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Project Summary

Toxicological Studies with Herbicides, Selected EPA Priority Pollutants and Related Chemicals in Aquatic Organisms

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Toxicological studies were conducted in two areas: (1) the toxicity, bioconcentration potential and metabolism of five herbicides in fish; and (2) the toxicity and/or metabolism of priority pollutants and related chemicals in various aquatic organisms.

The test herbicides included alachlor [2-chloro-2',6'-diethyl-N- (methoxymethyl) acetanilide], bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), dinoseb [2-(*sec*-butyl)-4,6-dinitrophenol], diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], and propanil (3,4-dichloropropionanilide). Acute toxicity (through 192 hr), early life-stage toxicity (58-64 day), and bioconcentration studies were conducted with fathead minnows (*Pimephales promelas*) in Lake Superior water. Herbicide metabolism was investigated in rainbow trout (*Salmo gairdneri*) both *in vivo* and *in vitro*.

Twenty-two chemicals from the EPA priority pollutant list were studied for their acute and/or chronic toxicity to selected freshwater organisms. These included 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2,-tetrachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, hexachlorobutadiene, di-*n*-butylphthalate, pentachlorophenol, heptachlor, chlordane, toxaphene, arsenic^{+3}, chromium^{+6}, lead^{+2}, mercury^{+2}, nickel^{+2}, silver^{+1}, selenium^{+4}, and cyanide. Freshwater species tested included the fathead minnow, rainbow trout, bluegill sunfish (*Lepomis macrochirus*), flagfish (*Jordanella floridae*), *Daphnia magna*, scud (*Gammarus pseudolimnaeus*), midge (*Tanytarsus dissimilis*) and green alga (*Selenastrum capricornutum*). Toxicity tests were also conducted with pentachloroethane, hexachloroethane, 1,2,4-trichlorobenzene, pentachlorobenzene, methanol and dimethylformamide. The uptake by fish of di-*n*-butylphthalate from water, its metabolism and elimination were investigated. Comparative metabolism of 1,1,2-trichloroethane, chlorobenzene, 1,1,2-trichloroethylene, chloroform, and carbon tetrachloride was studied in rainbow trout and *Daphnia*.

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

Introduction

Many different agricultural and industrial chemicals enter aquatic ecosystems each year. For the protection of aquatic biota, it is important to evaluate the hazard potential of these chemicals. Toxicological studies collectively repre-

sent one component of a hazard evaluation scheme.

Extensive toxicological investigations have been carried out for only a small fraction of the thousands of chemicals in production. Generally, the most extensive aquatic toxicological data bases have been generated on a select few chemicals, where a combination of use, production volume, frequency of occurrence in water and/or biota, environmental persistence, and known adverse effects have dictated a need for toxicological data.

It is important to develop data bases on such chemicals to the extent that judicious environmental risk documents can be prepared. The major focus of this research project consisted of toxicological studies on individual chemicals where data bases were considered insufficient for hazard evaluations.

Methodology

Flow-through toxicity tests with fish and scuds were conducted in proportional diluters using either Lake Superior water or dechlorinated city water. Toxicity tests with *Daphnia magna* were static tests using Lake Superior water in which the toxicant solutions were renewed at 24 hr intervals. Midge acute toxicity tests were conducted in chambers in which toxicant solutions in Lake Superior water were continuously replaced from reservoirs above the test chambers at a slow drip rate.

In the acute tests, observations were made at selected time intervals for mortalities and gross behavioral effects. LC_{50} and EC_{50} values were calculated by several methods (trimmed Spearman-Karber, probit, moving average, or binomial) depending upon the characteristics of the data. In the fish early life-stage toxicity tests, observations were made for mortalities, abnormal development, and growth. Data were analyzed by one-way analysis of variance in conjunction with Dunnett's procedure to determine "no-effect" concentration ranges In *Daphnia* chronic life-cycle studies, observations were made of mortalities, number of offspring produced, and growth. Data were analyzed to determine "no-effect" concentration ranges.

