FINAL REPORT

VALIDATION OF SEDIMENT QUALITY CRITERIA DERIVED FROM EQUILIBRIUM PARTITIONING THEORY

To

DYNAMAC CORPORATION

ROCKVILLE, MARYLAND

By

LAKE SUPERIOR RESEARCH INSTITUTE

UNIVERSITY OF WISCONSIN-SUPERIOR

SUPERIOR, WISCONSIN

EPA CONTRACT NO. 68-C0-0070

WORK ASSIGNMENT NO. 1-21

Subcontractor Work Assignment Investigator: Daniel Call

Dynamac Work Assignment Director: Mark Sutherland

EPA Work Assignment Manager: Gerald Ankley

SUMMARY

This work was in support of the development of sediment quality criteria (SQC) by the U.S. Environmental Protection Agency (EPA). Two different tasks were associated with this work:

(1) A spiking experiment with the pesticide chlorpyrifos was conducted using two test sediments with different organic carbon concentrations and a benthic test species (Chironomus tentans). Exposures of the test organisms were for ten days and the endpoints of survival and growth were evaluated. Test sediments were analyzed for organic carbon and chlorpyrifos concentrations, and sediment pore water was isolated and also analyzed for chlorpyrifos. Data from these studies will enable determination of whether it is possible to use equilibrium partitioning theory to predict the bioavailability of chlorpyrifos in test sediments and thereby develop SQC for this pesticide.

(2) Sediment samples from a DDT-contaminated site were supplied to the contractor, and benthos in the samples were sorted and identified to species. These data were summarized and analyzed statistically. This task is in support of an ongoing effort by EPA to validate proposed SQC for DDT in the field.

These tasks were successfully completed and reports submitted to the EPA Work Assignment Manager, Gerald Ankley. This final report consists of two separate reports on each of the two tasks.

A STUDY OF THE PARTITIONING OF THE PESTICIDE, CHLORPYRIFOS, FROM LAKE SEDIMENTS TO PORE WATER AND THE TOXICITY OF SEDIMENT-SPIKED CHLORPYRIFOS TO *CHIRONOMUS TENTANS*

by

Daniel J. Call, Julie A. Swenson, and Michael D. Kahl

Lake Superior Research Institute

University of Wisconsin

Superior, WI 54880

January 12, 1993

CONTENTS

FIGURES

TABLES

I. INTRODUCTION

The equilibrium partitioning theory (EPT) approach in conjunction with existing water quality criteria (WQC) has been adopted by EPA for establishing sediment quality criteria (SQC) for nonionic organic chemicals (Di Toro et al., 1991). Chlorpyrifos is one of several pesticides for which SQC development is currently in progress.

This study was conducted to evaluate the EPT approach with chlorpyrifos. The EPT approach theorizes that an equilibrium is established between the concentration of a chemical associated with the bulk sediment and the concentration in sediment pore water. Further, the concentration dissolved in pore water is believed to be the bioavailable form and, consequently, the toxic form of the chemical. Bioavailability, bioaccumulation and toxicity of some sediment contaminants are better correlated with pore water concentrations than with bulk sediment concentrations (Swartz et al., 1990). In this study, chlorpyrifos was incorporated into a sediment-water test system, and an equilibrium established between sediment and pore water chlorpyrifos concentrations. A 10-day sediment toxicity test was performed with an infaunal benthic organism, Chironomus tentans, and the 10-day LC50 obtained in the sediment-water system compared to a 10-day LC50 obtained in a water-only system.

II. MATERIALS AND METHODS

A. Water-Only Toxicity Tests. Two benthic species, Chironomus tentans and Hyalella azteca, were used in 10-day toxicity tests with chlorpyrifos. Tests were performed using an intermittent-flow diluter system in which the chlorpyrifos concentrations in the exposure chambers were regularly renewed. Toxicant concentrations in the exposure chambers were measured on days 0, 4, 7 and 10 of the exposure period.

The exposure chambers were 300 mL high form beakers containing two screened holes (1.6 cm diameter) in the walls for water exchange. These beakers were placed into larger diluter exposure chambers. Duplicate beaker exposures were made. A replicated control for each test species received dilution water only, with no chlorpyrifos. Five exposure levels had mean concentrations between 0.015 and 0.828 μg/L. The working stock solution of approximately 32 μg/L was diluted from a column-generated stock solution.

In the test with Hyalella azteca, stainless steel screen was placed vertically in each beaker to provide a substrate. In the test with Chironomus tentans, a light layer (3.0 mL) of silica sand was sprinkled on the bottom of each beaker to provide a substrate for case building. The tests were performed at a nominal water temperature of 20° C using dechlorinated water from the City of Superior. Temperature, dissolved oxygen, pH, hardness, alkalinity, and conductivity were regularly measured during the test.

Ten animals were used per replicate. Hyalella azteca were 10 days old at the start of the exposure, and second instar C. tentans were 10 days old post-hatch. The test animals were fed daily. C. tentans received the daily equivalent of 4.0 mg dry Tetrafin^R in a slurry for each replicate. Hyalella received 0.8 mg of YCT slurry daily per replicate.

At the end of the 10-day exposure, observations for the replicates were pooled prior to statistical treatment. Survival was the sole endpoint in the *H. azteca* test. Survival and dry weight were the endpoints evaluated in the *C. tentans* test. Test data for the survival endpoint were adjusted as necessary for control mortality using Abbott's formula (Finney, 1971; Weber, 1991), and then analyzed by the Trimmed Spearman-Karber method for determination of LC50 values (Hamilton et al., 1977). Survival and growth data were also analyzed by one-way ANOVA in conjunction with a t-test with Bonferonni adjustment to determine the lowest observable effect concentration (LOEC) and the no observable effect concentration (NOEC).

B. Lake Sediment Total Organic Carbon (TOC) Analysis. Two lake sediment samples collected in the fall of 1991 from West Bearskin and Pequaywan Lakes, Minnesota, and maintained at 4 C in a cold room at the Environmental Research Laboratory-Duluth (ERL-Duluth) were analyzed for TOC content. An aliquot of each sediment sample was air-dried overnight under laboratory hood exhaust and ground to a fine powder in an agate mortar and pestle. A 300 to 500 mg portion of powdered sediment was weighed into a tared glass vial and ten percent hydrochloric acid was added dropwise to the vials, until foaming ceased, to eliminate inorganic carbon. The acid treated samples were dried overnight at 103° C, cooled in a desiccator, reweighed and ground in an agate mortar and pestle. A two to three mg portion of the powdered sample was weighed into a platinum sample boat and advanced into a Dorhman DC80 carbon analyzer furnace. The sample organic components were oxidized at 800° to CO_2 and measured by a linearized non-dispersive infrared detector. The results are reported as percent total organic carbon.

C. Preliminary Sediment-Spiked Chlorpyrifos Toxicity Test with *Chironomus tentans*. The sensitivities of H. azteca and C. tentans to chlorpyrifos were similar based upon 10-day toxicity tests in water only. It was decided that C. tentans would be used for further toxicity studies.

Based upon the mean measured TOC concentration of the higher TOC sediment (Pequaywan Lake) in conjunction with (a) sediment dry matter percent, (b) the log K_{ow} for chlorpyrifos, and (c) the water-only LC50 information, the following equilibrium partitioning equation (Pavlou and Weston, 1984; Pavlou, 1987; DiToro et al., 1991) was applied to determine the amount of chlorpyrifos to spike into the sediment to achieve exposures at high, medium and low levels:

$$C_p = C_s/(K_{oc} \times f_{oc})$$

where C_p = concentration of chlorpyrifos in pore water

C_s = concentration of chlorpyrifos in sediment (dry weight basis)

K_{oc} = chlorpyrifos partition coefficient for sediment organic carbon

f_{oc} = mass fraction of organic carbon of sediment

The log K_{oc} was determined from the log K_{ow} (octanol/water partition coefficient) by the equation:

Chlorpyrifos Log K_{oc} = 0.00028 + 0.983 (Log K_{ow}) (Di Toro et al., 1991).

