The toxicity of *1,2*-dichlorobenzene, *1,4*-dichlorobenzene, *1,2,3*-trichlorobenzene and *1,2,4,5*-tetrachlorobenzene to two species of freshwater benthic invertebrates in spiked-sediment toxicity tests

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EXECUTIVE SUMMARY

The toxicity of two isomers of dichlorobenzene, the congener *1,2,3*-trichlorobenzene and the congener *1,2,4,5*-tetrachlorobenzene to two species of freshwater benthic invertebrates (the mayfly *Hexagenia* spp. and the oligochaete worm *Tubifex tubifex)* was determined in chronic, spiked, whole-sediment toxicity tests. Concentrations of each congener in the overlying water, pore water and bulk sediment were measured analytically at test initiation (T=0) and test termination (T=21- or 28-d, respectively) and the total concentration for exposure for each bioassay was estimated assuming first order degradation/dissipation kinetics. Concentrations are therefore expressed in this document as the geometric mean of exposure during the bioassay due to the decrease in concentration of each chlorobenzene over the course of the experiments. The bulk sediment consistently contained the highest concentration of each chlorobenzene followed by lower concentrations in the pore water and the overlying water, respectively.

Acute toxicity was not observed for either species nor for any chemical tested with the exception of a small but statistically significant reduction in percent survival of the mayfly when exposed to a concentration of 3.7×10^4 ng/g *1,4*-dichlorobenzene. Sublethal effects on the growth of *Hexagenia* (*i.e.*, lower biomass measured as mg dry weight/individual in a treated sediment compared to control sediment(s)) were observed at the highest concentrations for three out of four chlorobenzenes *i.e.*, LOELs of 3.7×10^4 ng/g for *1,4*-dichlorobenzene and 1.1×10^5 ng/g for *1,2,3*-trichlorobenzene and *1,2,4,5*-tetrachlorobenzene. These reduced levels of growth were, however, within the range of values reported for the same species exposed to clean, reference sediments with similar particle size distributions and organic carbon content collected from the Laurentian Great Lakes. Reproduction by the oligochaete worm was also signficantly affected by several of the chlorobenzenes and reduced production of total young occured at mean exposure concentrations of 1.3×10^5 ng/g *1,2*-dichlorobenzene, 1.0×10^5 ng/g *1,4*-dichlorobenzene and 2.1×10^5 ng/g *1,2,3*-trichlorobenzene. This reduction in young was below the values reported for *T. tubifex* in clean, reference sediments with similar particle size distributions and organic carbon content. The congener *1,2,4,5*-tetrachlorobenzene had minor effects on growth of the mayfly and no effects on reproduction by the oligochaete worm.

The concentrations of chlorobenzenes which produced a toxic response in these two species of benthic invertebrates were lower than the maximum measured concentrations of these compounds reported in natural freshwater sediments by at least one order of magnitude and often two. For example, maximum measured concentrations of the chlorobenzenes in sediments collected from the Great Lakes have been reported as: 1.7×10^3 ng *1,2*-dichlorobenzene/g (St. Clair River; Government of Canada 1993a); 0.5×10^3 ng *1,4*-dichlorobenzene/g (St. Clair River; Oliver and Pubsley 1986; Government of Canada 1993b); 3.4×10^3 ng *1,2,3*-trichlorobenzene (MOEE 1995, unpublished data); 1.7×10^5 ng total trichlorobenzenes/g (an extreme case of all three isomers near the outfall of a storm

sewer near the Love Canal dump site in New York; Government of Canada 1993c); 7.0 X 10^3 tetrachlorobenzene/g (St. Clair River; Oliver and Pugsley 1992; Government of Canada 1993d).

In conclusion, the two isomers of dichlorobenzene, *1,2,3*-trichlorobenzene and *1,2,4,5*-tetrachlorobenzene had few effects of survival, growth and/or reproduction of two species of benthic invertebrates at environmentally relevant concentrations.

INTRODUCTION

The chlorobenzenes (dichlorobenzene, trichlorobenzene, tetrachlorobenzene and pentachlorobenzene) are a group of chemicals which have recently been assessed under the *Canadian Environmental Protection Act* (CEPA) (Government of Canada 1993a,b,c,d) for their effects on ecosystem and human health. All assessments concluded that "although significant exposure of benthic organisms in sediment may be occurring in specific aquatic ecosystems in Canada, adequate data on the toxicological effects [of these compounds] on these organisms were not identified". It was therefore not possible in these assessments to determine whether concentrations of these substances in sediments could result in harmful effects to biota which inhabit the benthos.

Benthic invertebrates have traditionally been considered the best overall indicators of contamination in sediments and several species have been recommended as suitable organisms for toxicological testing (Giesy *et al.* 1992; ASTM 1993). As a group, these organisms are in direct contact with particles of sediment as well as the interstitial water and they have have been shown to be sensitive to a wide variety of contaminants in toxicological studies (Burton *et al.* 1992; Phipps *et al.* 1995). The amphipod, *Hyalella azteca*, and the chironomids, *Chironomus tentans* or *C. riparius*, have received the most attention in studies with freshwater sediments (ASTM 1993; USEPA 1994) but other organisms such as the mayfly, *Hexagenia* spp. (Hanes *et al.* 1990; Bedard *et al.* 1992) and oligochaete worms, *(Lumbriculus variegatus* or *Tubifex tubifex)* (Reynoldson *et al.* 1991; Phipps *et al.* 1993) have also been used in studies on contaminated freshwater sediments, especially when samples are from fine-grained, nearshore areas in the Laurentian Great Lakes (Reynoldson *et al.* 1994, 1995).

The objective of this research was to determine the toxicity of several chlorobenzenes, *i.e.*, two isomers of dichlorobenzene *(1,2*-dichlorobenzene and *1,4*-dichlorobenzene), *1,2,3*-trichlorobenzene and *1,2,4,5*-tetrachlorobenzene in freshwater sediment using two species of benthic invertebrates, the mayfly, *Hexagenia* spp. and the oligochaete worm, *Tubifex tubifex.* The toxicity tests were chronic (21-d and 28-d, respectively) and the endpoints measured were survival and growth or reproduction depending on species.

Materials and Methods

Procedures for Spiking of Sediment

All sediment toxicity tests were conducted with a formulated sediment which consisted of a 4:1 mixture by wet volume of sieved (250 μm) natural sediment (collected from a wetland near Long Point, Lake Erie) and a mixture of 50% kaolin (Allen R) clay and 50% fine silica sand #75 (Hamr *et al.* 1994; Stephenson *et al.* 1995).

The resulting particle size distribution and organic/inorganic content of the formulated sediment is given in Table 1.

Table 1. Mean particle size distribution and carbon content of formulated sediment used in toxicity tests with chlorobenzenes.

% Sand	% Silt	% Clay	% Organic carbon	% Inorganic carbon
20.8 ± 1.9	44.6 ± 3.2	34.6 ± 1.6	3.93 ± 0.56	2.26 ± 0.38

The chlorobenzenes used in the study were purchased from Aldrich Chemical Co. and consisted of the following isomers: *1,2*- and *1,4*-dichlororbenzene (99+% purity); *1,2,3*-trichlorobenzene (99% purity); and *1,2,4,5*-tetrachlorobenzene (98% purity). Nominal concentrations of 0 (control), acetone control, 0.5, 5.0, 50 and 500 μg/g dry weight of sediment (determined by oven-drying the sediment at 60° C for 24 hours) were prepared for each isomer with the exception of *1,2,4,5*-tetrachlorobenzene. The highest concentration for this compound was 150 μg/g (d.w.) due to precipitation of the compound in solution at higher concentrations. The choice of the nominal concentrations was based on calculations to bracket the highest environmental concentrations reported in Government of Canada background material for each of the compounds (1993a,b,c,d) and estimated concentrations of each chlorobenzene in pore water based on the equilibrium partitioning theory (EqP) (Di Toro *et al.* 1991).

For each concentration of each chlorobenzene, 1 L (≈1215 g) of formulated sediment was poured into a 1 L square-sided glass container and an appropriate aliquot of a chlorobenzene stock solution dissolved in acetone was added. The glass containers were then fitted with plastic lids and placed on a side-to-side shaker at 175 agitations per minute for 2 hours. Following shaking, the spiked sediment was portioned into test beakers (125 g sediment for *T. tubifex* (300 mL beaker) and 180 g sediment for *Hexagenia* (1 L beaker). Overlying water (carbon-filtered, aerated, City of Burlington tap water; pH 7.8-8.3, conductivity 439-578 uohm/cm, hardness 119-137 mg/L) was then gently added (125 mL for *T. tubifex* and 650 mL for *Hexagenia*). The beakers were allowed to equilibrate for 2 weeks at 4°C in the dark before initiation of the toxicity test; loose-fitting plastic lids (petri dishes) covered each of the beakers and no aeration was provided during the equilibration period. Each concentration was replicated six times; of these six replicates, four were used for biological effects and two were analyzed chemically for the concentrations of each chlorobenzene in bulk sediment, pore water and overlying water. Of the samples taken for chemical analyses, one beaker was sampled at test initiation before the addition of animals (T = 0) and one beaker was sampled at test completion. The latter beaker(s) contained animals but the animals were not chemically analyzed for body burdens.

