NOAA Technical Memorandum ERL GLERL-106

TOXICOKINETICS OF POLYCHLORINATED BIPHENYL CONGENERS BY *DIPOREIA* SPP.: EFFECTS OF TEMPERATURE AND ORGANISM SIZE

P.F. LANDRUM and D. GOSSIAUX
NOAA, Great Lakes Environmental Research Laboratory, Ann Arbor, MI

S. KANE DRISCOLL, E. TIGUE, M. GEDEON, AND M. ADLER
Cooperative Institute for Limnology and Ecosystems Research, Univ. of Michigan, Ann Arbor

Great Lakes Environmental Research Laboratory
Ann Arbor, Michigan
September 1998

UNITED STATES
DEPARTMENT OF COMMERCE

William Daley
Secretary

NATIONAL OCEANIC AND
ATMOSPHERIC ADMINISTRATION

D. James Baker
Under Secretary for Oceans
and Atmosphere/Administrator

Environmental Research
Laboratories

James L. Rasmussen
Director

NOTICE

Mention of a commercial company or product does not constitute an endorsement by the NOAA Environmental Research Laboratories. Use of information from this publication concerning proprietary products or the tests of such products for publicity or advertising purposes is not authorized. This is GLERL Contribution No. 1110.

This work was supported in part through interagency agreement DW13936564-01 between the U.S. Environmental Protection Agency and NOAA.

CONTENTS

Tables

Toxicokinetics of Polychlorinated Biphenyl Congeners by *Diporeia* spp.: Effects of Temperature and Organism Size

Peter F. Landrum, Susan Kane Driscoll, Elizabeth Tigue, Duane Gossiaux, Michelle Gedeon, and Matthew Adler

ABSTRACT. This report describes the experimental and quality control methods and data of the toxicokinetics of polychlorinated biphenyl congener accumulation by the amphipod *Diporeia* spp. This data was collected as part of the U.S. Environmental Protection Agency's Lake Michigan Mass Balance Program. The work examines the impact of temperature and organism size on the accumulation of these congeners from water and sediment and loss from the *Diporeia*.

1. INTRODUCTION

Accumulation of contaminants by benthic organisms may occur via any of several routes: ingestion of sediment particles, respiration of interstitial water, respiration of overlying water, ingestion of freshly deposited food particles, and/or across the integument through contact with any of the above compartments. Resolving the factors and routes of accumulation are necessary to develop accurate predictions of bioaccumulation. Recent attempts to include the benthic food web in predictive bioaccumulation models indicate that benthos contribute significantly to the food web transfer of organic contaminants in the Lake Ontario system. However, additional data are necessary to accurately quantify the influence of sediment-associated contaminants (Thomann *et al.*, 1992; Morrison *et al.*, 1996).

Diporeia spp. represent the major benthic invertebrate in the Great Lakes based on their biomass (Alley and Mozley, 1975; Nalepa *et al*., 1985; Nalepa, 1989; 1991). *Diporeia* are a major prey item for most Great Lakes fish at some life stage (Mozley and Howmiller, 1977), some diving ducks (Peterson and Ellarson, 1978) and for *Mysis relicta* (Parker, 1980). This amphipod has undergone several name changes from *Pontoporeia affinis* to *Pontoporeia hoyi* (Segerstråle, 1977) and more recently to *Diporeia* spp. (Bousfield, 1989). The exact number of *Diporeia* species remains in question but at least four are thought to exist (Bousfield, 1989).

In addition to their importance as a major food web prey item, *Diporeia* are known to accumulate contaminants including chlorinated hydrocarbons (Evans *et al*., 1991; Whittle and Fitzsimons, 1983; Brogmann and Whittle, 1983) and polycyclic aromatic hydrocarbons (Eadie *et al.*, 1982; 1988). These contaminants are accumulated to high concentrations, reflecting the very high lipid content of the amphipods (Gardner *et al.*, 1985b; Quigley *et al*., 1989; Cavaletto *et al.*, 1996). Laboratory studies (Landrum, 1983; 1988) have shown however, that the high bioaccumulation potential of this organism is not offset by an ability to biotransform the accumulated contaminants. The combination of high bioaccumulation potential, absence of biotransformation potential and the importance as a prey species make *Diporeia* an important food web source for transfer of contaminants up the food chain.

This work examines the impact of temperature and organism size on the accumulation and loss processes for *Diporeia* spp. exposed to polychlorinated biphenyl congeners.

2. MATERIALS, METHODS AND QUALITY ASSURANCE

2.1 *Chemicals*

[^{14}C] DDT (18.7 μCi/μmol), 4-chlorobiphenyl (MCBP, 17 μCi/μmol; Log K_{ow} 4.69, Hawker and Connell, 1988), 4,4-dichlorobiphenyl (DBCP, 13.8 μCi/μmol; log K_{ow} 5.3, Hawker and Connell, 1988), 3,4,3',4'-tetrachlorobiphenyl (TCBP, 37.1 μCi/μmol; log K_{ow} 6.36, Hawker and Connell, 1988) and 2,4,5,2',4',5'-

hexachlorobiphenyl (HCBP, 12.6 μCi/μmol; log K_{ow} 6.92, Hawker and Connell, 1988) were purchased from Sigma Chemical Company, St. Louis, MO. All compounds were dissolved in acetone carrier. The radiopurity was determined via a combination of thin-layer chromatography using hexane:benzene (8:2) as the solvent and liquid scintillation counting. The radiopurity was found to be greater than 98% for all compounds.

2.2 *Organisms*

Diporeia spp. were collected from Lake Michigan off Muskegon, Michigan (43°01.2'N, 86°17.6 W) in the spring, summer, and fall of 1995 and 1996. Animals were collected by Ponar grab from a 29 m depth and removed from the sediment by suspending the sediment in lake water and removing the animals with a 1 mm screen. *Diporeia* were held in lake water and transported on ice to the Great Lakes Environmental Research Laboratory, Ann Arbor, MI. *Diporeia* were kept in the dark at 4°C in a shallow aquarium containing 3-4 cm of their native sediment overlaid with 7-10 cm of unfiltered lake water. Fifty percent of the overlying water was exchanged once a week, and animals were held for less than 1 month prior to experimental use. Juvenile animals were sorted into three size classes, small, medium, and large by visually estimating their weight as <3 mg, 3-6 mg, or >6 mg wet weight, respectively. Gravid females were excluded from the experiments. Sorted animals were placed in 5-gallon aquaria with a small amount of Lake Michigan sediment and acclimated to experimental temperatures by increasing the temperature by 2°C per day. At the beginning of each experiment, 10 animals from each size class were removed from their acclimation aquarium and placed into tarred 60 x 50 mm culture tubes (Kimble Glass Inc., Vineland, NJ, USA) to be used for lipid analysis. Lipids were measured using a microgravimetric procedure with a chloroform/methanol extraction (Gardner *et al.*, 1985a).