A Log K_{ow} value of 4.82 was used for the initial sediment spiking determinations. More recent studies by the Athens Laboratory (U.S. EPA, Athens, GA), in which log K_{ow} determinations were made by the a slow-stir flask method (DeBruijn et al., 1989), have determined the appropriate Log K_{ow} to be 5.25.

A preliminary 10-day toxicity test was then conducted with Pequaywan Lake sediment spiked at low, medium and high concentrations, in duplicate. The medium concentration of spiked sediment was designed to yield a pore water concentration of chlorpyrifos that would closely approximate the water-only LC50 of 0.070 µg/L. A chlorpyrifos spiking solution of 78.21 µg/mL was prepared, and used to plate or shell-coat the walls of 1 gallon capacity glass jars in the following manner:

Sample	mL Chlorpyrifos Spiking Solution Added	Approximate mL of Acetone Added	Nominal Sediment Chlorpyrifos Conc. (µg/kg, dry weight)
Control	0	40	0
Low	1	39	69.8
Medium	4	36	279.3
High	16	24	1,117
High (duplicate jar)	16	24	1,117

The spiking solution and acetone were added to the jars, and the solvent was evaporated off over a steam bath in a hood while turning the glass jars by hand. The glass jars were held in a nearly horizontal position while turning to ensure a uniform plating of the jar walls with the pesticide.

Following evaporation of solvent, 4.0 kg of homogenized wet sediment from Pequaywan Lake was placed into each jar. The jars were placed on a rolling mill (Wheaton Co., Millville, NJ) maintained in a 4 C cold room, and continuously turned at a speed of 8 revolutions per minute until the sediment was used in the toxicity tests. The preliminary toxicity test was initiated after 39 days of rolling.

Pequaywan Lake sediment was determined to consist of a mean dry weight of 28.0 percent (n=4). Based upon the quantities of chlorpyrifos plated onto the jar walls, a sediment wet weight of 4.0 kg, and a sediment fractional dry weight of 0.280, nominal chlorpyrifos concentrations of 69.8, 279.3 and 1117 µg/kg in the sediment (dry weight basis) were prepared. A duplicate jar and toxicity test was performed at the high exposure level.

Samples of sediment, pore water, and overlying water were collected on days 0 and 10 of the toxicity test for analysis of chlorpyrifos concentration. Pore water was obtained by centrifugation at 10,000 x g for 30 min. at 4 C. All samples were stored in the refrigerator until analysis. A sample of the control sediment was spiked just prior to the chemical analyses to determine analytical recovery of chlorpyrifos. Duplicate analyses of one of the exposures were performed to determine the reproducibility of the data.

D. Definitive Sediment-Spiked Chlorpyrifos Toxicity Test with *Chironomus tentans*. The preliminary toxicity test with chlorpyrifos-spiked sediment indicated that a large loss (from 80 to 96 percent) of chlorpyrifos had occurred during the 39 days of equilibration on the rolling mill. Consequently, spiking levels were calculated to ensure that the definitive test would encompass a range of concentrations such that at least one exposure level would have a concentration of chlorpyrifos in pore water that approximated the water-only LC50 of 0.70 µg/L, and that this would pertain to both Pequaywan Lake and West Bearskin Lake sediments. Chlorpyrifos was coated onto the walls of 1 gallon capacity glass jars following the same procedure as described for the preliminary study. Stock solutions of 1,000.9 (spiking solution no. 1) and 78.21 (spiking solution no. 2) µg/mL were used to coat the jars as shown in Table 1.

TABLE 1. Volumes of chlorpyrifos spiking solutions used in 10-day definitive toxicity tests with *Chironomus tentans* and resultant nominal sediment concentrations.

Sample	mL Chlorpyrifos Spiking Solution [a] No. 1 Added	mL Chlorpyrifos Spiking Solution [a] No. 2 Added	Approximate mL of Acetone Added	Nominal Sediment Chlorpyrifos Concentration (µg/kg, dry wt.)
PEQUAYWAN LAKE SEDIMENT				
PEQ1 (Control)	0	0	40	0.00
PEQ2	0	3	37	209.5
PEQ3	0.5	1	38.5	517.0
PEQ4	2	0	38	1,787
PEQ5	6	3	31	5,571
PEQ6	21	0	19	18,770
WEST BEARSKIN LAKE SEDIMENT				
WBS1 (Control)	0	0	40	0.00
WBS2	0	2	38	75.35
WBS3	0	5	35	188.4
WBS4	1	2	37	557.5
WBS5	4	0	36	1,929
WBS6	13	0	27	6,268

[a] Spiking solutions No. 1 and 2 contained chlorpyrifos concentrations of 1.000.9 and 78.21 µg/mL, respectively.

West Bearskin Lake had a mean dry weight of 51.9 percent (n=4). Based upon the quantities of chlorpyrifos plated onto the jar walls, a sediment weight of 4.0 kg, and sediment fractional dry weights of 0.280 and 0.519 for Pequaywan and West Bearskin Lakes, respectively, nominal bulk sediment concentrations were calculated. For Pequaywan Lake, nominal sediment (dry wt.) chlorpyrifos concentrations were 0, 209.5, 517.0, 1,787, 5,571 and 18,770 µg/kg. For West Bearskin Lake, nominal sediment (dry wt.) chlorpyrifos concentrations were 0, 75.35, 188.4, 557.5, 1,929 and 6,268 µg/kg.

Due to concern regarding the possibility of missing a pore water concentration that approximated the 10-day LC50, a 0.3 dilution factor was used in the definitive study to provide a broader range of pore water exposure concentrations than would be provided by a 0.5 dilution factor.

For Pequaywan Lake sediments, with a mean measured f_{oc} of 0.0847 and a preliminary calculated K_{oc} of 54,954 (final calculated K_{oc} with new ERL-Athens K_{ow} data was 144,544), a sediment concentration (dry weight basis) of 326 µg/kg would theoretically yield a pore water concentration of 0.070 µg/L from the equilibrium partitioning equation, i.e., $C_p = C_s/(K_{oc} * f_{oc})$. Using a K_{ow} of 144,544, the corresponding chlorpyrifos concentration in dry sediment was 857 µg/kg.

For West Bearskin Lake, with a mean measured f_{oc} of 0.0302 and preliminary calculated K_{oc} of 54,954, a sediment concentration (dry weight basis) of 116.6 µg/kg would theoretically yield a pore water concentration of 0.070 µg/L. Using a K_{ow} of 144,544, the corresponding chlorpyrifos concentration in dry sediment was 306 µg/kg.

Prior to the start of the definitive test, it was necessary to determine if the test system was in equilibrium; i.e., if the concentrations of chlorpyrifos in the sediment and pore water were uniform over time. Samples of sediment and pore water were collected on days 13, 20, and 35 of constant rolling. Pore water was prepared by centrifugation at 10,000 x g for 30 min. at 4 C. The data indicated that the system was in equilibrium by day 35. (See Results, Table 9).

The definitive toxicity test was initiated with the addition of organisms after 42 days of mixing on the roller mill. Tests were performed with 300 mL high form beakers having screened holes for water exchange, as described previously for the water-only and preliminary sediment toxicity tests. Tests were performed in triplicate, with each beaker receiving 100 mL of sediment. Ten healthy-appearing second instar chironomids (12 days old post-hatch) from the University culture facility were added to each beaker at the start of the test. Each beaker received an equal volume of food each day, as in the water-only test.

Renewal water was supplied continuously to the rectangular chambers that housed the beakers. Water quality parameters of temperature, dissolved oxygen, and pH were recorded regularly on a daily basis. Hardness and alkalinity were measured in the overlying water on days 0 and 10 in the control, medium and high exposure chambers.

