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The Aquatic Toxicity of Organic Compounds to Embryo-Larval Stages of Fish and Amphibians

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RESEARCH REPORT NO. 133

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1982

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LEXINGTON, KENTUCKY

United States Department of the Interior
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March 1982

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ABSTRACT

Aquatic toxicity tests were conducted on 11 organic compounds considered hazardous to water resources. The toxicity of each compound was evaluated using embryo-larval stages of two to eight fish and amphibian species. Exposure was initiated at fertilization and maintained through 4 days posthatching. The animal test species exhibited varying degrees of sensitivity to the selected toxicants. Combined frequencies for mortality and teratogenesis at 4 days posthatching gave LC_{50} ranges of 3.66 to 8.25 mg/L for benzene, 1.16 to 22.42 mg/L for carbon tetrachloride, 0.11 to 1.20 mg/L for chlorobenzene, 2.03 to >68 mg/L for chloroform, 3.01 to 5.56 mg/L for 1,2-dichlorobenzene, 2.54 to ~34 mg/L for 1,2-dichloroethane, 13.16 to >48 mg/L for methylene chloride, 0.002 to 0.64 mg/L for nitrobenzene, 0.04 to ~32 mg/L for phenol, 0.02 to 0.85 mg/L for toluene, and 3.53 to 3.77 mg/L for m-xylene. The species which exhibited the greatest susceptibility to organic compounds were the rainbow trout, Rana pipiens, and Rana temporaria. The more sensitive amphibian species generally were those which normally are restricted to aquatic or moist terrestrial habitats, whereas the more tolerant amphibians included those semi-aquatic and terrestrial species which appear to be more broadly adapted ecologically. Of the 11 test compounds, nitrobenzene, toluene, chlorobenzene, and phenol were the most toxic. The least toxic organics included dichloroethane and methylene chloride. For three chlorinated alkanes, including methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), and carbon tetrachloride (CCl_4), toxicity was found to increase with the degree of chlorination. Concerning several aromatic hydrocarbons, benzene always was found to be less toxic than its monosubstituted analogs. Toxicity of the 11 compounds was further evaluated by calculating toxicant concentrations which produced embryo-larval mortality and/or teratogenesis at frequencies of 10% (LC_{10}) and 1% (LC_1). The LC_1 values, used to estimate toxicity thresholds, ranged from <0.1 for nitrobenzene to 69.9 µg/L for methylene chloride. A limited number of toxicity tests were performed to determine whether embryo-larval bioassays are suitable to assess effects of transitory chemical exposures, such as those resulting from intermittent discharges or accidental spills of chemicals into water resources. Results indicated that Rana pipiens embryos were sufficiently sensitive to quantify effects produced by short-term exposures to chloroform. Animals tested during the earliest embryonic stage appeared to be less tolerant than organisms exposed later in development.

Descriptors	Identifiers	
Embryos	Benzene	Phenol
Larvae	Carbon Tetrachloride	Toluene
Terata	Chlorobenzene	Xylene
Organic Compounds	Chloroform	Amphibians
Water Quality	Dichlorobenzene	Fish
	Dichloroethane	Aquatic Toxicity Tests
	Methylene Chloride	Embryo-Larval Tests
	Nitrobenzene	Predictive Toxicology

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CHAPTER I

INTRODUCTION

In recent years, it has become apparent that organic trace contaminants pose a formidable hazard to aquatic ecosystems. This is particularly true for aromatic and chlorinated hydrocarbons, many of which are characterized by long environmental half-lives, high tendencies for bioaccumulation in animal tissues, appreciable levels of biomagnification through aquatic food chains, and a propensity for extreme toxicity to embryos and other reproductive stages (1-8). However, well-documented freshwater criteria are lacking for most organic toxicants. Therefore, the major objective of this investigation was to evaluate the toxicity of 11 selected organic compounds to sensitive life-cycle stages of certain fish and amphibian species. The organics chosen for study included benzene, carbon tetrachloride, chloroform, chlorobenzene, 1,2-dichlorobenzene, 1,2-dichloroethane, methylene chloride, nitrobenzene, phenol, toluene, and m-xylene. All are important industrial chemicals and have contaminated natural waters as a result of point-source discharges or accidental spills. With the exception of m-xylene, these compounds appear among the initial list of 65 priority toxicants recently reviewed by the Environmental Protection Agency, and all except chlorobenzene were included in the NSF(RANN) Research Priority List of Organic Compounds Considered Hazardous to the Environment and Human Health (8, 9).