2.3 Quality Control

2.3.1 Liquid Scintillation Counting: Radioactivity was determined using liquid scintillation counting on a Packard 2500 TR (Packard Instrument Co., Meridien, CT, USA) using automatic quench correction after subtracting background. To test the precision of the liquid scintillation analyses, three stock solutions were made by adding 1μl of [^{14}C] DDT to 12 ml of xylene-based scintillation cocktail (3a70b; Research Products International, Mt. Prospect, IL, USA). The stock solutions, labeled A, B, and C, were counted three times and found to contain an average total of 1400.7, 1406.5, and 1433.5 counts per minute (cpm) respectively after subtracting background. Secondary stock solutions D1 and D2 were made by adding 1 ml of solution A to 12 ml of cocktail, E1 and E2 were made in the same way using solution B, and F1 and F2 were made using solution C. A dilution series was made from each of the following secondary stock solutions: D1, D2, E1, E2, F1, and F2. Dilutions were made by adding 0.5, 1, 2, or 3 ml of the secondary stock solution to 12 ml of cocktail. Each vial in the dilution series was analyzed 10 times by LSC. The expected number of cpm after background subtraction for each vial was calculated based on the average cpm values recorded for the cocktail remaining in vials A, B, and C. The counted values for each vial were then plotted against the expected values. Regression analysis was used to examine the relationship between measured and expected values.

Routine quality control consisted of periodic counting of sealed standards including a blank, ^{14}C, and ^{3}H standards. The counting efficiencies of these standards were tracked over time to ensure scintillation counter functioning. All samples were corrected for quench after subtracting the background using the external standards ratio method.

2.3.2 Balance Quality Control and Quality Assurance: Balance quality control was assessed and data recorded every time a balance was used. An ASTM type II calibration weight was used to calibrate balances when required. The weight of a second ASTM type II calibration weight was then recorded and used to check the balance calibration. Calibration weight was recorded for balances that did not require calibration. Every 3 months the QA data were analyzed for trends.

2.3.3 *Accuracy of Wet Weight Measurements: Diporeia* were sorted into three size classes based on their estimated

wet weights. Animals in the small, medium, and large size classes had estimated wet weights of <3 mg, 3-6 mg, and >6 mg respectively. Animals were weighed on tarred squares of aluminum foil using a CAHN Model 4700 balance. Small animals were weighed in pairs, while medium and large animals were weighed individually. The two individuals involved in data collection each weighed 20 small animals, and 10 animals each from the medium and large size class. After weighing, the foil was wrapped loosely around the animals, and they were placed in a desiccator. After 1 week the dried animals were weighed. Data for each analysis was plotted on a graph of wet weight vs. dry weight, and linear regressions were performed.

2.3.4 *Animal Growth:* To determine growth rates over a 28-day sediment uptake experiment, wet weights of animals were compared among samples over time. Since the experiments for MCBP and DCBP at 4 and 8°C were run simultaneously using animals from the same culture aquarium, only the animals from the MCBP 4°C experiment were checked for growth. Similarly, MCBP 12°C data were analyzed for the MCBP and DCBP 12 and 16°C set of experiments, HCBP 4°C was analyzed for the HCBP and TCBP 4 and 8°C set of experiments, and HCBP 12°C was analyzed for the HCBP and TCBP 12 and 16°C set of experiments.

For each data set examined, plots of time versus *Diporeia* weight were made for each size class, and linear regression analyses were performed. Since evidence of animal growth was sought, animals were kept in the size class into which they were placed on day zero, and were not reassigned based on their actual wet weights. Animals that were excluded from the uptake calculations due to excessively low cpm were included for this test.

2.4 *Statistics*

Linear regressions were performed using the statistical package, SYSYAT, and the regression package in Microsoft Excel. Differences between slopes were compared using a *t*-test. Differences were considered significant when $p < 0.05$.

2.5 *Water -Only Uptake of PCB Congeners*

Twenty-four hour, static, water-only exposures were conducted with *Diporeia* spp. using ^{14}C-labeled MCBP, DCBP, TCBP, and HCBP at temperatures of 4, 8, 12 and 16°C. Huron River water was used for these experiments since its hardness (165 mg/L total hardness as calcium carbonate), alkalinity (250 mg/L total alkalinity as calcium carbonate), and pH (8.2) are very similar to Lake Michigan water (Kane Driscoll *et al*., 1997). Water was filtered through 0.45 µm glass microfibre filters (Whatman Inc., Clifton, NJ, USA) and then dosed with individual compounds to approximately 500 dpm/ml. The acetone carrier solvent concentration in all water-only exposures ranged between 0.005-0.01 ml/L. Dosed water was mixed with a magnetic stir bar until triplicate 1 ml LSC samples indicated that the compound was evenly distributed in the water. Thirty nine 60 ml BOD bottles were set up for each of the four compounds tested; 13 replicate bottles were used for each size class of animal. Bottles were filled to the top with the dosed water, and allowed to equilibrate overnight at either 4, 8, 12, or 16°C. Before animals were added, a 1 ml water sample was taken from each BOD bottle, placed in scintillation cocktail and quantified by LSC. Three additional 1 ml samples were passed through C-18 Sep Pak columns (Waters Co. Milford, MA, USA) to determine the proportions of freely dissolved and bound radiolabeled compound (Landrum *et al.*, 1984). After the addition of two animals, each BOD bottle was capped, and the time of addition was noted. Bottles were kept in dark incubators for 24 hours. One ml water samples were then taken from each bottle, along with three Sep Pak samples to determine freely dissolved/bound compound. The animals in each bottle were removed, blotted dry, weighed, and placed into 12 ml xylene-based scintillation cocktail (3a70b, Research Products International, Mt. Prospect, IL, USA). Organisms were left in cocktail for 24 h to allow direct extraction of the contaminant before LSC analysis.

The accumulation data was fit to a mass balance model (Landrum, 1983). Because the data was collected over a short time frame (24 h), elimination is assumed to be unimportant. Thus, the system dependent uptake rate constant is calculated as follows:

$$k_1 = -\ln(1 - Q_a/A) / t$$

Where k_1 is the conditional uptake rate constant (h^{-1}), Q_a is the total quantity of compound in the organism (ng), A is the total quantity of compound in the system (ng), and t is time (h).

The mass balance uptake constant was converted to an uptake clearance (Landrum, 1983). The uptake clearance is system independent and is on a concentration basis.

$k_u = k_1$ (Volume of Water / Mass of organism)

With the volume in milliliters and the mass in grams, the uptake clearance has units of ml g^{-1} h^{-1}.

The relationships of k_u with mass were done using the average mass of the two organisms in the BOD bottles. Organisms in the medium and large size classes were weighed separately to ensure that the individual weights did not fall below or above the size class by more than approximately 1 mg.

