., A. J. Wilson, Jr., J. I. Lowe, J. M. Patrick, Jr., and J. C. Oberheu. 1973. Accumulation and movement of mirex in selected estuaries of South Carolina, 1969-71. Pesticides Monitoring Journal, 7(1): 6-26.

MONS 204291

UNITED STATES GOVERNMENT

Memorandum

Received May 17, 1974.

TO : Phocion S. Park

DATE: May 14, 1974

FROM : Alan V. Nebeker

SUBJECT: PCB

Enclosed are portions of my statement on PCB toxicity to freshwater organisms that were misplaced in Washington, D.C.

Sorry for the inconvenience,

Alan V. Nebeker



Buy U.S. Savings Bonds Regularly on the Payroll Savings Plan

MONS 204292

Exhibit 4

(4)

POLYCLORINATED BIPHENYL RESEARCH
a Static Bioassay using Arochlor[R] 1016 and Related Experiments

by Ken Moorman
Bemidji State College
Bemidji, Minnesota

Intern at
National Water Quality Laboratory
Duluth, Minnesota

MONS 204293

1st wk count Nov. 14
2nd wk count Nov. 21
3rd wk count Nov. 23

9101		1			10ppb	2			25ppb	3			50ppb	4			75ppb
	1st wk	5	5	5	5	5	5	5	5	5	6	5	5	5	5	5	5
	2nd wk	4	3	0	4	4	5	5	5	3	4	1	5	5	5	5	5
	3rd wk	12	23	0	6	17	21	22	21	0	21	0	43	0	10	18	10
	T. Adj.	2	3	0	4	3	3	5	5	3	4	1	5	5	5	5	5
	T. Wk	82	72	0	78	60	65	83	90	33	55	35	79	38	67	73	65
	T. Adj.	11				16				13				20			
	T. Y	273				379				268				271			

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

17

Acetone Cont.					
	1st wk	5	5	5	5
	2nd wk	2	4	4	0
	3rd wk	7	0	22	0
	T. Adj.	1	3	4	0
	T. Wk	19	66	77	0
	T. Adj.	8			
	T. Y	194			

1242		9			10ppb	10			25ppb	11			50ppb	12			75ppb
	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
	3rd wk	1	0	0	0	0	0	0	0	0	33	2	0	12	0	0	0
	T. Adj.	4	2	0	0	3	0	0	0	5	5	4	3	4	0	4	3
	T. Wk	38	11	0	0	11	0	17	0	38	66	33	2	61	0	42	2
	T. Adj.	6				4				17				11			
	T. Y	50				28				179				122			

18

Water Cont.					
	1st wk	5	5	5	5
	2nd wk	4	5	5	5
	3rd wk	14	28	14	29
	T. Adj.	4	5	5	5
	T. Wk	84	87	109	85
	T. Adj.	19			
	T. Y	451			

1242		13			100ppb	14			125ppb	15			150ppb	16			200ppb
	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								
	3rd wk	8	0	0	0	0	0	0	0								
	T. Adj.	1	4	5	4	0	2	0	0								
	T. Wk	9	4	13	2	0	0	0	0								
	T. Adj.	1				2				0				0			
	T. Y	36				0				0				0			

Wild Yeast

MONS 204294

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND MONITORING
NATIONAL WATER QUALITY LABORATORY

Exhibit 11

REPLY TO:
6201 Congdon Boulevard
Duluth, Minnesota 55804

FIELD STATIONS:
Newtown, Cincinnati, Ohio
Corvallis, Oregon
Monticello, Minnesota

30 March 1972

(Hebeker Final Abstracts here)

Dear Newsletter Subscribers:

Attached is the fourth issue of the PCB Newsletter. I have again tried to put your contributions in with as little change as possible. I have placed the news items into rather arbitrary categories, as Analytical Methods, Environmental Occurrence and Fate, Etc., to make finding the subject areas you are interested in more convenient.

Sincerely,

Ginny Snarski

Virginia M. Snarski
Research Aquatic Biologist

Attachment:
PCB Newsletter (fourth issue)

MONS 204295

Exhibit 11

19 (11)

Foster L. Mayer, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Chronic Toxicology of PCBs to Fish

TL50 values (5-30 days) from continuous flow exposures of Aroclors® 1242, 1248, 1254, and 1260 to three species of fish have been determined. Comparative toxicity data for DDT was also determined.

Compound	Species	TL50[1] (ug/l) for 5-30 days					
		5	10	15	20	25	30
DDT	RBT[2]	4.22	1.39	0.51	0.41	0.22	-
	BG[3]	1.18	1.43	1.63	1.27	0.59	0.35
	CCF[4]	>1.0	1.54	2.33	1.32	0.87	0.57
1242	RBT	67	48	18	10	12	-
	BG	125	125	164	125	120	84
	CCF	>100	125	219	150	132	87
1248	RBT	54	38	16	6.4	3.4	-
	BG	136	115	111	106	100	78
	CCF	>100	121	121	115	104	75
1254	RBT	142	160	64	39	27	-
	BG	>200	>200	303	260	239	177
	CCF	>200	303	286	293	181	139
1260	RBT	232	326	143	78	49	51
	BG	>400	>400	>400	>400	>400	>400
	CCF	>400	535	482	512	465	433

[1] temperature, 17°C

[2] Rainbow trout

[3] Bluegills

[4] Channel Catfish

MONS 204296

Exhibit 13

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 reasearch 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 reieved 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 204297

195

Exhibit 13

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.

MONS 204298

Exhibit 14

United States Department of the Interior

IN REPLY REFER TO:

FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
Fish-Pesticide Research Laboratory
Route No. 1
Columbia, Missouri 65201

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 204299

197

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.

Lennon, R. E., and C. R. Walker. (1964). Laboratories and methods for screening fish-control chemicals. U. S. Bur. Sport Fish. & Wildl. Inveat. in Fish Control No. 1 and Circ. 185:1-15.

Litchfield, J. T. and F. Wilcoxon. (1949). A simplified method of evaluating dose-effect experiments. J. Phatmacol. Exper. Therap., 96:99.

Mount, D. I. and W. A. Brungs. (1967). A simplified dosing apparatus for fish toxicology studies. Water Research, I:21-29.

MONS 204300

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[1/] eggs	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.

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				
							Terminal Value		TI	TI
Species	Size (g)	Temp (°C)	pH	Alkal (mg/l)	Hard. (mg/l)	96 hr. 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 204302

STATEMENT OF THOMAS W. DUKE

My name is Thomas W. Duke. I am the director of the Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory at Gulf Breeze, Florida. I have directed research activities at this laboratory since 1968. The mission of the Gulf Breeze Laboratory is to study the effects of toxic organics on marine organisms.

My educational background is as follows: I received my B.S. degree in Zoology from Texas A & M University in 1953, my M.S. degree in Oceanography from Texas A & M University in 1960 and my Ph.D. in Oceanography from Texas A & M University in 1962. A list of my scientific publications is appended to this testimony.

My testimony will be taken from completed and ongoing research, as well as pertinent results of related studies in the literature. My testimony is concerned with the routes, rates and reservoirs of Aroclor® 1254 in Escambia Bay, Florida and with the effects of various Aroclors on marine organisms as determined in the laboratory.

The following exhibits are included in my testimony:

1. Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970. A Polychlorinated Biphenyl (Aroclor® 1254) in the Water, Sediment, and Biota of Escambia Bay, Florida. Bulletin of Environmental Contamination and Toxicology, Volume 5, No. 2, pages 171-180.

2. Nimmo, D. R., D. J. Hansen, J. A. Couch, N. R. Cooley, P. R. Parrish, and J. I. Lowe. 1974. Toxicity of Aroclor® 1254 and Its Physiological Activity in Several Estuarine Organisms. Archives Environmental Contamination and Toxicology. (In press)

3. Nimmo, D. R., P. D. Wilson, R. R. Blackman and A. J. Wilson, Jr. 1971. Polychlorinated Biphenyl Absorbed from Sediments by Fiddler Crabs and Pink Shrimp. Nature, Volume 231, No. 5297, pages 50-52.

4. Wilson, A. J., Jr. 1972. Escambia Bay PCB Survey. Unpublished data.

5. Nimmo, D. R., R. R. Blackman, A. J. Wilson, Jr. and J. Forester. 1971. Toxicity and Distribution of Aroclor® 1254 in the Pink Shrimp Penaeus duorarum. Marine Biology, Volume 11, No. 3, pages 191-197.

6. Nimmo, D. R., J. Forester, P. T. Heitmuller and G. H. Cook. 1974. Accumulation of Aroclor® 1254 in Grass Shrimp (Palaemonetes pugio) in Laboratory and Field Exposures. Bulletin of Environmental Contamination and Toxicology. (In press)

7. Staff, Gulf Breeze Environmental Research Laboratory. 1969-1974. Aroclor® 1254 in Escambia Bay System. Unpublished data.

