ACUTE TOXICITIES OF NINE CHLORINATED ORGANIC COMPOUNDS TO SELECTED FRESHWATER ORGANISMS

P.J. Shubat\(^1\), S.H. Poirier, M.L. Knuth, D.E. Hammermeister, A.R. Lima\(^2\), L.T. Brooke, D.J. Call and T.A. Felhaber\(^3\)

Center for Lake Superior Environmental Studies
University of Wisconsin-Superior
Superior, WI 54880

\(^1\) Present address: Dept. of Fisheries and Wildlife, Oregon State Univ., Corvallis, Oregon 97331.
\(^2\) Present address: 3841 Amber Lane, Deerfield, Wisconsin 53531.
\(^3\) Present address: Dept. of Geochemistry, Univ. of Capetown, Rondebosch 7700, Capetown, South Africa.
ABSTRACT

Acute flow-through toxicity tests were conducted with nine chlorinated organic compounds and four species of freshwater organisms. Eight compounds (1,2-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, hexachlorobenzene, 1,3-hexachlorobutadiene, hexachloroethane, tetrachloroethylene, and pentachlorophenol) were selected from a list of priority pollutants identified by the U.S. Environmental Protection Agency for which water quality criteria would be written. Pentachlorobenzene was also tested. Test organisms included rainbow trout (Salmo gairdneri), bluegills (Lepomis macrochirus), midge larvae (Paratanytarsus parthenogeneticus), and gammarids (Gammarus pseudolimnaeus).

LC$_{50}$ values were determined for all compounds except hexachlorobenzene, which did not produce sufficient mortalities at the highest exposure levels. LC$_{50}$ values (96 h) for trout exposed to 1,2-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and hexachloroethane were 1.58, 1.12, 1.53, and 0.94 mg/L, respectively. Pentachlorobenzene did not produce 50 percent mortality in trout after 96 h of exposure; however, two 144 h LC$_{50}$ values of 0.25 and 0.25 mg/L were obtained in separate tests.

1,3-Hexachlorobutadiene tests were conducted with bluegills and rainbow trout and yielded identical 96 h LC$_{50}$ values of 0.32 mg/L. One gammarid exposure was conducted with pentachlorophenol and yielded a 0.28 mg/L 96 h LC$_{50}$. Midge larvae were less sensitive than rainbow trout or gammarids when tested with the same compounds. LC$_{50}$ values (48 h) of 12.0, 13.0, 46.0, 5.85 and 30.8 mg/L were obtained for 1,2-dichlorobenzene, 1,4-dichlorobenzene, pentachlorophenol, hexachloroethane, and tetrachloroethylene, respectively, with midge larvae. EC$_{50}$ values were also calculated.
INTRODUCTION

In the past decade we have seen a considerable increase in the concern over toxic substances. Federal statutes were established in the 1970s for controlling the entrance of toxic pollutants into the aquatic environment. Foremost among these was the Water Pollution Control Act (PL92-500) which required the U.S. Environmental Protection Agency (EPA) to publish a list of toxic pollutants and establish effluent standards. Legal and administrative difficulties slowed efforts to identify toxic pollutants until a 1976 court settlement between the EPA and several environmental plaintiffs resulted in the formation of a "priority pollutant" list. Ultimately, 129 chemicals were listed for which technology based effluent limitations and guidelines would be required (Keith and Teilliard, 1979). The court settlement also required that water quality criteria be developed for each of the "priority pollutants" and used to establish effluent regulations on 21 point source categories (Barrett, 1978).

This study was undertaken to complete the minimum data requirements for eight chlorinated "priority pollutants" so that national water quality criteria could be developed by EPA. LC$_{50}$ values for these tests have been reported in preliminary form in the U.S. EPA's "Water Quality Criteria" series of reports where they are referenced as "1980 laboratory data" (U.S. EPA, 1980a, b, c, d, e, f). Measured flow-through toxicity tests were conducted in accordance with testing guidelines (American Society for Testing and Materials, 1980) to study the acute effects of 1,2-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, hexachlorobenzene, 1,3-hexachlorobutadiene, hexachloroethane, tetrachloroethylene and pentachlorophenol on two fish species [rainbow trout (Salmo gairdneri) and bluegill sunfish
(Lepomis macrochirus), one benthic insect [midge larvae (Paratanytarsus parthenogenetica)], and one benthic crustacean (Gammarus pseudolimnaeus). The toxicity of pentachlorobenzene to rainbow trout was similarly studied.