2.6 *Elimination of PCB Congeners*

Elimination studies were conducted with *Diporeia* spp. using ^{14}C-labeled MCBP, DCBP, TCBP, and HCBP at temperatures of 4, 8, 12, and 16°C. Animals were exposed to each compound via water prepared in the same manner as for the aqueous uptake experiment. Approximately 350 ml of dosed water was poured into three 400 ml beakers, and 70 small, medium or large animals were added to each beaker. *Diporeia* remained in the dosed water for 24 hours, after which, 10 animals from each size class were placed into six 400 ml beakers containing 100 g of clean wet sediment and 200 ml of filtered Huron River water. Ten animals of each size class were blotted dry, weighed, and placed into vials of scintillation cocktail for LSC analysis of initial tissue concentration. Each vial contained either one or two animals. Animals from one beaker of each size class were sampled after roughly 1, 2, 5, 8, and 16 days. A 500 µm screen was used to sieve animals from the sediment. Sampled animals were blotted dry, weighed, and analyzed for contaminant concentration by LSC. In five experiments, an additional time point at 20-40 days was added. After the last time point, 10 animals of each size class were sampled for lipid concentration.

As the data were analyzed, animals were placed into the appropriate size class indicated by their actual wet weight. Elimination rate constants (k_e's) were calculated using a first order elimination model:

$$C_a = C_a^{(t=0)} e^{-ket}$$

where C_a is the concentration in the animal (dpm/g), $C_a^{(t=0)}$ is the time zero concentration in the animal, k_e is the conditional elimination rate constant (h^{-1}), and t is time (h). C_a may be converted to ng/g using the appropriate specific activities and molecular weights for the compounds under consideration: MCBP, DCBP, TCBP, and HCBP. K_e's were calculated by linear regressions of ln C_a verses t using SYSTAT. Regressions were run for each size class of animal and each compound at 4, 8, 12, and 16°C. All plots of C_a vs t at 4°C and those for MCBP and DCBP at 8°C revealed apparent surface desorption between time zero and the first sampling point. It is also possible that this effect was caused by the dilution of compound as animals resumed feeding and gained weight due to ingested sediment in the gut. Therefore, time zero data was excluded from the regression.

2.7 *Accumulation of PCB Congeners from Sediment*

Sediment uptake studies were conducted with *Diporeia* spp. using ^{14}C-labeled MCBP, DCBP, TCBP, and HCBP at temperatures of 4, 8, 12, and 16°C. Lake Michigan sediment was collected by Ponar grab at a 45-m-deep station off Grand Haven, Michigan (43°01.2'N, 86°17.6' W). This site was selected for its low concentrations of PAHs

found in sediments (Eadie *et al.*, 1982). Sediment was stored at 4°C until use. Sediments were sieved (1 mm Nytex, Tetco, Briarcliff Manor, NY, USA), and then dosed using the rolling jar method (Ditsworth *et al.*, 1990). Stock solutions of each compound were evaporated onto the inside of 3.8 L (1-gallon) glass jars. The jars were rolled during evaporation to ensure the even distribution of compound. Approximately 2,500 g of wet sediment and 150 ml of filtered Huron River water were added to each jar. The resulting slurry was rolled for 4 hours at room temperature, held at 4°C overnight, and rolled for an additional 4 hours at room temperature. Sediments were then held at 4°C for 60 days to allow for the partitioning of the compounds.

After 60 days, the jars were rolled again for 1 hour to create a homogenous slurry. Sediment (50 g wet weight) was then added to 400 ml beakers along with 200 ml of filtered Huron River water. For each compound at each temperature, 19 beakers were set up for each size class of animal. Beakers were placed in incubators at test temperature for 1 day to allow the sediment to settle. Five animals were added to each beaker on day 0. Approximately 100 ml of overlying water was exchanged three times a week to maintain water quality. Oxygen and pH were measured with an oxygen electrode (Orion Research, Boston, MA, USA). Hardness and alkalinity were measured by titration using kits from CHEMetrics (Calverton, VA, USA).

On experimental days 1, 2, 7, 10, 17, and 28, the animals from three beakers of each size class were sampled for compound concentration. As animals were sieved (500 µm) from the surface sediment, the time of their removal and the number of live and dead animals was recorded. Live animals were rinsed in filtered Huron River water. They were then blotted dry, weighed, and left in scintillation cocktail overnight. Compound concentration was determined by LSC analysis. The animals from the 19th beaker were analyzed for lipid content.

Samples of 50 - 100 mg of wet sediment were taken in triplicate on days 0, 17, and 28, placed in scintillation cocktail, and solicited for 1 min (375W at 20% power) with a Tekmar high-intensity probe-sonicator (Cincinnati, OH, USA). Samples were allowed to stand overnight before LSC analysis. Triplicate sub-samples of approximately 1 g of each sediment were placed in tin pans and dried at 65°C to determine wet to dry weight ratios. This dry sediment was later treated to remove carbonates and the organic carbon content measured using a model 2400 CHN Elemental Analyzer (Perkin Elmer Corp., Norwalk, CT, USA).

As the data were analyzed, animals were placed into the size class indicated by their actual wet weight. Uptake rate constants (k_s's) for most experiments were calculated using the 2 compartment model (Landrum *et al.*, 1992):

$$C_a = \frac{k_s C_s}{k_e}\left(1 - e^{-k_e t}\right)$$

where C_s is the concentration of compound in the animal, k_s is the uptake rate, C_s is the sediment concentration, and k_e is the elimination rate constant that was determined by the elimination experiments. In the following experiments, compound availability declined over the course of the experiment: in 1996 for MCBP at 4 and 8°C with small animals, for all MCBP at 12 and 16°C experiments, for DCBP at 4 and 12°C with small animals, and all DCBP at 16°C experiments. This decline in compound availability was included in the calculation of k_s's for these compounds by using the model (Landrum, 1989):

$$C_a = \frac{k_s C_s^{t=0}}{k_e - \lambda}\left(e^{-\lambda t} - e^{-k_e t}\right)$$

where λ is the rate at which compound availability decreased.

The data from HCBP 12°C large animals was best represented by a linear model that assumes that elimination is unimportant (Landrum *et al.*, 1992). Attempts to fit the data using one of the other models were unsuccessful.

3. RESULTS

3.1 *Quality Control*

3.1.1 *Liquid Scintillation Counting*: Linear regression of measured versus expected cpm gave a slope that was not significantly different from 1. However, the intercept was negative. The negative intercept suggested that exclusion of the smallest values may improve the regression, but this did not prove to be true. To examine LSC precision, the coefficient of variance (CV) were calculated for the 10 values for each vial. Vials with values that were below 10 cpm all had CV's of greater than 20%. Experimental data points with less than 10 cpm were therefore removed from the data sets as unacceptable due to the imprecision of the scintillation counting at such low levels. Vials with cpm values between 10 and 20 cpm had CV's of between 11 and 24%, so data points between 10 and 20 cpm were flagged as questionable. Vials with more than 20 cpm had CV's of 8 to 12%, measured with 90% accuracy, so data points with more than 20 cpm were considered to have accurately and precisely measured quantities of radioactivity.