Toxicity Tests

Toxicity tests were conducted with two species of freshwater benthic invertebrates, the mayfly, *Hexagenia* (either *limbata* or *rigida* or both) and the oligochaete tubificid worm, *Tubifex tubifex*. Eggs of the mayfly *Hexa*genia spp. were collected during late June and July in 1994 according to the method of Hanes and Ciborowski (1992) and stored at 8 ° C until used in toxicity tests. Oganisms were cultured using the procedure of Bedard *et al.* (1992). The culture of *T. tubif*ex is described in Reynoldson *et al.* (1991) and bioassay procedures for both species are described in Day *et al.* (1994) and Reynoldson *et al.* (1995). *Hexagenia* nymphs were 1.5 to 2 months old (approximately 5 to 10 mg wet weight) and *T. tubifex* adults were 8-9 weeks old at test initiation.

Twenty-four hours prior to test initiation, the beakers containing the spiked sediment and overlying water were removed from 4° C, placed in an environmental chamber at 23±1° C with a 16L:8D photoperiod, and each beaker was aerated gently with a Pasteur pipette and air bubbler. Tests were initiated with the random addition of 10 organisms per jar for *Hexagenia* spp. and 4 organisms per beaker for *T. tubifex*.

Tests were static with the periodic addition of distilled water (≈ 25 mL/650 mL for *Hexagenia* and 20 mL/100 mL for *T. tubifex* over the course of each bioassay) to replace water lost due to evaporation. Each beaker was covered with a plastic petri dish with a central hole for aeration using a Pasteur pipette and air line. *T. tubifex* were fed 80 mg Nutrafin^{R} at test initiation and *Hexagenia* were fed 0.5 mL of a mixture of brewer's yeast, Cerophyll^{R} (Agri-Tech, Kansas City, MO) and Nutrafin^{R} at test initiation and twice weekly during the test. Dissolved oxygen concentrations and pH were measured at the beginning, middle and end of each exposure period; ammonia was measured at test conclusion. Tests for the biological samples were terminated after 21 d for *Hexagen*ia and 28 d for *T. tubifex* by passing the sediment samples through a 500 µm mesh sieve after all samples for water chemistry had been taken. Sediment from the *T. tubifex* test was passed through an additional 250 µm mesh sieve at test completion for collection of cocoons and small young.

Endpoints measured in the tests were survival and biomass of *Hexagenia* and survival and reproduction measured as total number of cocoons and young per four adults of *T. tubifex*. Mean dry weights of *Hexagenia* spp. were determined after drying the surviving animals from each treatment replicate as a group to a constant weight in a drying oven (60° C).

Dose-response data for each of the congeners were analyzed for normality and homogeneity of variance followed by ANOVA. When statistically significant differences were noted among the responses for each concentration, comparison of means was conducted using Dunnett's test and the solvent control (using Sigmastat^{R}). All levels of signficance are at the $P<0.05$ unless otherwise stated.

Chemical Analysis

Sample Collection at Test Initiation, T = 0

Prior to the addition of animals to the beakers, samples of the overlying water, pore water and bulk sediment were taken form one beaker per concentration. Replication of chemical samples was not possible due to limtied budget. Overlying water samples were collected by decanting the water from each beaker carefully into cleaned, solvent rinsed glass bottles. The wet sediment was transferred to polyethylene centrifuge bottles and the pore water separated by refrigerated centrifugation at 10,000 RPM and 4°C. The pore water was transferred into 250 mL separatory funnels and allowed to reach room temperature prior to extraction. The sediment was transferred to cleaned solvent-rinsed jars and refrigerated at 4 °C until extraction.

Extraction and Clean-up

The extraction and clean up procedures used in this report were modified from the standard procedures of the Organic Contaminants Laboratory, Aquatic Ecosystem Restoration Branch, National Water Research Institute, Burlington, Ontario, Canada.

Overlying Water and Pore Water:

A brominated benzene was added to the samples prior to extraction to monitor any losses that occurred throughout the procedure and to adjust for instrument discrimination. The overlying water samples were extracted three times with 50 mL of dichloromethane and passed through fired sodium sulphate. After the addition of 25 mL of hexane, the extracts were evaporated to approximately 5 mL by rotary evaporation and quantitatively transferred to 15 mL centrifuge tubes. The extracts were then evaporated to approximately 1 mL under dry nitrogen at 35° C. The 1 mL extract was then subjected to clean-up on a small pasteur pipette column of 6 cm of fully activated Florisil toped with 1 cm of fired sodium sulphate. A total of 10 mL of hexane eluate was collected and evaporated to 1 ml under dry nitrogen at 35 ° C. The extracts were then transferred to a GC vial.

Sediment :

A brominated benzene was added to the sample prior to extraction to monitor any losses that occurred throughout the procedure and to adjust for instrument discrimination. A 2-4 g subsample of wet sediment mixed with 10 g of fired sodium sulphate in a glass thimble was soxhlet extracted for 12 h with dichloromethane. After the addition of 25 mL of hexane, the extracts were evaporated to approximately 5 mL by rotary evaporation and quantitatively transferred to 15 mL centrifuge tubes. The

extracts were then evaporated to approximately 1 mL under dry nitrogen at 35 ° C and stored as above. Representative samples of sediment were used to determine the percent moisture and the final results calculated on a dry weight basis.

Analysis and Quantitation

Samples were analyzed on a Hewlett-Packard 5890 GC equipped with a 5871A MSD detector, a 5673 autosampler, electronic pressure control (EPC) and a 30 m HP-5MS column (Hewlett-Packard, 0.25mm i.d./0.25 um film). The carrier gas was helium with a constant flow of 1.38 ml/min. The spectrometer was tuned using perfluorotributlyamine prior to each set of samples using the autotune procedure provided by the manufacturer. The mass spectrometer was set in SIM mode and the following ions (Table 2) monitored for each compound set.

Table 2. Ions monitored in mass spectrometer.

	Ions Monitored		Ions Monitored
Dichlorobenzene	***146***, 111	Dibromobenzene	***236***, 238
Trichlorobenzene	***180***, 182	Tribromobenzene	***314***, 316
Tetrachlorobenzene	***214***	Tetrabromobenzene	***314***

Samples (1 uL) were injected using a 1 min. splitless injection with the injector at 250° C and the transfer line at 290° C. The oven was held at 60° C for 2 min., heated at 10° C/min to 90° C, and then heated at 3° C/min to 150° C, temperature programmed at 20° C/min to 285° C and held for a further 5 min. The filament and multiplier were turned on at 5 min. and data were acquired for 6 min. for the di-chlorobenzenes, 14 min. for the trichlorobenzenes and 25 min. for the tetra-chlorobenzenes. An external standard was run every 6 samples and the response factors and retention times adjusted. The final data were adjusted to the recoveries of the brominated surrogate standards (Table 3).

Table 3. Average percent recovery of 1,3-DBB internal control standard.

	1,2- dichlorobenzene	*1,4-* dichlorobenzene	*1,2,3-* trichlorobenzene	*1,2,4,5-*tetra-chlorobenzene
Overlying water	74.9 ± 29.6	87.9 ± 15.7	87.7 ± 24.2	100.5 ± 19.9
Pore water	90.5 ± 87	88.9 ± 10.9	93.8 ± 17.2	91.8 ± 24.2
Bulk sediment	77.0 ± 14.2	82.9 ± 14.8	93.3 ± 21.7	83.8 ± 31.3

Minimum peak areas were determined using representative chromatograms and a nominal detection limit calculated as follows:

Table 4. Detection limits for chemical analyses.

	Nominal Detection Limit
Dichlorobenzene	20 ng/mL or g
Trichlorobenzene	10 ng/mL or g
Tetrechlorobenzene	160 ng/mL or g

The detection limit is dependent on the amount of sample used for the analysis, *i.e.*, if the sample size was 40 mL, the detection limit for dichlorobenzene would be 20 ng/40 mL= 0.5 ng/mL. An average value for the volume of overlying water, pore water, and sediment can be used to calculate the detection limit for each test species. The detection limits for each matrix and for each toxicity test are presented in Table 5.

RESULTS:

Chemical Analysis

The concentrations of each isomer in the overlying water, pore water and bulk sediment at T = 0 (placement of organisms in beakers) and T = 21 d or 28 d (termination of test with *Hexagenia* spp. and *T. tubifex*, respectively) are presented in Tables 7 to 14. The geometric mean of each concentration assuming first-order kinetics over time is also presented in each table.

The bulk sediment contained the highest concentration of each chlorobenzene followed by concentrations in the porewater and the overlying water, independent of the

isomer used for spiking. Total concentrations of each isomer at T = 0 were lower than the nominal concentration and ranged from approximately 31-52% of nominal for *1,2*-dichlorobenzene, 24-51% of nominal for *1,4*-dichlorobenzene, 36-69% of nominal for *1,2,3*-trichlorobenzene and 89-118% of nominal for *1,2,4,5*-tetrachlorobenzene. Collection of samples for the analyses of each chemical in the various phases occured after the equilibration period of 2 weeks; therefore, concentrations lower than nominal can be expected due to losses of each compounds through volatilization or degradation.

