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Acute Toxicity of Selected Organic Compounds to Fathead Minnows

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Ecological Research Series

ACUTE TOXICITY OF SELECTED ORGANIC COMPOUNDS TO FATHEAD MINNOWS

Environmental Research Laboratory
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FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota develops methods, conducts laboratory and field studies, and extrapolates research findings

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This report describes the short-term toxicity of 26 organic compounds to fathead minnows. The laboratory tests were limited to 4-days' duration.

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ABSTRACT

Static nonrenewal laboratory bioassays were conducted with 26 organic compounds commonly used by industry. The selected compounds represented the five following chemical classes: acids, alcohols, hydrocarbons, ketones and aldehydes, and phenols. Juvenile fathead minnows (*Pimephales promelas*) were the test animal, and test duration was limited to 96 hr. Lake Superior water served as the diluent source for all test compounds. Additional tests were conducted with a reconstituted diluent water for five compounds. The alcohol compounds were generally 10 to 100 times less toxic than chemicals tested in the other four chemical classes. Compounds in the other four classes had a similar toxicity range. The most lethal chemical tested was pentachlorophenol (96-hr LC50 of 0.6 mg/l.) and the least lethal was ethanol (96-hr LC50 of 13,480 mg/l.). Most of the minnow deaths occurred during the first 24 hr. Comparative chemical tests in the two diluent waters gave similar lethal responses.

This report was submitted in partial fulfillment of Project Number 16-AAE by the Environmental Research Laboratory-Duluth. Experimental work was completed in July 1974.

CONTENTS

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SECTION I

INTRODUCTION

Industrial production estimates for 1973 of the top 50 inorganic and organic chemicals (based on volume) exceeded 400 billion pounds. Production volumes of the organics, as a group, increased over previous years (Anderson, 1974). There has been and still exists a demand for information concerning the harmful effects of organic chemicals on aquatic life. The Battelle Columbus Laboratories (1971) and Kemp et al. (1973) have compiled extensive lists of the effects of chemicals on aquatic life. MacPhee and Ruelle (1969) studied the effects of 1,888 chemicals on four fish species, but the tests were limited to 24 hours' duration and to chemical concentrations of 10 mg/l. or less. Canadian investigators have published comprehensive reviews on the toxicity of individual chemicals commonly found in various effluents. Clarke (1974) summarized the toxicity of chemical components from inorganic chemical industries. The toxicity of effluents containing large inclusions of organic chemicals has been reviewed by Hunka (1974) for the plastics industries, Thompson (1974) for the textiles industries, and Smith (1974) for petrochemical plants.

The Environmental Research Laboratory-Duluth received from Donaldson (1972) a list of 257 organic compounds that he identified from industrial wastewaters. Twenty-six compounds were selected for testing based on their reported importance to industry (Kirk and Othmer, 1963-1969; Hawley, 1971) and on their use as parent compounds from which derivatives are commonly made. Since very little information was available on the comparative toxicity of chemical groups, the 26 compounds were grouped into the following five classes: alcohols, acids, hydrocarbons, ketones and aldehydes, and phenols. This report then gives the concentrations of each chemical lethal to fathead minnows during short-term 96-hr static tests.

SECTION II

CONCLUSIONS

1. The alcohols tested were generally 10 to 100 times less toxic to fathead minnows than the acids, hydrocarbons, ketones and aldehydes, and phenols. A loss in equilibrium generally preceded death in all chemical groups groups tested except the acids.

2. Pentachlorophenol was the most toxic compound tested and ethanol the least toxic.

3. Most of the deaths during the 4-day tests occurred within the first 24 hr and may have been related to the methods employed.

SECTION III

RECOMMENDATIONS

Information is lacking on the individual long-term consequences to aquatic life of the chemicals tested in this study. Industrial effluents are typically represented by a mixture of many chemicals. Studies are therefore needed on their combined interactive effects on both a short- and long-term exposure basis.