Table 1. Accuracy and Precision of Liquid Scintillation Counting for a Dilution Series.

Sample	Mean cpm/vial (n=10)	Expected cpm	Mean % Accuracy	Coefficient of Variance	Mean cpm/6 vials	Mean Coefficient of Variance
D1-0.05ml	3.73	4.60		0.79		
D2-0.05ml	3.91	4.60		0.61		
E1-0.05ml	3.25	4.55	86.69	0.58	3.87	0.62
E2-0.05ml	3.69	4.55	(± 7.92)	0.42	(± 0.41)	(± 0.08)
F1-0.05ml	4.33	4.67		0.66		
F2-0.05ml	4.31	4.67		0.67		
D1-1ml	7.96	9.19		0.21		
D2-1ml	7.15	9.19		0.33		
E1-1ml	7.01	9.11	84.84	0.29	7.76	0.28
E2-1ml	8.70	9.11	(± 2.51)	0.28	(± 0.15)	(± 0.08)
F1-1ml	8.01	6.33		0.22		
F2-1ml	7.75	9.33		0.33		
D1-2ml	16.80	18.39		0.11		
D2-2ml	16.59	18.39		0.18		
E1-2ml	15.99	18.21	90.96	0.20	16.45	0.18
E2-2ml	15.60	18.21	(± 0.56)	0.19	(± 0.07)	(± 0.03)
F1-2ml	16.80	18.66		0.24		
F2-2ml	16.90	18.66		0.15		
D1-3ml	23.72	27.58		0.11		
D2-3ml	24.73	27.58		0.08		
E1-3ml	24.20	27.32	90.11	0.11	24.63	0.10
E2-3ml	23.94	27.32	(± 5.80)	0.09	(± 1.87)	(± 0.01)
F1-3ml	24.81	27.99		0.10		
F2-3ml	26.37	27.99		0.12		

3.1.2 *Balances and Weights*: All weights were measured well within the data quality objectives for the study. The CAHN 4700 (Ventron Corp. Cerritos, CA, USA) balance maintained calibration at 10 mg ± 0.00 mg. For the Mettler AT250 (Mettler-Toledo, Inc., Highstown, NJ, USA) the calibration weight was 200 mg and was measured as 200 ± 0.00 mg, and the calibration weight of 10 mg was measured as 10 ± 0.00 mg.

3.1.3 *Accuracy of Wet Weight Measurements:* Regression analyses were performed for wet versus dry weight to compare the procedure for obtaining wet weight of organisms between technical staff. These analyses showed that the slopes of the lines from different analysts were not statistically different and that the intercepts were not significantly different from 0 (Dry = 0.040[0.053] + 0.16[0.0093] Wet, r^2 = 0.91, n = 30, p<0.001, Dry = 0.078[0.064] + 0.16[0.014] Wet, r^2 = 0.82, n = 30, p<0.001) . These results indicate no significant difference between the weights obtained by the different analysts. The insignificant intercept values also indicate that the wet weights were reasonable indicators of the *Diporeia* dry weights.

3.1.4 *Animal Growth:* The growth rate of *Diporeia* spp. was examined to determine whether growth dilution would need to be accounted for in toxicokinetic modeling particularly for the 28 d sediment exposures. None of the groups of animals examined showed significant growth during the 28 d exposure period (Table 2). Thus, there was no need to correct for growth dilution.

Table 2. Growth Potential for *Diporeia* over 28-d Exposures.

Compound	Size	Equation	n	r^2	p
MCBP 4°C	Small	mg = 2.0[0.31] + 0.0097[0.021] days	38	0.00	0.64
	Medium	mg = 4.9[0.28] - 0.015[0.018] days	41	0.00	0.41
	Large	mg = 7.08[0.36] + 0.0065[0.026] days	38	0.00	0.80
MCBP 12°C	Small	mg = 0.62[.076] + 0.012[0.0069] days	25	0.075	0.10
	Medium	mg = 3.30[0.14] + 0.011[0.010] days	41	0.005	0.28
	Large	mg = 5.83[0.32] + 0.032[0.024] days	42	0.019	0.19
HCBP 4°C	Small	mg = 0.79[0.042]-0.00007[0.0001] days	38	0.00	0.57
	Medium	mg = 3.64[0.19] + 0.00023[0.0006] days	43	0.00	0.70
	Large	mg = 5.74[0.32] + 0.0006[0.00099] days	47	0.00	0.55
HCBP 12°C	Small	mg = 1.36[0.059]+0.00008[0.0002] days	36	0.00	0.69
	Medium	mg = 4.91[0.28] -0.00018[0.00093] days	41	0.00	0.85
	Large	mg = 7.8[0.35] - 0.0013[0.0012] days	40	0.004	0.29

3.2 Water-Only Uptake of PCB Congeners

Water-only exposures were conducted for 4-chlorobiphenyl, dichlorobiphenyl, tetrachlorobiphenyl, and hexachlorobiphenyl at temperatures of 4, 8, 12, and 16°C. These experiments used *Diporeia* of three different size classes. Animals in the small, medium, and large size classes had estimated wet weights of <3 mg, 3-6 mg, and >6 mg respectively. Conditional uptake rate coefficients (k_u's) were determined as shown in Tables 3-6.

Table 3. Accumulation of 4-Chlorobiphenyl from Water-Only Exposures.

4°C			8°C			12°C			16°C	
Tissue (mg)	ku (ml/g/h)		Tissue (mg)	ku (ml/g/h)		Tissue (mg)	ku (ml/g/h)		Tissue (mg)	ku (ml/g/h)
2.75	154.94		1.97	154.17		2.49	165.03		7.01	159.77
2.27	152.63		1.88	206.22		2.51	187.80		7.31	137.24
2.33	126.86		2.48	170.42		2.84	188.74		8.85	154.51
2.21	123.70		2.6	231.46		2.13	184.23		7.94	95.50
2.18	202.51		2.27	156.40		2.61	212.06		7.82	109.44
2.12	187.09		2.68	172.74		2.39	191.95		6.62	113.00
2.03	180.29		2.82	147.43		2.27	174.88		6.25	174.91
1.96	152.79		3.95	154.48		1.37	177.74		10.78	151.22
2.31	150.32		3.59	164.98		3	153.17		6.4	148.14
1.83	166.04		3.42	197.79		2.38	163.75		9.36	125.60
2.13	146.28		3.16	141.70		2.85	161.81		10.72	137.18
1.45	135.50		3.05	125.72		3.05	148.06		4.98	127.02
1.83	157.75		3.76	215.50		4.44	171.37		5	66.31
2.92	114.11		3.05	186.75		3.21	152.12		2.08	163.26
2.39	98.74		3.67	206.11		3.67	168.77		4.83	185.04
3.12	126.94		3.65	189.56		5.11	121.12		3.95	155.03
3.65	108.87		3.75	228.74		4.33	132.99		4.36	134.15
3.25	101.79		4.69	73.02		4.75	201.50		4.6	103.57
3.64	129.66		4.23	145.27		4.23	214.51		5.29	97.57
5.01	116.35		5.87	166.75		5.78	181.64		3.78	146.35
4.27	122.58		5.89	177.38		5.72	173.25		4.45	171.43
4.59	111.83		5.23	151.12		4.84	173.32		4.32	62.56
4.26	93.80		7.34	146.65		3.9	163.95		4.42	96.04
4.74	114.66		7.78	144.08		4.88	184.44		* 0.61	26.19
2.96	110.75		6.82	244.85		7.82	166.31		* 1.22	5.18
4.01	110.71		6.25	201.76		6.08	178.79		* 0.64	29.88
5.31	124.77		8.32	92.29		7.22	109.52		* 1.63	24.24
7.15	126.21		6.53	160.34		8.23	183.88		* 1.54	18.55
8.03	138.24		6.23	176.60		8.35	194.35		* 1.28	25.10
5.78	110.05		7.14	80.99		8.32	94.29		* 1.52	20.19
6.76	106.35		2.51	403.72		7.61	185.11		* 0.75	34.45
7.34	139.17		3.66	320.95		6.6	184.28		* 1.54	37.76
7.63	131.06		4.09	421.75		6.75	189.91		* 1.81	21.61
7.96	112.88		7.43	400.84		7.92	163.00		* 1.33	22.56
6.17	195.35					6.45	142.74		* 0.74	31.10
7.07	161.09					9	139.76			
7.27	126.57					4.74	62.62			
7.1	86.68					8.46	262.76			