8. Proceedings. 1971. Second Session of Conference in the Matter of Pollution of the Interstate Waters of Escambia River Basin (Alabama-Florida) and the Intrastate Portions of the Escambia Basin and Bay within the State of Florida. Second Session.

MONS 204304

9. Proceedings. 1972. Third Session of Conference in the Matter of Pollution of the Interstate Waters of Escambia River Basin and the Intrastate Portions of the Escambia Basin and Bay within the State of Florida. Third Session.

10. Staff, Gulf Breeze Environmental Research Laboratory. 1969-1974. Aroclor®1254 in Selected Oysters from Escambia Bay, Florida. Unpublished data.

11. Cooley, N. R., J. M. Keltner, Jr. and J. Forester. 1973. The Polychlorinated Biphenyls, Aroclors®1248 and 1260: Effect on and Accumulation by Tetrahymena pyriformis. Journal of Protozoology, Volume 20, No. 3, pages 443-445.

12. Lowe, Jack I. 1969. Gulf Breeze Environmental Research Laboratory - Bioassay Screening Test on Aroclor® 1248 and 1260. Unpublished data.

13. Cooley, N. R., Keltner, J. M., Jr. and J. Forester. 1972. Mirex and Aroclor®1254: Effect on and Accumulation by Tetrahymena pyriformis Strain W. Journal of Protozoology, Volume 19, No. 4, pages 636-638.

14. Wildish, D. J. 1972. Polychlorinated Biphenyls (PCB) in Sea Water and Their Effect on Reproduction of Gammarus oceanicus. Bulletin of Environmental Contamination and Toxicology, Volume 7, No. 2/3, pages 182-187.

15. Wildish, D. J. and V. Zitko. 1971. Uptake of Polychlorinated Biphenyls from Sea Water by Gammarus oceanicus. Marine Biology, Volume 9, No. 3, pages 213-218.

MONS 204305

16. Parrish, P. R. 1972. Gulf Breeze Environmental Research Laboratory - Bioassay Screening Test on Aroclors® 1242 and 1254. Unpublished data.

17. Bourquin, A. W. 1974. Growth Responses of Estuarine Bacteria to Mirex, Heptachlor and Polychlorinated Biphenyls. Unpublished manuscript.

18. Kinter, W. B., L. S. Merkens, R. H. Janicki and A. M. Guarino. 1972. Studies on the Mechanism of Toxicity of DDT and Polychlorinated Biphenyls (PCB's): Disruption of Osmoregulation in Marine Fish. Environmental Health Perspectives, Volume 1, pages 169-173.

19. Hansen, D. J., P. R. Parrish and J. Forester. 1974. Aroclor®1016: Toxicity to and Uptake by Estuarine Animals. Environmental Research. (In press)

20. Staff, Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory. 1974. Toxicity of Aroclors 1260, 1254, 1248, 1242 and 1016 to Salt Water Organisms in 48- and 96-hour Bioassays. Unpublished summary.

21. Lowe, J. I., P. R. Parrish, J. M. Patrick, Jr. and J. Forester. 1972. Effects of the Polychlorinated Biphenyl Aroclor®1254 on the American Oyster Crassostrea virginica. Marine Biology, Volume 17, No. 3, pages 208-214.

22. Parrish, Patrick R. 1974. Aroclor®1254, DDT and DDD, and Dieldrin: Accumulation and Loss by American Oysters (Crassostrea virginica) Exposed Continuously for 56 Weeks. Proceedings of National Shellfisheries. (In press)

23. Couch, John A. 1974. Free and Occluded Virus, Similar to Baculovirus, in Hepatopancreas of Pink Shrimp. Nature, Volume 147, No. 5438, pages 229-231.

24. Nimmo, D. R. and L. H. Bahner. 1973. Physiological Consequences of Polychlorinated Biphenyl and Salinity-stress in Penaeid Shrimp. Symposium on Pollution and the Physiological Ecology of Estuarine and Coastal Water Organisms, November 14-17, 1973, Hobcaw Barony, South Carolina.

25. Wildish, D. J. 1970. The Toxicity of Polychlorinated Biphenyls (PCB) in Sea Water to Grammarus Oceanicus. Bulletin of Environmental Contamination and Toxicology, Volume 5, No. 3, pages 202-204.

26. Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and P. D. Wilson. 1971. Chronic Toxicity, Uptake, and Retention of Aroclor® 1254 in Two Estuarine Fishes. Bulletin of Environmental Contamination and Toxicology, Volume 6, No. 2, pages 113-119.

27. Schimmel, Steven C., David J. Hansen, and Jerrold Forester. 1974. Effects of Aroclor® 1254 on Laboratory-reared Embryos and Fry of Sheepshead Minnows (Cyprinodon variegatus). Transactions of the American Fisheries Society. (In press)

28. Hansen, D. J., S. C. Schimmel and J. Forester. 1974. Aroclor® 1254 in Eggs of Sheepshead Minnows: Effect on Fertilization Success and Survival of Embryos and Fry. Proceedings of the Southeastern Game and Fish Commission. (In press)

29. Johansson, N., S. Jensen and M. Olsson. 1970. PCB - Indications of Effects on Fish. In "PCB Conference, Wenner-Gren Center," September 29, 1970, pages 59-68. National Environmental Protection Board, Stockholm.

30. Hansen, David J. 1974. Aroclor® 1254: Effect on Developing Estuarine Animal Communities in the Laboratory. Contributions to Marine Science. (In press)

31. Hansen, D. J., S. C. Schimmel and J. Forester. 1974. Effects of Aroclor® 1016 on Embryo, Fry, Juvenile and Adult Sheepshead Minnows (Cyprinodon variegatus). Manuscript approved by Gulf Breeze Environmental Research Laboratory.

32. Staff, Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory. 1974. Toxicity of Aroclors 1254 and 1016 to Salt Water Organisms in Flow-through Bioassays Exceeding 96-hour Duration. Unpublished summary.

ROUTES, RATES AND RESERVOIRS OF AROCLOR® 1254 IN ESCAMBIA BAY, FLORIDA

We detected the polychlorinated biphenyl, Aroclor® 1254, in the water, sediment and biota of Escambia Bay in 1969 (Exhibit 1) and continue to follow the movement and accumulation of this chemical within the bay system. Only one source of the chemical, the Monsanto chemical plant on the Escambia River, has been located at this time. The Aroclor reportedly entered the plant's effluent through accidental leakage of a heat exchange fluid. The leakage was stopped after the company was notified of the appearance of PCBs in the bay system. However, a small amount of this chemical continues to enter the system, possibly from leaching from contaminated sediments into the plant's effluent ditch. Several conclusions have been reached from this study:

(1) Much of the chemical entering the water of Escambia River appeared in particulate form in the water samples

(2) Aroclor® 1254 was distributed from the point of entry in the Escambia River to sediment and biota in the Escambia Bay system by physical or biological transport

(3) Some of the organisms containing residues of Aroclor® 1254 are used as seafood by man

(4) Although the quantity of the Aroclor in the Escambia River was reduced drastically, residues continue

to appear in oysters approximately 8 miles downstream.

Exhibit 1 summarizes the history of the discovery of the chemical in the bay. Exhibit 2, Table 1, gives Aroclor® 1254 concentrations in water, sediment and biota from September 1969 to December 1971 after the leakage was stopped. By comparison, samples of sediments had high concentrations ranging from non-detectable to 30.0 mg/kg (dry weight). In the initial survey (1970) of sediments from the river and bay, concentrations were high at the source of the pollutant (Exhibit 3, Fig. 1; see below the industry) and also at the mouth of the river (Exhibit 3, Fig. 1). Other surveys (1970-1972) show that concentrations of Aroclor® 1254 in sediments from three locations in the river and bay appear to decrease with time (Exhibit 2, Fig. 3). Also, the concentrations in sediment strata appear to decrease with depth. While the number of samples was inadequate for absolute comparison, these data indicate a trend. Data from the latest survey (1972) taken in the channel (Exhibit 4, page 4, see Transect A-middle) indicate that in November, 1972, the sediments above the trestle still had considerable amounts of Aroclor® 1254. Concentrations ranged from 0.16 to 9.0 mg/kg (dry.weight).

The sediment reservoir of Aroclor® 1254 is probably a source of this chemical to the biota of the bay because residues continued to appear after the amount in the water was reduced drastically. For example, the initial survey of biota

(Exhibit 1, Table 1.) showed biota with levels as high as 12.0 mg/kg. In surveys conducted in 1969-70, shrimp collected far from the original source had whole body concentrations as high as 14 mg/kg wet weight (Exhibit 2, Fig. 4). Concentrations of Aroclor found in species collected in Escambia and East Bays are shown in Exhibit 2, Fig. 5. The biota in East Bay are about 55 kilometers from the original source of the chemical, and yet have detectable levels of the chemical. There is also a higher concentration of Aroclor®1254 in higher trophic levels in both East and Escambia Bays' biota which could implicate a food chain transfer from sediment to large animals.

