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Project Summary

Aquatic Toxicity Tests to Characterize the Hazard of Volatile Organic Chemicals in Water: A Toxicity Data Summary

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This summary presents acute and chronic toxicity test data and bioconcentration factors compiled over a two-year period on fish and invertebrates exposed to several representative chemicals from five chemical classes (chlorinated ethanes, chlorinated benzenes, chlorinated ethylenes, chlorinated dienes, and chlorinated propanes).

The fathead minnows and *Daphnia* were quite similar in their sensitivities (acute and chronic) to each chemical class, while the rainbow trout were considerably more sensitive to all classes during acute tests, except for the chlorinated diene exposures, where they were more resistant. The ranking of acute and chronic sensitivity was generally the same for each chemical within each class of chemicals for all three species tested.

Both the acute and chronic toxicity of all chemicals within a class increased as the number of chlorines in the chemical structure increased.

Bioconcentration factors for fathead minnows were determined for four of the chemical classes tested. Hexachlorobenzene was bioconcentrated the most (23,000x), and tetrachloroethane was bioconcentrated the least (8x). Again, as with the toxicity experiments, the greater the number of chlorines on the molecule the greater the bioconcentration within each class of chemicals.

This Project Summary was developed by EPA's Environmental Research Laboratory, Duluth, MN, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The objectives of this study were to evaluate the use of sensitive aquatic toxicity tests in determining the relative hazard of volatile organic compounds; and to compare the sensitivities of three aquatic species to several homologous series of organic chemicals. Investigations were divided into two major areas. First, preliminary studies were conducted to determine similarities and differences in metabolism of selected xenobiotics between aquatic and mammalian organisms. The second phase, reported here, represents the largest effort, which was to develop methods for testing volatile chemicals and to determine the differences in toxicity between daphnids (*Daphnia magna*) and fish to selected volatile chemicals.

Both acute and chronic toxicity tests were completed with fathead minnows (*Pimephales promelas*) and daphnids. Only acute tests were performed using rainbow trout (*Salmo gairdneri*). Test chemicals were selected from the following classes of compounds: chlorinated ethanes; chlorinated benzenes; chlorinated ethylenes; chlorinated dienes and chlorinated propanes. Bioconcentration factors for fathead minnows exposed to

10 chemicals from the above classes were also determined.

The full report represents an overview of the results from these investigations. Most data have been or are scheduled to be published in scientific journals.

Methods and Procedures

Bioassay-Chemical

Routine toxicity test methods and chemical procedures followed closely those described by Mount and Brungs (1967), APHA (1975), USEPA (1975), Phipps et al (1981), and Benoit et al (1982)

All tests, except for the static *Daphnia* exposures, were performed using proportional diluters. Lake Superior water was used for all tests. Nominal water temperatures were 12, 20, and 25° C for rainbow trout, *Daphnia*, and fathead minnows, respectively Acute tests were for 96 hrs for fatheads and rainbow trout and 48 hrs for *Daphnia*. Chronic fish (embryo-larval) tests were conducted for 32 days, and chronic *Daphnia* tests were of 28-day duration

Chemical characteristics of the control and experimental waters were as follows: dissolved oxygen 7.0-9.6 mg/l; pH 6.7-7.6, total hardness (as $CaCO_3$) 43-57 mg/l, and alkalinity 35-53 mg/l Several statistical procedures (Spearman-Karber, ANOV, Probit analyses) were used to test the significance of the results.

Chemicals selected for testing ranged in purity from 95-99 percent Solvents used in the extraction process were of glass-distilled, gas-chromatography grade. The effectiveness of the extraction procedure was examined by determining the percent recovery of a known amount of each chemical in the experimental water. All toxicant concentrations in water and tissues were analyzed by gas chromatography Further description of these analytical procedures, according to chemical, appear in the full report.

Development of Early Life Stage, Mini-Diluter Apparatus

In order to successfully and safely test volatile chemicals, it was necessary to develop specialized exposure systems and methods for monitoring air and water leaving these systems. Interest in developing fish early life-stage (ELS) toxicity tests led to the design of a compact continuous flow mini-diluter exposure system which accurately delivered as little as 3 liters of test water per hour to each of five concentrations plus a control This system can be used to test the effects of either single chemicals or treated complex effluents on young fish in the laboratory or in the field. The small ELS test apparatus takes less space and requires smaller volumes of test water, which is a critical factor when effluents are shipped to the laboratory or on-site toxicity tests are conducted. Smaller volumes of test water also reduce filtration costs for the removal of hazardous test chemicals before discharging waste water to the sewer.