Toxicity tests were conducted using a flow-through system designed to stabilize exposure concentrations of organic compounds with varying degrees of volatility or solubility. Embryo-larval stages of the rainbow trout,

fathead minnow, and six species of amphibians were selected as test organisms, and exposure was initiated at fertilization and maintained through 4 days post-hatching. Specific objectives of this study included the following:

(1) Evaluate the comparative toxicity of 11 organic pollutants on fish and amphibian developmental stages (e.g., mortality, teratogenesis).

(2) Estimate toxicity thresholds for early life-cycle stages of aquatic organisms.

(3) Compare sensitivity of fish and amphibian species and determine whether species tolerance varies in a predictable manner with ecological adaptations or other criteria which could be applied in extrapolating test results to natural aquatic ecosystems.

(4) Determine to what extent the toxicity of the selected organic compounds may vary with specific aspects of molecular configuration (e.g., substitution groups).

(5) Administer a limited number of short-term toxicant exposures to eggs and early larvae in an effort to estimate the effects of spills or other intermittent discharges of organic contaminants on aquatic biota.

CHAPTER II

RESEARCH PROCEDURES

Test animals. Fish used in this study were the rainbow trout (Salmo gairdneri) and fathead minnow (Pimephales promelas). These species were chosen for their economic importance, seasonal availability, suitable egg production, and for variations in ecological and geographic distribution, including warmwater and coldwater habitats. Gravid rainbow trout were procured from the Wytheville National Fish Hatchery, Wytheville, Virginia or the Erwin National Fish Hatchery, Erwin, Tennessee. Eggs and sperm were obtained by the artificial milking and spawning procedures of Leitritz and Lewis (10). Fertilization was accomplished by mixing eggs and milt for 20 minutes. Freshly fertilized fathead minnow eggs were collected from the EPA Newtown Fish Toxicology Laboratory, Cincinnati, Ohio.

The amphibian species tested were Ambystoma gracile (northwestern salamander), Rana pipiens (leopard frog), Rana temporaria (European common frog), and Xenopus laevis (African clawed frog). In addition, toxicological data on Rana palustris (pickerel frog) and Bufo fowleri (Fowler's toad), taken from an earlier study (11), were included for purposes of discussion. These species were selected to represent different patterns of reproduction and variations in ecological habitat and geographical distribution to determine whether such factors correlate with susceptibility to organic toxicants. Rana pipiens were procured from Nasco, Oshkosh, Wisconsin, and A. gracile and R. temporaria were supplied by Charles Sullivan, Nashville, Tennessee. Eggs from R. pipiens and R. temporaria were obtained by inducing ovulation with pituitary extract, as described by Rugh (12). Ambystoma gracile were hormonally induced with pituitary extract and

human chorionic gonadotropin, following the procedures of Westerman (personal communication). Embryos of X. laevis were taken from cultures maintained in our laboratory. Freshly fertilized eggs from R. palustris and B. fowleri were collected locally from the Frankfort National Fish Hatchery in Frankfort, Kentucky.

Selection of organic toxicants. Toxicity tests were performed with benzene, carbon tetrachloride, chloroform, chlorobenzene, 1,2-dichlorobenzene, 1,2-dichloroethane, methylene chloride, nitrobenzene, phenol, toluene, and m-xylene. These organics are known to affect important waterways in the eastern U.S. (8, 13, 14), and all except xylene appear on the initial list of 65 priority toxicants identified by EPA (9). All compounds selected for testing were reagent grade.

Dilution water. The reconstituted dilution water used in the embryo-larval tests was prepared by the addition of reagent-grade calcium, magnesium, sodium, and potassium salts to distilled, double deionized water. Physicochemical characteristics are given in Table 1. Concentrations of cations and anions were within ranges published for freshwater resources in Arizona (15), Kentucky (16), and other areas of the U.S. (17, 18). Total chloride content, total dissolved solids, and the concentration of sodium plus potassium were under maximum levels of 170 mg/L, 400 mg/L, and 85 mg/L observed for 95% of U.S. waters found to support a good, mixed fish fauna (19). Specific conductivity compared favorably with the values of 150 to 500 µmhos/cm recommended for fish propagation (17), and osmolarity was well under the maximum limit of 50 mOsm/kg water suggested for fresh waters (20). Total alkalinity and pH also were within optimum ranges for aquatic habitats (17, 20, 21). In tests with trout embryos, dissolved oxygen was maintained between 9.2 and 9.8 mg/L. A minimum of 7 mg/L has been

Table 1. Reconstituted test water.