Three separate studies have been conducted to study the capacity of benthic animals to accumulate Aroclor® 1254 from contaminated sediments. The first of these (Exhibit 3) showed that shrimp placed on various naturally-occurring substrates containing this PCB accumulated the chemical in proportion to the concentration of the chemical in the sediment. The second study showed that penaeid shrimp (Penaeus duorarum), placed on Aroclor®1254-contaminated sediments, in upper Escambia Bay, accumulated the material (Exhibit 5, page 197). Grass shrimp, Palaemonetes pugio, exposed for 3 months to sediments in the bay accumulated Aroclor®1254 to about 0.4 mg/kg whole body (Exhibit 6) in the third study.

Additional data on the levels of Aroclor® 1254 in the water of the Escambia Bay system are shown in Exhibit 7. We analyzed both filtered and unfiltered samples of surface and bottom water and monitored water from the new outfall that the industry constructed in 1971. Duplicate samples were also analyzed periodically, and in general, there was good agreement. The water column was fairly homogeneous judging from the consistency of surface to bottom samples. But in filtered samples of water, little Aroclor® 1254 was recoverable. This observation probably means that most of the chemical was attached to suspended particulates in the river water. Other evidence of this is shown in Exhibit 8, Fig. 1; Exhibit 9, Fig. 1, where waters in Escambia Bay show only trace levels (0.3 to 0.05 µg/l), whereas the levels in the sediments are relatively high (Exhibit 8, Fig. 2; Exhibit 9, Fig. 2).

The Aroclor® 1254 concentrations from a new outfall constructed by the industrial plant in 1971 are listed in Exhibit 7. Levels of this PCB continue to be above 0.03 µg/l but well below those found in the initial surveys (1969). Some Aroclor® 1254 is now found in the river above the industry and we assume this is due to the effects of high tides and winds moving surface waters slightly up the river.

We originally discovered Aroclor® 1254 in the bay in oysters from Devil's Point Light and we have continued to

monitor these mollusks (Exhibit 10). The increases in Aroclor® 1254 levels in May to July each year are probably due to the increase in egg mass which could have a high affinity to these chemicals because of a high lipid content. The levels drop shortly after the oysters spawn. This phenomenon also is observed in the laboratory. If the data from Devil's Point Light are analyzed by linear regression analysis it is projected that these mollusks would reach the <0.03 µg/l level by 1985. This prediction assumes that no more chemical is put into the system and that climate, hydrologic or other factors remain the same.

EFFECTS OF AROCLORS ON MARINE ORGANISMS

At the time Aroclor® 1254 was discovered in Escambia Bay, essentially no data existed on the effects of this chemical or other PCBs on marine organisms. Since it is difficult and sometimes impossible to determine toxic effects of specific chemicals in the natural environment because of the presence of other chemicals and fluctuating environmental conditions, we conducted specific toxicity test with Aroclor® 1254 and other Aroclors under controlled conditions in the laboratory. In order to make our bioassay information as meaningful as possible, we conducted most of our tests with indigenous organisms from Escambia Bay or similar areas, and utilized flowing seawater when possible. The toxicity of the test chemicals was determined for specific marine organisms for a given period of time. Detailed test conditions are given in the exhibits.

Residue analyses were conducted where possible to determine if the animals concentrated the chemical from the water in which they were tested. This is an important aspect of these bioassays because Aroclor® 1254 occurred in animals from Escambia Bay that are used by man for food. Obviously, these animals could serve as a vector for the movement of Aroclor® 1254 from site of application to humans. If residues in marine organisms were to exceed levels deemed harmful for human consumption, the fishery resource could not be harvested and would be adversely affected. Consequently, the well-being of commercial fishermen and those with related occupations would be adversely affected.

The following conclusions were reached from laboratory bioassay experiments:

(1) All of the Aroclors tested are acutely toxic for certain estuarine organisms.

(2) Bioassays lasting longer than 96 hours demonstrate that short-term or acute tests underestimate toxicities of some of the Aroclors.

(3) Bioassays lasting over 96 hours indicate that Aroclor® 1254 is toxic to commercially valuable shrimps at concentrations less than 1 µg/l and its toxicity is greatest to young shrimp and to shrimp that are subjected to stress.

(4) Fishes, particularly sheepshead minnows, are extremely sensitive to Aroclor® 1254. Concentrations

of about 0.1 µg/l in water are lethal to fry and affect reproduction of this fish. About 1 µg/g in eggs of the fish is lethal to fry hatching in water free of this chemical.

(5) The acute or short-term toxicity of Aroclor® 1016 to estuarine organisms is similar to other Aroclors but it appears less toxic to fishes in long exposures than does Aroclor® 1254.

BIOASSAYS OF 96 HOURS OR LESS DURATION

Aroclor 1260: Acute bioassays lasting as long as 96 hours show that Aroclor® 1260 is toxic to a wide variety of animals. Growth of the ciliate, Tetrahymena pyriformis, was reduced after 96 hours in growth media containing 1,000 µg/l of Aroclor® 1260 (Exhibit 11). Pinfish were apparently unaffected and 10% of the pink shrimp died in 100 µg/l of the PCB (Exhibit 12) during a 48-hour test. Oysters, however, were sensitive to Aroclor® 1260 with growth diminished by 44% in 10 µg/l and 52% in 100 µg/l in a 96-hour test.

Aroclor 1254: Acute bioassays indicate that Aroclor® 1254 may be more toxic to some estuarine organisms than Aroclor® 1260. Growth rates of the ciliate protozoan, T. pyriformis, were significantly reduced by 96 hours exposure to 1 µg/l (part per billion) of Aroclor® 1254 in 10 ml of growth medium (Exhibit 13). A concentration of 100 µg/l of Aroclor® 1254 in 48-hour flowing water bioassays appeared to have no effect on

juvenile pinfish, Lagodon rhomboides, but killed 100% of the juvenile pink shrimp, Penaeus duorarum. Shell growth of oysters, Crassostrea virginica, exposed for 96 hours was completely inhibited by 100 µg/l and decreased 41% over that of unexposed controls by 10 µg/l of Aroclor® 1254 (Exhibit 11). All four species accumulated Aroclor® 1254 with quantities in oysters, pinfish, and pink shrimp exposed to 10 µg/l in excess of 1 µg/g (part per million). The Aroclor® 1254 is toxic to the amphipod, Gammarus oceanicus, (Exhibit 14). The amphipod also accumulates Aroclor® 1254 from water (Exhibit 15).

Aroclor 1248: The toxicity of Aroclor® 1248 to T. pyriformis (Exhibit 2) is about 1,000 times less than that of Aroclor® 1254. Its toxicity to shrimp, oysters, and pinfish was similar to that of Aroclor® 1254 (Exhibit 12).

Aroclor 1242: Bioassays lasting 96 hours demonstrate that this PCB is toxic to oysters and shrimps at concentrations of 100 µg/l or less (Exhibit 16). This PCB, like the others tested, had no apparent effect on pinfish. Aroclor® 1242 inhibits the growth of certain strains of estuarine bacteria (Exhibit 17).

Aroclor 1221: In static bioassays lasting 96 hours, 80% of the killifish, Fundulus heteroclitus, exposed to 25,000 µg/l of Aroclor® 1221 died (Exhibit 18). All control and 7,500 µg/l exposed fish survived the 96-hour experiment. Lethal concentrations of Aroclor® 1221 did decrease the ability of this killifish to osmoregulate. The osmolarity and Na concentration of the serum was consistently increased in fish exposed

to lethal levels of Aroclor®1221.

Aroclor 1016: Bioassays lasting 96 hours show that Aroclor®1016, like other PCBs, is acutely toxic to many estuarine organisms at concentrations of 100 µg/l or less (Exhibit 19). About 10 µg/l was estimated to be lethal to 50% of the oysters, brown shrimp (Penaeus aztecus) and grass shrimp (Palaemonetes pugio) in flowing water bioassays. Only 18% of the pinfish exposed to 100 µg/l died in the 96-hour exposure. All four of these species accumulated the PCB with the quantities accumulated being related to concentrations in the test water and apparently not to species of animal. Aroclor®1016 inhibits the growth of certain strains of estuarine bacteria (Exhibit 17).

Data on the acute toxicity PCBs tested is summarized in Exhibit 20.