Although chemical analyses of each compound was not replicated in any given bioassay, the same batch of spiked sediment was used for each concentration for each of the two invertebrates used in the bioassays. Therefore, comparisons of measured concentrations in each environmental matrice (overlying water, pore water and bulk sediment) for individual compounds provides an indication of the reliability of the chemical analyses. For example, concentrations of 144,496 ng/g and 145,339 ng/g of *1,4*-dichlorobenzene were measured in the bulk sediment for the *Hexagenia* and the *T. tubifex* bioassay, respectively (Tables 9 and 10).

Each chlorobenzene decreased in concentration over the course of the experiment in all environmental matrices. At T=21 d in the *Hexagenia* spp. toxicity test, *1,2*-dichlorobenzene ranged from 5.6-19.8% of concentrations at T = 0; *1,4*-dichlorobenzene ranged from 6.2-18.5%; *1,2,3*-trichlorobenzene ranged from 13.5-21.4%; and *1,2,4,5*-tetrachlorobenzene from 36.7-112%. At T = 28 d in the *T. tubifex* toxicity test, *1,2*-dichlorobenzene ranged from 37.1-41.8% of concentrations at T = 0; *1,4*-dichlorobenzene ranged from 27.1-48.9%; *1,2,3*-trichlorobenzene ranged from 34.9-89.1% of concentrations at T=0; and *1,2,4,5*-tetrachlorobenzene from 71.7-82.1%.

Toxicity

The results for the benthic invertebrate toxicity tests with the two species exposed to various concentrations of chlorobenzenes spiked into a formulated sediment are presented in Figures 1 to 8. Concentrations of each chlorobenzene shown on the graphs are expressed as the geometric mean of the total concentration in all three matrices (*i.e.*, overlying water, pore water and bulk sediment).

1,2-dichlorobenzene

Survival and growth of the mayfly, *Hexagenia* spp., was not reduced by any concentration of *1,2*-dichlorobenzene (Fig. 1). Both parameters were within one standard deviation of values determined in reference, 'clean' sediments collected from the Great Lakes with particle size distributions and TOC similar to the sediment used in the bioassays in this study (Fig 1; Table 6).

All adults of *T. tubifex* placed into spiked sediment also survived exposure.

Production of cocoons by this species was not affected by the contaminant but the highest concentration of *1,2*-dichlorobenzene (geometric mean 131,000 ng/g) caused a significant reduction in total young compared to lower concentrations (Fig. 2). However, this reduction in young was not significantly lower than values reported in reference sediments in the Great Lakes (Table 6).

1,4-dichlorobenzene

Survival and growth of the mayfly, *Hexagenia* spp., was reduced slightly in comparison to the solvent control at the highest concentration of 37,000 ng *1,4*-dichlorobenzene/g ((geometric mean) (Fig. 3). However, this reduction was well within the values for survival and growth of this species in reference' sediments from the Laurentian Great lakes (see above). All other concentrations had no signficiant effect on this species.

The highest concentration of this compound (geometric mean 105,555 ng/g) significantly reduced the production of total young by*T. tubifex* (Fig. 4) although survival of adult worms and production of cocoons were not affected by this concentration. The reduction in total young at 105,000 ng/g was also significantly below the values for reference sediment with similar characteristics.

1,2,3-trichlorobenzene

Survival of *Hexagenia* spp. exposed to concentrations of *1,2,3*-trichlorobenzene as high as 112,000 ng/g (geometric mean) was not affected but growth at this concentration was slightlybut signficantly reduced compared to the solvent control (Fig. 5). Again, this slight reduction in growth was still above the average level of growth for this species in clean Great Lakes' sediments.

All adult worms survived exposure to this compound in spiked sediment. Production of total young by *T. tubifex* was signficantly reduced by a concentration of 214,000 ng/g (geometric mean) but all lower concentrations had no effect on either production of cocoons or young (Fig. 6).

1,2,4,5-tetrachlorobenzene

There were few toxic effects from this compound on either species of invertebrate, Both survival of the mayfly (Fig. 7) and survival and production of young by the tubificid worm (Fig. 8) were not affected by the highest concentrations of 112,000 and 161,000 ng/g (geometric mean), respectively. Growth of the mayfly was slightly, but significantly reduced at a concentration of 112,000 ng/g compared to the solvent control but growth was still higher than average values for reference sediments.

DISCUSSION

There was very little toxicity observed for any of the isomers or congeners of the various chlorobenzenes used in this study. None of the compounds were acutely lethal at the highest concentrations tested. Significant sublethal effects were only observed for three compounds, *1,2*-dichlorobenzene, *1,4*-dichlorobenzene and *1,2,3*-trichlorobenzene and with one species, *T.tubifex,* at concentrations of 1.3×10^5, 1.0×10^5 and 2.1×10^5 ng/g (geometric mean concentrations over the 28-d bioassay). Maximum measured concentrations of the chlorobenzenes in sediments collected from the Great Lakes have been reported as: 1700 ng *1,2*-dichlorobenzene/g (St. Clair River; Government of Canada 1993a); 524 ng *1,4*-dichlorobenzene/g (St. Clair River; Oliver and Pugsley 1986; Government of Canada 1993b); 1.7×10^5 ng trichlorobenzenes/g (an extreme case of all three isomers near the outfall of a storm sewer near the Love Canal dump site in New York; Government of Canada 1993c); 7000 ng tetrachlorobenzene/g (St. Clair River; Oliver and Pugsley 1992; Government of Canada 1993d). In general, survival of either mayflies or tubificid worms was not affected by high concentrations of various isomers and congeners of the chlorobenzenes with the exception of *1,4*-dichlorobenzene which reduced survival of the mayfly at 37,000 ng/g. However, survival was still >70% at this concentration and just slightly below the range of survival rates in clean, Laurentian Great Lakes reference sediments.

Any observed sublethal toxicity (*i.e.*, reductions in growth or reproduction after chronic exposure) was slight especially with regard to growth of the mayfly. However, production of young by *T. tubifex* at the highest concentrations tested for several compounds was signficant. These effects occurred at concentrations that exceeded 1.0×10^5 ng/g (dry weight of sediment) for any of the compounds tested.

There are few data in the scientific literature regarding the effects of the chlorobenzenes on benthic invertebrates and therefore comparisons are limited. van Leeuwen *et al.* (1992) used quantitative structure-activity relationships and equilibrium partitioning modelling to predict the concentrations of *1,2*-dichlorobenzene, *1,4*-dichlorobenzene and total trichlorobenzene at which 95% of the species in marine or freshwater benthic communities are unlikely to be affected. The authors calculated an effect threshold level of approximately 5300-5700 ng/g (d.w.) for sediments with an organic carbon content of 5% no matter what the compound. More specifically, Clark *et al.* (1987) conducted 10-d bioassays with grass shrimp and amphioxus using spiked sediment containing 0.5% to 1.0% organic matter. The authors found that a nominal concentration of 1.0×10^4 ng *1,2,4*-trichlorobenzene/g had no lethal effect on grass shrimp and 2.0×10^5 ng/g was the LC50 for amphioxus. Tagatz *et al.* (1985) found no significant effect on the stucture of macrobenthic marine animal communities that colonized aquaria containing a sandy sediment spiked with *1,2,4*-trichlorobenzene at a nominal concentration of 10,000 ng/g after 8 weeks. The lowest measured

concentration of this compound that significantly reduced the average number of individuals was 1.0 X 10^5 ng/g (measured values - range 5.2 - 7.9 X 10^5 ng/g) for arthropods and annelids. Our results reflect this same level of low toxicity for all compounds tested.

The most toxic compound was *1,4*-dichlorobenzene which had some signficant effects on survival and growth of *Hexagenia* and production of young by *T. tubifex* at 3.7 X 10^4 and 1.0 X 10^5 ng/g (geometric mean concentration). Tetrachlorobenzene had no effect on either species and was the least toxic.

The extent of the bioturbation of the sediment by each of the benthic invertebrates and the volatility of each compound played a role in the removal of the compounds from sediment. For example, the dichlorobenzenes are the most volatile of the chlorobenzene tested in this study and this volatility was reflected in the losses of the two isomers from the spiked sediments during the equilibration period of two weeks prior to test initiation. In addition, the constant gill activity of the mayfly as it filters both pore water and overlying water through its burrows, resulted in a greater loss of the two isomers of dichlorobenzene as well as the other two chlorobenzenes from the sediment compared to the losses produced by the movements of the tubificid worm.

In conclusion, the two isomers of dichlorobenzene, the *1,2,3*-trichlorobenzene isomer and *1,2,4,5*-tetrachlorobenzene isomer had few effects on survival and growth of the mayfly, *Hexagenia* spp. and survival and reproduction of the oligochaete worm, *T. tubifex.* Slight reductions in the survival and growth of *Hexagenia* occurred at concentrations that were several orders of magnitude higher than the average reported concentrations in sediment in the Great Lakes (or other areas in Canada). Reduced production of young by the tubificid worm, *T. tubifex*, occurred at the highest concentration tested for each compound with the exception of *1,2,4,5*-tetrachlorobenzene.