Hardness as $CaCO_3$:	50 mg/L	100 mg/L	200 mg/L
DISSOLVED SALTS[1], mg/L			
$CaCl_2$	37.5	75.0	150
$MgSO_4 \cdot 7H_2O$	37.5	75.0	150
$NaHCO_3$	100	100	100
KCl	5	5	5
CHEMICAL COMPOSITION, mg/L			
Ca	13.6	27.1	54.2
Mg	3.7	7.4	14.8
Na	27.4	27.4	27.4
K	2.6	2.6	2.6
Cl	26.3	52.3	98.2
HCO_3	72.6	72.6	72.6
SO_4	14.6	29.2	58.5
PHYSICOCHEMICAL CHARACTERISTICS[2]			
Hardness, mg/L as $CaCO_3$	53.3 ± 1.3	101.6 ± 4.4	197.5 ± 5.8
pH	7.84 ± 0.02	7.70 ± 0.01	7.78 ± 0.02
Total alkalinity, as mg/L as $CaCO_3$	66.7 ± 0.4	65.0 ± 0.4	65.3 ± 0.6
Conductivity, µmhos/cm	133.6 ± 1.4	176.0 ± 1.0	282.0 ± 1.9
Osmolarity, mOsm/Kg H_2O	8.9 ± 0.2	10.8 ± 0.3	12.7 ± 0.4
Total dissolved solids, mg/L	121.4 ± 4.4	171.8 ± 2.0	336.7 ± 7.8
Dissolved oxygen, mg/L at 13.5°C	9.9 ± 0.2	10.1 ± 0.2	10.1 ± 0.2

[1]Prepared in distilled, deionized water with a specific conductivity of 0.25 µmhos or less.

[2]Measurements made at 25°C except where noted. Mean with standard error determined for 10 replicates.

recommended for trout and salmon spawning waters (20). This reconstituted water has been used previously in embryo-larval tests with a broad array of inorganic and organic toxicants, and results have compared closely with those obtained when toxicants were administered in natural waters of similar composition (22, 23). Reconstituted water was used in this study to provide reproducible test conditions required for evaluating the comparative toxicity of the test compounds and the relative sensitivity of the animal species. Natural waters often are subject to substantial seasonal fluctuations in composition (e.g., dissolved solids, hardness, pH) and frequently contain background contaminants.

Test conditions and expression of data. Each of the eleven organic compounds was administered at four to seven concentrations, and toxicity tests were conducted with medium hard water (i.e., 100 mg/L as $CaCO_3$). Exposure was initiated within 30 minutes of fertilization for trout, A. gracile, R. pipiens, R. temporaria, and X. laevis and 2 to 8 hrs postspawning for the other three species. Average hatching times were 23, 5.5, 5, 5, 5, 4.5, 3, and 2 days for trout, A. gracile, fathead minnows, R. pipiens, R. temporaria, R. palustris, B. fowleri, and X. laevis, respectively. Toxicity tests were performed in temperature-regulated environmental rooms. Test water was monitored at regular intervals for temperature, dissolved oxygen, water hardness, pH, and conductivity, using a tele-thermometer with thermocouple (YSI model 42SC), oxygen meter (YSI model 51A), divalent cation electrode (Orion model 93-32), digital pH meter (Corning model 110), and conductivity meter (Radiometer model DCM 2e). The general water quality characteristics observed during the embryo-larval toxicity tests are summarized in Table 2.

Control eggs were cultured simultaneously with experimentals and under identical conditions, except for the omission of toxicants. Eggs were examined

Table 2. Water quality characteristics observed during embryo-larval toxicity tests.