MATERIALS AND METHODS

Biological Procedures

Fish tests were conducted with rainbow trout (Salmo gairdneri) from Fattig Fish Hatchery, Brady, NE and Lake Mills Hatchery, Madison, WI and with bluegills (Lepomis macrochirus) from Newtown Fish Toxicology Station, Cincinnati, OH. Fish were treated with formaldehyde after arrival and held in Lake Superior water for a minimum of fourteen days before testing. Fish were fed appropriately sized trout food pellets (Glencoe Mills, Inc., Glencoe, MN) until 24 h before testing and were not fed during the test. Tests were conducted for a minimum of 96 h. The mean weight of fish varied from test to test, ranging from 0.46 g to 6.8 g for rainbow trout and 0.42 g to 1.5 g for bluegills.

Ten fish were randomly assigned to test chambers and observed for loss of equilibrium (effect) and mortality. Observations were recorded at 0, 3, 6, 12 and 24 h and every 24 h thereafter for the duration of the test. The criterion for death was cessation of opercular movement.

One test was conducted with Gammarus pseudolimnaeus collected from the Eau Claire River near Gordon, WI. Organisms were acclimated in 16-17°C Lake Superior water 23 days before testing. Deciduous tree leaves that had been soaked in lake water provided cover and food during acclimation. Gammarids were not fed during the 96 h test. Adult gammarids, mean weight of 0.05 g, were randomly placed in test chambers and observed for mortality at 0, 6, 12
and 24 h and every 24 h thereafter for the duration of the test. Complete immobilization was the criterion for death.

Midge tests were conducted with Paratanytarsus parthenogeneticus cultured in the ERL-D laboratory. Twenty-four h before beginning a test, third instar midges were randomly transferred to each of 8-12 exposure dishes. Each dish contained 200 mL of food (Anderson, 1980) and lake water in a ratio of 2.5 mL:1L with a fine layer of sand for case building. Midges were examined for normal movement and case building after 24 h acclimation either at room temperature (23-24 °C) or in 20 °C water bath. Prior to adding toxicant, water was siphoned off to a depth of 0.5 cm, leaving undisturbed a layer of food that had settled during acclimation.

Observations were made after toxicant addition and were recorded for 0, 2, 4, 6, 12, 24, and 48 h. The criterion for death was immobility when gently prodded. Effect was also noted and included slowed reactions, convulsive movements, and evacuation of cases.

Exposure Systems

Rainbow trout, bluegill, and gammarid tests were conducted on three modified proportional diluters (Mount & Brungs, 1967) with flow-splitting chambers (Benoit and Puglisi, 1973) which divided each of five toxicant concentrations and control into two duplicate exposure tanks. Rainbow trout were exposed in 6.3L glass aquaria and received an average of 9.2 volume additions per day. Bluegills were exposed in 7.7L glass aquaria and received 10.3 volume additions per day. Gammarids were exposed in 3.9L glass aquaria and received 11.5 volume additions per day.

Dimethylformamide was used as a carrier solvent in the pentachlorobenzene and hexachlorobenzene exposures to facilitate toxicant dissolution. Hexa-
chlorobenzene tests with rainbow trout and bluegills were conducted in duplicate with two concentrations and a solvent control. The pentachlorobenzene rainbow trout test was conducted twice with five concentrations and a solvent control tested each time. Fluid metering pumps were used to deliver an equal amount of solvent to each exposure and control tank. Fish were exposed in 27 L glass aquaria and received an average of 4.6 volume additions per day. Fluorescent lights and a controlled 16 h photoperiod provided lighting of 20-46 ft candles for all diluters.

Midges were acclimated and exposed in 200 mL covered crystallizing dishes modified for flow-through exposure. Tests with 1,2-dichlorobenzene, 1,4-dichlorobenzene, hexachloroethane and tetrachloroethylene were conducted under flow-through conditions in a 20°C water bath. The toxicant was dripped into each dish from 500 mL separatory funnels at a rate of 2 mL/min for a minimum of 48 h. Tests with pentachlorophenol and hexachlorobenzene were conducted at room temp (23-24°C) under static conditions. For these tests, a 200 mL volume of toxicant solution was dripped into each exposure dish, siphoned off, and replaced with another 200 mL of solution. Dishes were covered with a glass plate and no further additions were made for the duration of the 48 h test. Duplicate tests were conducted with lake water controls and five toxicant concentrations with the exception of hexachlorobenzene which was tested with two toxicant concentrations, a solvent control and a lake water control. Indirect lighting provided a 16 h (1.9 ft candles) photoperiod in the test enclosure.