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LIST OF FIGURES

Table 5. Detection limits for chemical analysis of environmental matrices in bioassays with *Hexagenia* spp. and *Tubifex tubifex*

Hexagenia spp.			
			Detection Limit
Overlying water	700 mL	Dichlorobenzene Trichlorobenzene Tetrachlorobenzene	0.03 ng/mL 0.01 ng/mL 0.2 ng/mL
Pore water	80 mL	Dichlorobenzene Trichlorobenzene Tetrachlorobenzene	0.2 ng/mL 0.1 ng/mL 2 ng/mL
Sediment	2 g (dry weight)	Dichlorobenzene Trichlorobenzene Tetrachlorobenzene	10 ng/g 5 ng/g 80 ng/g

Tubifex tubifex			
			Detection Limit
Overlying water	140 mL	Dichlorobenzene Trichlorobenzene Tetrachlorobenzene	0.1 ng/mL 0.07 ng/mL 1 ng/mL
Pore water	50 mL	Dichlorobenzene Trichlorobenzene Tetrachlorobenzene	0.4 ng/mL 0.2 ng/mL 3.2 ng/mL
Sediment	2 g (dry weight)	Dichlorobenzene Trichlorobenzene Tetrachlorobenzene	10 ng/g 5 ng/g 80 ng/g

Table 6. Percent survival and growth of *Hexagenia*-and cocoon and total young production of *T. tubifex* in reference sediments from the Great Lakes with particle size distribution and total organic carbon similar to formulated sediment used in bioassays.

Site No.	Lake	% Sand	% Silt	% Clay	TOC	% Hex. Surv.	Hex. Gwth.	TTCC	TTTY
105	Erie	14.3	48.1	37.5	2.2	100	3.44	36	67
109	Erie	15.3	48.9	35.8	2.6	100	3.18	38	94
112	Erie	24.6	40.5	34.9	2.4	94	3.62	38	51
113	Erie	19.3	46.6	34.2	1.8	100	5.9	41	49
304	Erie	19.8	41.1	39.2	6.16	100	7.77	46	107
601	Huron	17.5	45.2	37.4	5.68	83.3	4.9	38	104
Mean ± S.D.		18.5 ± 3.7	45.0 ± 3.6	36.5 ± 1.9	3.47 ± 1.92	96.2 ± 6.8	4.80 ± 1.78	39.3 ± 3.5	78.9 ± 2.6

Table 7. Concentrations of *1,2*-dichlorobenzene in overlying water, pore water and bulk sediment in whole-sediment toxicity test with the mayfly, *Hexagenia* spp.

Hexagenia spp.			
Nominal Concentration at Time of Spiking (ng/g dry weight sediment)	Time = 0 Concentration of 1,2-DCB	Time = 21 d Concentration of 1,2-DCB	Geometric Mean ng/mL or ng/g
Overlying water:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	1	n.d.	n.d.
5,000	17	n.d.	n.d.
50,000	247	0.6	13
500,000	2,536	16	201
Porewater:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	3	n.d.	0.5
5,000	130	0.4	7
50,000	478	4	42
500,000	5,139	137	839
Bulk Sediment	(ng/g)	(ng/g)	(ng/g)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d
500	217	18	63
5,000	2,095	79	407
50,000	14,708	615	3,008
500,000	166,729	9,613	40,035

n.d. = below limits of detection

Table 8. Concentrations of *1,2*-dichlorobenzene in overlying water, pore water and bulk sediment in whole-sediment toxicity test with the oligochaete, *T. tubifex*

Tubifex tubifex			
Nominal Concentration at Time of Spiking (ng/g dry weight sediment)	Time = 0 Concentration of 1,2-DCB	Time = 28-d Concentration of 1,2-DCB	Geometric Mean ng/mL or ng/g
Overlying water:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	1	0.1	0.3
5,000	10	0.3	2
50,000	126	40	71
500,000	1,725	90	394
Porewater:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	4	n.d.	1
5,000	36	5	14
50,000	523	68	188
500,000	5,198	2,312	3,467
Bulk Sediment:	(ng/g)	(ng/g)	(ng.g)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	198	110	148
5,000	2,228	855	1,380
50,000	25,259	9,498	1,549
500,000	196,167	82,510	127,223

n.d. = below limits of detection

Table 9. Concentrations of *1,4*-dichlorobenzene in overlying water, pore water and bulk sediment in whole-sediment toxicity test with the mayfly, *Hexagenia* spp.

Hexagenia spp.			
Nominal Concentration at Time of Spiking (ng/g dry weight sediment)	Time = 0 Concentration of 1,4-DCB	Time = 21-d Concentration of 1,4-DCB	Geometric Mean ng/mL or ng/g
Overlying water:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	2	0.1	n.d.
5,000	8	0.1	1
50,000	177	0.6	10
500,000	507	11	75
Porewater:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	2	0.3	n.d.
5,000	38	0.4	4
50,000	407	5	45
500,000	5,325	143	873
Bulk Sediment:	(ng/g)	(ng/g)	(ng/g)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	165	72	109
5,000	1,645	312	716
50,000	11,608	1,408	4,043
500,000	144,496	9,185	36,471

n.d. = below limits of detection

Table 10. Concentrations of *1,4*-dichlorobenzene in overlying water, pore water and bulk sediment in whole-sediment toxicity test with the oligochaete, *T. tubifex*

Tubifex tubifex			
Nominal Concentration at Time of Spiking (ng/g dry weight sediment)	Time = 0 Concentration of 1,2-DCB	Time = 28-d Concentration of 1,2-DCB	Geometric Mean ng/mL or ng/g
Overlying water:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	0.7	0.1	0.1
5,000	11	0.4	3
50,000	14	6	75
500,000	915	80	-
Porewater:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	0.6	0.2	0.3
500	3	0.9	2
5,000	87	8	26
50,000	578	99	240
500,000	4,025	1,706	2,620
Bulk Sediment:	(ng/g)	(ng/g)	(ng.g)
Control	n.d.	n.d.	n.d.
Control solvent	13	n.d.	n.d.
500	256	159	202
5,000	2,367	1,109	1,620
50,000	16,715	4,583	8,752
500,000	145,339	71,729	102,103

n.d. = non detectable

Table 11. Concentrations of *1,2,3*-trihlorobenzene in overlying water, pore water and bulk sediment in whole-sediment toxicity test with the mayfly, *Hexagenia* spp.

Hexagenia spp.			
Nominal Concentration at Time of Spiking (ng/g dry weight sediment)	Time = 0 Concentration of 1,4-DCB	Time = 21-d Concentration of 1,4-DCB	Geometric Mean ng/mL or ng/g
Overlying water:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	0.4	0.02	n.d.
5,000	7	0.3	1
50,000	62	2	12
500,000	492	162	282
Porewater:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	1	0.3	0.6
5,000	10	3	5
50,000	162	20	57
500,000	1,044	869	952
Bulk Sediment:	(ng/g)	(ng/g)	(ng/g)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	311	34	103
5,000	2,134	433	961
50,000	23,312	3,123	8,532
500,000	240,993	50,822	110,669

n.d. = non detectable

Table 12. Concentrations of *1,2,3*-trichlorobenzene in overlying water, pore water and bulk sediment in whole-sediment toxicity test with the oligochaete, *T. tubifex*

Tubifex tubifex spp.			
Nominal Concentration at Time of Spiking (ng/g dry weight sediment)	Time = 0 Concentration of 1,2-DCB	Time = 28-d Concentration of 1,2-DCB	Geometric Mean ng/mL or ng/g
Overlying water:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	1	n.d.	n.d.
5,000	11	n.d.	n.d.
50,000	148	0.5	9
500,000	1,709	5	89
Porewater:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	0.3	n.d.	n.d.
5,000	5	n.d.	n.d.
50,000	384	158	246
500,000	4,054	1,635	2,574
Bulk Sediment:	(ng/g)	(ng/g)	(ng.g)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	304	82	158
5,000	1,768	805	1,193
50,000	33,755	11,831	19,983
500,000	222,251	201,605	211,676

n.d. = below limits of detection

Table 13. Concentrations of *1,2,4,5*-tetrachlorobenzene in overlying water, pore water and bulk sediment in whole-sediment toxicity test with the mayfly, *Hexagenia* spp.

Hexagenia spp.			
Nominal Concentration at Time of Spiking (ng/g dry weight sediment)	Time = 0 Concentration of 1,4-DCB	Time = 21-d Concentration of 1,4-DCB	Geometric Mean ng/mL or ng/g
Overlying water:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	n.d.	n.d.	n.d.
5,000	3	n.d.	n.d.
50,000	93	5	21
150,000	479	25	109
Porewater:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	n.d.	n.d.	n.d.
5,000	6	n.d.	n.d.
50,000	178	43	88
150,000	448	195	296
Bulk Sediment:	(ng/g)	(ng/g)	(ng/g)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	n.d.	n.d.	n.d.
5,000	4,197	1,543	2,545
50,000	26,993	30,347	28,621
150,000	139,206	89,529	111,638