SECTION IV

METHODS AND MATERIALS

Juvenile fathead minnows obtained from the Environmental Research Laboratory-Duluth cultures served as the test animal. All fish used for the test were from 4 to 8 weeks old and varied in length from 1.1 to 3.1 cm. Fish were acclimated in flowing water 11 cm deep in a holding trough (360 cm long by 58 cm wide by 31 cm deep) for at least 48 hr before the tests. Water temperature was 18-22° C. During acclimation the fish were fed Glencoe trout chow no. 3 granules.

The organic compounds tested and the supplier are given in Table 1. All compounds were reagent-grade chemicals. Test solutions were prepared by adding a weighed amount of chemical to a 9-l. glass carboy containing 4 l. of Lake Superior water. Solution components were thoroughly mixed by shaking and were then poured into two glass battery jars for preparation of the toxicant test concentrations. All toxicant concentrations are nominal; none were analyzed to determine concentration levels. A fiberglass trough of the same dimensions and water depth as used for acclimation served as a water bath for maintaining the test solutions at 18°-22° C.

The static tests were conducted in 3-l. cylindrical glass battery jars containing 2 l. of test water. Exploratory tests were conducted for many of the compounds to determine the lethal range before attempting a definitive test. Three to five widely spaced test concentrations differing by a factor of 10 or more and a control were used. These tests were usually limited to 24 hours' duration.

TABLE 1. ORGANIC COMPOUNDS TESTED AND SOURCE OF SUPPLY

Chemical	Formula[a]	Company
Acetic acid	CH_3COOH	Burkick and Jackson Lab. Inc.
Acetophenone	$C_6H_5COCH_3$	Curtin Matheson Scientific Inc.
Adipic acid	$COOH(CH_2)_4COOH$	Curtin Matheson Scientific Inc.
Benzyl alcohol	$C_6H_5CH_2OH$	Curtin Matheson Scientific Inc.
1-Butanol	$CH_3(CH_2)_2CH_2OH$	Burkick and Jackson Lab. Inc.
d-Camphor	$C_{10}H_{16}O$	Curtin Matheson Scientific Inc.
Caproic acid	$CH_3(CH_2)_4COOH$	Aldrich Chemical Co.
p-Cresol	$CH_3C_6H_4OH$	Curtin Matheson Scientific Inc.
Cyclohexane	C_6H_{12}	Burkick and Jackson Lab. Inc.
Cyclohexanol	$C_6H_{11}OH$	Curtin Matheson Scientific Inc.
Ethanol	C_2H_5OH	U.S. Industrial Chemical Co.
Eugenol	$C_3H_5C_6H_3(OH)OCH_3$	Aldrich Chemical Co.
Furfural	C_4H_3OCHO	Curtin Matheson Scientific Inc.
Indan	$CHCHCHCHCCCH_2CH_2CH_2$	Curtin Matheson Scientific Inc.
Isopropanol	$(CH_3)_2CHOH$	Burkick and Jackson Lab. Inc.
1-Methylnapthalene	$C_{10}H_7CH_3$	Curtin Matheson Scientific Inc.
m-Nitrotoluene	$NO_2C_6H_4CH_3$	Curtin Matheson Scientific Inc.
Oleic acid	$CH_3(CH_2)_7CH:CH(CH_2)_7COOH$	Coleman and Bell
Pentachlorophenol	C_6Cl_5OH	Aldrich Chemical Co.
Phenol	C_6H_5OH	Mallinckrodt Chemical Works
Quinoline	$CHCHCHCHCCNCHCHCH$	Curtin Matheson Scientific Inc.
Styrene monomer	$C_6H_5CH:CH_2$	Curtin Matheson Scientific Inc.
Valeric acid	$CH_3(CH_2)_3COOH$	Aldrich Chemical Co.
Vanillin	$(CH_3O)(OH)C_6H_3CHO$	Curtin Matheson Scientific Inc.
Xylene	$C_6H_4(CH_3)_2$	Aldrich Chemical Co.
3,4 Xylenol	$(CH_3)_2C_6H_3OH$	Aldrich Chemical Co.