Algal toxicity tests were run in flasks under uniform conditions of light, temperature, and aeration. Dry weights were measured after 96 hr of exposure, and the concentration that inhibited growth by 50 percent was determined by the trimmed Spearman-Karber method.

Studies on uptake, bioconcentration, elimination, and metabolism of selected organics in fish and *Daphnia* were conducted using ^{14}C radiolabeled compounds. Five herbicides (alachlor, bromacil, dinoseb, diuron, and propanil) and the plasticizer, di-*n*-butylphthalate, were studied in fish. The compounds 1,1,2-trichloroethane, chlorobenzene, 1,1,2-trichloroethylene, chloroform, and carbon tetrachloride were studied for their relative tendencies to be metabolized by fish and *Daphnia*.

Results and Conclusions

Results of the acute toxicity, early life-stage toxicity, bioconcentration studies for the five herbicides are summarized in Table 1 Dinoseb was the most toxic herbicide on an acute basis, while propanil was most toxic on the basis of a longer exposure encompassing the early lifestages. Parent herbicides did not appreciably accumulate in fish tissue. Metabolism studies indicated that fish quite readily metabolized the herbicides. Mass spectral analysis of extracts from the bile of fish exposed to diuron and propanil aided in the characterization of metabolites

Toxicity data for EPA priority pollutants are presented in Table 2. LC_{50} values were not determined for hexachlorobenzene due to insufficient mortalities at concentrations in water approaching solubility.

Radiolabeled di-*n*-butylphthalate was rapidly accumulated and metabolized by fathead minnows. It was concentrated in fish tissue approximately 600 times its concentration in the water. The radiolabel, which consisted largely of metabolites, was slowly eliminated from the fish upon transfer to clean water.

Chloroform was more readily metabolized *in vitro* by rainbow trout and *Daphnia* than was carbon tetrachloride, chlorobenzene, 1,1,2-trichloroethylene or 1,1,2-trichloroethane. Metabolism by enzyme systems from both species resulted in similar percentages of total ^{14}C that were hexane-unextractable for carbon tetrachloride, chlorobenzene, and 1,1,2-trichloroethylene. A greater percentage of total ^{14}C-chloroform was hexane-unextractable with the *Daphnia* enzyme system than with the trout system; while the converse applied for 1,1,2-trichloroethane. Higher percentages of total ^{14}C were protein bound with trout systems than with *Daphnia* systems for all compounds. Assays indicated that both species possess active mixed function oxidase systems.

Table 1. *Toxicity and Bioconcentration Potential of Five Herbicides With the Fathead Minnow*

Herbicide	Acute Toxicity (96 Hr. LC_{50} and 95% C.I., mg · L^{-1})	Early Life-Stage Toxicity (58-64 Day "No Effect" Concentration Range, µg · L^{-1})	Bioconcentration Factor
Alachlor	5.0 (4.5-5.6)	520-1,000	6X
Bromacil	182 (177-188)	1,000	<3X
Dinoseb	0.7 (0.6-0.7)	14.5-48.5	1X
Diuron	14.2 (13.4-15.0)	33.4-78.0	2X
Propanil	8.6 (7.7-9.5)	0.4-0.6	2X

Table 2. *Toxicities of Selected EPA Priority Pollutants to Freshwater Organisms*