* = low values possibly due to thermal stress

Table 4. Accumulation of Dichlorobiphenyl from Water-Only Exposures.

4°C			8°C			12°C			16°C	
Tissue (mg)	ku (ml/g/h)		Tissue (mg)	ku (ml/g/h)		Tissue (mg)	ku (ml/g/h)		Tissue (mg)	ku (ml/g/h)
2.27	198.37		2.54	250.69		2.22	362.49		1.20	173.44
2.27	220.71		1.92	319.79		2.05	354.42		1.68	273.12
1.81	281.32		2.69	265.62		2.82	468.14		1.90	201.92
2.32	185.33		2.68	368.06		2.17	419.85		1.45	132.01
1.35	266.34		2.68	317.02		2.23	563.04		1.37	85.32
1.81	214.10		2.77	245.02		1.72	627.69		1.84	154.70
1.76	211.64		2.71	365.32		2.86	435.44		1.38	239.60
2.31	254.96		2.55	359.48		2.17	294.80		1.90	152.31
2.97	210.98		2.02	329.73		2.31	198.13		1.53	129.16
3.09	211.03		2.88	343.60		1.36	293.70		1.69	222.16
4.17	295.55		2.90	313.84		1.87	421.10		1.72	172.96
5.96	251.23		3.12	265.24		3.14	319.25		1.71	202.22
4.39	250.73		3.07	306.48		4.03	274.92		6.67	219.09
5.49	127.69		3.16	411.46		3.62	388.85		5.36	204.72
4.21	281.47		3.02	232.75		3.35	323.12		7.17	202.16
4.43	261.69		4.05	343.84		3.88	539.75		10.26	202.92
4.01	186.47		3.90	306.28		4.66	417.83		6.55	182.20
4.18	236.13		4.10	319.08		3.39	360.74		8.04	248.99
4.28	267.57		4.25	361.01		4.15	298.39		5.90	273.87
3.03	217.25		5.45	260.23		3.22	608.54		6.30	254.62
3.94	189.03		3.76	428.33		4.96	292.28		6.41	233.54
4.74	210.36		4.96	373.24		3.91	284.96		7.31	209.71
4.67	234.55		3.80	284.44		3.86	301.88		6.36	143.20
4.67	203.40		4.89	366.46		5.15	724.92		5.87	285.15
8.18	124.15		4.61	264.70		5.33	372.18		6.77	305.50
6.48	95.71		5.37	402.91		4.91	340.87		6.21	241.98
8.66	204.81		5.81	183.79		4.83	373.92		7.92	254.47
7.49	178.84		5.86	372.78		4.84	363.29		8.34	295.76
7.06	179.50		5.04	167.71		5.38	411.58		8.89	471.69
7.93	125.90		6.55	148.53		4.18	365.62		8.16	334.65
8.97	116.45		7.21	299.51		3.88	334.53		7.80	271.65
6.02	200.62		6.87	215.54		3.46	484.88		6.11	93.54
8.94	159.01		7.26	346.80		4.63	306.24		8.00	363.19
7.28	151.81		6.41	287.27		6.56	222.91		8.24	393.66
			6.50	192.49		8.73	300.71		10.16	718.29
			6.73	271.01		8.46	185.36		11.67	130.96
			6.99	242.27		7.96	265.60		9.82	334.35
			6.24	202.13		7.70	212.72		10.79	384.03
			3.43	659.27		3.03	153.32		10.19	211.95

Table 5. Accumulation of Tetrachlorobiphenyl from Water-Only Exposures.

4°C		8°C		12°C		16°C	
Tissue (mg)	ku (ml/g/h)	Tissue (mg)	ku (ml/g/h)	Tissue (mg)	ku (ml/g/h)	Tissue (mg)	ku (ml/g/h)
1.71	50.91	2.39	114.48	2.89	203.70	1.52	324.44
2.44	86.87	2.21	138.79	2.69	270.10	1.84	283.38
2.84	67.30	2.80	116.89	2.12	246.56	1.78	379.68
2.02	60.32	2.67	106.47	3.57	172.24	1.83	309.62
1.73	73.33	2.47	159.73	2.28	393.08	2.02	254.31
2.04	89.31	2.28	156.93	1.97	295.29	1.93	231.44
2.08	78.68	2.46	72.37	2.06	271.48	1.42	401.33
2.11	72.66	2.53	120.69	2.40	316.20	2.89	351.83
2.75	77.45	2.66	135.11	1.89	277.63	1.84	734.30
1.97	87.69	2.48	89.24	2.48	273.35	2.30	599.07
2.12	72.25	2.44	138.87	2.31	245.02	2.29	371.53
2.57	70.95	2.28	135.84	2.14	226.43	1.87	408.10
1.95	78.64	1.78	142.23	3.57	172.24	3.57	285.02
3.75	59.64	4.14	99.94	5.23	167.76	3.30	355.00
3.28	29.62	4.37	132.22	3.77	197.58	3.31	332.01
3.73	62.65	4.66	68.02	3.35	205.88	3.03	326.23
3.73	42.84	5.02	79.59	5.37	221.25	4.73	318.98
3.51	64.16	4.36	86.61	5.50	227.36	3.92	557.38
5.20	48.26	4.94	84.18	3.44	252.51	3.50	329.25
4.68	54.17	3.53	120.73	4.51	272.40	3.05	307.56
4.05	54.52	4.79	80.93	3.60	252.10	3.89	454.34
2.81	63.60	5.43	64.74	3.87	216.37	4.02	333.93
4.22	42.49	4.78	68.60	5.13	207.61	3.29	361.84
4.33	65.63	4.36	87.08	3.47	244.48	3.29	423.05
4.90	61.96	3.42	94.64	4.35	285.21	4.87	342.84
4.40	54.13	5.88	72.12	5.95	153.46	5.92	238.25
6.36	40.76	6.47	53.86	5.72	126.22	4.68	369.52
7.38	37.45	6.28	51.85	5.36	165.33	3.26	316.42
6.45	46.78	7.12	51.54	7.19	215.95	5.14	464.68
8.11	44.40	6.46	59.35	6.50	159.30	4.09	284.64
6.85	44.67	6.90	53.45	6.91	165.69	4.32	349.28
6.82	48.91	8.24	65.13	6.36	175.25	4.41	335.65
7.66	42.02	6.75	37.16	6.90	136.93	4.97	490.44
6.24	53.01	7.35	53.02	9.88	85.37	5.16	163.87
7.46	40.45	7.99	55.75	6.44	216.22	5.28	289.47
6.50	40.89	7.73	43.32	6.86	185.40	4.52	261.66
8.92	28.81	7.39	58.84	9.36	142.45	6.57	231.03
6.46	46.08	8.62	56.20			5.85	207.48
5.96	52.82					6.62	208.38
						5.91	196.99
						7.41	217.62
						8.14	207.26
						5.79	263.73
						7.64	219.13
						6.54	257.27

