Exhibit 7

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

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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 Peakall and Lincer (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

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TABLE I

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

Organism	Organisms per sample 1/	Water concentration ppb x̄ ± SE 2/	Organism concentration (4 day exposure) ppm x̄ ± 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	-	-	-
Phenton midge *Chaoborus punctipennis*	5	1.3 ± 0.1	30 ± 1.6	22,000	23,000	23,800	24,800	-
Scud *Cammarus 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	5,800
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.

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

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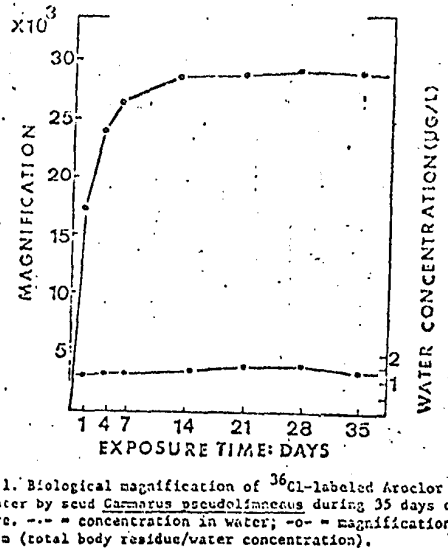


Figure 1. Biological magnification of ^{36}Cl-labeled Aroclor® 1254 from water by scud Gammarus pseudolimnaeus during 35 days of exposure. -•- = concentration in water; -o- = magnification in organism (total body residue/water concentration).

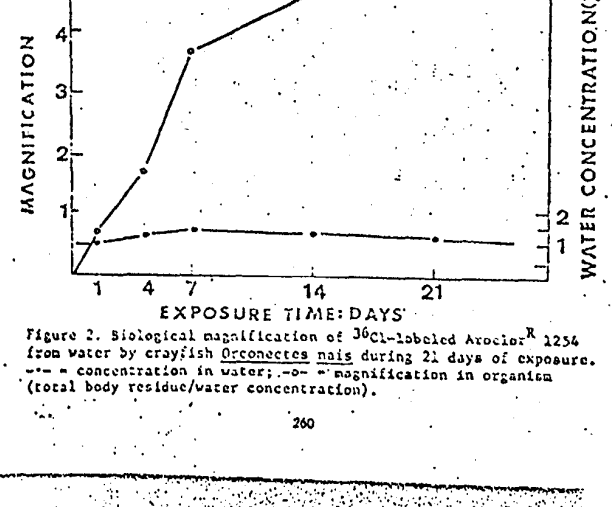


Figure 2. Biological magnification of ^{36}Cl-labeled Aroclor® 1254 from water by crayfish Orconectes nais during 21 days of exposure. -•- = concentration in water; -o- = magnification in organism (total body residue/water concentration).

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48,000 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; 1.00-1.11 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.

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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

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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.

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