Compound	Species	Test Conditions (Mean ± Standard Error)				
		Temperature (°C)	Dissolved Oxygen (mg/L)	Water Hardness (mg/L as $CaCO_3$)	pH	Conductivity (µmhos/cm)
Benzene	*R. pipiens*	20.2 ± 0.5	7.5 ± 0.1	96.6 ± 1.0	7.7 ± 0.02	150.0 ± 1.5
	A. gracile	20.2 ± 0.5	7.5 ± 0.1	96.6 ± 1.0	7.7 ± 0.02	150.0 ± 1.5
	S. gairdneri	13.0 ± 0.1	9.8 ± 0.0	104.3 ± 1.4	8.0 ± 0.03	147.3 ± 1.3
Carbon tetrachloride	*R. temporaria*	18.6 ± 0.3	7.3 ± 0.1	95.9 ± 0.9	7.7 ± 0.02	149.5 ± 1.0
	R. pipiens	18.6 ± 0.3	7.3 ± 0.1	95.9 ± 0.9	7.7 ± 0.02	149.5 ± 1.0
	S. gairdneri	13.3 ± 0.3	9.2 ± 0.1	104.2 ± 1.6	7.9 ± 0.04	143.6 ± 0.8
	A. gracile	18.6 ± 0.3	7.3 ± 0.1	95.9 ± 0.9	7.7 ± 0.02	149.5 ± 1.0
	P. promelas	20.4 ± 0.6	6.4 ± 0.0	96.1 ± 1.3	7.8 ± 0.02	154.6 ± 0.9
	X. laevis	18.6 ± 0.3	7.3 ± 0.1	95.9 ± 0.9	7.7 ± 0.02	149.5 ± 1.0
Chlorobenzene	*S. gairdneri*	14.3 ± 0.2	9.7 ± 0.1	103.6 ± 1.2	7.8 ± 0.02	140.1 ± 0.9
	A. gracile	20.2 ± 0.5	7.5 ± 0.1	98.8 ± 0.7	7.8 ± 0.02	153.7 ± 0.6
	R. pipiens	20.2 ± 0.5	7.5 ± 0.1	98.8 ± 0.7	7.8 ± 0.02	153.7 ± 0.6
Chloroform	*R. temporaria*	18.6 ± 0.3	7.3 ± 0.1	93.8 ± 0.9	7.7 ± 0.02	144.8 ± 0.9
	A. gracile	18.6 ± 0.3	7.3 ± 0.1	93.8 ± 0.9	7.7 ± 0.02	144.8 ± 0.9
	P. promelas	20.4 ± 0.6	6.4 ± 0.0	93.8 ± 1.2	7.7 ± 0.02	150.1 ± 0.9
	X. laevis	18.6 ± 0.3	7.3 ± 0.1	93.8 ± 0.9	7.7 ± 0.02	144.8 ± 0.9

Table 2 - continued.

Compound	Species	Test Conditions (Mean ± Standard Error)				
		Temperature (°C)	Dissolved Oxygen (mg/L)	Water Hardness (mg/L as $CaCO_3$)	pH	Conductivity (µmhos/cm)
Dichlorobenzene	*S. gairdneri*	13.1 ± 0.1	9.4 ± 0.0	96.0 ± 0.3	7.8 ± 0.01	141.4 ± 0.3
	R. pipiens	20.3 ± 0.4	8.7 ± 0.1	105.4 ± 0.6	7.3 ± 0.02	158.5 ± 0.6
Dichloroethane	*A. gracile*	20.2 ± 0.5	8.1 ± 0.1	98.2 ± 1.1	8.4 ± 0.03	152.6 ± 1.0
	R. pipiens	20.2 ± 0.5	8.1 ± 0.1	98.2 ± 1.1	8.4 ± 0.03	152.6 ± 1.0
	S. gairdneri	13.1 ± 0.1	9.5 ± 0.1	93.9 ± 0.4	7.8 ± 0.01	148.9 ± 0.8
Methylene Chloride	*S. gairdneri*	13.3 ± 0.3	9.4 ± 0.1	106.0 ± 1.5	7.8 ± 0.07	155.3 ± 2.6
	R. temporaria	18.6 ± 0.3	7.4 ± 0.1	97.9 ± 0.7	7.7 ± 0.02	148.2 ± 0.7
	A. gracile	18.6 ± 0.3	7.4 ± 0.1	97.9 ± 0.7	7.7 ± 0.02	148.2 ± 0.7
	P. promelas	20.4 ± 0.6	6.5 ± 0.0	95.3 ± 0.8	7.8 ± 0.02	151.0 ± 0.6
	X. laevis	18.6 ± 0.3	7.4 ± 0.1	97.9 ± 0.7	7.7 ± 0.02	148.2 ± 0.7
	R. pipiens	20.3 ± 0.4	7.5 ± 0.1	95.8 ± 0.2	7.9 ± 0.01	158.2 ± 0.3
Nitrobenzene	*S. gairdneri*	14.2 ± 0.1	9.8 ± 0.1	112.1 ± 1.2	7.9 ± 0.02	151.9 ± 1.7
	R. pipiens	17.9 ± 0.1	7.8 ± 0.0	96.4 ± 0.5	8.0 ± 0.02	156.6 ± 0.7
Phenol	*R. temporaria*	21.7 ± 0.4	7.3 ± 0.1	99.6 ± 0.8	7.7 ± 0.02	152.3 ± 1.4
	A. gracile	21.7 ± 0.4	7.3 ± 0.1	99.6 ± 0.8	7.7 ± 0.02	152.3 ± 1.4
	X. laevis	21.7 ± 0.4	7.3 ± 0.1	99.6 ± 0.8	7.7 ± 0.02	152.3 ± 1.4
	P. promelas	20.4 ± 0.4	6.6 ± 0.0	100.0 ± 1.1	7.8 ± 0.02	157.2 ± 1.2