Water Characteristics

Lake Superior water was used in all tests and modified only by heating or cooling to the desired temperatures. Tests were conducted at 11.9 ± 0.5°C
for rainbow trout, 24.2 ± 1.4°C for bluegills, 17.1 ± 0.5°C for gammarids, and 21.3 ± 2.0°C for midges. Water was monitored in the exposure tanks for dissolved oxygen, pH, acidity, total alkalinity and EDTA hardness following established guidelines (U.S. EPA, 1975). Dissolved oxygen was maintained at greater than 60% for all tests with an overall mean of 87.0 ± 7.7% saturation. Means for pH, total alkalinity, acidity and EDTA hardness were 7.5 ± 0.3,
48 ± 5 mg/L CaCO₃, 2.4 ± 1.2 mg/L CaCO₃, and 45 ± 3 mg/L CaCO₃, respectively. Measurements were made according to methods described by the American Public Health Association (1975).

Toxicant Solutions

Test chemicals obtained from Aldrich Chemical Co. (Milwaukee, WI) were >97% pure. Pentachlorophenol (Lot no. P260-4) was 99+ % pure.

Toxicant stock solutions of 1,2-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, 1,3-hexachlorobutadiene, hexachloroethane, and tetrachloroethylene were made continuously using a recirculating system similar to one described by Veith and Comstock (1975). A near saturation solution of 1,3-hexachlorobutadiene was made using a non-recirculating saturator described by Phipps et al. (1982).

Stock solutions of hexachlorobenzene and pentachlorobenzene were prepared with the solvent dimethylformamide (DMF), as the toxicant concentrations generated by saturators (4.7 and 340 µg/L, respectively) had no effect upon the test organisms. Rainbow trout, bluegills, and midges were exposed to two concentrations of hexachlorobenzene and a constant DMF concentration of 970 mg/L. Rainbow trout were exposed to five concentrations of pentachlorobenzene and a constant DMF concentration of 390 mg/L.
Stock solutions of pentachlorophenol in Lake Superior water were prepared with NaOH to facilitate dissolution (1:1, w/w).

Data Analysis

Lethal effects of the toxicants were determined by pooling data from replicate chambers and calculating LC$_{50}$ values at several time intervals for each test. EC$_{50}$ values were similarly calculated using loss of equilibrium for fish and abnormal movement for midges as the effect end-points. The average toxicant concentration of combined replicate chambers was used to calculate LC$_{50}$ and EC$_{50}$ concentrations using the trimmed Spearman-Karber Method (Hamilton et al., 1977).

Analytical Procedures

All toxicant concentrations in exposure tanks were analyzed by liquid-liquid extraction and subsequent electron capture gas-liquid chromatographic (GLC) analysis. Pentachlorophenol was methylated with diazomethane prior to GLC analysis (U.S. EPA, 1977). Samples were taken from all exposure tanks on the first and last day and from one duplicate tank on each of the other days.

Recoveries of hexachlorobenzene, pentachlorobenzene, 1,3-hexachlorobutadiene, tetrachloroethylene, hexachloroethane, 1,2- and 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and pentachlorophenol from Lake Superior water spiked with test compounds over the concentration ranges encountered during the tests were: 96.6 ± 3.2 (n=23), 94.3 ± 4.0 (n=21), 100.7 ± 5.5 (n=16), 89.9 ± 6.2 (n=23), 96.7 ± 2.9 (n=16), 103.7 ± 2.6 (n=15), 100.0 ± 3.0 (n=19), 98.8 ± 2.3 (n=18), and 97.2 ± 6.0 (n=6) percent, respectively.
RESULTS

Median lethal (LC$_{50}$) and median effect (EC$_{50}$) concentrations for 96 or 144 h exposures to 1,2- and 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, pentachlorobenzene, 1,3-hexachlorobutadiene, and hexachloroethane were estimated for rainbow trout. Estimates of the 96 h LC$_{50}$ values ranged from 0.320 mg/L for 1,3-hexachlorobutadiene-exposed fish to 1.58 mg/L for 1,2-dichlorobenzene-exposed fish (Table 1). Estimates of the 96 h EC$_{50}$ values ranged from 0.139 to 1.51 mg/L for the same compounds. A 96 h LC$_{50}$ value could not be calculated for pentachlorobenzene-exposed rainbow trout due to insufficient deaths at the highest exposure concentrations of 0.68 and 0.66 mg/L for the two tests. The tests were continued and 144 h LC$_{50}$ values of 0.267 and 0.249 mg/L were obtained. EC$_{50}$ estimates (144 h) for pentachlorobenzene-exposed rainbow trout were 0.123 and 0.200 mg/L for the two tests.