BIOASSAYS EXCEEDING 96 HOURS DURATION

Aroclor 1254:

Mollusca - Young oysters were continuously exposed to Aroclor®1254 for 24 or 30 weeks in flowing, unfiltered sea water (Exhibit 21). Growth rates (height and in water weights) were significantly reduced in oysters exposed for 24 weeks to 5 µg/l (3.9 µg/l measured), but apparently were not affected by 30 weeks exposure to 1 µg/l (0.64 µg/l measured). Survival of oysters was not affected by these concentrations of the chemical. Oysters accumulated as much as 101,000 X the concentration in the test water. Concentration was <0.3 µg/g in 5 µg/l exposed oysters after 28 weeks of depuration in PCB-free

water and $< 0.2 \mu g/g$ in $1 \mu g/l$ exposed oysters after 12 weeks of depuration. Oysters exposed to $5 \mu g/l$ for 24 weeks displayed general tissue alterations in the vesicular connective tissue around the digestive diverticula of the hepatopancreas.

In another experiment, oysters were exposed continuously, with no significant mortality, for 56 weeks to $0.01 \mu g/l$ of Aroclor® 1254 and showed a concentration factor of 165,000 (Exhibit 22). Maximum concentration of $1.65 \mu g/g$ occurred in oysters exposed for eight weeks. Patterns of accumulation and loss were seasonal. Residues decreased 45% to 81% in early July and late October, apparently as a result of spawning, and increased following these periods. This data indicates that life-history of oysters must be known when evaluating residue data from monitoring programs.

Crustacea - blue crabs. Juvenile blue crabs, Callinectes sapidus, exposed for 20 days to $5 \mu g/l$ (3.5 to $4.2 \mu g/l$ measured) of Aroclor® 1254 were apparently not affected (Exhibit 1). Average concentration in five crabs was $23 \mu g/g$. The Aroclor® 1254 was slow to flush from crabs. After one week in PCB-free water concentrations in a second group of five crabs averaged $22 \mu g/g$ and after 4 weeks six crabs contained $11 \mu g/g$.

Fiddler crabs. Fiddler crabs, Uca pugilator, were exposed to Aroclor® 1254 contaminated sediment from Escambia Bay, Florida to determine if the crabs would accumulate the Aroclor®

1254 from the sediment (Exhibit 3). Fiddler crabs accumulated Aroclor in quantities directly related to the amounts in sediments. The chemical was accumulated by ingestion or by its leaching into the water.

Pink shrimp. - Pink shrimp were exposed for 20 days to 5µg/l of Aroclor®1254 (3.5 to 4.2µg/l measured) in flowing unfiltered water (Exhibit 1). Seventy-two percent (18 of 25) died during the exposure, no controls died. The first shrimp died on the tenth day of exposure, and a few died each day during the next 10 days. Several died during molting. Symptoms of insecticide poisoning were not exhibited by dying shrimp.

A series of additional bioassays using pink shrimp showed that Aroclor®1254 is differentially toxic to juvenile and adult shrimp (Exhibit 5). In flowing water bioassays, a measured concentration of Aroclor of 0.94µg/l killed 51% of the juvenile shrimp (2.5 to 3.8 cm) within 15 days. Exposure to 3.5µg/l (measured concentration) for 35 days resulted in a mortality of 50% of a group of (9.5 to 12.5 cm) adult shrimp. Dying shrimp exhibited no apparent outward symptoms of poisoning. Mortality was delayed, they died at the rate of one or two per day and seemed most susceptible during molting. Shrimp obtained the PCB from water and food and concentrated it in the greatest amounts in the hepatopancreas. Shrimp placed in PCB-free water lost about 40% of their total body burden of PCB within 5 weeks. If Aroclor®1254 caused shrimp mortality in the environment, it would be unlikely that it could be observed because:

(1) shrimp would die over an extended period of time, (2) dying shrimp would decompose rapidly or be eaten by predators, and (3) dead shrimp would not be visible, because unlike fish, they do not float on the surface of the water.

Pathological examinations of exposed pink shrimp demonstrated that, even if there were no outward symptoms of poisoning, there were alterations at the cellular and sub-cellular level. The hepatopancreas of shrimp exposed to Aroclor® 1254 did exhibit dramatic tissue changes which includes the presence of pyramidal crystalloids in the nuclei of epithelial cells (Exhibit 2). Along with the presence of this crystalloid were rod-shaped, free (non-occluded) and occluded virons which appear similar to the Baculovirus group of invertebrate viruses (Exhibit 17). Aroclor® 1254 may facilitate the transmission or enhance the expression of latent viral infections.

Brown shrimp - adult brown shrimp (Penaeus aztecus) were as sensitive to Aroclor® 1254 as adult pink shrimp (Exhibit 24). Three µg/l was lethal to both shrimps within 30 days. Brown shrimp exposed to a sublethal quantity of the Aroclor® 1254 for seven days died if the salinity of the test water decreased gradually in 8 hours from 30‰ to 10‰ and 7‰. Salinity changes such as this occur during tidal cycles. Osmoregulation seemed impaired because concentrations of most major ions in sera of PCB-exposed shrimp became significantly less as the

ambient salinity decreased. This was reflected as a decrease in the sum of the major ions (e.g. Na, Ca, Mg, K, Cu, and Cl) and particularly in the sodium, chloride, and calcium ions.

Grass shrimp - grass shrimp, Palaemonetes pugio, were exposed to measured concentrations of Aroclor® 1254 ranging from 0.17 to 12.5 µg/l for 7 or 16 days (Exhibit 6). A concentration of 4.0 µg/l produced significant mortality but mortality of 1.3 µg/l exposed shrimp was not significantly different from that of control shrimp. Shrimp exposed for seven days to from 0.17 to 9.1 µg/l contained Aroclor in amounts ranging from 3,200 to 11,000 X the measured concentration in the test water. Grass shrimp accumulated the Aroclor® 1254 in ever increasing amounts in exposures lasting as long as 35 days.

Amphipod - the amphipod, Gammarus oceanicus, was exposed to nominal concentrations of Aroclor® 1254 of from 1 to 10,000 µg/l for up to 30 days (Exhibit 25). Aroclor® 1254 was lethal at concentrations as low as 10 µg/l. Some Gammarus had severely necrosed branchiae. Branchial necrosis was found in some animals exposed to 1 µg/l of the Aroclor® 1254.

Fishes - Spot. In three separate flow-through bioassays, 5 g/l of Aroclor® 1254 (measured concentration with ± 20% of nominal) was lethal to juvenile spot (Leiostomus xanthurus) exposed in flowing water for 20, 26 or 45 days (Exhibit 26). Spot exposed to 1 µg/l showed no apparent effects. Affected

spot usually ceased feeding, became emaciated, and developed ragged fins and lesions on the body. In some instances, fish continued to die even though they were place in PCB-free water. Aroclor® 1254 was accumulated by exposed spot with amounts related to duration and extent of exposure and not apparently to mortality. Spot exposed to 1 µg/l of the Aroclor® 1254 for 56 days rapidly stored this chemical, maximum levels were attained in 14 to 28 days. Maximum amount stored in whole fish was 37,000 times that in the test water. After 84 days in PCB-free water, 39% of the Aroclor® 1254 remained in the fish.

Pinfish - in two separate bioassays lasting 14 or 35 days, 5 µg/l of Aroclor® 1254 was lethal to juvenile pinfish (Exhibit 26). Affected fish usually developed fungus-like lesions on the body, especially around the mouth. Concentration of the Aroclor® 1254 in whole pinfish exposed for 35 days to 5 µg/l was 109 µg/g (21,800 X the amount in the test water).

Sheepshead minnows. Sheepshead minnows are the most sensitive estuarine organism to Aroclor® 1254 that we have tested (Exhibit 27). Intermittent flow bioassays lasting three weeks were conducted with embryo, fry, juvenile and adult fish. Newly hatched fry were the most sensitive life-stage tested. Significant fry mortality occurred in 0.3 µg/l (0.16 µg/l measured) and 0.1 µg/l (0.06 µg/l measured) had no apparent effect. Fry did not die immediately upon hatching but were killed over most of the two weeks following hatching. Embryos

and juveniles of this fish were affected by 10 µg/l (3.5 µg/l measured) but this concentration was not lethal to adults. Many fry, juvenile and adult fish developed fin-rot.

Aroclor® 1254 in eggs from adult sheepshead minnows exposed in bioassays to as little as 0.3 µg/l (0.14 µg/l measured) of the Aroclor® 1254 can decrease survival of embryos and fry that are maintained in water free of this chemical (Exhibit 28). Fertilization success was unimpaired by concentrations in eggs as high as 201 µg/g but survival of embryos and fry was significantly reduced. Usually, fry from eggs containing 7.0 µg/g or more began dying 24-48 hours after hatching. Some preliminary research indicates that PCBs in eggs may decrease fertility and survival in early stages of embryonic development in Atlantic salmon, Salmo salar (Exhibit 29).