Table 2 - continued.

Compound	Species	Test Conditions (Mean ± Standard Error)				
		Temperature (°C)	Dissolved Oxygen (mg/L)	Water Hardness (mg/L as $CaCO_3$)	pH	Conductivity (µmhos/cm)
Toluene	*S. gairdneri*	14.3 ± 0.2	9.6 ± 0.1	106.3 ± 1.2	7.8 ± 0.02	141.8 ± 0.9
	R. pipiens	20.2 ± 0.5	7.5 ± 0.1	98.9 ± 0.9	7.7 ± 0.02	151.0 ± 0.8
	A. gracile	20.2 ± 0.5	7.5 ± 0.1	98.9 ± 0.9	7.7 ± 0.02	151.0 ± 0.8
m-Xylene	*R. pipiens*	20.3 ± 0.4	8.7 ± 0.1	105.4 ± 0.6	7.3 ± 0.02	158.5 ± 0.6
	S. gairdneri	13.1 ± 0.1	9.4 ± 0.0	96.0 ± 0.3	7.8 ± 0.02	141.4 ± 0.3

daily to gauge extent of development and to remove dead specimens. Sample size ranged from 50 to 125 eggs per exposure chamber. Percent survival of normal organisms was expressed as the frequency in experimental populations/controls and was determined at hatching and 4 days after hatching. Normal organisms were defined as those animals free of gross teratic defects. Percent egg hatchability included all embryos, normal or aberrant, which completed the hatching process. Teratogenesis was expressed as the percent of survivors affected by gross, debilitating abnormalities considered likely to result in eventual mortality (24). Terata were infrequent in control populations and seldom exceeded 1%. Teratic organisms were counted as lethals in analyzing dose-response data. Log probit analysis (25) was applied to combined frequencies of embryo-larval mortality and teratogenesis to determine control-adjusted LC_{50}, LC_{10}, and LC_1 values with 95% confidence limits. The LC_1, taken as the concentration which produced 1% control-adjusted impairment in test populations, was used to estimate the toxicity threshold for each compound. All LC values, whether expressed at hatching or 4 days posthatching, were based on accumulative test responses incurred from the onset of treatment.

Embryo-larval test system. Toxicity tests were conducted using the flow-through system illustrated in Figures 1 and 2. Graduated flow from a syringe pump was used to administer toxicant to a mixing chamber situated ahead of each egg exposure chamber. Dilution water was delivered to the mixing chamber by regulated flow from a peristaltic pump. Continuous aeration was supplied to the peristaltic pump reservoirs. Solutions from the two pump channels were mixed by mechanical stirring or homogenization, and delivered from the mixing unit to the test chamber under positive pressure. Toxicant exposure level was regulated by adjusting the mixing ratio between pumping units and/or by varying

the concentration of toxicant delivered from the syringe pump. Flow rates from syringe and peristaltic pumps were monitored using Gilmont flow meters and timed volumetric measurements. Flow rate was set at 200 mL/hr for 500-mL test chambers, giving a retention time of 2.5 hrs. The flow-through system was operated using Brinkmann multichannel peristaltic pumps (model 131900) and Sage syringe pumps (model 355). Sage pumps were fitted with modified syringe holders, as noted previously by Birge, et al. (26), and each unit was operated with up to six double-ground glass syringes. Syringe capacity varied from 0.25 mL to 100 mL, depending on the toxicant.