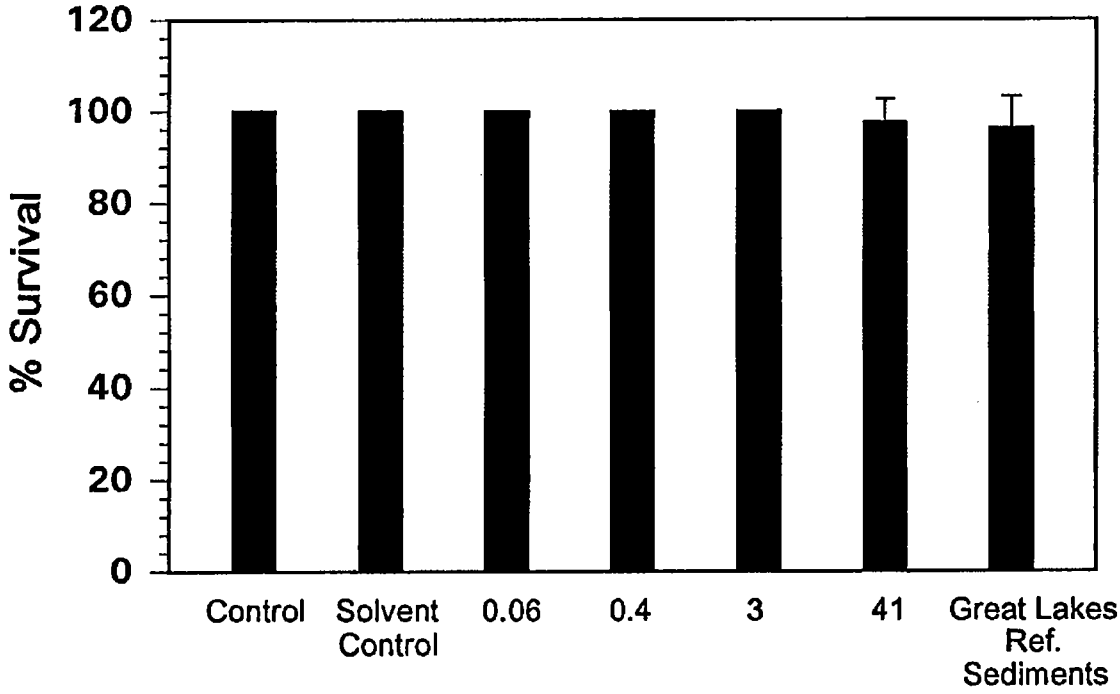
n.d. = below limits of detection

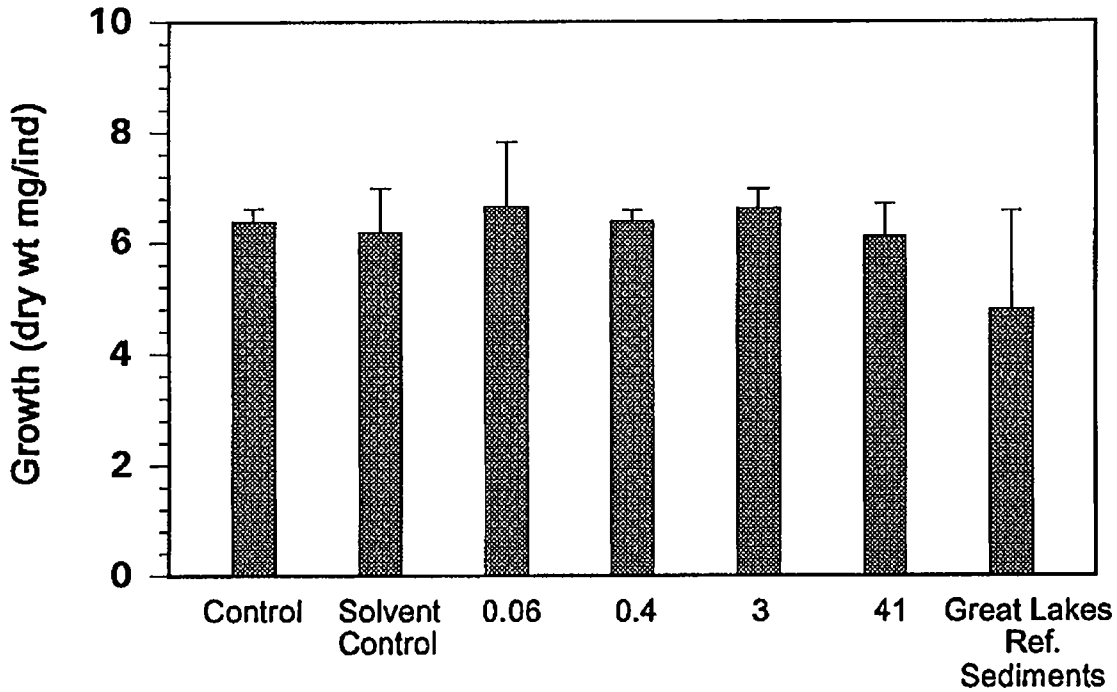
Table 14. Concentrations of *1,2,4,5*-tetrachlorobenzene in overlying water, pore water and bulk sediment in whole-sediment toxicity test with the oligochaete, *T. tubifex*

Tubifex tubifex spp.			
Nominal Concentration at Time of Spiking (ng/g dry weight sediment)	Time = 0 Concentration of 1,2-DCB	Time = 28-d Concentration of 1,2-DCB	Geometric Mean ng/mL or ng/g
Overlying water:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	n.d	n.d.	n.d.
5,000	n.d.	n.d.	n.d.
50,000	20	6	11
150,000	89	5	22
Porewater:	(ng/mL)	(ng/mL)	(ng/mL)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	n.d.	n.d.	n.d.
5,000	2	n.d.	n.d.
50,000	30	79	49
150,000	116	341	199
Bulk Sediment:	(ng/g)	(ng/g)	(ng.g)
Control	n.d.	n.d.	n.d.
Control solvent	n.d.	n.d.	n.d.
500	346	152	229
5,000	3,954	3,087	3,494
50,000	52,767	37,797	44,659
150,000	177,146	145,316	160,443

n.d. = non detectable

Hexagenia spp.



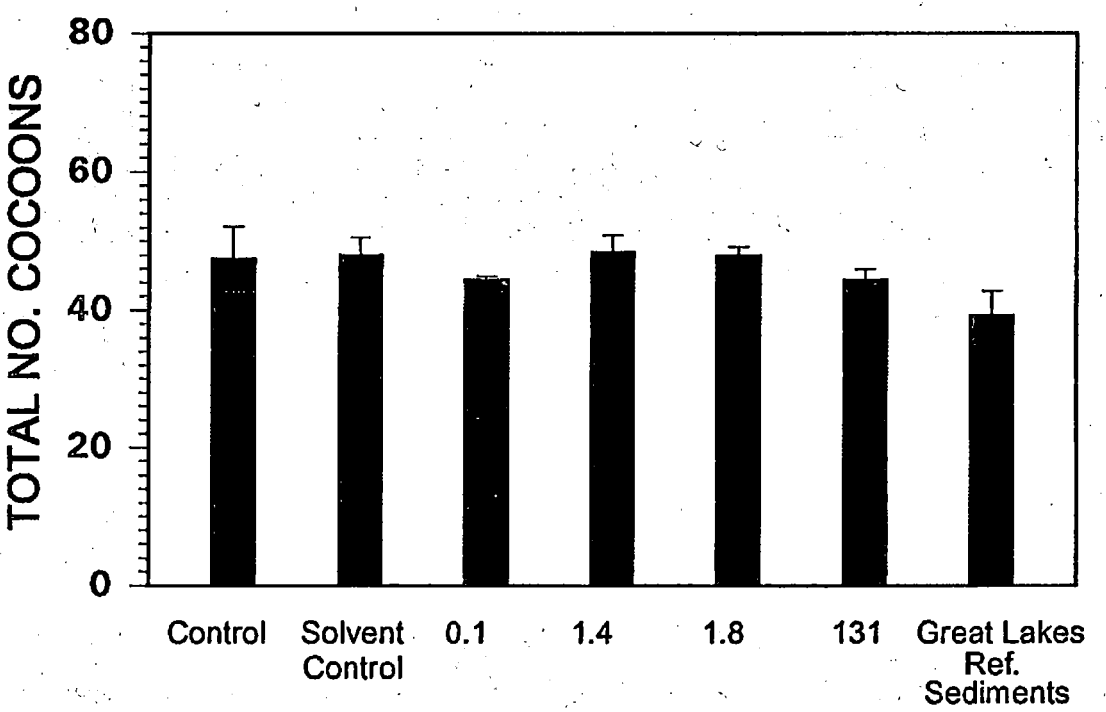


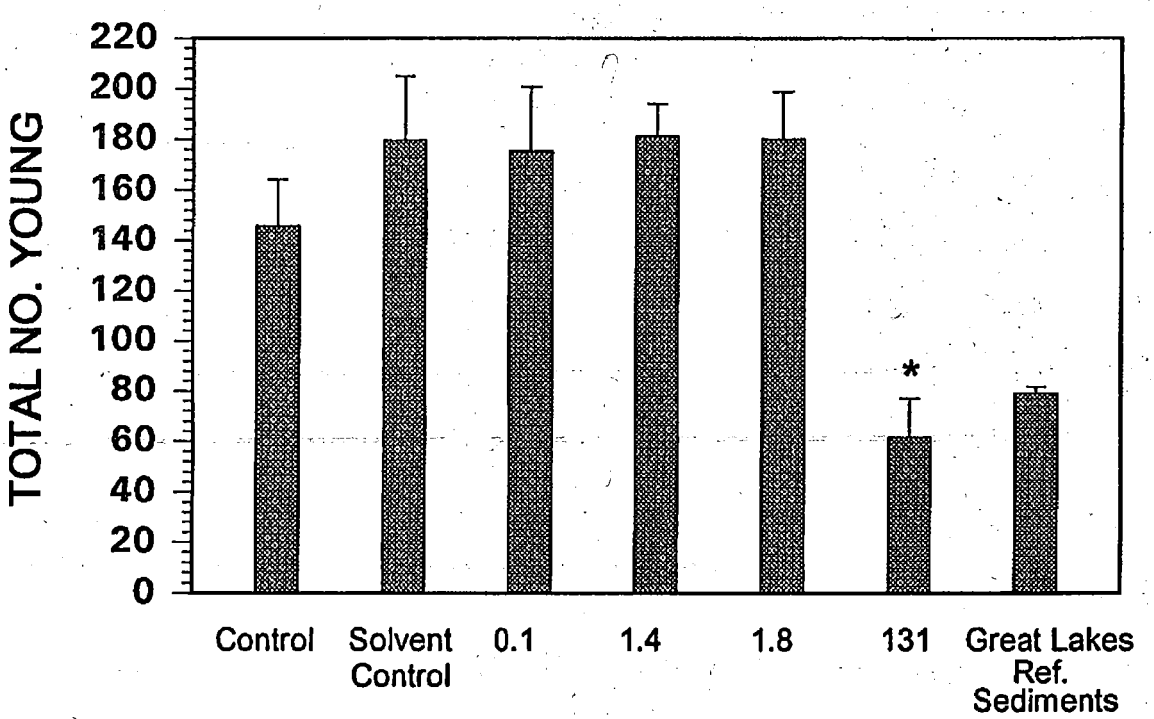
GEOMETRIC MEAN CONCENTRATION (*ug*/g)