Communities of Estuarine Organisms:

Aroclor® 1254 affected the composition of communities of estuarine animals that developed from planktonic larvae in salt water that flowed through 10 control aquaria and 10 aquaria contaminated with 0.1, 1, or 10 µg/l of the Aroclor® 1254 (Exhibit 30). Communities that developed in control aquaria and aquaria that received 0.1 µg/l of PCB in water for four months were dominated (>75%) by arthropods, primarily the amphipod Corophium volutator. In aquaria receiving 1 and 10 µg/l, the number of arthropods decreased and the number of chordates, primarily the tunicate Molgula manhattensis, increased; over 75% of the animals in 10 µg/l aquaria were tunicates. Numbers of phyla, species, and individuals (particularly

amphipods, bryozoans, crabs, and mollusks) were decreased, but there was no apparent effect on the abundance of annelids, brachipods, coelenterates, echinoderms or nemerteans. The Shannon-Weaver index of species diversity was not altered by Aroclor®1254.

Aroclor 1016 - Pinfish. The chronic toxicity and uptake of Aroclor®1016 by pinfish was tested in three separate flow-through bioassays lasting 42 days (Exhibit 19). Mortality of pinfish exposed to 32 µg/l (13 µg/l measured in one experiment and 21 µg/l in the second) or 100 µg/l (59 µg/l measured) was significantly greater than mortalities of unexposed pinfish. Mortality of 10 µg/l (about 7 µg/l measured) exposed fish was negligible. Color of most dying fish darkened, they stopped feeding and they swam erratically with their head inclined downward. Difficulty in swimming progressed until the fish swam upside down just prior to death. Pathological examination of 32 µg/l exposed fish revealed several liver and pancreatic alterations that distinguished them from control fish. Concentrations of Aroclor®1016 stored by the pinfish exposed for 42 days ranged from 11,000 to 24,000 times the nominal concentration in the test water. Concentrations in pinfish exposed to 1 µg/l for 56 days increased for three or four weeks and then stablized. When placed in PCB-free water for 56 days, concentrations (µg/g) in these fish decreased by 51%.

Sheepshead minnows: The chronic toxicity of Aroclor® 1016 to fry, juvenile and adult sheepshead minnows was investigated in intermittent flow bioassays lasting 28 days (Exhibit 31). Fry, juvenile, and adult fish were apparently not affected by nominal concentrations of 0.1, 0.32, 1.0, 3.2 or 10 µg/l but died in 32 and 100 µg/l of this chemical. Chemical analyses of the test water showed about 4 µg/l in the 10 µg/l aquaria, 15 µg/l in 32 µg/l aquaria and 42 µg/l in the aquaria contaminated with 100 µg/l of Aroclor® 1016. Responses to poisoning included darkened body coloration, uncoordinated swimming, cessation of feeding, lesions on the body of many juveniles and adults, and death. Sheepshead minnows accumulated the PCB in proportion to its concentration in the test water. Fry contained 2,500 to 8,100 X the nominal concentration in the test water, adults 4,700 to 14,000 X and juveniles 10,000 to 34,000 X. Eggs from exposed adults were fertilized and placed in PCB-free water. As much as 77 µg/g of the PCB in these eggs apparently did not affect survival of embryos and fry for two weeks.

Information on flow-through bioassays lasting longer than 96 hours is summarized in Exhibit 32.

Published Works:

Duke, Thomas W. 1961. Availability of sediment-sorbed radionuclides to the biota. Ph.D. Dissertation. Texas A & M University.

With Don Hood and Bernadette Stevenson. 1961. Measurement of toxicity of organic wastes to marine organisms. J. Water Pollution Control Federation 9: 982-996.

Duke, Thomas W. 1962. Observations on the movement of radionuclides in selected marine environments. Bull. Ecological Soc. of America 43: 120 (Abstract).

Duke, Thomas W., E. R. Ibert, and K. M. Rae. 1963. Availability of sediment-sorbed material to the biota. In First National Symposium on Radioecology, V. Schultz and W. Klement (eds.), Reinhold Publishing Company, pp. 171-175.

Duke, Thomas W. 1964. Use of radioisotopes in marine biological research. Proceedings of the Second Oak Ridge Radioisotope Conference. U. S. Atomic Energy Commission TID-7689, pp. 69-72.

Duke, Thomas W. 1965. Biogeochemcial cycling of radionuclides in the estuarine environment. Proceedings of the 17th Annual Conference Southeastern Association of Game and Fish Commission, pp. 315-323.

Duke, Thomas W., James N. Willis, and Thomas J. Price, 1965. Cycling of zinc in experimental marine environments. Association of Southeastern Biologists Bulletin 12: 45 (Abstract).

Duke, Thomas W., J. N. Willis, and T. J. Price. 1966. Cycling of trace elements in the estuarine environment. I. Movement and distribution of zinc 65 and stable zinc in experimental ponds. Chesapeake Science 7: 1-10.

Duke, Thomas W., J. P. Baptist, and D. E. Hoss. 1966. Bioaccumulation of radioactive gold as a sediment tracer in the estuarine environment. U.S. Fish and Wildlife Service, Fishery Bulletin 65(2): 427-436.

Duke, Thomas W. 1967. Possible routes of zinc 65 from an estuarine environment to man. J. Water Pollution Control Federation 39(4): 536-542.

Duke, Thomas W., James N. Willis, and Douglas A. Wolfe. 1967. Studies of the exchange of trace elements between estuarine sediments and water. Association of Southeastern Biologists Bulletin 14(2): 27 (Abstract).

MONS 204326

Published works: (continued)

Duke, T. W., J. N. Willis, T. J. Price, and K. Fischler. 1967. Influence of environmental factors on the concentration of Zn^{65} by an experimental community. Proceedings of the Second National Symposium on Radioecology held in Ann Arbor, Michigan, May 15-17, 1967.

Duke, T.W., and T.R. Rice. 1967. Cycling of nutrients in estuaries. Proceedings of the Gulf and Caribbean Fisheries Institute, 19th Annual Session, pp. 59-67.

Duke, Thomas W. 1968. Salt Water Radioecology. Wildlife in North Carolina 32(5): 4-6.

Duke, Thomas W., James N. Willis, and Douglas A. Wolfe. 1968. A technique for studying the exchange of trace elements between estuarine sediments and water. Limnology and Oceanography 13(3): 541-545.

With Kenneth R. Tenore, and Donald B. Horton. 1968. Effects of bottom substrate on the brackish water bivalve Rangia cuneata. Chesapeake Science 9(4): 238-248.

With T.R. Rice. 1969. Radioactivity in the Sea. In Encyclopedia of Marine Resources, Frank Firth (ed.), Van Nostrand Reinhold, pp. 566-569.

With D.W. Hayne, and T.J. Sheets. 1969. Pesticides in Estuaries. In, H.T. Odum, B.J. Copeland and Elizabeth McMahan. Coastal ecological systems of the United States. (A Report to the Federal Water Pollution Control Administration. Institute of Marine Science, University of North Carolina, Morehead City, N.C.).

Duke, Thomas W. 1970. Eatuarine Pesticide Research--Bureau of Commercial Fisheries. Proceedings of the Gulf and Caribbean Fisheries Institute, Twnety-second Annual Session, November, 1969, pp. 146-153.

Duke, T.W., J.I. Lowe, and A.J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor® 1254) in the water, sediment, and biota of Escambia Bay, Florida. Bulletin of Environmental Contamination & Toxicology 5(2): 171-180, 1970.

Duke, Thomas W. 1970. Effects of pesticides on estuarine organisms. Marine Pollution Bulletin 1(8): 126, August, 1970.

MONS 204327

Published works: (continued)

With T.R. Rice, J.P. Baptist, and F.A. Cross. 1970. Potential hazards from radioactive pollution of the estuary. Proceedings of the FAO Technical Conference on Marine Pollution and its Effects on the Living Resources and Fishing, Rome, Italy, 9-18 December 1970.

With Ford A. Cross and James N. Willis. 1970. Biogeochemistry of trace elements in a coastal plain estuary: Distribution of manganese, iron, and zinc in sediments, water, and polychaetous worms. Chesapeake Science, 11(4): 221-234.

With J. I. Lowe, P. R. Parrish, A. J. Wilson, Jr., and P. W. Wilson. 1971. Effects of mirex on selected estuarine organisms. Transactions of the 36th North American Wildlife and Natural Resources Conference, March 7-10, 1971, Portland, Oregon, pp. 171-186.

Duke, Thomas W. and A. J. Wilson, Jr. 1971. Chlorinated hydrocarbons in livers of fishes from the Northeastern Pacific Ocean. Pesticides Monitoring Journal 5(2): 228-232.