The toxicological characterization of environmental pollutants is frequently complicated by physical and chemical properties of the test compounds (22, 26-28). For example, volatility may preclude adequate regulation of exposure concentrations in aquatic test systems, especially when open chambers are used. Though emulsifiers or carrier solvents may be of some aid in testing hydrophobic organics, they generally introduce undesirable variables. Birge, et al. (11, 22) recently developed a continuous-flow system designed for testing volatile and insoluble organic compounds which are difficult to stabilize with conventional techniques. Designed for use with embryo-larval stages, a covered flow-through test chamber devoid of an air-water interface was used to minimize evaporative loss of volatile toxicants (e.g., chloroform, methylene chloride). Insoluble compounds (e.g., chlorobenzene) were suspended in influent water by mechanical homogenization and maintained by moderate agitation in the exposure chamber and regulation of retention time.

The covered exposure chamber was constructed from 3" Pyrex pipe joints, provided with a clamp-locking O-ring seal. By use of standard glass-blowing techniques, the pipe was cut and sealed to give a capacity of 0.5 L (Figure 3).

An outlet tube was annealed to the cover, with an inlet positioned near the bottom of the chamber. A stainless steel inlet screen was situated 3 cm above the bottom of the dish, dividing the chamber into an upper egg compartment and a lower stirring compartment. Eggs were supported on the inlet screen, and a Teflon-coated magnetic stirring bar was used in the lower compartment to provide moderate, continuous agitation of test solution. An upper outlet screen was used to retain test organisms. The outlet screen was held in place by a Pyrex pedestal, and the inlet screen was supported on the constricted upper wall of the stirring compartment (Figure 3). Access to test organisms was obtained by opening the watertight joint and removing the chamber cover. Prior to opening the chamber, a rapid-disconnect was used to remove the inlet line and drain the fluid level down to the O-ring seal. When perfused with a continuous flow of oxygen-saturated water, the sealed chamber was essentially free of standing air space.

As noted above, toxicant and dilution water were blended by either mechanical mixing or homogenization. A stoppered 125-mL side-arm flask, operated with a magnetic stirrer (Magnestir model S8290), was adequate for maintaining stable concentrations of water-soluble organic compounds (Figure 2). However, high-speed homogenization was required to suspend less soluble organics in test water. This was accomplished with an Oster homogenizer, equipped with a 400-mL glass container. The latter was provided with inlets for syringe and peristaltic pump lines and a side outlet for delivery of water-toxicant homogenate to the test chamber (Figures 3.1, 3.2). Pyrex tubing (3 mm O.D.) was used to extend pump inlet lines to a depth of 3 cm above the stirring blades. Though homogenization initially was maintained continuously, intermittent operation generally proved adequate. Blending time was regulated with an electronic timer and varied for

different organic compounds, depending on the stability of their aqueous suspensions. In addition, moderate agitation supplied to the exposure chamber and regulation of flow rate were used to prevent immiscible materials from partitioning out of test water.

Analytical procedures. Exposure concentrations of all organic toxicants were confirmed by daily analyses of test water, using gas-liquid chromatography (GLC), high pressure liquid chromatography (HPLC), or spectrophotometric methods. The GLC determinations were performed on a Hewlett Packard gas chromatograph (model 5838A) equipped with a purge and trap system (model 7675A) and a flame ionization detector. The HPLC analyses were conducted using a Beckman high pressure liquid chromatograph (model 320). Spectrophotometric quantification was accomplished on a Varian-Techtron spectrophotometer (model 635).

Carbon tetrachloride, chloroform, dichloroethane, and methylene chloride were analyzed directly from 1- to 15-mL aliquots of test water, using the GLC purge and trap system. Samples were purged with dry, pre-purified nitrogen at 10 mL/min. Each compound was adsorbed on a Tenax GC trap at ambient temperature, desorbed at 200°C, and analyzed at programmed temperatures of 50 to 105°C on a 2 m x 2 mm I.D. glass column. The stationary phase was 10% Carbowax 20M on 80/100 Anakrom U, and the detector temperature was 250°C. Nitrogen was used as the carrier gas and the flow rate was 10 mL/min. The detection limit for these compounds was 0.1 ug/L.