Table 6. Accumulation of Hexachlorobiphenyl from Water-Only Exposures.

4°C		8°C		12°C		16°C	
Tissue (mg)	ku (ml/g/h)	Tissue (mg)	ku (ml/g/h)	Tissue (mg)	ku (ml/g/h)	Tissue (mg)	ku (ml/g/h)
2.05	216.65	2.73	245.56	1.98	296.84	2.22	164.07
1.92	203.47	2.40	311.59	1.78	95.96	1.60	433.11
2.12	160.04	2.44	239.66	2.97	233.55	1.79	265.17
1.82	289.70	2.61	313.10	2.28	285.78	1.95	209.67
1.96	181.05	2.25	322.32	2.67	212.10	1.84	261.64
2.05	137.88	2.32	392.02	3.08	169.68	2.88	351.04
1.83	190.38	2.34	325.88	2.72	245.09	1.17	241.69
2.65	144.05	2.41	367.05	2.93	222.41	1.15	274.91
2.57	248.97	2.47	229.50	2.17	284.71	2.74	309.40
1.66	141.31	2.64	179.94	2.42	145.72	2.78	255.55
2.08	116.11	1.98	349.24	2.44	284.08	1.63	417.50
2.14	170.62	2.90	370.06	2.33	293.84	2.67	285.88
1.98	156.06	5.16	193.92	5.71	195.78	2.83	249.96
6.94	123.65	4.09	237.57	4.86	147.41	2.96	258.09
7.76	210.59	5.94	338.37	6.13	124.39	2.79	360.22
6.97	159.75	3.19	379.63	3.55	196.13	2.48	327.46
7.34	194.02	3.64	226.32	5.40	185.31	2.01	267.69
7.36	104.37	4.95	273.62	4.47	141.13	2.25	338.51
6.49	155.98	4.18	211.17	3.61	160.70	2.55	300.74
8.70	138.86	4.60	202.68	4.18	252.63	1.91	392.18
7.21	123.31	3.82	307.84	4.22	206.94	2.53	357.18
7.43	102.76	4.17	228.40	3.22	302.40	1.45	209.89
6.47	108.81	3.26	352.28	7.67	219.43	3.75	218.57
8.83	74.41	5.20	189.24	5.20	127.57	3.39	411.70
5.79	102.10	5.51	173.26	7.12	188.58	3.14	281.43
7.54	60.14	7.13	117.65	5.28	156.61	3.62	293.41
10.16	153.92	7.13	138.00	7.78	128.44	3.20	284.31
9.62	153.21	3.66	362.39	9.39	139.14	3.32	369.43
9.83	207.29	7.28	139.52	5.49	181.52	3.51	253.81
13.33	106.65	7.67	189.31	8.48	109.66	4.22	229.67
14.24	158.16	6.53	306.02	5.49	195.48	3.18	309.15
12.71	93.41	6.27	185.94	6.82	130.51	3.73	281.75
13.38	96.13	7.85	196.12	6.15	119.43	3.42	219.32
14.47	84.42	7.12	125.68	5.04	192.37	3.54	182.46
13.38	170.46	6.45	121.05	8.92	219.71	3.09	300.07
11.87	240.29	6.84	441.00			3.97	280.38
14.24	66.11	2.93	743.24			9.74	106.13
12.89	184.94					8.12	125.24
						9.10	87.51
						8.29	39.88
						10.15	124.89
						10.56	117.46
						6.60	123.01
						8.27	118.11
						7.63	193.52
						8.07	47.25
						8.01	123.22

3.3 Elimination of PCB Congeners

Elimination rate constants (k_e's) were calculated for 4-chlorobiphenyl, dichlorobiphenyl, tetrachlorobiphenyl, and hexachlorobiphenyl at temperatures of 4, 8, 12, and 16°C. The equations for the k_e's of small, medium, and large animals from each study are shown in Tables 7-10.

Table 7. 4-Chlorobiphenyl Elimination Rate Constants.

Temp. (°C)	Size Class	k_e	S.E.	n	r^2	P
4	small	0.0074	0.00069	30	0.80	< 0.001
	medium	0.0078	0.00060	49	0.78	< 0.001
	large	0.0031	0.00088	39	0.24	= 0.001
8	small	0.0081	0.00079	30	0.78	< 0.001
	medium	0.0061	0.00082	31	0.64	< 0.001
	large	0.0058	0.00043	28	0.87	< 0.001
12	small	0.0098	0.00076	35	0.83	< 0.001
	medium	0.0080	0.00060	35	0.84	< 0.001
	large	0.0046	0.00077	34	0.52	< 0.001
16	small	0.0019	0.00040	24	0.50	< 0.001
	medium	0.0026	0.00039	39	0.53	< 0.001
	large	0.0032	0.00036	41	0.66	< 0.001

Table 8. Dichlorobiphenyl Elimination Rate Constants.

Temp (°C)	Size Class	k_e	S.E.	n	r^2	P
4	small	0.0055	0.00042	29	0.86	< 0.001
	medium	0.0035	0.00031	47	0.73	< 0.001
	large	0.0015	0.00055	44	0.13	= 0.008
8	small	0.0033	0.00081	20	0.46	< 0.001
	medium	0.0030	0.00056	26	0.52	< 0.001
	large	0.0021	0.00044	24	0.49	< 0.001
12	small	0.0059	0.00066	22	0.79	< 0.001
	medium	0.0045	0.00043	38	0.75	< 0.001
	large	0.0036	0.00051	29	0.64	< 0.001
16	small	0.012	0.0018	56	0.46	< 0.001
	medium	0.0036	0.00056	45	0.48	< 0.001
	large	0.0019	0.00046	71	0.18	< 0.001

Table 9. Tetrachlorobiphenyl Elimination Rate Constants.