1,2-dichlorobenzene

Figure 1

Tubifex tubifex



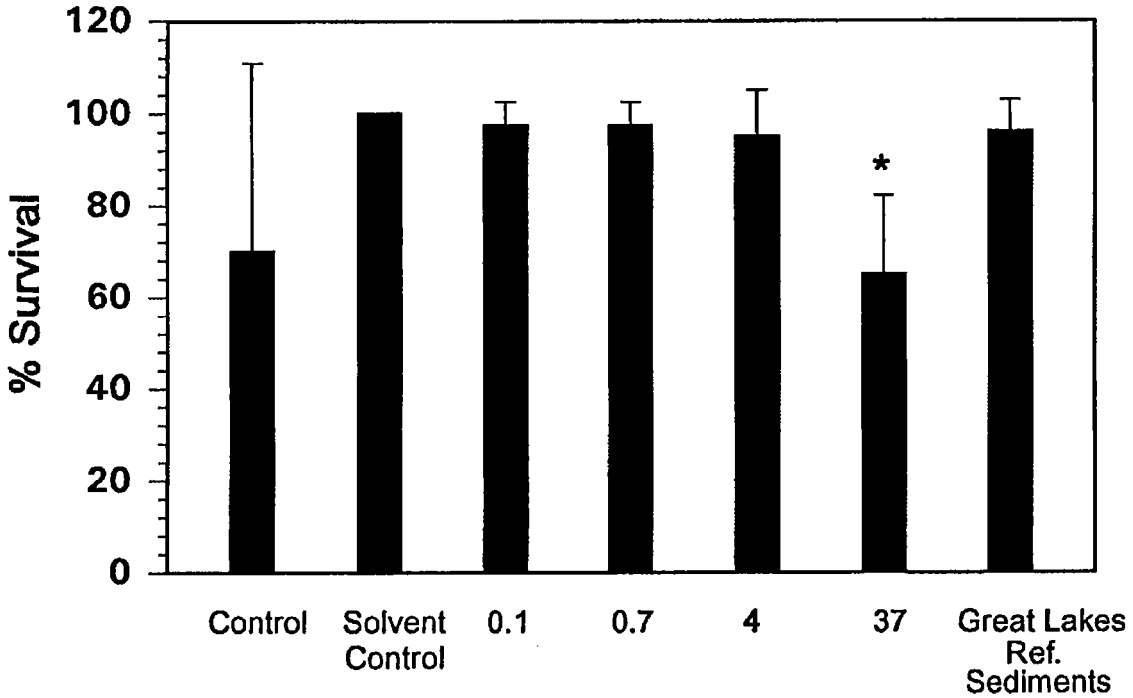


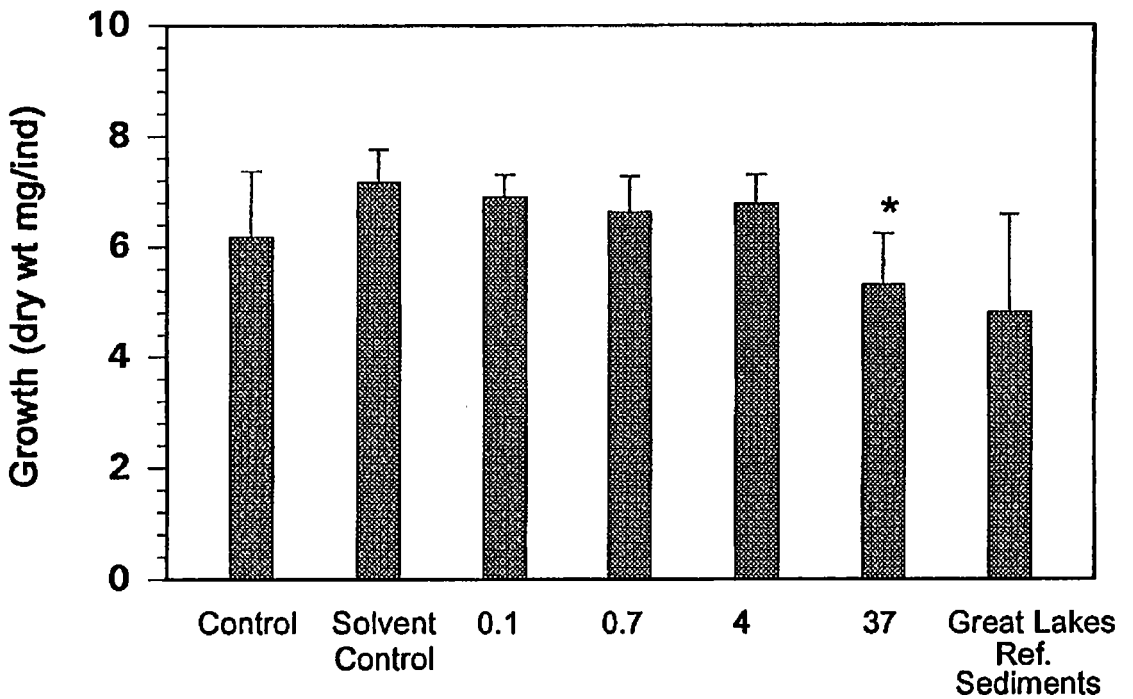
GEOMETRIC MEAN CONCENTRATION (*ug*/g)

1,2-dichlorobenzene

Figure 2

Hexagenia spp.





GEOMETRIC MEAN CONCENTRATION (*ug*/g)