With D. L. Coppage. 1971. Effects of pesticides in estuaries along the Gulf and Southeast Atlantic Coasts. Proceedings of the 2nd Gulf Coast Conference on Mosquito Suppression and Wildlife Management, New Orleans, Louisiana, October 20-22, 1971, pp. 24-31.

With C. G. Bookhout, A. J. Wilson, Jr., and J. I. Lowe. 1972. Effects of mirex on the larval development of two crabs. Water, Air, and Soil Pollution 1: 165-180.

With Borthwick, P. W., A. J. Wilson, Jr., J. I. Lowe, J. M. Patrick, Jr., and J. C. Oberheu. 1973. Accumulation and movement of mirex in selected estuaries of South Carolina, 1969-71. Pesticides Monitoring Journal, 7(1): 6-26.

W-1
D-1

Bulletin of Environmental Contamination and Toxicology
1970
Vol. 5, No 2
March-April

BULLETIN • Vol. 5, No. 2 • Pages 95-183 • March-April 1970

Table of Contents

THE DETERMINATION OF HCN RESIDUES ON GREENHOUSE TOMATOES
by Acie C. Waldron, Carl J. Robb and Jay P. Sicesman 95

COMPARISON OF SEVERAL LIQUID PHASES IN GAS LIQUID CHROMATOGRAPHY OF METHYL PARATHION AND METABOLITES
by P. S. Jaglan and F. A. Gunther 98

PERSISTENCE OF METHIDATHION IN SOILS
by L. W. Getzin 104

SINGLE COLUMN GAS LIQUID CHROMATOGRAPHY OF METHYL PARATHION AND METABOLITES USING TEMPERATURE PROGRAMMING
by P. S. Jaglan and F. A. Gunther 111

UBER DIE BINDUNG VON BLEI AN EISENHYDROXIDHALTIGE STOFFE IN LEBER, NIERE UND MILZ VERGIFTETER RINDER
by Harald A. Russel 115

MODIFICATION OF MICROSOMAL ENZYME ACTIVITY AND PARATHION TOXICITY IN RATS
by D. C. Villeneuve, W. E. J. Phillips and John Sysoluk 125

FACILE DEGRADATION OF P,P'-DDT VIA P,P'-DDD USING CHROMOUS CHLORIDE AS REDUCING AGENT
by A. S. Y. Chau and W. P. Cochrane 133

DDT RESIDUES IN FOREST SOILS
by W. N. Yule 139

TOXICITY TO FISH OF TWO ORGANIC REACTOR COOLANTS
by J. E. Guthrie and D. E. Acres 145

THE EFFECT OF O,P'-DDT ON JAPANESE QUAIL
by A. S. Cooke 157

MICROBIAL DEGRADATION OF THE ACARICIDE N'(4 CHLORO-O TOLYL) N,N-DIMETHYLFORMAMIDINE
by B. Thomas Johnson and Charles O. Knowles 158

AN ORIENTATIONAL SURVEY ON THE SIDE-EFFECTS AND ENVIRONMENTAL DISTRIBUTION OF INSECTICIDES USED IN TSETSE CONTROL IN AFRICA
by J. H. Koeman and J. H. Pennings 164

A POLYCHLORINATED BIPHENYL (AROCLOR 1254®) IN THE WATER, SEDIMENT, AND BIOTA OF ESCAMBIA BAY, FLORIDA
by T. W. Duke, J. I. Lowe and A. J. Wilson, Jr. 171

STARCH GEL ELECTROPHORESIS OF LIVER ESTERASES SELECTIVELY INHIBITED BY PESTICIDES
by C. E. Mendoza and G. V. Halma 181

SPRINGER-VERLAG NEW YORK • HEIDELBERG • BERLIN

MONS 204329

A Polychlorinated Biphenyl (Aroclor 1254®) in the Water, Sediment, and Biota of Escambia Bay, Florida[1]

By T. W. Duke, J. I. Lowe and A. J. Wilson, Jr.
Bureau of Commercial Fisheries, Center for Estuarine and Menhaden Research, Pesticide Field Station, Gulf Breeze, Florida

We have detected a polychlorinated biphenyl (PCB), Aroclor 1254, in the biota, sediment, and water of estuarine areas near Pensacola, Florida. Only one source of the chemical, an industrial plant on the Escambia River, has been located. However, the chemical occurs in tissues of pelagic and sessile organisms that are widely distributed within the estuary. This distribution of Aroclor 1254 could be due to dispersion of the chemical from the river by currents and biota to other parts of the system. Alternatively, it could have entered the system from more than one source. In this paper we report the occurrence of Aroclor 1254 in the estuarine environment and discuss its possible effects on some estuarine organisms.

PCB's are considered industrial pollutants and, unfortunately, are relatively persistent in an aquatic ecosystem. Aroclor 1254, one of a series of chlorinated biphenyl compounds manufactured by the Monsanto Company, is a light yellow viscous oil with a chlorine content of 54%. They are used in industry as plasticizers and resins for chlorinated-rubber-based lacquers, varnishes and paints, lubricants, heat transfer fluids and insulators. Also, PCB's are used as carriers for some insecticides. They are stable, insoluble in water and highly soluble in lipid. Structurally they resemble chlorinated hydrocarbon insecticides such as DDT. Thus, it is not surprising to find PCB's sorbed onto sediment and accumulated in the tissues of animals exposed to water containing this chemical.

Polychlorinated biphenyls occur in fish and wildlife from other parts of the United States, Great Britain and the Netherlands. Risebrough (1) reported these compounds to be widely distributed in marine ecosystems of the Pacific Ocean. Marine birds had higher concentrations of PCB's than fish, and animals from San

® Registered trademark, Monsanto Co., St. Louis, Mo.
[1] Contribution No. 101

Bulletin of Environmental Contamination & Toxicology
Vol. 4, No. 2, 1970, published by Springer-Verlag New York, Inc.

MONS 204330

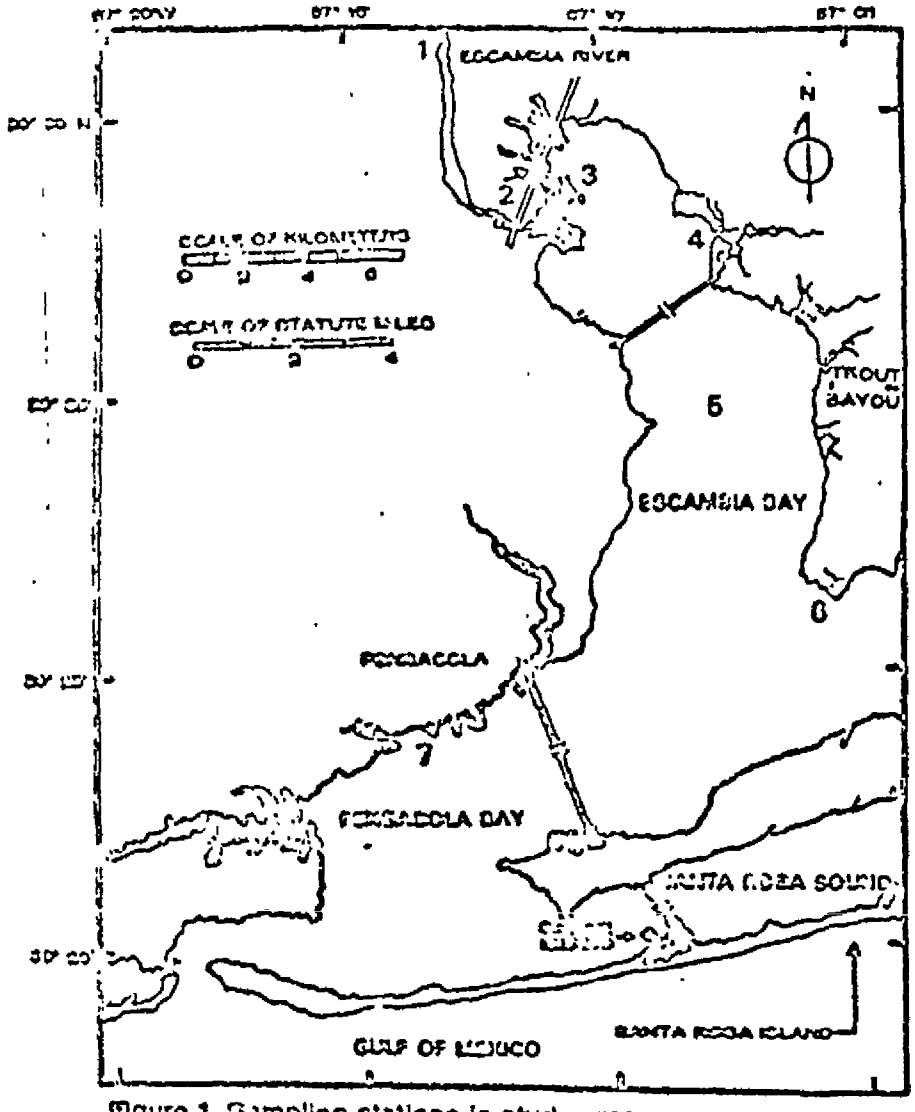


Figure 1. Sampling stations in study area.