After 10 days of exposure, the sediment from each beaker was screened and the survivors counted. Surviving larvae were dried at 105 C for 24 hr and individually weighed. LC50s were determined on the basis of three different chlorpyrifos concentrations: (1) the mean measured pore water concentrations of chlorpyrifos sampled on days 0 and 10, (2) the mean measured chlorpyrifos concentrations in bulk sediment (dry weight) on days 0 and 10, and (3) the theoretical pore water concentrations determined from mean measured chlorpyrifos concentrations on days 0 and 10 in bulk sediment (dry weight) and application of the equation, $Cp = Cs/(K_{oc} * f_{oc})$ (Di Toro et al., 1991). The Pequaywan

Lake-spiked sediment mortality data were first smoothed and then adjusted for control mortality by Abbott's formula (Finney, 1971; Weber, 1991), prior to LC50 estimation by the trimmed Spearman-Karber method (Hamilton et al., 1977). The West Bearskin Lake-spiked sediment mortality data were first adjusted for control mortality by Abbott's formula prior to LC50 estimation by the trimmed Spearman-Karber method. Larval dry weight data were analyzed by one-way ANOVA and a t-test with Bonferonni adjustment to determine, where possible, the NOEC and LOEC for both bulk sediment and pore water.

E. Chlorpyrifos Chemical Analyses. Chlorpyrifos was analyzed in overlying water, pore water, and bulk sediment in this study. In the water-only toxicity test with chlorpyrifos, concentrations were measured in the water of one replicate for each exposure concentration on days 0, 4, 7 and 10. In the preliminary and definitive exposures with chlorpyrifos spiked sediments, all three matrices were sampled on days 0 and 10. Three separate beakers with spiked sediment only (no test organisms) were used for collection of chemistry samples in the definitive test.

Overlying water samples of 100 mL were withdrawn from the exposure chambers using a volumetric pipette. The sample was placed in a QorPak bottle and 5 mL of isooctane were added. The sample was extracted for 45 minutes using a magnetic stir bar and stir plate. Samples were allowed to stand for 10 minutes to allow for phase separation, after which an aliquot was removed with a Pasteur pipette, transferred to a GC vial, sealed, and refrigerated until analyzed.

Pore water (~30-45 mL) was first obtained by centrifugation of wet sediment (~150 mL) at 10,000 x g at 4 C. Chlorpyrifos was then extracted from a 25 mL water sample by vigorous shaking for 5 min. with 5 mL of hexane. The hexane was concentrated to a lesser volume (~0.5 to 2.5 mL) in isooctane. Pore water samples produced an emulsion following the initial extraction. To attempt to break the emulsion, the sample was then centrifuged in a swinging bucket centrifuge at 4,000 rpm for 30 min. at 5 C. The pore water was drawn off with a Pasteur pipette, passed through hexane-rinsed Na_2SO_4 in a funnel, and eluted with 10 mL hexane. The remaining entire emulsion mat was then also placed on the Na_2SO_4 and eluted with ~40 mL hexane. The hexane was concentrated in isooctane to a smaller volume (0.5-2.5 mL) for analysis by gas chromatography (GC).

Extraction of chlorpyrifos in sediment samples was achieved using an exhaustive steam distillation procedure (Veith and Kiwas, 1977; Knuth and Heinis, 1992). A sample of wet sediment weighing between 10 and 40 g was placed in a 1 L Erlenmeyer flask along with 20 g Na_2SO_4 and 200 mL of deionized water. The flask was attached to a steam distillation column fitted with a foam trap, both purchased from Ace Glass, Inc. (Vineland, NJ). Ten mL of 80:20 hexane:isooctane were introduced into the column as the extraction solvent. The apparatus was placed on a hot plate/stirrer and the mixture in the flask was heated to boiling while being stirred using a large, wedge-shaped stir bar. The sample was allowed to reflux for three hours. The extract was collected in a 15-mL centrifuge tube and stored in the refrigerator to await further clean-up.

The sediment extracts were concentrated to 2 mL in isooctane and sulfur was removed by adding 1 mL of isopropanol and 1 mL of a reagent of tetrabutyl ammonium hydrogen sulfate saturated with sodium sulfite and mixing for 2 minutes with a Vortex Genie Mixer (Jensen et al., 1977). Five mL of deionized water were added and the solution was mixed for an additional 2 minutes with the Vortex Genie Mixer. The phases were separated by centrifuging the sample for 2 minutes, using a bench-top centrifuge. At this point, the isooctane layer was drawn off and quantitatively transferred to a clean 15 mL glass centrifuge tube with a final volume of 10 mL. Samples were stored in the refrigerator until required for GC analysis. Just prior to GC analysis, samples were concentrated or diluted to a volume which would bring them within the range of the standards being used for instrumental analysis.

GC analysis was performed on Hewlett-Packard instruments (Models 5880 or 5890) equipped with 25 and 60 m DB-5 capillary columns. The GCs were equipped with 63 Ni electron-capture detectors, and data were collected with a Nelson Turbochrom II data system. Specific operating conditions are specified in Appendix C (Table C-1). Standards were prepared to produce a standard curve and detector responses to sample extracts were within the overall response range of the standards.

Quality assurance samples included blanks, matrix spikes, and duplicate analyses of at least one sample at each time of sampling. Recovery efficiencies and measures of repeatability (percent agreement of duplicates) were determined.

To facilitate the reporting of chlorpyrifos concentrations on a dry weight basis, 5 to 10 g wet weight of each sediment was placed into a preweighed beaker. The beaker was placed into an oven and dried overnight at 103° C. The sample was cooled to room temperature in a desiccator, reweighed and percent dry weight was calculated. Measured sediment concentrations of chlorpyrifos are reported on a dry weight basis.

III. RESULTS

A. Water-Only Toxicity Tests. *C. tentans* larvae were exposed to mean measured chlorpyrifos concentrations of <0.028, 0.015, 0.040, 0.132, 0.380 and 0.828 μg/L for 10 days. For the two replicates combined, mean mortalities at the five exposure levels (Table 2) resulted in an LC50 of 0.070 μg/L. Due to the dose-response relationship, 95% confidence limits were not determined. Five percent of the 20 control animals died. Measured concentrations of chlorpyrifos throughout the test are presented in Appendix A (Table A-1). Test conditions and basic water quality measurements are given in Appendix B (Table B-1).

TABLE 2. Mortalities of *Chironomus tentans* in a 10-day exposure to chlorpyrifos in a water-only test system.

Mean Chlorpyrifos Concentration (μg/L)	Rep. A[a]	Rep. B[a]	Total Mortalities	Percent Mortality[b]	Adjusted Percent Mortality[c]
Control (<0.028)	1	0	1	5	0.0
0.015	1	1	2	10	5.3
0.040	1	1	2	10	5.3
0.132	10	10	20	100	100.0
0.380	10	10	20	100	100.0
0.828	10	10	20	100	100.0

a/ Test was initiated with 10 organisms per replicate.
b/ Replicates A and B were pooled.
c/ Adjusted for control deaths by use of Abbott's formula (Finney, 1971; Weber, 1991).

Mean C. tentans dry weight comparisons for the pooled replicates (Table 3) showed that both of the lower two exposures with survivors after 10 days had identical mean dry weights of 0.0013 g. This represented a significant decrease from the mean control weight of 0.0016 g.

TABLE 3. Dry weight analysis of Chironomus tentans following a 10-day exposure to chlorpyrifos in a water-only test system.

Mean Chlorpyrifos Concentration (μg/L)	n	Mean Dry Weight (g)	s.d.[a]	c.v. %[b]
Control (<0.028)	19	0.0016	0.0004	24.0
0.015	18	0.0013*	0.0003	26.2
0.040	18	0.0013*	0.0006	46.8
0.132	0	-	-	-
0.380	0	-	-	-
0.828	0	-	-	-

a/ Standard deviation.
b/ Coefficient of variation.
* Significantly different from controls at 0.05 confidence level.