The ELS test system has been tested and evaluated in the laboratory and on-site in a mobile trailer (Benoit et al., 1982). This apparatus has been used to conduct fathead minnow (*Pimephales promelas*) ELS exposures to various toxicants including volatile organic compounds, metals, pesticides, and treated complex effluents from metal plating, oil refinery, and sewage treatment plants The system has also been successfully used for testing macroinvertebrates. All embryo-larval fish tests described herein were conducted in this mini-diluter system.

Summary and Conclusions

It has long been known that aquatic animals are extremely sensitive to chemicals at very low µg/L to mg/L concentrations in the aqueous environment. This knowledge led us to the position of evaluating both acute and chronic toxicity tests with several sensitive aquatic species in an effort to determine the range of sensitivities and the possible application of the data screening out chemicals, which after short inexpensive tests with selected aquatic species are shown to be extremely toxic, and/or highly bioaccumulable

Phase one of these studies was designed to provide preliminary information on the metabolic capabilities of rainbow trout and daphnids These studies, coupled with earlier studies on mixed function oxidase activity in mammals and other animals, indicated the metabolic systems to be similar qualitatively, therefore, the mechanisms leading to toxicity and neoplasia, for example, are presumed to be similar in all organisms. Phase two was to determine the acute and chronic toxicity of five classes of chlorinated organic compounds to selected fish and invertebrate animals In addition, the bioconcentration potential of these chemicals was measured to determine possible food-chain problems involving man.

Daphnia showed generally the same order of acute and chronic sensitivity as the fish for all classes of chemicals tested. A comparison of species acute sensitivities (Table 1) indicated that *Daphnia* were slightly more resistant in most cases than the fathead minnow, whereas the rainbow trout was considerably more sensitive than either the fathead minnow or *Daphnia* to all compounds except hexachlorobutadiene

One of the more interesting findings of the acute studies was that the toxicity of the ethanes, benzenes, and ethylenes increased as the number of chlorines on the molecule increased. This was true for both fathead minnows and *Daphnia* (Table 1).

These data indicate that either fathead minnows or *Daphnia* would provide essentially the same acute values for these particular chemicals. These chemicals are not considered to be very toxic to aquatic species, since their 96-hr LC50s are one to two orders of magnitude above those environmental chemicals considered as extremely toxic.

Fifteen chronic toxicity tests with fish were also conducted on chemicals in the five chemical classes. The order of sensitivity for all chemicals tested chronically (Table 2) was the same as that established for acute toxicity (Table 1). Six chronic values were also determined for *Daphnia* and in most cases the sensitivity was similar except for the ethanes, where there seemed to be considerable variation between the fathead and *Daphnia* results. Again, chronic toxicity increased considerably for both species as the number of chlorines on the molecule increased (Table 2).

The bioconcentration potentials of these chemicals were determined by establishing a bioconcentration factor (BCF) C_{Fish}/C_{Water}) for fathead minnows exposed for 32 days to each chemical during an early life-stage toxicity test. These BCFs were then compared to BCF values for other species of fish found in the literature (Table 3). In the two classes where comparisons were possible (chlorinated benzenes and ethanes), bioconcentration as well as toxicity increased as the number of chlorines on the molecule increased. The literature values for other fathead minnow studies as well as studies with bluegill, sunfish (*Lepomis macrochirus*), and guppies (*Poecilia reticulata*) (Table 3) all agree very closely with the BCFs generated here. This indicates that age, size, and species of fish have little effect on the BCF generated over a 32-day period of water exposures. Based on BCF values, hexachlorobenzene, hexachlorobutadiene, and 1,2,3,4-tetrachlorobenzene are the chemicals from the groups

Table 1. *Summary of Acute Toxicity Data for Fathead Minnows, Rainbow Trout, and* Daphnia

Compound	Fathead Minnow 96-hr LC50 (mg/l)	Rainbow Trout 96-hr LC50 (mg/l)	Daphnia 48-hr LC50 (mg/l)
Chlorinated Ethanes			
Hexachloroethane	1.53	0 84	2 90
Pentachloroethane	7 30	[a]	7 32
1,1,2,2-Tetrachloroethane	20 30	[a]	62.10
1,1,2-Trichloroethane	81 70	[a]	186.0
1,2-Dichloroethane	117 80	[a]	268.0
Chlorinated Benzenes			
Hexachlorobenzene	[b]	[b]	[a]
Pentachlorobenzene	[b]	[b]	[a]
1,2,3,4-Tetrachlorobenzene	1 07	[a]	[a]
1,2,4-Trichlorobenzene	·2 76	1.52	2.09
1,3-Dichlorobenzene	7 79	1.61	7.43
1,4-Dichlorobenzene	4 16	1.12	[a]
Chlorinated Ethylenes			
Tetrachloroethylene	13 50	4 99	18 10
1,1,2-Trichloroethylene	44 10	[a]	[a]
Chlorinated Propanes			
1,2-Dichloropropane	139 30	[a]	[a]
1,3-Dichloropropane	131 10	[a]	[a]
Chlorinated Butadienes			
Hexachlorobutadiene	0.10	0 32	[a]