[a]From Hawley (1971).

Definitive tests were started by placing 10 fish into each battery jar so that 20 individuals were tested at each concentration. A glass cover was placed over each jar to reduce evaporation. The test waters were not aerated. Fish were not fed during the tests.

Complete immobilization of the fish was considered as the biological endpoint and equated with death. Fish mortality was measured after 1, 24, 48, and 96 hr. Standard graphical procedures were followed for determining concentrations that would result in 50% mortality (American Public Health Association, 1971).

Five organic compounds were also tested in soft reconstituted water. The objective was to compare the toxic response in two different diluent waters. The animals were acclimated in Lake Superior water. The reconstituted water was prepared by adding $NaHCO_3$, $CaSO_4 \cdot 2H_2O$, $MgSO_4$, and KCl salts to distilled water in quantities specified by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Replicate tests were conducted with vanillin in both diluent waters. Comparative tests were also conducted with vanillin in reconstituted water for minnows acclimated in lake and reconstituted waters. Vanillin was chosen for these additional tests because of its low handling hazard to human beings (Hawley, 1971).

Analyses of the test waters for dissolved oxygen and pH were made at the beginning and one or two times in the course of the test. Dissolved oxygen measurements were made with a YSI oxygen meter, pH measurements with a Corning pH meter. Water temperature was measured daily in two of the test containers with a mercury thermometer.

SECTION V

RESULTS AND DISCUSSION

Concentrations toxic to the minnows in Lake Superior water and in reconstituted water are presented in Tables 2 and 3, respectively. For five chemicals the LC50 values did not change by more than 10% after 1 hr of exposure (1-butanol, isopropanol, cyclohexane, xylene, and eugenol). The 24- and 96-hr LC50 levels for 17 of 26 compounds were alike. Many of the chemicals noticeably affected minnow equilibrium before death. Loss of equilibrium frequently disappeared for minnows surviving beyond 24-48 hr. Equilibrium losses were not generally observed for the acid compounds tested. The lethal and locomotor observations can be related to the static method employed and the compound's vapor pressure. These results support the recommendation by the American Public Health Association (1971, p. 570) that test solutions of volatile or unstable compounds be renewed every 24 hr or less.

Toxicity results obtained in reconstituted water for one organic compound from four of the five classes were nearly identical to those obtained in Lake Superior diluent water (Tables 2 and 3). Toxic responses by fathead minnows when acclimated in both lake and reconstituted water before tests with vanillin were also similar. Except for the 1-hr LC50 values, all remaining replicate vanillin LC50 values in both diluent waters were similar.

Tests with dissolved oxygen concentrations less than 4.0 mg/l. and pH values less than 5.9, are denoted in Tables 2 and 3. Earlier work has shown that at these concentrations low oxygen and pH themselves can cause adverse effects on fathead minnows in long-term toxicity tests (Brungs, 1971; Mount, 1973).

Alcohols, with the exception of benzyl alcohol, were 10 to 100 times less toxic than compounds tested in the other four chemical classes (Tables 2 and 3). Toxicity of the alcohols increased with increasing