Chemical	Organism	LC_{50} Concentration $mg \cdot L^{-1}$ (Test Duration)		"No Effect" Concentration Range $mg \cdot L^{-1}$ (Test Duration)	
1,2-Dichloroethane	Daphnia magna	268,315[a]	(48 hr)	10.6-20.7	(28 days)
1,1,2-Trichloroethane	Daphnia magna	186,174[a]	(48 hr)	13.2-26.0	(28 days)
1,1,2,2,-Tetrachloroethane	Daphnia magna	62.1,56.9[a]	(48 hr)	6.85-14.4	(28 days)
Tetrachloroethylene	Daphnia magna	18.1,9.09[a]	(48 hr)	0.505-1.11	(28 days)
"	Tanytarsus dissimilis	30 8	(48 hr)		
"	Salmo gairdneri	4.99	(96 hr)		
Tetrachloroethylene/DMF	Salmo gairdneri	5.84	(96 hr)		
1,2-Dichlorobenzene	Tanytarsus dissimilis	12.0	(48 hr)		
"	Salmo gairdneri	1.58	(96 hr)		
1,3-Dichlorobenzene	Daphnia magna	7.43,7.23[a]	(48 hr)	0.689-1.45	(28 days)
1,4-Dichlorobenzene	Tanytarsus dissimilis	13.0	(48 hr)		
"	Salmo gairdneri	1.12	(96 hr)		
Hexachlorobenzene/DMF	Tanytarsus dissimilis	$>H_2O$ Sol.	(48 hr)		
"	Salmo gairdneri	$>H_2O$ Sol.	(96 hr)		
"	Lepomis macrochirus	$>H_2O$ Sol.	(96 hr)		
Hexachlorobutadiene	Salmo gairdneri	0.320	(96 hr)		
"	Lepomis macrochirus	0.324	(96 hr)		
Di-n-butylphthalate	Daphnia magna	3.7	(48 hr)		
Pentachlorophenol	Tanytarsus dissimilis	46.0	(48 hr)		
"	Gammarus pseudolimnaeus	0.280	(96 hr)		
Heptachlor	Selenastrum capricornutum	0.0381, 0.0282[b,c]	(96 hr)		
Chlordane	Daphnia magna	0.035	(48 hr)		
Toxaphene	Selenastrum capricornutum	0.38[b]	(96 hr)		
Arsenic^{+3}	Gammarus pseudolimnaeus	0.875	(96 hr)		
"	Daphnia magna	1.54, 4.83[d]	(48 hr)	0.63-1.32	(28 days)
"	Pimephales promelas	14.2	(96 hr)	2.1-4.3	(30 days)
Arsenic $^{+3}$	Jordanella floridae	14.4	(96 hr)	2.1-4.1	(30 days)
Selenium^{+4}	Tanytarsus dissimilis	42.5	(48 hr)		
Chromium^{+6}	Tanytarsus dissimilis	57.3	(48 hr)		
"	Gammarus pseudolimnaeus	0.0671	(96 hr)		
Lead^{+2}	Tanytarsus dissimilis	224	(48 hr)		
"	Gammarus pseudolimnaeus	0.140	(96 hr)		
Mercury^{+2}	Pimephales promelas	0.150	(96 hr)	<0.00021	(35 days)
Nickel^{+2}	Daphnia magna	0.915	(48 hr)		
Silver^{+1}	Tanytarsus dissimilis	3.17	(48 hr)		
"	Gammarus pseudolimnaeus	0.0045	(96 hr)		
"	Pimephales promelas	0.0107	(96 hr)		
"	Jordanella floridae	0.0092	(96 hr)		
CN^-	Tanytarsus dissimilis	2.36, 2.49[e]	(48 hr)		

[a] *Results from assays in which organisms were unfed and fed, respectively.*
[b] *Calculated concentration that inhibited algal growth by 50 percent.*
[c] EC_{50} *values for two separate tests.*
[d] EC_{50} *values from assays in which organisms were unfed and fed, respectively.*
[e] *Expressed as HCN and free* CN^-, *respectively.*

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This Project Summary covers two reports, entitled:
*"**Toxicity and Metabolism Studies with EPA Priority Pollutants and Related** Chemicals in Freshwater Organisms," (Order No. PB 83-263 665; Cost: $14.50, subject to change)*
"Toxicity, Bioconcentration, and Metabolism of Five Herbicides in Freshwater Fish," (Order No. PB 83-263 681; Cost: $13.00, subject to change
The above reports are available only from:
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