Since the lowest chlorpyrifos exposure resulted in a significant reduction in dry weight, it was not possible to obtain an NOEC (no observable effect concentration). The LOEC (lowest observable effect concentration) was less than 0.015 μg/L.

H. azteca juveniles were exposed to mean measured concentrations of <0.028, 0.015, 0.040, 0.132, 0.380 and 0.828 μg/L for 10 days. For the two replicates combined, mean mortalities at the five exposure levels (Table 4) resulted in an LC50 of 0.086 μg/L. Confidence limits (95%) were 0.072-0.103 μg/L. None of the 20 control animals died. Measured concentrations of chlorpyrifos throughout the test are presented in Appendix A (Table A-1). Test conditions and basic water quality measurements are given in Appendix B (Table B-1).

TABLE 4. Mortalities of Hyalella azteca in a 10-day exposure to chlorpyrifos in a water-only test system.

Mean Chlorpyrifos Concentration (μg/L)	Rep. A[a]	Rep. B[a]	Total Mortalities	Percent Mortality[b]
Control (<0.028)	0	0	0	0
0.015	0	0	0	0
0.040	0	0	0	0
0.132	10	7	17	85
0.380	10	10	20	100
0.828	10	10	20	100

[a] Test was initiated with 10 organisms per replicate.
[b] Replicates A and B were pooled.

B. Lake Sediment TOC Analysis. TOC was analyzed on four separate samples each for sediment from Pequaywan Lake and West Bearskin Lake (September-collected). Mean TOC levels were 8.47 and 3.02 percent for Pequaywan and West Bearskin Lakes, respectively (Table 5).

TABLE 5. Total organic carbon (% TOC) in sediment samples from Pequaywan and West Bearskin Lakes.

Lake	Sample Number 1	2	3	4	Mean % TOC	s.d.
Pequaywan	8.26	8.78	7.59	9.25	8.47	0.71
West Bearskin (Sept.-collected)	2.82	3.33	3.13	2.78	3.02	0.26

C. Preliminary Sediment-Spiked Chlorpyrifos Toxicity Test With *Chironomus tentans*. Pequaywan Lake sediment was spiked with chlorpyrifos at low, medium and high levels to yield nominal sediment concentrations (dry weight) of 69.88, 279.5 and 1,118 $\mu g/kg$. The spiked sediment was allowed to equilibrate for 44 days on the rolling mill before being sampled and analyzed for chlorpyrifos concentration on days 44 and 50. Bulk sediment concentrations off the rolling mill are presented in Table 6, and from the preliminary toxicity test in Table 7. Good agreement between samples taken from the roller mill jars and from the exposure systems on days 0 and 10 of the test in combination with very low recoveries from nominal concentrations in the sediment indicated that from 80 to 96 percent of the initial chlorpyrifos had been lost during the equilibration period.

The high exposure level of the toxicity test was replicated with a duplicate jar of sediment. The sediment in the toxicity test from the duplicate high level jar was higher in chlorpyrifos concentration than the other high level jar at both days 0 and 10 of the test. This observation proved to be useful in assessing the results from the toxicity test.

Results (Table 8) from the preliminary chlorpyrifos-spiked sediment toxicity test revealed a low level of mortality overall (0-25%) with the exception of the duplicate jar high level exposure, where 45% of the test animals died. This duplicate jar contained an average of 1.46 times more chlorpyrifos that the other high exposure.

TABLE 6. Chlorpyrifos concentrations (µg/kg, dry wt.)[a/] in sediment subsampled from the roller mill for the preliminary test.

Sample	Nominal Concentration (µg/kg)	Measured Concentration after 44 Days (µg/kg)	Percent of Nominal after 44 Days	Measured Concentration after 50 Days (µg/kg)	Percent of Nominal after 50 Days
Control	0.00	<0.585	-	<0.585	-
Low	69.88	5.66	7.1	3.97	4.6
Medium	279.5	16.64	5.1	14.02	4.1
High	1,118	138.0	10.6	219.7	16.3

a/ Concentrations were corrected for a mean analytical recovery of 76.8 ± 7.5 percent (n=4).

TABLE 7. Chlorpyrifos concentrations (µg/kg, dry wt.)[a/] in sediment from the preliminary toxicity test with *C. tentans*.

Sample	Nominal Concentration (µg/kg)	Measured Concentration at Day 0 of Toxicity Test	Percent of Nominal at Day 0	Measured Concentration at Day 10 of Toxicity Test	Percent of Nominal at Day 10
Control	0.00	<0.585	-	<0.585	-
Low	69.88	5.14	6.3	7.49	8.7
Medium	279.5	19.94	6.4	35.67	10.2
High	1,118	174.0	12.7	220.3	15.5
High Dup.	1,118	284.6	21.0	292.5	20.3

a/ Concentrations were corrected for a mean analytical recovery of 76.8 ± 7.5 percent (n=4).

TABLE 8. Mortalities of <u>Chironomus tentans</u> in chlorpyrifos-spiked sediment from Pequaywan Lake in a 10-day preliminary exposure.

Mean Measured Chlorpyrifos Concentration in Sediment (μg/kg Dry Weight Basis)	Number of Mortalities Rep. A[a/]	Rep. B[a/]	Percent[b] Mortality
Control (< -)	3	0*	10.0
Low (6.32)	3	2	25.0
Medium (27.80)	2	1	15.0
High (197.2)	0	0	0.0
High Duplicate Jar (288.6)	4	5	45.0

a/ Exposures were initiated with 10 organisms per replicate.
b/ Percent mortality is based on pooled data for replicates A and B.
* Eleven individuals were found in this replicate at test termination.

Pore water concentrations of chlorpyrifos were not reliably determined for the preliminary exposure due to the fact that methods development in handling emulsions was still in progress. Therefore, pore water concentrations for this test were predicted based on the equation, $Cp = C_s/(K_{oc} * f_{oc})$. Predicted pore water concentrations were 0.00136, 0.0060, 0.042, and 0.062 μg/L for the low, medium, high and high duplicate jar concentrations, respectively, using the log K_{ow} value of 4.82 in the calculation. These calculated pore water concentrations and mortality data were compared to the corresponding data in the water-only exposure, where 10% mortality occurred at 0.039 μg/L and 100% mortality at 0.128 μg/L. The water-only LC50 was 0.070 μg/L. From these data and comparisons, the nominal concentrations for the definitive exposure regime with chlorpyrifos-spiked sediment were determined.

D. <u>Definitive Chlorpyrifos-Spiked Sediment Toxicity Test With *Chironomus tentans*.</u> Based on the chemistry and survival data from the preliminary test, the nominal sediment concentration range for the definitive test for each of the two sediments was increased above the initially intended range. The range was also expanded by using a 0.3 dilution factor rather than a factor of 0.5, as originally intended. For Pequaywan Lake sediment, chlorpyrifos was spiked to provide a nominal dry weight concentration range from 210 to 18,770 μg/kg, representing increases from 3.0 to 16.8 times an earlier plan that had not accounted for the major observed loss of chlorpyrifos. For West Bearskin Lake sediment, chlorpyrifos was spiked to provide a nominal dry weight concentration range from 75.4 to 6,270 μg/kg, representing increases from 3.0 to 15.7 times the original plan.

The spiked sediment was sampled after 13, 20 and 35 days on the rolling mill to determine if an equilibrium in chlorpyrifos concentrations had occurred between sediment and pore water. Recovery-corrected concentrations of chlorpyrifos in the sediment and pore water (Table 9) indicated that an equilibrium had been achieved over 35 days. A definitive toxicity test was started after 42 days of equilibration.

The chlorpyrifos concentrations remained more stable with time than in the preliminary test. Some losses from nominal bulk sediment concentrations occurred in the lower exposures, particulary PEQ2 with a loss of approximately 84%, but the higher exposures were quite stable.