Temp (°C)	Size Class	k_e	S.E.	n	r^2	P
4	small	0.000062	0.00047	27	0.0	= 0.90
	medium	0.00098	0.00047	43	0.07	= 0.04
	large	0.00038	0.00054	39	0.0	= 0.49
8	small	0.00062	0.00031	30	0.09	= 0.058
	medium	0.00076	0.00025	38	0.18	= 0.005
	large	0.00041	0.00046	37	0.0	= 0.38
12	small	0.0011	0.00037	27	0.23	= 0.007
	medium	0.0011	0.00049	30	0.11	= 0.040
	large	0.00006	0.00050	30	0.0	= 0.91
16	small	0.0015	0.00026	29	0.54	< 0.001
	medium	0.00082	0.00014	84	0.29	< 0.001
	large	0.000034	0.00017	54	0.0	= 0.84

Table 10. Hexachlorobiphenyl Elimination Rate Constants.

Temp (°C)	Size Class	k_e	S.E.	n	r^2	P
4	small	0.00058	0.00033	24	0.08	= 0.09
	medium	0.00061	0.00035	46	0.04	= 0.09
	large	0.00017	0.00034	39	0.0	= 0.63
8	small	0.00032	0.00030	28	0.01	=0.28
	medium	0.00014	0.00036	31	0.0	= 0.70
	large	0.00054	0.00034	28	0.05	= 0.13
12	small	0.00047	0.00034	30	0.03	= 0.18
	medium	0.0012	0.00031	38	0.28	< 0.001
	large	0.00037	0.00029	21	0.03	= 0.21
16	small	0.00051	0.00051	14	0.0	= 0.34
	medium	0.00013	0.00015	75	0.0	= 0.37
	large	0.000026	0.00015	30	0.0	= 0.86

3.4 Accumulation of PCB Congeners from Sediment

Sediment uptake rate constants (k_s's) were determined for small, medium and large *Diporeia* exposed to 4-chlorobiphenyl, dichlorobiphenyl, tetrachlorobiphenyl, and hexachlorobiphenyl at temperatures of 4, 8, 12, and 16°C (Table 11). Throughout the course of these studies the average pH of the overlying water was 8.1 (±0.18), and the average dissolved oxygen content of the water was 7.4 mg/L (±0.21). Dissolved oxygen in the overlying water was above 50% saturation at all times. The lipid and organic carbon contents of the sediment were also determined (Tables 12 and 13).

Table 11. Sediment Uptake Rate Constants (k_s) for 4-Chlorobiphenyl (MCBP), Dichlorobiphenyl (DCBP), Tetrachlorobiphenyl (TCBP) and Hexachlorobiphenyl (HCBP).

Temperature Size	ks (g $g^{-1}h^{-1}$) 1995	Error se	ks (g g^{-1} h^{-1}) 1996	Error se	ks (g $g^{-1}h^{-1}$) 1995	Error se	ks(g g^{-1} h^{-1}) 1996	Error se
	MCBP		**MCBP**		**DBCP**		**DBCP**	
4°C Small	0.015	0.002	0.015	0.0016	0.013	0.0008	0.018	0.0014
4° Medium	0.019	0.001			0.01	0.0004		
4°C Large	0.017	0.001			0.0065	0.0006		
8°C Small			0.025	0.004	0.014	0.003	0.079[b]	0.014
8°C Medium	0.017	0.002			0.011	0.0007		
8°C Large	0.017	0.001			0.009	0.0007		
12°C Small	0.004[a]	0.0005			0.018	0.004	0.037	0.0049
12°C Medium	0.0069[a]	0.00047			0.025	0.0071		
12°C Large	0.0045[a]	0.00068			0.014	0.0025	0.016	0.003
16°C Small			0.025	0.0037	0.021	0.0037	0.045	0.0058
16°C Medium	0.019	0.0029	0.053[b]	0.0049	0.031	0.0031		
16°C Large	0.013	0.0018			0.016	0.0011		
	TCBP		**TCBP**		**HCBP**		**HCBP**	
4°C Small	0.016	0.0043	0.012	0.003	0.019	0.0025	0.019	0.0025
4°C Medium	0.0039	0.00025			0.0072	0.00036		
4°C Large	0.0038	0.00036			0.0057	0.0007		
8°C Small	0.017	0.0041			0.031	0.0027		
8°C Medium	0.013	0.0014			0.017	0.0017		
8°C Large	0.0085	0.0012			0.0093	0.00094	0.017	0.0022
12°C Small	0.022	0.0053			0.031	0.0041	0.039	0.0057
12°C Medium	0.0087	0.00094			0.014	0.0012		
12°C Large	0.0049	0.00043			0.008	0.00057	0.022	0.0031
16°C Small	0.033	0.0083	0.034	0.0063	0.059[b]	0.012		
16°C Medium	0.025	0.0053	0.026	0.0045	0.02	0.0028		
16°C Large	0.017	0.0031	0.022	0.0029	0.019	0.0025		

a. Inexplicably small values.
b. Much larger than expected values.

Table 12. Percent Lipid in *Diporeia* spp. Used for Sediment Accumulation Studies.

Compound Size Class	Mean % Lipid 4ºC	Mean % Lipid 8ºC	Mean % Lipid 12ºC	Mean % Lipid 16ºC
4-Chlorobiphenyl				
Small	11.6	11	8.1	7.8
Medium	23.6	19.4	14.3	18.3
Large	25.1	24.5	19.2	17
Dichlorobiphenyl				
Small	12.2	13.6	7.8	7.7
Medium	21.7	18.5	15.7	17.8
Large	27.8	25.8	18.1	17
Tetrachlorobiphenyl				
Small	11.2	9.6	8	7.5
Medium	16.4	18	10.4	11.1
Large	18.5	16.8	16.1	16.6
Hexachlorobiphenyl				
Small	9.8	9.5	8.9	8
Medium	13.8	17.2	13.9	11.4
Large	19.1	18.4	15.3	15.5

Table 13. Percent Organic Carbon in Sediments Used for Accumulation Studies.

Year	Compound	Temperature (°C)	Mean % Organic Carbon	n
1995	4-Chlorobiphenyl and Dichlorobiphenyl	4C & 8C	0.37 (± 0.026)	11
	4-Chlorobiphenyl and Dichlorobiphenyl	12C & 16C	0.315 (±0.024)	10
	Tetrachlorobiphenyl and Hexachlorobiphenyl	4C & 8C	0.379 (±0.016)	17
	Tetrachlorobiphenyl and Hexachlorobiphenyl	12C & 16C	0.524 (±0.094)	11
1996	4-Chlorobiphenyl	4,8,12,16°C	0.422 (±0.047)	28
	Dichlorobiphenyl	4,8,12,16°C	0.359 (±0.017)	27
	Tetrachlorobiphenyl	4,8,12,16°C	0.479 (±0.018)	55
	Hexachlorobiphenyl	4,8,12,16°C	0.511 (±0.048)	67

4. REFERENCES

Alley, W.P., and S.C. Mozley. Seasonal abundance and spatial distribution of Lake Michigan macrobenthos, 1964-67. Special Report No. 54 of the Great Lakes Research Division, The University of Michigan, 103 pp. (1975).