Rainbow trout and bluegills were tested with hexachlorobenzene but no lethality or noticeable effects were found at the highest exposure level of 0.0047 mg/L using a recirculating saturator system. The tests were repeated using DMF to facilitate the dissolution of hexachlorobenzene, and concentrations of 0.081 and 0.078 mg/L, respectively, were obtained. No lethality or effects were observed at these elevated concentrations.

Bluegills were also exposed to 1,3-hexachlorobutadiene. The 96 h LC$_{50}$ and EC$_{50}$ values were 0.324 and 0.126 mg/L, respectively. Bluegill response to 1,3-hexachlorobutadiene was very similar to the rainbow trout response (Table 1).

Gammarids were exposed to one of the tested compounds, pentachlorophenol, for 96 h with a resultant estimated LC$_{50}$ value of 0.280 mg/L. No observations were made for sublethal effects.

Midges were exposed to six compounds for 48 h at various concentrations with estimates made of LC$_{50}$ and EC$_{50}$ values (Table 2). Three chlorinated
benzenes (1,2- and 1,4-dichlorobenzene, and hexachlorobenzene), pentachlorophenol, hexachloroethane, and tetrachloroethylene were used to expose midge larvae. All tested compounds were acutely lethal to midges except hexachlorobenzene which had no effect at the highest test concentration of 0.058 mg/L obtained using DMF. Larvae reacted to the lowest concentrations of 1,2- and 1,4-dichlorobenzene tested, 2.00 and 1.80 mg/L, respectively, within 24 h of exposure. Reactions were convulsive movements (in contrast to normal sinuating movements) and evacuation of cases. The most toxic compound tested was hexachloroethane with an estimated LC$_{50}$ value of 5.85 mg/L. EC$_{50}$ (48 h) values ranged from 1.82 mg/L for hexachloroethane-exposed midges to 44.0 mg/L for pentachlorophenol-exposed midges. Midges were more tolerant of the test compounds than were the fish or gammarid species used.

Pentachlorophenol tests were conducted with stock solutions prepared with NaOH. The highest exposure level for the midge test had a pH of 8.48 which was higher than that of any other test conducted.

Control mortalities did not exceed 10% in any of the tests.

**DISCUSSION**

**Chlorinated Benzenes**

Test results indicate that the concentration of chlorinated benzenes necessary to cause a loss of equilibrium at 96 h in rainbow trout decreases with increased chlorination (Table 1). Other researchers have demonstrated this trend with mortality data on flow-through exposures of fathead minnows (*Pimephales promelas*) to 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1,2,3,4-tetrachlorobenzene where 96 h LC$_{50}$ values were 4.00, 2.87, and 1.07 mg/L, respectively (EPA, 1980a). Buccafusco et al. (1981) and Könemann (1981)
have also shown increasing toxicity of the di-, tri-, and pentachlorobenzene series in static exposures with bluegills and guppies (*Poecilia reticulata*).

This relationship was not evident with mortality data, as mortalities increased in the order: 1,2-dichlorobenzene, <1,2,4-trichlorobenzene, <1,4-dichlorobenzene. This same order was reported by Calamari *et al.* (1983) for two species of fish (*Salmo gairdneri* and *Brachydanio rerio*) in 48 h exposures. LC$_{50}$ values for *Salmo* were 2.3, 1.95 and 1.18 mg/L for 1,2-dichlorobenzene, 1,2,4-trichlorobenzene, and 1,4-dichlorobenzene, respectively; and for *Brachydanio* were 6.8, 6.3, and 4.25 mg/L, respectively. Monochlorobenzene was less toxic than di- or tri-chlorobenzenes, with 48 h LC$_{50}$ values of 4.1 and 10.5 mg/L for *Salmo* and *Brachydanio*, respectively (Calamari *et al.* 1983).