TABLE 9. Concentrations of chlorpyrifos in Pequaywan Lake sediment (dry weight) and pore water during equilibration on the rolling mill.

Nominal Chlorpyrifos Exposure Level (μg/kg Dry wt. basis)[b/]	Days of Equilibration 13	20	35
	Sediment Concentration (μg/kg)		
PEQ1 - Control (0)	<1.17	<1.17	<1.17
PEQ3 - 596	678	810	305
PEQ4 - 2,383	2,753	2,340	2,489
PEQ5 - 7,059	6,963	7,485	-[a/]
PEQ6 - 23,251	25,907	23,208	29,915
	Pore Water Concentration (μg/L)		
PEQ1 - Control (0)	<0.021	<0.021	<0.021
PEQ3 - 596	1.16	0.60	0.42
PEQ4 - 2,383	4.27	4.43	2.00
PEQ5 - 7,059	12.86	13.85	12.37
PEQ6 - 23,251	35.25	38.65	44.34

a/ Not determined.
b/ Nominal concentrations are based upon actual dry weights of each sample analyzed.

Results from the 10-day definitive toxicity test with Pequaywan Lake sediment are presented in Tables 10 and 11. LC50s were determined from the mortality data presented and chlorpyrifos concentration based upon (1) measured chlorpyrifos concentrations in the sediment (dry weight), (2) measured chlorpyrifos concentrations in the pore water, and (3) calculated chlorpyrifos concentrations in the pore water based upon measured concentrations in the sediment (dry wt.).

LC50s were 520 μg/kg, 0.76 μg/L and 0.043 μg/L when calculated on the basis of chlorpyrifos concentration in dry sediment, measured pore water and calculated pore water, respectively. The LC50 of 0.043 μg/L determined from chlorpyrifos pore water concentrations that were calculated from measured dry sediment concentrations compared more

TABLE 10. Mortalities of <u>Chironomus tentans</u> in chlorpyrifos-spiked sediment from Pequaywan Lake in definitive toxicity test.

Exposure Level	Number of Mortalities Rep. A[a]	Rep. B[a]	Rep. C[a]	Mortality[b]
PEQ1 (Control)	0	0[c]	2	6.7
PEQ2	1	0	0	3.3
PEQ3	4	3	5	40.0
PEQ4	10	10	8	93.3
PEQ5	10	10	10	100.0
PEQ6	10	10	10	100.0

[a] Exposures were initiated with 10 organisms per replicate.
[b] Percent mortality is based on pooled data for the three replicates.
[c] Eleven individuals were recovered at termination of test.

TABLE 11. Chlorpyrifos concentrations and mortality data for <u>Chironomus tentans</u> in definitive toxicity test with Pequaywan Lake sediment.

	Measured Chlorpyrifos in Dry Sediment[a] (µg/kg)	Measured Chlorpyrifos in Pore Water[a] (µg/L)	Calculated Chlorpyrifos in Pore Water[a,b] (µg/L)	Actual Percent Mortality[c]	Smoothed and Adjusted Percent Mortality[d]
PEQ1 (Control)	<1.17	<0.021	<0.0001	6.5	0.0
PEQ2	34.5	0.14	<0.003	3.3	0.0
PEQ3	480	0.58	0.039	40.0	36.8
PEQ4	2,149	2.31	0.176	93.3	92.9
PEQ5	6,636	9.36	0.542	100.0	100.0
PEQ6	23,656	26.2	1.93	100.0	100.0

[a] Mean measured recovery-corrected concentration for samples collected on days 0 and 10.
[b] Chlorpyrifos concentrations were calculated from the equation, $C_p = C_s/(K_{oc} * f_{oc})$, where C_s = mean measured chlorpyrifos concentration in dry sediment, K_{oc} = 144,544 and f_{oc} = 0.0847.
[c] Based upon 30 organisms total at each exposure level and with replicates A, B and C pooled.
[d] Mortalities smoothed and adjusted for control deaths by use of Abbott's formula (Finney, 1971; Weber, 1991).

favorably with the water-only LC50 of 0.070 μgL than did the LC50 based upon measured pore water concentrations. The dose-response relationship is given in Figure 1. Linear graphical interpolation between the two exposure levels that bracketed 50 percent mortality resulted in an estimate of the LC50 of 0.055 μg/L. The Spearman-Karber estimate was lower at 0.043 μg/L.

For the two exposure levels that bracketed the actual 50 percent mortality level (i.e., PEQ3 and PEQ4), the measured chlorpyrifos concentrations in the pore water were 16.9 and 10.6 times greater for the measured concentration than for the calculated concentration. This suggested that the concentrations measured in pore water consisted of more than the truly dissolved form of chlorpyrifos, likely represented in large part by organic carbon- associated chlorpyrifos. Pore water TOC content is being further investigated with these sediments under a separate contractual agreement. The level of agreement between the calculated pore water LC50 and the water-only LC50 is supportive of the EPT assumption that the truly dissolved form of a chemical is the bioavailable and toxic form. These data indicate that sediment dry weight measurements of chlorpyrifos can quite accurately predict toxic pore water concentrations, provided that K_{oc}, f_{oc} and water-only toxicity data are available.

Larval dry weight data were statistically analyzed (Table 12). Mean weights were similar between the control and the two lowest exposures. The two surviving larvae at the next higher exposure (PEQ4) had a mean dry weight that was 50 percent less than that of the controls, but was not significantly less. No larvae survived at the two