Borgmann, U., and D.M. Whittle. Particle size conversion efficiency and contaminant concentrations in Lake Ontario biota. *Can. J. Fish. Aquat. Sci*. 40:328-336 (1983).

Bousfield, E.L. Revised morphological relationship within the amphipod genera *Pontoporeia* and *Gammaracanthus* and the glacial relict significance of their postglacial distributions. *Can. J. Fish. Aquat. Sci*. 46:1714-1725 (1989).

CAVALETTO, J.F., T.F. NALEPA, R. Dermott, W.S. GARDNER, M.A. QUIGLEY, and G.A. LANG. Seasonal variation of lipid composition, weight, and length in juvenile *Diporeia* spp. (amphipoda) from lakes Michigan and Ontario. *Can. J. Fish. Aquat. Sci*. 53:2044-2051 (1996).

Ditsworth, G.R., and D.W. Schults. Preparation of benthic substrates for sediment toxicity testing. *Environ. Toxicol. Chem.* 11:847-858 (1990).

EADIE, B. J., P. F. LANDRUM, and W. R. FAUST. Polycyclic aromatic hydrocarbons in sediments, pore water and the amphipod, *Pontoporeia hoyi* from Lake Michigan. *Chemosphere* 11:847-858 (1982).

EADIE, B. J., P. F. LANDRUM, and W. R. FAUST. Existence of a seasonal cycle of PAH concentration in the amphipod, *Pontoporeia hoyi*. In *Polynuclear Aromatic Hydrocarbons: A Decade of Research*, M. Cooke and A.J. Dennis (eds.). Battelle Press, Columbus, OH, 195-209 (1988).

Evans, M.S., G.E. Noguchi, and C.P. Rice. The biomagnification of polychlorinated biphenyls, toxaphene, and DDT compounds in a Lake Michigan USA offshore food web. *Arch. Environ. Contam. Toxicol*. 20:87-93 (1991).

GARDNER, W.S., W.A. Frez, E.A. Cichocki, and C.C. Parrish. Micromethod for lipid analysis in aquatic invertebrates. *Limnol. Oceanogr*. 30:1099-1105 (1985a).

GARDNER, W.S., T.F. NALEPA, W.A. Frez, E.A. Cichocki, and P.F. LANDRUM. Seasonal patterns of lipid content of Lake Michigan macroinvertebrates. *Can. J. Fish Aquat. Sci* 42:1827-1832 (1985b).

Hawker, D.W., and D.W. Connell. Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environ. Sci. Technol*. 22:382-387 (1988).

Kane Driscoll, S., P.F. LANDRUM, and E. Tigue. Accumulation and toxicokinetics of fluoranthene in water-only exposures with freshwater amphipods. *Environ. Toxicol. Chem*. 16:754-761 (1997).

LANDRUM, P.F. The effect of co-contaminants on the bioavailability of polycyclic aromatic hydrocarbons to *Pontoporeia hoyi*. In *Polynuclear Aromatic Hydrocarbons: Formation, Metabolism and Measurement 7th International Symposium*. M. Cooke and A.J. Dennis (eds.). Battelle Press, Columbus, OH, 731-743 (1983).

LANDRUM, P.F. Toxicokinetics of Polycyclic Aromatic Hydrocarbons in the amphipod, *Pontoporeia hoyi*: Role of Season and Temperature. *Aquatic Toxicol*. 12:245-271 (1988).

LANDRUM, P.F. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod, *Pontoporeia hoyi. Environ. Sci. Technol*. 23:588-595 (1989).

LANDRUM, P.F., H. Lee II, and M.J. Lydy. Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environ. Toxicol. Chem*. 11:1709-1726 (1992).

LANDRUM, P.F., S.R. Nihart, B.J. EADIE, and W.S. GARDNER. Reverse-phase separation method for determin ing pollutant binding to Aldrich humic acid and dissolved organic carbon of natural waters. *Environ. Sci. Technol*. 18:187-192 (1984).

LANDRUM, P. F., and C. R. Stubblefield. Role of respiration in the accumulation of organic xenobiotics by the amphipod, *Pontoporeia hoyi. Environ. Toxicol. Chem.* 10:1019-1028 (1991).

Morrison, H.A., F.A.P.C. Gobas, R. Lazar, and G.D. Haffner. Development and verification of a bioaccumulation model for organic contaminants in benthic invertebrates. *Environ. Sci. Technol*. 30:3377-3384 (1996).

Mozley, S.C., and R.P. Howmiller. Environmental Status of the Lake Michigan Region. Volume 6, ANL/ES-40, Argonne National Laboratory, Argonne, IL, (1977).

NALEPA, T.F. Estimates of macroinvertebrate biomass in Lake Michigan. *J. Great Lakes Res*. 15:437-443 (1989).

NALEPA, T.F. Status and Trends of the Lake Ontario Macrobenthos. *Can. J. Fish. Aquat. Sci*. 48:1558-1567 (1991).

NALEPA, T.F., M.A. QUIGLEY, K.F. Childs, J.M. Gauvin, T.S. Heatlie, M.P. Parker, and L. Vanover. Macrobenthos of Southern Lake Michigan, 1980-81. NOAA DR ERL GLERL-28, 273pp. (1985)

Parker, J.I. Predation by *Mysis relicta* on *Pontoporeia hoyi*: A food chain link of potential importance in the Great Lakes. *J. Great Lakes Res*. 6:164-166 (1980).

Peterson, S.R., and R.S. Ellarson. P,P'-DDE, polychlorinated biphenyls and endrin in oldsquaws in North America 1969-1973. *Pestis. Monit. J.* 11:170-183 (1978).

QUIGLEY, M.A., J.F. CAVALETTO and W.S. GARDNER. Lipid composition related to size and maturity of the amphipod *Pontoporeia hoyi. J. Great Lakes Res*. 15:601-610 (1989).

Segerstråle, S.G. The taxonomic status and prehistory of the glacial relict *Pontoporeia* (Crustacea Amphipoda) living in North American lakes. *Commentat. Biol.* (Soc. Sci. Fenn.) 89:1-18 (1977).

Thomann, R.V., J.P. Connnolly, and T.F. Parkerton. An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. *Environ. Toxicol. Chem*. 11:615-629 (1992).

Whittle, D.M., and J.D. Fitzsimons. The influence of the Niagara River on contaminant burdens of Lake Ontario Biota. *J. Great Lakes Res*. 9:295-302 (1983).