[a] *Not tested*
[b] *Not toxic at water saturation*

Table 2. *Summary of Fathead Minnow and* Daphnia *Chronic Toxicity Data*

Compound	Fathead Minnow 32-day (ELS) MATC (µg/l)	Daphnia 28-day[a] Chronic (µg/l)
Chlorinated Ethanes		
Hexachloroethane	69-207	-
Pentachloroethane	900-1,400	-
1,1,2,2-Tetrachloroethane	1,400-4,000	6,850-14,400
1,1,2-Trichloroethane	6,000-14,800	13,200-26,000
1,2-Dichloroethane	29,000-59,000	10,600-20,700
Chlorinated Benzenes		
Hexachlorobenzene	4.76[b]	-
Pentachlorobenzene	54 9[b]	-
1,2,3,4-Tetrachlorobenzene	245-412	-
1,2,4-Trichlorobenzene	499-1,008	363-694
1,3-Dichlorobenzene	1,000-2,267	689-1,450
1,4-Dichlorobenzene	565-1,040	-
Chlorinated Ethylenes		
Tetrachloroethylene	500-1,400	505-1,110
1,1,2-Trichloroethylene	-	-
Chlorinated Propanes		
1,2-Dichloropropane	6,000-11,000	-
1,3-Dichloropropane	8,000-16,000	-
Chlorinated Butadienes		
Hexachlorobutadiene	6 5-13.0	-

[a] *Effect—no effect concentrations*
[b] *Water saturation—no effects noted*

tested that pose the greatest bioconcentration threat to the environment.

The acute toxicity tests run with both fish and invertebrates established a relative order of toxicity for the individual chemicals that was nearly identical to the order seen in the more sensitive chronic exposures. Therefore, the short 96-hr LC50 fish exposures or the 48-hr *Daphnia* exposures could be used to establish a ranking of chemicals found in water that will permit the researcher to initially concentrate the expensive chronic testing on the more toxic materials.

The *Daphnia* acute test may be better than a fish acute test, since it takes only 48-hrs and does not require the more difficult flow-through system required for a fish 96-hour LC50 estimate, yet it gives the same relative order of chemical sensitivity for these classes of chemicals (Tables 1 and 2).

Early life-stage toxicity tests with fish (32-days) and/or 28-day *Daphnia* chronics would provide a more sensitive test and the fish exposures would also allow the determination of a BCF, which would provide further information on the environmental impact of a chemical.

The usefulness of aquatic tests as an early warning system for toxic chemicals in these classes may be somewhat limited, because of their low toxicity and low ambient water concentrations. However, this approach for more toxic chemicals has considerable promise as an early warning system for higher animals including man.

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Table 3. *A Comparison of Bioconcentration Factors for Chemicals Tested in Present Study in Fathead Minnows vs. Other Species of Fish in Other Studies*

Chemicals	Present Study Fathead Minnows [a] BCF	Log BCF	Literature Values Fathead Minnow[b]	Bluegill[b]	Guppy[c]
Chlorinated Ethanes					
Hexachloroethane	703.4	2 85	-	138	-
Pentachloroethane	60.4	1.78	-	68	-
1,1,2,2-Tetrachloroethane	8.1	0.91	-	8	-
Chlorinated Ethylenes					
Tetrachloroethylene	61.5	1.79	-	49	-
Chlorinated Butadienes					
Hexachloro-1,3-butadiene	6,988.6	3.84	-	-	-
Chlorinated Benzenes					
Hexachlorobenzene	23,391 5	4.37	21,878	-	14,454
1,2,3,4-Tetrachlorobenzene	2,567.3	3.41	-	1,820	3,631
1,2,4-Trichlorobenzene	398.5	2 60	1,698	-	646
1,3-Dichlorobenzene	97.9	1.99	-	66	-
1,4-Dichlorobenzene	112.4	2 05	-	60	91

[a] *32-day exposure ELS toxicity test.*
[b] *30-day old fish exposed for 30 days.*
[c] *Adult fish exposed for 30 days.*

Standard practice for conducting acute toxicity tests with fishes and macroinvertebrates, and amphibians. EPA-660/3-75-009. Duluth, MN. 67 p.

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The complete report, entitled "Aquatic Toxicity Tests to Characterize the Hazard of Volatile Organic Chemicals in Water: A Toxicity Data Summary," (Order No. PB 84-141 506; Cost: $13.00, subject to change) will be available only from:
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