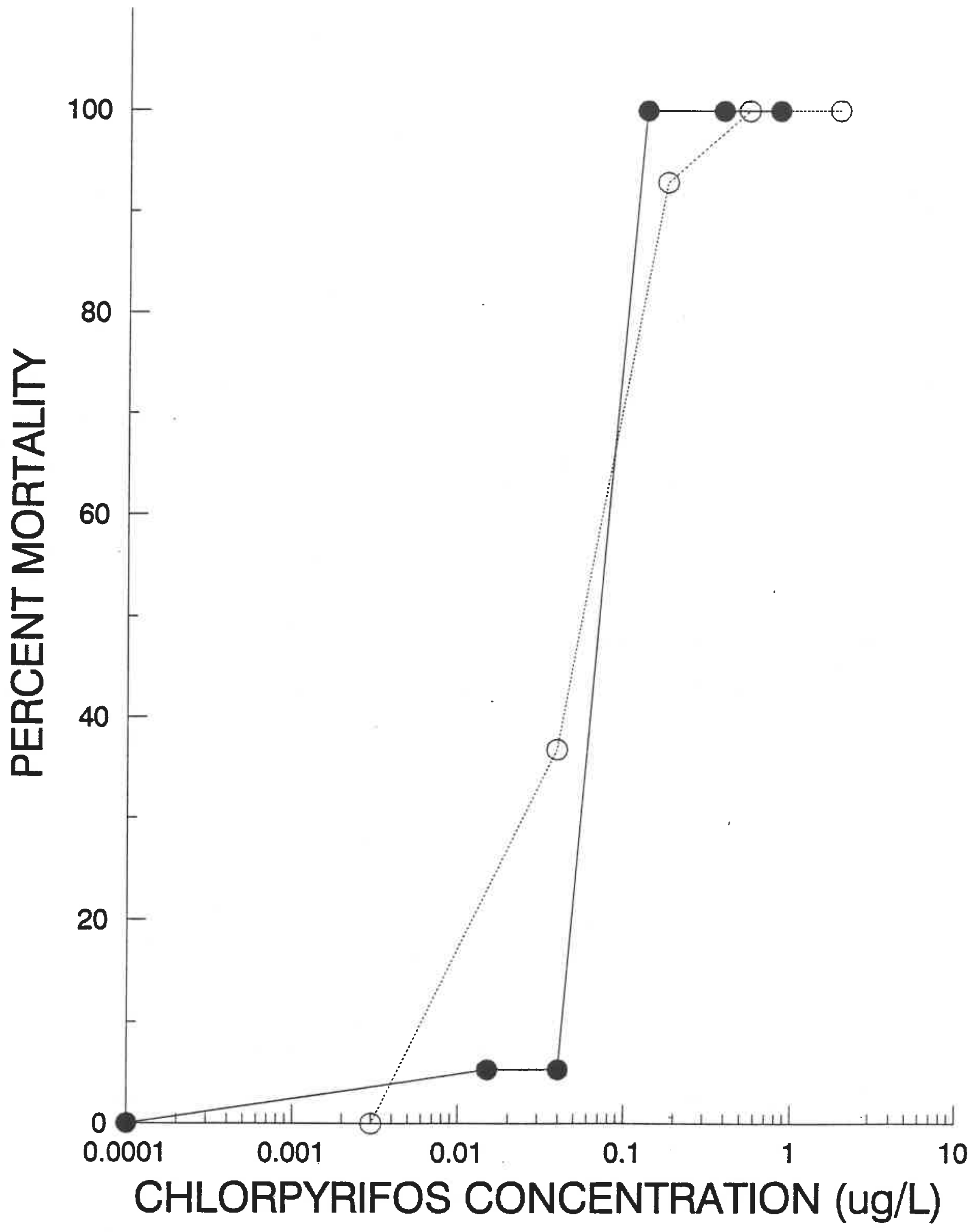

Figure 1. Plots of percent mortality versus chlorpyrifos concentration in water ($\log_{10}$ scale) for water-only toxicity test (solid line) and for calculated pore water chlorpyrifos concentrations in toxicity test with sediment from Pequaywan Lake (dotted line).

TABLE 12. Chlorpyrifos concentrations and larval dry weight data for <u>Chironomus tentans</u> in definitive 10-day toxicity test with Pequaywan Lake sediment.

Calculated Chlorpyrifos in Porewater[a]	n	Mean Larval Weight (g)	s.d.[b]	c.v. %[c]
PEQ1 (<0.000)	29	0.0015	0.0006	41.3
PEQ2 (<0.003)	28	0.0015	0.0006	39.2
PEQ3 (0.039)	18	0.0017	0.0006	34.6
PEQ4 (0.176)	2	0.0010	0.0006	67.0
PEQ5 (0.542)	0	-	-	-
PEQ6 (1.93)	0	-	-	-

[a] Chlorpyrifos concentrations were calculated from the equation, $C_p = C_s/(K_{oc} * f_{oc})$, where C_s = mean measured concentration in dry sediment, K_{oc} = 144,544, and f_{oc} = 0.0847.
[b] Standard deviation.
[c] Coefficient of variation.

highest exposures. These dry weight data did not allow for determination of an LOEC (i.e., lowest observable effect concentration) or an NOEC (i.e., no observable effect concentration).

Results from the 10-day definitive toxicity test with sediment from West Bearskin Lake are presented in Tables 13 and 14. LC50s were determined from the mortality data presented and chlorpyrifos concentrations in dry sediment, measured pore water, and calculated pore water, as described previously for Pequaywan Lake sediment. LC50s were 440 μg/kg, 1.43 μg/L and 0.099 μg/L when calculated on the basis of chlorpyrifos concentrations in dry sediment, measured pore water and calculated pore water, respectively.

The LC50 of 0.099 μg/L determined from chlorpyrifos pore water concentrations that were calculated from measured dry sediment concentrations compared more favorably with the water-only LC50 of

TABLE 13. Mortalities of *Chironomus tentans* in chlorpyrifos-spiked sediment from West Bearskin Lake in definitive toxicity test.

Exposure Level	Number of Mortalities Rep. A[a]	Rep. B[a]	Rep. C[a]	Percent Mortality[b]
WBS1 (Control)	1	2	2	16.7
WBS2	2	2	1	16.7
WBS3	3	3	1	23.3
WBS4	3	3	6	40.0
WBS5	10	10	8	93.3
WBS6	10	10	10	100.0

a/ Exposures were initiated with 10 organisms per replicate.
b/ Percent mortality is based on pooled data for the three replicates.

TABLE 14. Chlorpyrifos concentrations and mortality data for *Chironomus tentans* in definitive toxicity test with West Bearskin Lake sediment.

	Measured Chlorpyrifos in Dry Sediment[a] ($\mu g/kg$)	Measured Chlorpyrifos in Porewater[a] ($\mu g/L$)	Calculated Chlorpyrifos in Porewater[a,b] ($\mu g/L$)	Actual Percent Mortality[c]	Adjusted Percent Mortality[d]
WBS1 (Control)	<1.17	<0.021	<0.0003	16.7	0.0
WBS2	5.78	0.02	0.001	16.7	0.0
WBS3	27.1	0.09	0.006	23.3	7.9
WBS4	462	1.79	0.106	40.0	28.0
WBS5	1,330	3.24	0.305	93.3	92.0
WBS6	6,354	42.5	1.46	100.0	100.0

a/ Mean measured recovery-corrected concentration for samples collected on days 0 and 10.
b/ Chlorpyrifos concentrations were calculated from the equation, $C_p = C_s/(K_{oc} * f_{oc})$, where C_s = mean measured chlorpyrifos concentration in dry sediment, K_{oc} = 144,544 and f_{oc} = 0.0302.
c/ Based upon 30 organisms total at each exposure level with replicates A, B and C pooled.
d/ Adjusted for control deaths by use of Abbott's formula (Finney, 1971; Weber, 1991).

0.070 μg/L than did the LC50 based upon measured pore water concentrations. The dose-response relationship is presented in Figure 2. Linear interpolation between the two exposure levels that bracketed the 50 percent mortality level yielded an LC50 of 0.152 μg/L. The Spearman-Karber estimate of 0.099 μg/L was based on a linear best fit for the data points. For both sediments, the Spearman-Karber LC50 estimate was lower than the linear graphical interpolation estimate.

For the two exposure levels that bracketed the actual 50 percent mortality level (i.e., WBS4 and WBS5), the measured chlorpyrifos concentrations in the pore water were 16.9 and 10.6 times greater than for the calculated concentration. As with Pequaywan Lake sediment, this indicated that measured concentrations consisted of more than the truly dissolved form.

Larval dry weight data (Table 15) showed similar mean dry weights for the controls and three lowest exposure levels. The second highest exposure (WBS5) had a mean dry weight that was 50 percent less than that of the control group, but with only two survivors, it did not test significant. No survivors remained at the highest exposure. As with the Pequaywan Lake test results, these data also did not allow for determination of LOEC and NOEC values.