The solubility of hexachlorobenzene in Lake Superior water was estimated to be 4.7 µg/L using a recirculating saturator system. Reported values of the water solubility of hexachlorobenzene are 5.0 µg/L (Geyer *et al.*, 1981; CRC Press, 1972-3) and 5.4 µg/L (Hashimoto *et al.*, 1982). None of the three species tested - rainbow trout, bluegills, or midge larvae - were sensitive to the compound at 4.7 µg/L. It was possible to elevate concentrations up to approximately 17 times greater than the estimated water solubility with the solvent DMF. The existence of a true solution at these elevated levels is questionable; however, bluegill and trout tests were conducted. One bluegill died, possibly due to handling, but none of the trout responded to elevated levels of toxicant. Midges were exposed to concentrations as much as 12 times saturation with no noticeable response. Laska *et al.* (1978) reported that hexachlorobenzene was not acutely toxic to crayfish (*Procambarus clarkii*) or largemouth bass (*Micropterus salmoides*) at maximum concentrations of 27.3 and 25.8 µg/L, respectively. Hexachlorobenzene did not show any toxicity at concentrations approaching 30 µg/L to *Salmo gairdneri, Brachydanio rerio*,
or Daphnia magna (Calamari et al., 1983). Parrish et al. (1974) reported that hexachlorobenzene was not acutely toxic to four estuarine species tested at measured concentrations to 25 μg/L. However, in one study (Johnson and Finley, 1980), 96 hr LC$_{50}$ values of 22, 14, 12, and 12 mg/L were reported for fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), bluegills and largemouth bass, respectively. Static exposures were conducted with a carrier solvent.

**Species Sensitivity**

Separate tests with seven compounds were conducted with a variety of species, making it possible to compare EC$_{50}$ and LC$_{50}$ endpoints between species. Midge larvae and rainbow trout were exposed to 1,2-dichlorobenzene, 1,4-dichlorobenzene, and hexachloroethane (Tables 1 and 2). Midge larvae were affected by the two tested dichlorobenzenes at concentrations comparable to rainbow trout 96 h median lethal concentrations. Complete immobilization of the midges occurred at concentrations that were 6- to 12-fold greater than rainbow trout 96 h LC$_{50}$ values. Midge larvae were also exposed to tetrachloroethylene and results (EC$_{50}$ = 6.77 mg/L) can be compared to previously published results of a rainbow trout test (Shubat et al. 1982) in which a 96 h LC$_{50}$ of 4.99 mg/L was found. A 48 h EC$_{50}$ could not be calculated for midge larvae exposed to 1,4-dichlorobenzene, as it was less than 1.81 mg/L, the lowest concentration tested. For each of these four compounds, midges reacted (vacated cases and moved convulsively) when exposed to concentrations causing a loss of equilibrium and mortality in trout. The midge 48 h EC$_{50}$ value was a good indicator of the rainbow trout 96 h LC$_{50}$ values for these four compounds.

Very little difference was observed in the sensitivity of bluegills and rainbow trout when exposed to 1,3-hexachlorobutadiene. Almost identical LC$_{50}$
values of 0.324 and 0.320 mg/L were calculated for rainbow trout and bluegills, respectively.

Buccafusco et al. (1981) conducted 96 h static exposures of bluegills to many of the "priority pollutants" presented here. Their LC$_{50}$ estimations were based on nominal additions of toxicant and many of the exposure vessels contained undissolved chemical. LC$_{50}$ values reported were 5.60 mg/L of 1,2-dichlorobenzene, 4.30 mg/L of 1,4-dichlorobenzene, 3.40 mg/L of 1,2,4-trichlorobenzene, and 0.250 mg/L of pentachlorobenzene. With the exception of pentachlorobenzene, our flow-through rainbow trout exposures resulted in lower LC$_{50}$ values (Table 1). LC$_{50}$ values determined by Buccafusco et al. (1981) for bluebills exposed to hexachloroethane (0.980 mg/L) and pentachlorobenzene are in good agreement with our flow-through rainbow trout LC$_{50}$ values. However, they reported a precipitate formed in both tests with bluegills.

Midges and gammarids exposed to pentachlorophenol showed the greatest difference in sensitivity, gammarids being the more sensitive animal by a factor of 160. Differences between these pentachlorophenol tests included pH and test design (flow-through for gammarids and a modified static exposure for midges). A pH effect on midges cannot be ruled out in this test as the pH at the highest exposure level (8.48) exceeded the highest tested level (pH 7.8) at which normal development occurs (Bell, 1970).

Hexachlorobenzene was also tested with a variety of species with no toxicant-related effects occurring with any of the species tested.