***1,4*-dichlorobenzene**

Figure 3

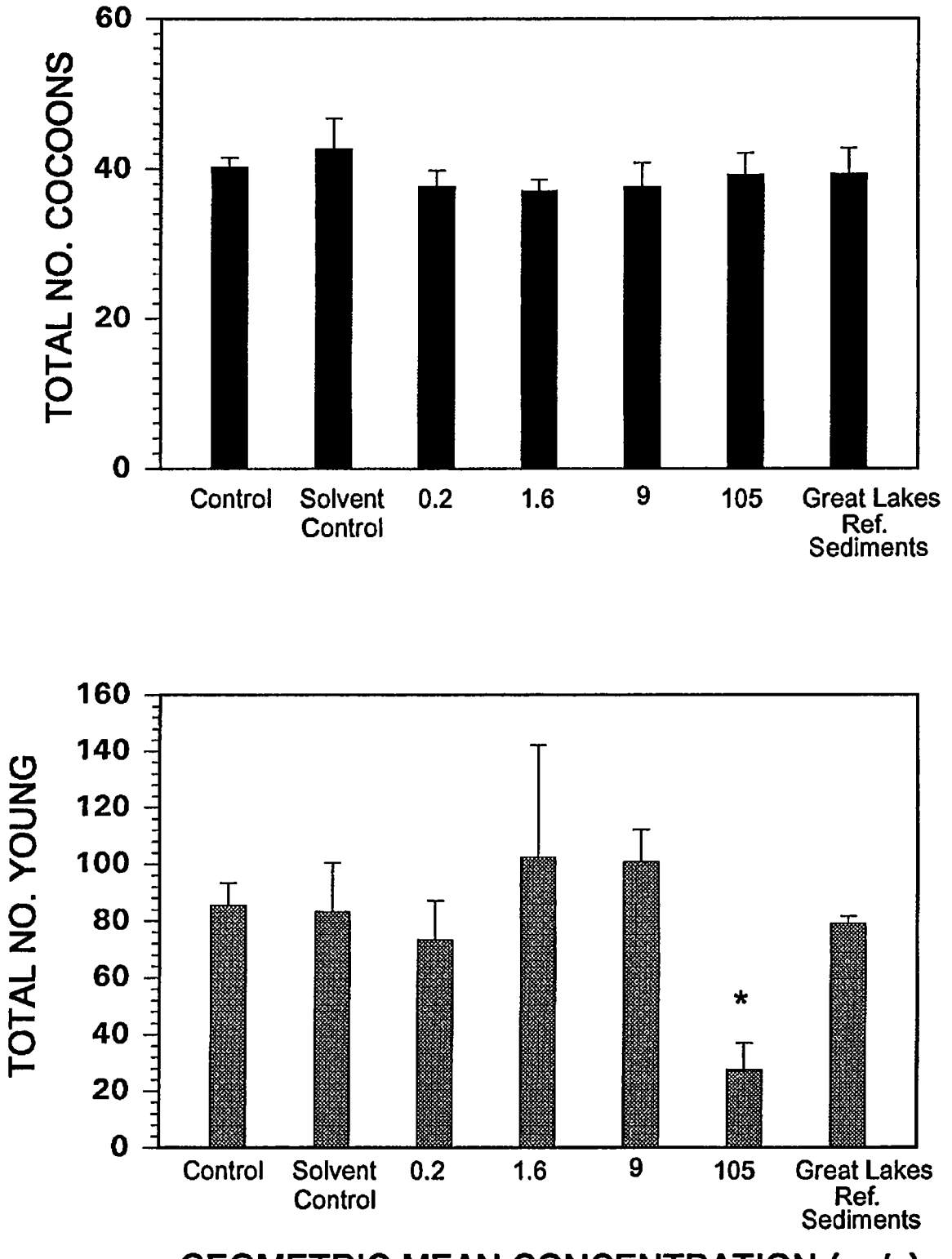
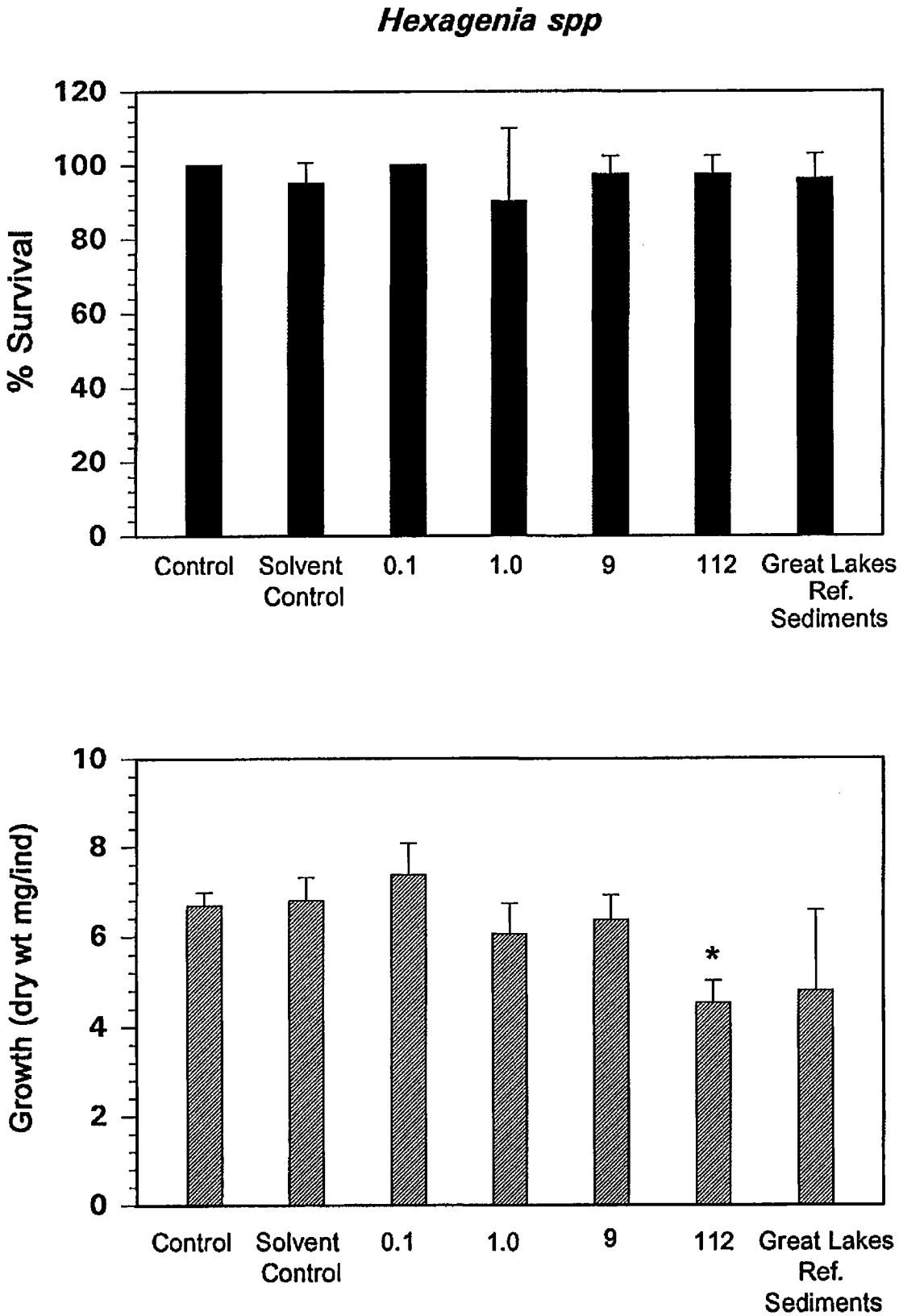

Tubifex tubifex
TOTAL NO. COCOONS
60
40
20
0
Control
Solvent Control
0.2
1.6
9
105
Great Lakes Ref. Sediments
TOTAL NO. YOUNG
160
140
120
100
80
60
40
20
0
*
Control
Solvent Control
0.2
1.6
9
105
Great Lakes Ref. Sediments
GEOMETRIC MEAN CONCENTRATION (ug/g)
1,4-dichlorobenzene

Figure 4



GEOMETRIC MEAN CONCENTRATION (*ug*/g)

1,2,3-trichlorobenzene

Figure 5

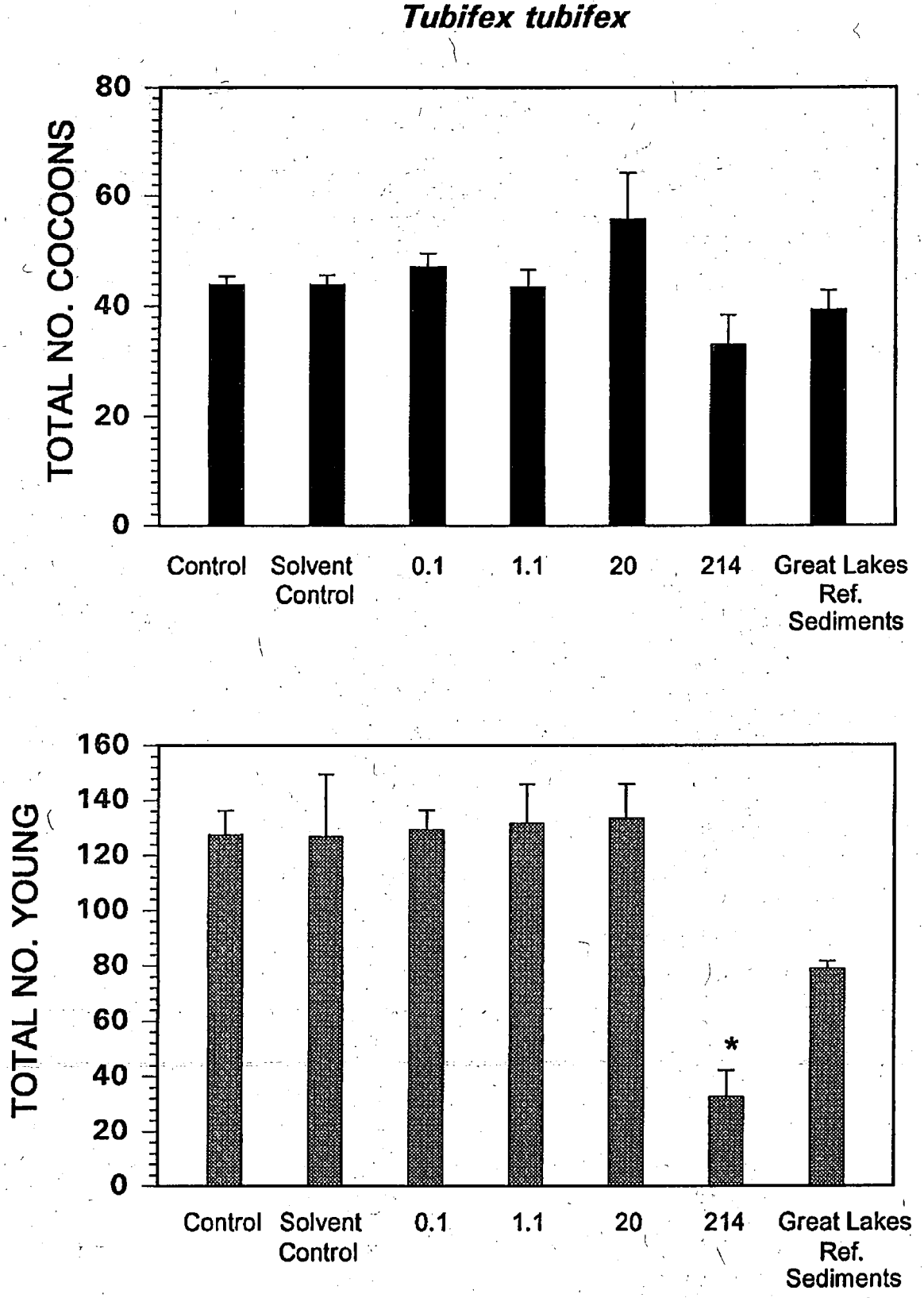

Tubifex tubifex
TOTAL NO. COCOONS
80
60
40
20
0
Control
Solvent Control
0.1
1.1
20
214
Great Lakes Ref. Sediments
TOTAL NO. YOUNG
160
140
120
100
80
60
40
20
0
*
Control
Solvent Control
0.1
1.1
20
214
Great Lakes Ref. Sediments
GEOMETRIC MEAN CONCENTRATION (ug/g)
1,2,3-trichlorobenzene