MONS 204331

Francisco Bay contained more of the chemicals than those from the ocean. A recent survey by this laboratory of the pesticide content of estuarine animals from Charleston, South Carolina, revealed less than 0.1 ppm (mg/Kg) of PCB in fish and blue crabs. Also, fish and shellfish from Texas and Georgia, collected as part of the Bureau of Commercial Fisheries National Monitoring Program, contained residues of these chemicals, as did coastal birds and bird eggs from England (2) and mussels, fish, and birds from The Netherlands (3).

Field Studies

We first detected residues of Aroclor 1254 in oysters in April 1969 at a newly established pesticide monitoring station in Escambia Bay. Subsequent sampling in this area showed residues of the chemical in water, sediment, fish, blue crabs, and shrimp. We traced one source of Aroclor 1254 to the outfall of a local industry (located 6 miles upstream from Station 1, Figure 1) after analyzing 30 water samples from Escambia River and Bay. The Aroclor reportedly entered the plant's effluent through accidental leakage of a heat-exchange fluid.

Methods. Samples of biota, water, and sediments were collected from April through October, 1969. Some of the animals were dead or moribund when collected. They were taken from the water immediately after "fish-kills" that were reportedly due to lack of oxygen in the water. Others were captured alive by trawl. Water was collected in 1-gallon glass jugs. Sediment was taken with a modified grab sampler.

The tissues of fish, crabs, oysters, and shrimp were mixed with anhydrous sodium sulfate in a blender. The mixture was extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and partitioned with acetonitrile. The acetonitrile was evaporated just to dryness and the residue transferred to a Florisil column (4) with petroleum ether. Aroclor 1254 was eluted from the column with 6% ethyl ether-in-petroleum ether.

Sediment samples were dried at room temperature and extracted for 4 hours with 10% acetone in petroleum ether in a Soxhlet apparatus. Extracts were evaporated just to dryness and the residues eluted from a Florisil column.

Water samples were extracted with petroleum ether. The extracts were dried with sodium sulfate and reduced to an appropriate volume.

MONS 204332

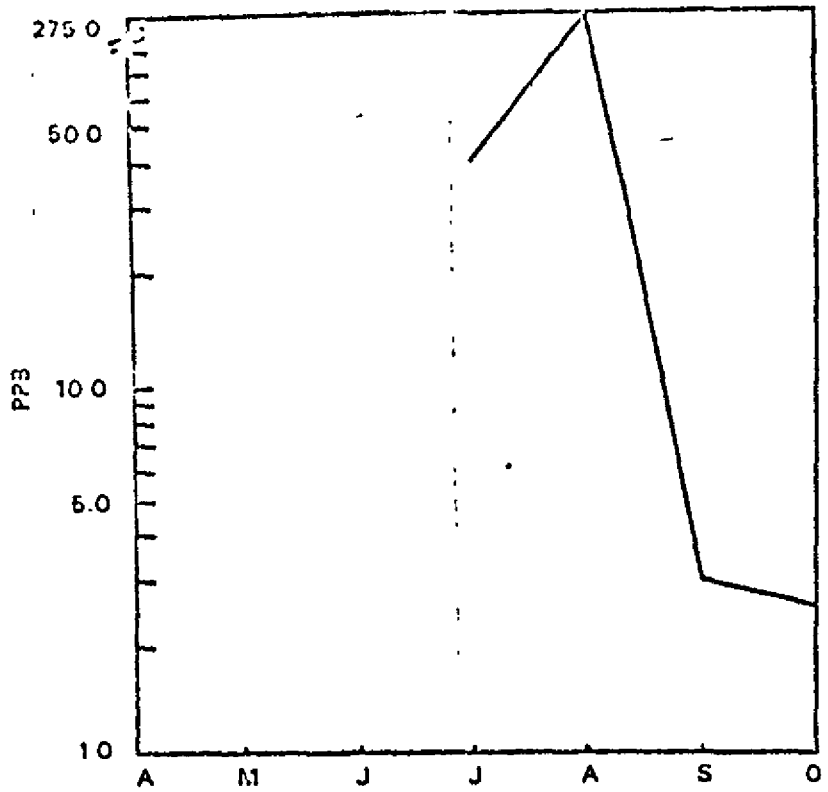


Figure 2A. Aroclor 1254 in water from outfall in Escambia River.

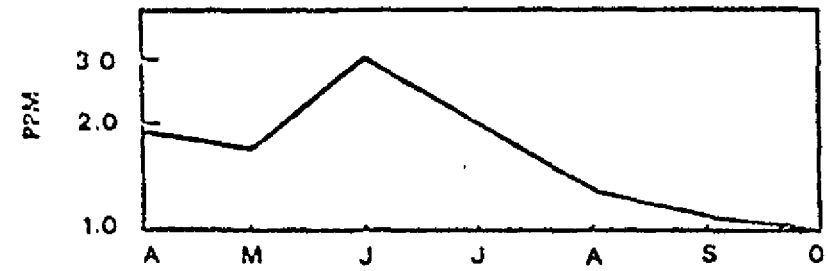


Figure 2B. Aroclor 1254 in live oysters from Escambia Bay (Station 5).

MONS 204333

The extracts of all substrates were identified and measured by electron capture gas chromatography. Three columns of different polarity (DC-200, QF-1, mixed DC-200 and QF-1) were used to confirm identification. In a few samples, thin layer chromatography was employed for additional confirmation and to assess the amount of DDT present. Interference from DDT was negligible, due to the relatively high residues of Aroclor 1254 found in the samples. Quantitation of this multiple peak compound was approximated by averaging the peak height of five major peaks. Laboratory tests indicated recovery rates above 60%. Data in this report do not include a correction factor for percentage recovery.

Residues of Aroclor 1254 in biota, water, and sediment of Escambia Bay. Evidently, Aroclor 1254 moved from the water of Escambia River to biota and sediment in the Bay and contiguous waters (Table I). Oysters are excellent indicators of the presence of the Aroclor in the environment and reflect, in general, the amount of the chemical in the water (Figure 2B). Fish, shrimp, and crabs contained higher concentrations of the chemical than oysters but because of their mobility they are not as useful as monitors for a particular area. Highest concentrations of Aroclor in water during the sampling period occurred at the outfall in Escambia River in August and decreased abruptly when leakage from the plant was corrected (Figure 2A). Less than 0.1 ppb (µg/L) of the Aroclor occurred in the water at Station 2 (Figure 1) but it was not detected in lower bay water. Continued presence of the chemical in river water presumably is a result of leaching from sediments. Aroclor residues in sediment samples taken near the outfall reached 486 ppm in August but have decreased since that time.

Laboratory Studies

The presence of PCB's in terrestrial and aquatic animal tissues is well documented, but to our knowledge, there are no published data on the toxicity of these compounds to marine life. In order to evaluate the concentrations of Aroclor found in water and animal tissues in relation to toxicity, we conducted bioassays under controlled conditions in the laboratory. Fish, shrimp, and oysters were exposed to Aroclor 1254 in acute (96 hours or less) laboratory bioassays. Also, juvenile pink shrimp, Penaeus duorarum, and blue crabs, Callinectes sapidus, were exposed to concentrations of Aroclor 1254 for 20 days. The animals were analyzed for residues of this PCB.

Bioassay procedures. The acute toxicity of Aroclor 1254 to marine animals was determined by exposing separate populations of juvenile fish, shrimp, and oysters to three concentrations (100.0,

MONS 204334

TABLE I

Residues of Aroclor 1254 in samples from Escambia Bay and River

Sample (shrimp collected live; crab and fish were dead or moribund)*	Number of animals or organs in sample	Date collected (D-M-Y)	Location (See Fig. 1)	Residues of Aroclor 1254 ppm
Fish				
Flounder liver	1	5-6-69	Mulatto Bayou (4)	76.
Flounder muscle	1	"	"	4.5
Flounder gills	1	"	"	19.
Croaker	3	"	"	12.
Menhaden	5	"	"	11.
Pinfish	5	"	"	10.
Menhaden	5	1-7-69	"	12.
Speckled trout gills	1	14-8-69	Pensacola Bay (7)	7.5
Speckled trout liver	1	"	"	21.
Speckled trout	3	31-8-69	Northern Escambia Bay (3)	20.
Flounder liver	1	"	"	184.
Menhaden	6	10-10-69	Mulatto Bayou (4)	5.7
Crustaceans				
Shrimp	10	1-7-69	Mulatto Bayou (4)	1.5
Shrimp	5	31-8-69	Northern Escambia Bay (3)	2.5
Blue crab	6	13-8-69	"	7.0
Blue crab	5	31-8-69	"	6.3
Blue crab	6	9-9-69	Mulatto Bayou (4)	1.0
Sediment				
"		8-7-69	Highway 90 (2)	1.7
"		15-8-69	Garcon Point (6)	<.01
"		"	Outfall 6 miles north of (1)	486.