TABLE 2. LETHAL CONCENTRATIONS OF 26 ORGANIC COMPOUNDS TO FATHEAD MINNOWS IN LAKE SUPERIOR WATER

Test chemical	LC50 concentration (in mg/l.)				
	1-hr	24-hr	48-hr	72-hr	96-hr
Acids					
Acetic[b]	>315	122	92	88	88
Adipic[b]	>300	172	114	97	97
Caproic[b]	140	88	88	88	88
Oleic[a]	>1,000	285	252	205	205
Valeric[b]	>100	>100	77	77	77
Alcohols					
Benzyl	770	770	770	480	460
1-Butanol	1,950	1,950	1,950	1,950	1,910
Cyclohexanol	1,550	1,033	1,033	1,033	1,033
Ethanol[a]	>18,000	>18,000	13,480	13,480	13,480
Isopropanol	11,830	11,160	11,130	11,130	11,130
Hydrocarbons					
Cyclohexane	95	93	93	93	93
Indan	39	14	14	14	14
1-Methylnapthalene	39	9	9	9	9
m-Nitrotoluene	43	30	30	30	30
Quinoline	78	46	46	46	46
Styrene	100	32	32	32	32
Styrene[c]	40	30	29	29	29
Xylene	46	42	42	42	42
Ketones and Aldehydes					
Acetophenone	>200	>200	163	158	155
d-Camphor	145	112	111	110	110
Furfural	>50	48	37	32	32
Vanillin-Test 1[a]	>173	131	123	121	121
Vanillin-Test 2[a]	370	125	116	112	112
Phenols					
p-Cresol[a]	>30	26	21	21	19
Eugenol	24	24	24	24	24
Pentachlorophenol	8	0.6	0.6	0.6	0.6
Phenol[b]	>50	>50	>50	33	32
3,4 Xylenol	>20	>20	15	14	14

[a]Dissolved oxygen measured ≤4.0 mg/l. during test.

[b]pH measured ≤5.9 units during test.

[c]Acetone added to diluent water.

TABLE 3. LETHAL CONCENTRATIONS OF FIVE ORGANIC COMPOUNDS TO FATHEAD MINNOWS IN RECONSTITUTED WATER

Test chemical	LC50 concentration (in mg/l.)				
	1-hr	24-hr	48-hr	72-hr	96-hr
Acetic acid[b]	175	106	106	79	79
1-Butanol	1,940	1,940	1,940	1,940	1,940
Cyclohexane	126	117	117	117	117
Furfural	>50	>50	37	33	32
Vanillin-Test 1[a,c]	348	100	97	88	88
Vanillin-Test 2[a,c]	>173	127	121	121	121
Vanillin[a,d]	>173	125	116	116	116

[a]Dissolved oxygen measured $\leq$4.0 mg/l. during test.

[b]pH measured $\leq$5.9 units during test.

[c]Acclimated in Lake Superior water.

[d]Acclimated in reconstituted water.

molecular weight. Oleic acid additions to the diluent lake water did not completely dissolve. Hawley (1971) lists this compound as insoluble in water. Actual LC50 values for oleic acid may be lower. The toxicity values obtained for styrene were similar with and without the additions of acetone (at a concentration of 500 mg/l.) in the diluent lake water except after 1-hr exposure (Table 1). Pentachlorophenol was the most toxic compound tested and had a lethal concentration 50 times greater than phenol and about 25 times greater than 3,4 dimethyl phenol (xylenol). Kopperman et al. (1974), in comparable tests with Daphnia magna, found that 4-chlorophenol was 37 times more toxic than phenol and concluded that phenol toxicity was increased significantly with increasing halogen content.

The following chemical concentrations were within 50% of the lethal concentrations reported (as TLm or LC50 values) in the toxicological reviews of Battelle Columbus Laboratories (1971), Hunka (1974), and Smith (1974) for 96-hr fish static bioassays: acetic, adipic, and caproic acids, ethyl alcohol, cyclohexane, styrene, xylene, cresol, furfural, and phenol. Lethal levels for cyclohexane were two to three times higher than cited in Hunka (1974) in "clear" water. Time required to produce death in a study by MacPhee and Ruelle (1969) for 10 mg/l. concentrations of furfural and xylenol were three to four times less than in our study. Test water temperature was reported to be about 12° C. No further comparable information pertaining to fish was found for the remaining chemicals tested.

The U.S. Environmental Protection Agency (1974) assembled a list of chemicals regarded as hazardous substances based on their reported short-term toxicity and potential for being discharged or spilled into waterways. Ten of the compounds tested in our study were on this list. It may never be possible to evaluate with short-term tests the toxicity of all industry-related organic compounds and derivatives to aquatic life. Future work could be pointed towards methodology which may predict toxicity.

SECTION VI

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