Figure 6

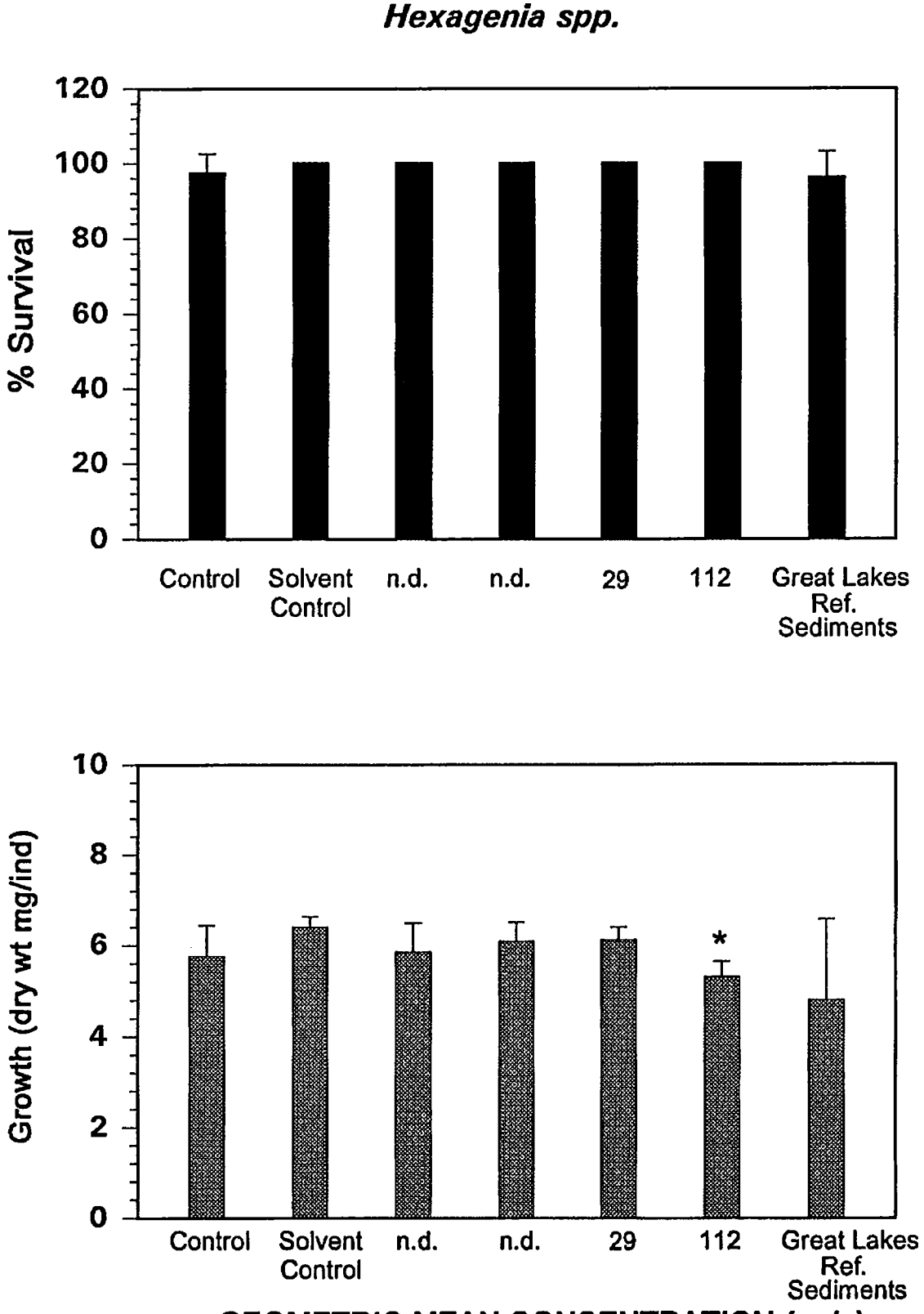

Hexagenia spp.
% Survival
120
100
80
60
40
20
0
Control
Solvent Control
n.d.
n.d.
29
112
Great Lakes Ref. Sediments
Growth (dry wt mg/ind)
10
8
6
4
2
0
*
Control
Solvent Control
n.d.
n.d.
29
112
Great Lakes Ref. Sediments
GEOMETRIC MEAN CONCENTRATION (ug/g)
1,2,4,5-tetrachlorobenzene

Figure 7

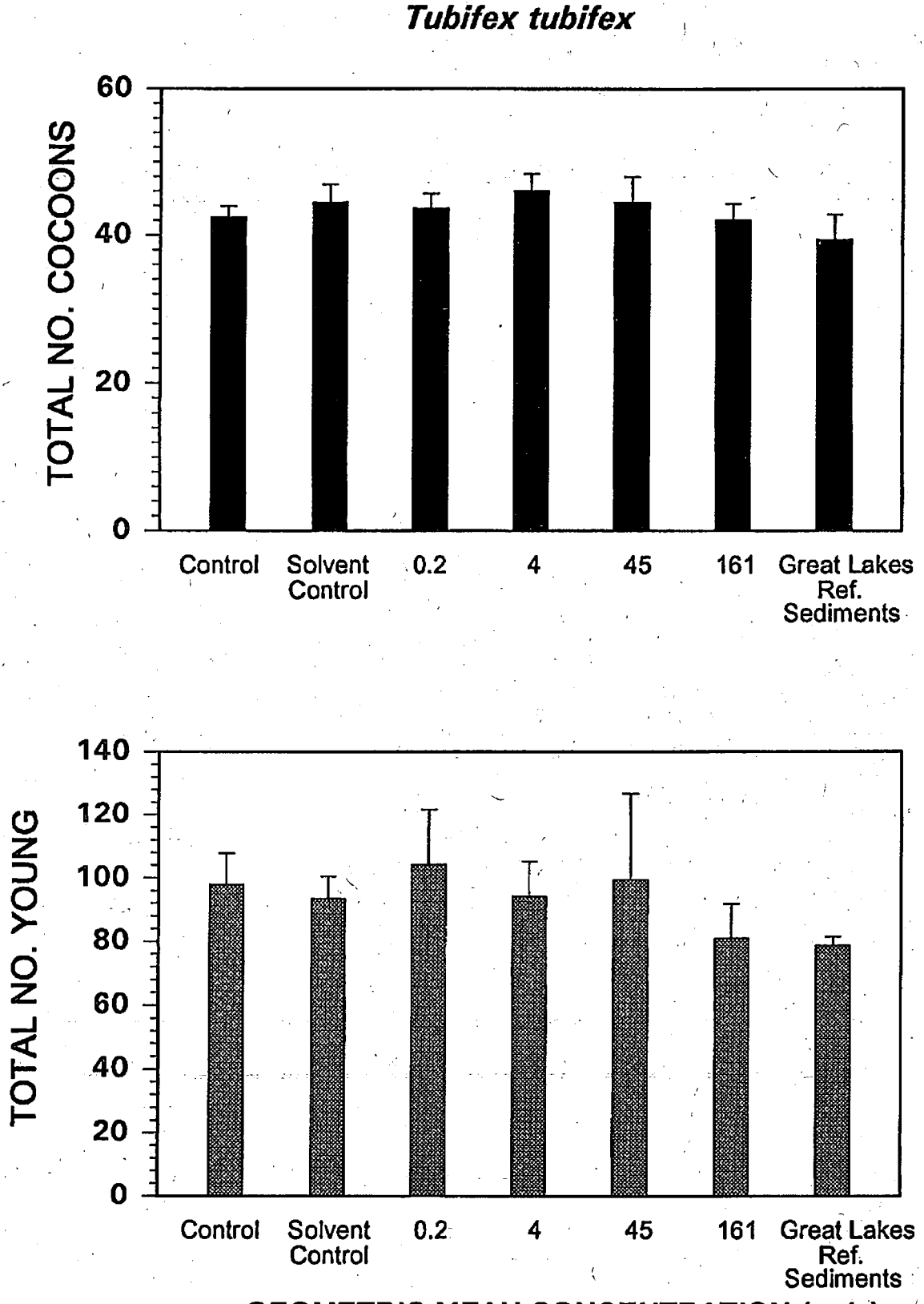

Tubifex tubifex
TOTAL NO. COCOONS
60
40
20
0
Control
Solvent Control
0.2
4
45
161
Great Lakes Ref. Sediments
TOTAL NO. YOUNG
140
120
100
80
60
40
20
0
Control
Solvent Control
0.2
4
45
161
Great Lakes Ref. Sediments
GEOMETRIC MEAN CONCENTRATION (ug/g)
1,2,4,5-tetrachlorobenzene

Figure 8