* Scientific names: trout (Cynoscion nebulosus), croaker (Micropogon undulatus), menhaden (Brevoortia patronus), pinfish (Lagodon rhomboides), flounder (Paralichthys sp.), shrimp (Penaeus setiferus) and (Penaeus azteeus), and blue crab (Callinectes ranidus).

MONS 204335

10.0, and 1.0 ppb) of this PCB for 48 hours (96 hours for the oysters). The bioassay techniques used are those routinely employed at this laboratory to study the relative toxicity of pesticides to marine life. The animals are held in aquaria with flowing sea water to which acetone stock solutions of the pollutant are added (with stopcocks or metering pumps) to give the desired test concentration (5,6).

Bioassay animals were captured in local waters and acclimated to laboratory conditions. Ten pinfish, Lagodon rhomboides (1" - 1 1/2" long), 10 pink shrimp, Penaeus duorarum (2" - 3"), and 12 oysters, Crassostrea virginica (1" - 1 1/2") were exposed in separate aquaria to each concentration. Frequent observations were made on the fish and shrimp and percentage mortality recorded at the end of 24- and 48-hour periods. Rate of shell growth of each experimental oyster was measured at the end of 96 hours and percentage decrease (as compared to controls) calculated. The valve edges of the young oysters were ground smooth before the test. We have found that the rate of shell deposition in young, fast growing oysters is a convenient and objective method for evaluating effects of chemical pollutants on this mollusk (7).

The 20-day bioassays with juvenile crabs and shrimp were made under conditions similar to those used in the acute bioassays except the animals were confined in compartmented aquaria and acetone stock solutions of the Aroclor were added to the water with a dual channel metering pump. The desired concentration of Aroclor was 5.0 ppb in both experiments, and was selected on the basis of residues found in water samples from the lower reaches of Escambia River. Water samples taken from experimental aquaria at periodic intervals during the 20-day tests ranged from 3.5 to 4.2 ppb of Aroclor 1254. Control aquaria received the same amount of acetone (1 ml/min) as the experimental. Four hundred liters of sea water per hour flowed through each aquarium.

Shrimp were exposed from July 24 to August 13, 1969 and crabs from August 21 to September 10. Mean water temperature and salinity were 27°C and 28‰ during the first period and 28°C and 27‰ during the latter period.

Twenty-five juvenile pink shrimp (1 1/2" - 2 1/2") were used in each of two (control and experimental) compartmented aquaria and 20 blue crabs (1/2" - 2") in each of two aquaria. Individual shrimp and crabs must be isolated during a long-term experiment because of cannibalism. They are especially vulnerable to predation immediately after molting. All animals were fed pieces of fish at least twice a week.

MONS 204336

Results of acute bioassays. Tables II, III, and IV summarize the results of acute toxicity tests and the amount of Aroclor 1254 in the animals. This PCB appeared to have no effect on juvenile pinfish exposed to 100 ppb of the chemical for 48 hours. Residue analysis of these fish (whole body) revealed 17 ppm of the compound. As indicated in the tables, shrimp are susceptible to relatively low levels of Aroclor 1254. Shell growth of oysters exposed to 100 ppb of the PCB for 96 hours was completely inhibited. However, the oysters did survive and their shell growth equaled that of controls after 3 weeks in water that contained no detectable PCB's. Exposure at 100 and 10 ppb were repeated with almost identical results to those shown in Table IV. All animals concentrated the PCB.

Results of 20-day bioassays. Seventy-two percent (18 of 25) of the juvenile shrimp exposed to 5.0 ppb Aroclor 1254 died during the 20-day exposure. The first shrimp died on the tenth day of exposure, and a few died each day during the next 10 days. A composite sample of these shrimp contained 16 ppm of the PCB. The experiment was terminated after 20 days in order to have live shrimp for residue analysis. A composite sample of the seven surviving shrimp contained 33 ppm of the PCB. The shrimp did not exhibit typical symptoms of insecticide poisoning before death; that is, extreme irritability followed by loss of equilibrium. Several of the shrimp died immediately after molting. None of 25 control shrimp died during the 20-day experiment. Residue analysis of a composite sample of seven control shrimp revealed no PCB.

Juvenile blue crabs were not as sensitive as shrimp to Aroclor 1254. Only one of 20 crabs died during the 20-day exposure to 5.0 ppb. One control crab also died. The most striking aspect of this experiment was the relatively large amount of PCB concentrated in tissues of live crabs during the 20-day exposure and the high levels of PCB still present after the crabs were held in clean flowing sea water for 4 weeks. Five crabs removed for analysis after exposure contained an average whole body residue of 23 ppm Aroclor 1254 (range 18 to 27 ppm). Residues in five crabs held in clean water for 1 week averaged 22 ppm (range 10 to 37 ppm). Six crabs held in clean water for 4 weeks, following the 20-day exposure, contained an average of 11 ppm (range 3.0 to 14 ppm), an example of the persistence of this compound in animal tissue.

Implications of Study

From our data, we have gained insight into the movement of Aroclor 1254 in an estuary and its accumulation by the biota. Our labora-

MONS 204337

TABLE II

Acute toxicity of Aroclor 1254 to juvenile pinfish[1]

Test conc. ppb (ug/l)	Number of animals per concentration	Mortality - % 24 hrs	48 hrs	Residues after 48 hrs exposure ppm (mg/kg)
100.0	10	0	0	17.00
10.0	10	0	0	3.80
1.0	10	0	0	0.98

[1] Mean temperature and salinity of sea water 16°C and 26‰

TABLE III

Acute toxicity of Aroclor 1254 to juvenile pink shrimp[1]

Test conc. ppb (ug/l)	Number of animals per concentration	Mortality - % 24 hrs	48 hrs	Residues after 48 hrs exposure ppm (mg/kg)
100.0	10	80	100	3.90
10.0	10	0	0	1.30
1.0	10	0	0	0.14

[1] Mean temperature and salinity of sea water 16°C and 26‰.

TABLE IV

Acute toxicity of Aroclor 1254 to oysters[1]

Test conc. ppb (ug/l)	Number of animals per concentration	Decrease in rate of shell growth in 96 hrs %	Residues after 96 hrs exposure ppm (mg/kg)
100.0	12	100	--
10.0	12	41	33.0*
1.0	12	19	8.1*

[1] Mean temperature and salinity of sea water 19°C and 31‰

* Residues after 4 days in water that contained no detectable PCB's.

MONS 204338

tory studies showed that juvenile shrimp were the most sensitive, and were killed when exposed to 5.0 ppb of Aroclor 1254 in flowing sea water. The Aroclor content in water from Escambia Bay, even near the mouth of the river, contained less than 1 ppb. Shrimp collected from the bay contained a maximum of 2.5 ppm. Thus, the shrimp in Escambia Bay probably were not exposed to lethal levels of the chemical during the sampling period.

This study demonstrates the urgent need for continued surveillance of our estuaries in order to preserve these nursery grounds for our valuable fishery resources. Also, the study shows a need for conducting long-term tests on the effect of sublethal concentrations of Aroclor 1254 on estuarine organisms in sensitive stages of their life history.

Acknowledgments

We thank Lt. Lewis Zangas, Division of Marine Resources, Florida Department of Natural Resources; Mr. Joe Blanchard, Florida Game and Freshwater Fish Commission; Dr. Thomas S. Hopkins, Chairman of the Faculty of Biology, University of West Florida; and Mr. William T. Young, Florida Department of Air and Water Pollution Control, who provided many of the samples for this study.

References

1. R. W. Risebrough, Chemical Fallout, First Rochester Conference on Toxicity (1969).
2. D. C. Holmes, J. H. Simmons, and J. O'G. Tatton, Nature, 216, 227 (1967).
3. J. H. Koeman, M. C. Ten Noever De Brauw, and R. H. DeVos, Nature 221, 1126 (1969).
4. Paul A. Mills, J. H. Onley, and R. A. Gaither, J. Assoc. Offic. Agr. Chem., 46, 182 (1963).
5. United States Department of the Interior. Annual Report of the Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Florida for the Fiscal Year Ending June 30, 1965. Circular 247, p. 3 (1966).
6. Jack I. Lowe, Trans. Amer. Fish. Soc. 93, 396 (1964).
7. Philip A. Butler, Biological Problems in Water Pollution. Third Seminar. U.S. Department of Health, Education, and Welfare, Public Health Service, 92 (1962).

MONS 204339