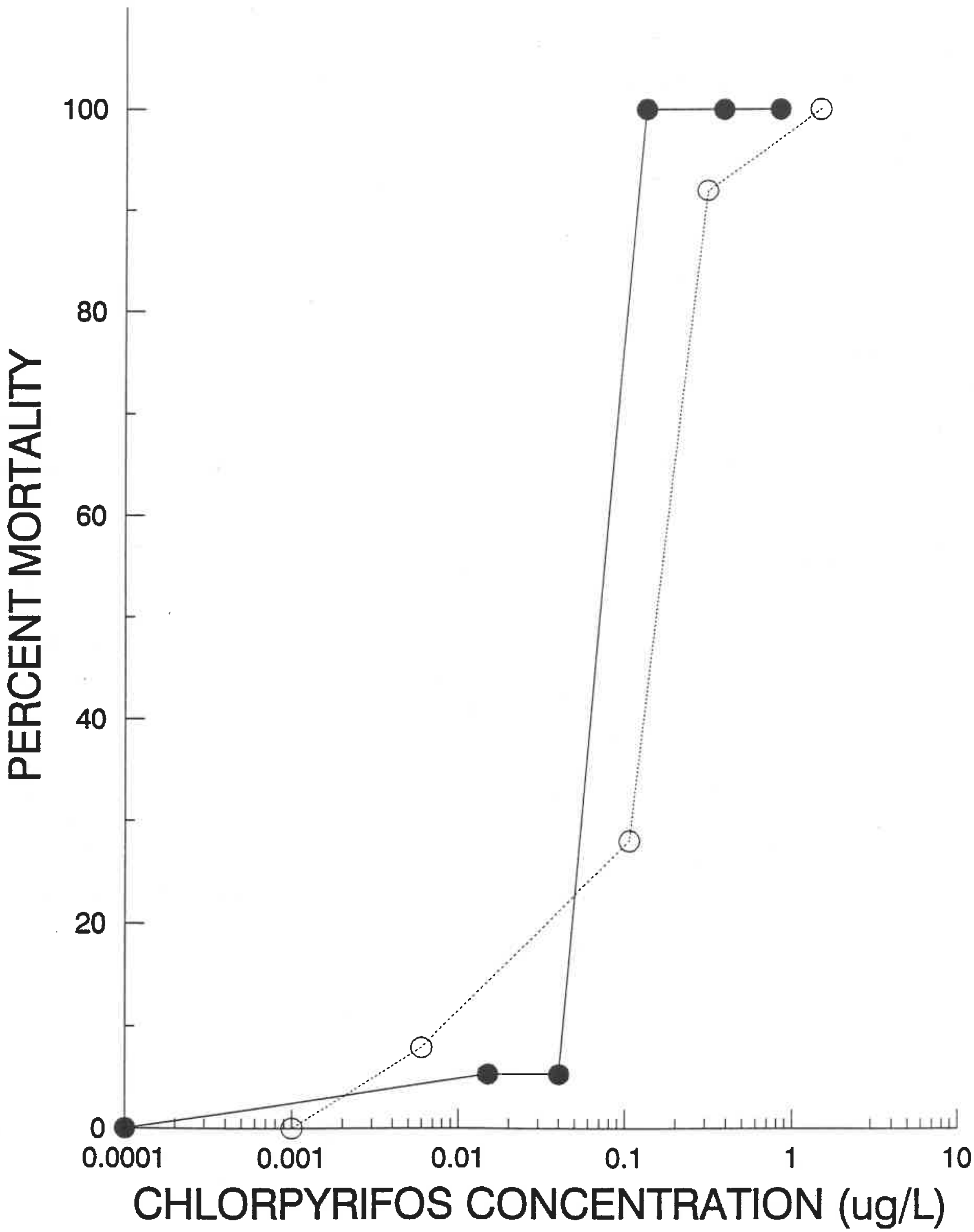

Figure 2. Plots of percent mortality versus chlorpyrifos concentration in water (log_{10} scale) for water-only toxicity test (solid line) and for calculated pore water chlorpyrifos concentrations in toxicity test with sediment from West Bearskin Lake (dotted line).

TABLE 15. Chlorpyrifos concentrations and larval dry weight data for <u>Chironomus tentans</u> in definitive 10-day toxicity test with West Bearskin Lake sediment.

Calculated Chlorpyrifos in Pore Water[a/]	n	Mean Larval Weight (g)	s.d.[b/]	c.v. %[c/]
WBS1 (<0.000)	25	0.0015	0.0006	40.1
WBS2 (0.001)	25	0.0015	0.0006	44.1
WBS3 (0.006)	23	0.0017	0.0006	36.1
WBS4 (0.106)	18	0.0017	0.0006	37.0
WBS5 (0.305)	2	0.0010	0.0000	0.0
WBS6 (1.46)	0	-	-	-

a/ Chlorpyrifos concentrations were calculated from the equation, $C_p = C_s/(K_{oc} * f_{oc})$, where C_s = mean measured concentration in dry sediment, K_{oc} = 144,544, and f_{oc} = 0.0302.
b/ Standard deviation.
c/ Coefficient of variation.

Measured concentrations of chlorpyrifos in overlying water were below detection limits for most samples (Table 16). Measurable quantities were observed at the two highest exposure levels for Pequaywan Lake spiked sediment and at the highest exposure level only for West Bearskin Lake spiked sediment. The fact that significant mortalities occurred at lower exposure levels for both sediments where chlorpyrifos concentrations in the overlying water were below detection indicated that overlying water was not an important route of chlorpyrifos exposure to chironomids at these levels.

E. <u>Chemical Quality Assurance/Quality Control</u>. Analytical recoveries of chlorpyrifos and agreement of duplicate analyses were determined for each matrix of concern. These data are summarized in Table 17. The data presented in the report were corrected for analytical recoveries.

TABLE 16. Concentrations of chlorpyrifos in overlying water (μg/L) in definitive 10-day toxicity tests with *Chironomus tentans*.

Day	PEQ1 (Control)	PEQ2	PEQUAYWAN LAKE PEQ3	PEQ4	PEQ5	PEQ6
0	<d.l.[a]	<d.l.	<d.l.	<d.l.	0.32	0.26
10	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	0.15

Day	WBS1 (Control)	WBS2	WEST BEARSKIN LAKE WBS3	WBS4	WBS5	WBS6
0	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	0.091
10	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	0.055

[a] Concentrations were below the analytical detection limit of 0.052 μg/L.

IV. CONCLUSIONS

The LC50 for chlorpyrifos with *Chironomus tentans* in a 10-day water-only toxicity test was 0.070 μg/L, as determined by the trimmed Spearman-Karber method. In chlorpyrifos-spiked Pequaywan Lake sediment, the LC50 based upon pore water concentrations calculated from measured dry sediment concentrations of chlorpyrifos and application of the equilibrium partitioning theory (EPT) equation, $C_p = C_s/(K_{oc} * f_{oc})$, more closely approximated the water-only LC50 than did the LC50 based upon actual measured concentrations of chlorpyrifos in the pore water. The LC50 based upon the calculated pore water concentrations was 0.043 μg/L, as determined by the trimmed Spearman-Karber method. For measured concentrations of chlorpyrifos in pore water, the LC50 was 0.76 μg/L. The higher LC50 value for measured chlorpyrifos in pore water was thought to be due largely to organic carbon-associated chlorpyrifos in the pore water samples.

TABLE 17. Analytical recoveries of spiked chlorpyrifos samples and agreement of duplicate analyses in the different study matrices.

Test and Matrix	Mean Percent Recovery (±s.d.)	Mean Percent Agreement of Duplicates (±s.d.)
Water-Only Toxicity Test		
Water	102.7 ± 10.2 (n=4)	96.4 ± 3.7 (n=4)
Preliminary Sediment Toxicity Test		
Bulk sediment	76.8 ± 7.5 (n=4)	97.2 ± 2.2 (n=2)
Definitive Sediment Toxicity Tests		
Sediment (roller mill samples)	73.2 ± 2.9 (n=3)	-
Porewater (roller mill samples)	88.6 ± 22.7 (n=3)	-
Pequaywan Lake bulk sediment	69.4 ± 0.4 (n=2)	90.4 ± 11.4 (n=2)
West Bearskin Lake bulk sediment	64.6 ± 0.7 (n=2)	97.2 ± 3.6 (n=2)
Pore water (Pequaywan and West Bearskin Lakes combined)	65.8 ± 15.2 (n=4)	68.4 ± 4.8 (n=4)
Overlying water (Pequaywan and West Bearskin Lakes combined)	99.8 ± 22.0 (n=4)	69.7 (n=1)

In chlorpyrifos-spiked West Bearskin Lake sediment, the LC50 based upon pore water concentrations calculated from measured dry sediment concentrations and application of the above EPT equation also more closely approximated the water-only test LC50 than did the LC50 based upon actual measured concentrations of chlorpyrifos in the pore water. The LC50 based upon calculated pore water concentrations was 0.099 µg/L, as determined by the trimmed Spearman-Karber method. The LC50 based upon measured concentrations of chlorpyrifos in the pore water was 1.43 µg/L. The elevated LC50 obtained with measured pore water chlorpyrifos concentrations was again attributed to quantities of chlorpyrifos present in association with organic carbon in the pore water samples.