With the exception of 1,3-hexachlorobutadiene, the compounds used in these tests caused both trout and bluegills to lose equilibrium during the first 12 hours of exposure and lie on the bottoms of the tanks. Deaths followed within another 12 hours. With 1,3-hexachlorobutadiene, both rainbow trout and bluegills
showed responses described by Laska et al. (1978) and Leeuwangh et al. (1975) at concentrations as low as 50 µg/L for trout and 200 µg/L for bluegills. Effects observed included swimming in tight circles, inverting, and eventually lying on the bottom of the tank. Fish were affected within 24 hours and deaths slowly increased through 96 hours.

ACKNOWLEDGEMENTS

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**TABLE 1.**

Rainbow trout (*Salmo gairdneri*), bluegill (*Lepomis macrochirus*) and gammarid (*Gammarus pseudolimnaeus*) LC$_{50}$ and EC$_{50}$ values with 95% confidence intervals for eight chlorinated organic compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>96 H LC$_{50}$ (mg/L)</th>
<th>96 H EC$_{50}$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-dichlorobenzene</td>
<td>Rainbow trout</td>
<td>1.58 (1.44-1.73)</td>
<td>1.51 (1.41-1.62)</td>
</tr>
<tr>
<td>1,4-dichlorobenzene</td>
<td>Rainbow trout</td>
<td>1.12 (1.05-1.20)</td>
<td>1.10 (1.05-1.16)</td>
</tr>
<tr>
<td>1,2,4-trichlorobenzene</td>
<td>Rainbow trout</td>
<td>1.53 (1.35-1.73)</td>
<td>0.790 (NC)</td>
</tr>
<tr>
<td>Pentachlorobenzene/DMF (I)</td>
<td>Rainbow trout</td>
<td>0.267 (0.205-0.347)*</td>
<td>0.123 (0.098-0.154)</td>
</tr>
<tr>
<td>Pentachlorobenzene/DMF (II)</td>
<td>Rainbow trout</td>
<td>0.249 (0.057-1.10)*</td>
<td>0.200 (0.163-0.245)</td>
</tr>
<tr>
<td>Hexachloroethane</td>
<td>Rainbow trout</td>
<td>0.944 (0.854-1.04)</td>
<td>0.840 (0.751-0.941)</td>
</tr>
<tr>
<td>1,3-Hexachlorobutadiene</td>
<td>Rainbow trout</td>
<td>0.320 (0.268-0.381)</td>
<td>0.139 (0.128-0.152)</td>
</tr>
<tr>
<td>1,3-Hexachlorobutadiene</td>
<td>Bluegills</td>
<td>0.324 (0.312-0.337)</td>
<td>0.126 (0.110-0.144)</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>Gammarid</td>
<td>0.280 (0.240-0.330)</td>
<td>NO</td>
</tr>
<tr>
<td>Hexachlorobenzene/DMF</td>
<td>Rainbow trout</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>Hexachlorobenzene/DMF</td>
<td>Bluegills</td>
<td>NF</td>
<td>NF</td>
</tr>
</tbody>
</table>

NC - Not calculable; greater than 50% effect in lowest concentration.

* - 144 h LC$_{50}$; less than 50% mortality in highest concentration at 96 h.

NO - No observations made for effect.

NF - Not found; less than 50% mortality or effect throughout test.

DMF - Dimethylformamide used as a carrier solvent.
TABLE 2.

Midge (Paratanytarsus parthenogeneticus) 48 h LC$_{50}$ and EC$_{50}$ values with 95% confidence intervals for six chlorinated organic compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>48 H LC$_{50}$ (mg/L)</th>
<th>48 H EC$_{50}$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-dichlorobenzene</td>
<td>12.0 (10.0-14.5)</td>
<td>2.36 (1.74-3.19)</td>
</tr>
<tr>
<td>1,4-dichlorobenzene</td>
<td>13.0 (10.9-15.6)</td>
<td>NC</td>
</tr>
<tr>
<td>Hexachlorobenzene/DMF</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>46.1 (39.1-54.4)</td>
<td>44.0 (37.3-52.0)</td>
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<tr>
<td>Hexachloroethane</td>
<td>5.85 (3.77-9.09)</td>
<td>1.82 (1.61-2.04)</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>30.8 (28.7-33.0)</td>
<td>6.77 (6.12-7.50)</td>
</tr>
</tbody>
</table>

NC - not calculable; greater than 50% effect in lowest concentration.

NF - not found; less than 50% mortality or effect in highest concentration.

DMF - dimethylformamide used as a carrier solvent.
REFERENCES