LC50s based upon measured dry bulk sediment concentrations were 520 and 440 µg/kg for Pequaywan and West Bearskin Lakes, respectively. Organic carbon content of the two sediments was 8.47 and 3.02 percent, respectively.

Concentrations of chlorpyrifos in water overlying the spiked sediments were below the analytical detection limit of 0.052 µg/L for most samples analyzed at days 0 and 10 from both Pequaywan and West Bearskin Lakes. The absence of chlorpyrifos in overlying water at exposure levels with significant mortalities (e.g. exposure levels 3 and 4 for Pequaywan Lake and exposure levels 4 and 5 for West Bearskin Lake) indicated that overlying water was not an important route of exposure to chlorpyrifos at those levels.

The water-only LC50 for chlorpyrifos and the calculated pore water LC50s (calculated from measured dry weight bulk sediment concentrations and the EPT equation) differed by factors of 1.63 and 1.41 for Pequaywan and West Bearskin lakes, respectively. This level of agreement is supportive of the EPT approach. The study has illustrated the importance of a measure or estimate of the truly dissolved form of a chemical in sediment pore water when comparing toxicity from contaminated sediments with toxicity from water-only exposures.

V. REFERENCES

DeBruijn, J., F. Busser, W. Seinen and J. Hermens. 1989. Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the "slow-stirring" method. Environ. Toxicol. Chem. 8:499-512.

DiToro, D.M., C.Z. Zarba, D.J. Hansen, W.J. Berry, R.C. Swartz, C.E. Cowan, S.P. Pavlou, H.E. Allen, N.A. Thomas and P.R. Paquin. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environ. Toxicol. Chem. 10:1541-1583.

Finney, D.J. 1971. Probit analysis. 3rd ed. Cambridge University Press, London. 333 pp.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11:714-719.

Jensen, S., L. Renberg and L. Reutergardh. 1977. Residue analysis of sediment and sewage sludge for organochlorines in the presence of organic sulfur. Anal. Chem. 49:316-318.

Knuth, M.L. and L.J. Heinis. 1992. Dissipation and persistence of chlorpyrifos within littoral enclosures. J. Agric. Food Chem. 40:1257-1263.

Pavlou, S.P. 1987. The use of the equilibrium partitioning approach in determining safe levels of contaminants in marine sediments. pp. 388-412 In: K.L. Dickson, A.W. Maki and W.A. Brungs, eds. Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. Pergamon Press, Elmsford, NY.

Pavlou, S.P. and D.P. Weston. 1984. Initial evaluation of alternatives for development of sediment related criteria for toxic contaminants in marine waters (Puget Sound). Phase II. Development and testing of the sediment-water partitioning approach. Final Report. EPA Contract No. 68-01-6388. U.S. Environmental Protection Agency, Washington, D.C.

Swartz, R.C., D.W. Schultz, T.H. DeWitt, G.R. Ditsworth and J.O. Lamberson. 1990. Toxicity of fluoranthene in sediment to marine amphipods: a test of the equilibrium partitioning approach to sediment quality criteria. Environ. Toxicol. Chem. 9:1071-1080.

Veith, G.D. and L.W. Kiwas. 1977. An exhaustive steam distillation and solvent extraction unit for pesticides and industrial chemicals. Bull. Environ. Contam. Toxicol. 17:631-636.

Weber, C.I. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-600/4-90-027. Environmental Monitoring Systems Laboratory-Cincinnati, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

APPENDICES

APPENDIX A. Measured recovery-corrected concentrations (μg/L) of chlorpyrifos in water-only toxicity tests with *Chironomus tentans* and *Hyalella azteca*.

APPENDIX B. Water quality parameters in water-only toxicity tests and definitive sediment toxicity tests with chlorpyrifos (mean, standard deviation and range)

APPENDIX C. Gas chromatographic operating conditions for analysis of chlorpyrifos

APPENDIX A

TABLE A-1. Measured recovery-corrected concentrations[a] ($\mu g/L$) of chlorpyrifos in water-only toxicity tests with *Chironomus tentans* and *Hyalella azteca*.

Exposure Day	Exposure Chamber 1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
0	<0.028	<0.028	0.015	0.017	0.045	0.042	0.148	0.131	0.384	0.407	0.906	0.970
4	[b]	<0.028	-	0.020	-	0.039	-	0.152	-	0.416	-	0.825
7	<0.028	-	0.015	-	0.040	-	0.131	-	0.383	-	0.930	-
10	<0.028	<0.028	0.011	0.012	0.038	0.034	0.117	0.112	0.352	0.337	0.657	0.679
Replicate Mean	<0.028	<0.028	0.014	0.016	0.041	0.038	0.132	0.132	0.373	0.387	0.831	0.825
± s.d.	0.000	0.000	0.002	0.004	0.004	0.004	0.016	0.020	0.018	0.043	0.151	0.146
Treatment Mean	<0.028		0.015		0.040		0.132		0.380		0.828	
± s.d.	0.000		0.003		0.004		0.016		0.031		0.133	

[a] Concentrations were corrected for a mean analytical recovery of 102.7 percent.

[b] Not analyzed.

APPENDIX B

TABLE B-1. Water quality parameters in water-only toxicity tests and definitive sediment toxicity tests with chlorpyrifos (mean, standard deviation and range)

Test	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Water-only	19.8 ± 0.6 (18.5 - 21.0) n=112	8.9 ± 0.2 (8.0 - 9.5) n=112	8.0 ± 0.5 (7.1 - 9.1) n=112	47.3 ± 1.2 (46.0 - 48.0) n=3	48.0 ± 0.0 (48.0 - 48.0) n=3
Pequaywan Lake sediment	20.7 ± 0.6 (18.7 - 21.7) n=180	6.1 ± 1.0 (3.2 - 7.8) n=192	7.3 ± 0.2 (7.0 - 7.8) n=192	49.9 ± 1.4 (48.4 - 52.0) n=8	47.1 ± 1.6 (44.0 - 48.8) n=8
West Bearskin Lake sediment	20.6 ± 0.8 (18.2 - 21.6) n=54	6.2 ± 0.8 (3.3 - 7.7) n=162	7.4 ± 0.1 (7.2 - 7.8) n=192	50.4 ± 1.3 (48.4 - 52.4) n=8	49.4 ± 4.5 (46.0 - 60.0) n=8

APPENDIX C

TABLE C-1. Gas chromatographic operating conditions for analysis of chlorpyrifos

GC Conditions for Water-Only Test Analysis

GC: Hewlett Packard 5890
Column: J&W Brand 60 meter, DB-5
Injection Volume: 2 μL
Injector Temperature: 250°C
Detector Temperature: 350°C
Initial Temperature: 60°C
Initial Hold Time: 1 minute
Ramp: 20°C/minute
Final Temperature: 190°C
Final Hold Time: 0 minutes
Ramp A: 1.5°C/minute
Final Temperature A: 203°C
Final Hold Time A: 0 minutes
Ramp B: 30°C/minute
Final Temperature B: 300°C
Final Time B: 5 minutes
Chlorpyrifos Retention Time: 15.88 minutes

GC Conditions for Sediment, Pore Water and Overlying Water Analyses

GC: Hewlett Packard 5880
Column: J&W 25 meter, DB-5
Injector Temperature: 250°C
Detector Temperature: 275°C
Initial Oven Temperature: 80°C
Initial Hold Time: 1 minute
Ramp: 4°C/minute
Final Temperature: 176°C
Final Hold Time: 0 minutes
Ramp A: 20°C/minute
Final Temperature A: 300°C
Final Hold Time A: 2 minutes
Retention Time: 21.85 minutes