Benzene, chlorobenzene, dichlorobenzene, toluene, and xylene were determined by either GLC or HPLC. The latter method was used to analyze toxicant concentrations at and above 1 mg/L. Water samples (20 µL-2 mL) were eluted on a 25 cm x 4.6 mm octyldecyl sulfate column (ODS-18) with a 90:10 methanol:water mixture at a flow rate of 1.5 mL/min. Quantification was accomplished at 254 nm. Exposure levels below 1 mg/L were analyzed directly from 10-mL samples of test water,

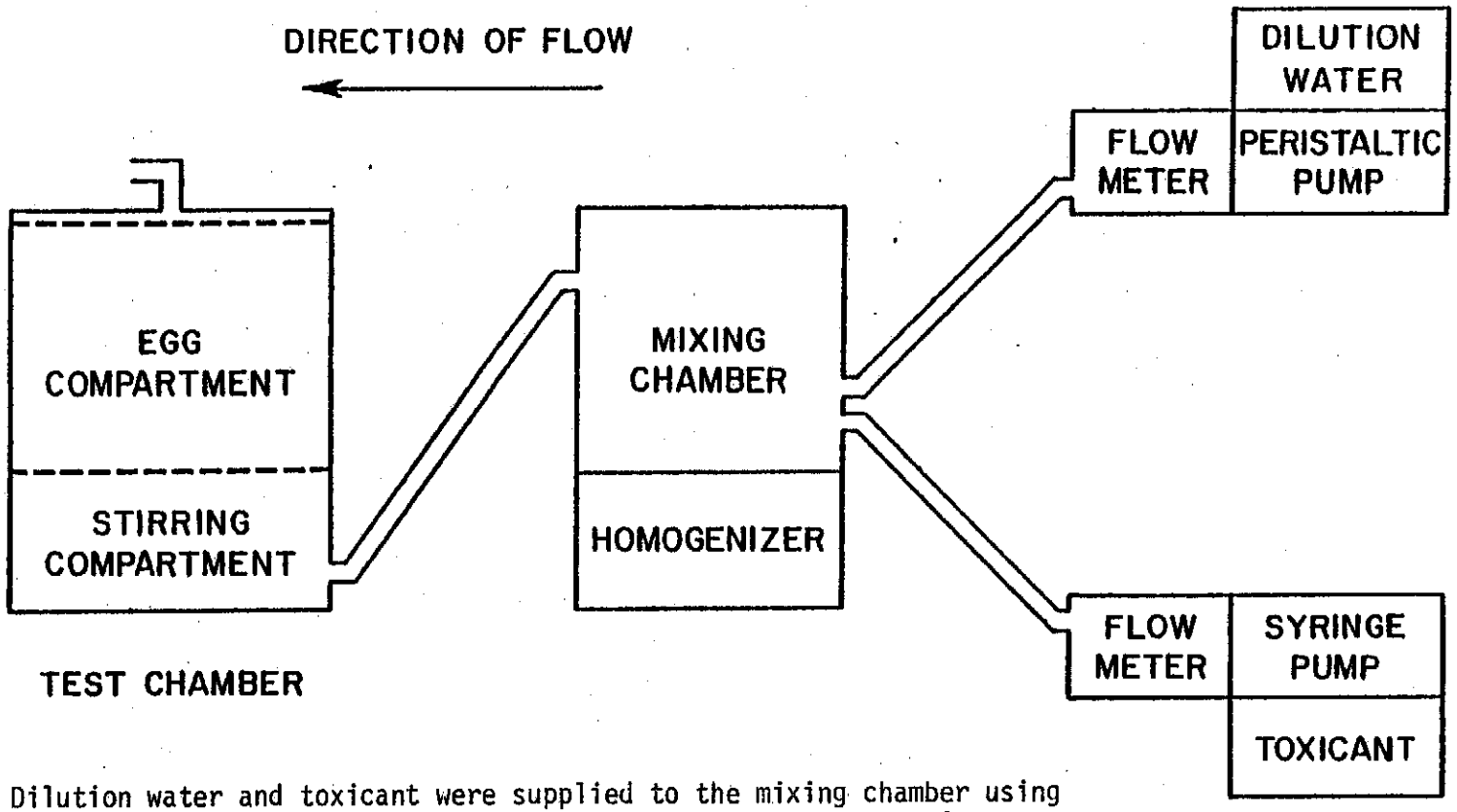
using the purge and trap procedures previously described. Column temperature was programmed from 70 to 105°C. The detection limit was 0.5 µg/L.

High pressure liquid chromatography also was used to analyze nitrobenzene. Techniques were similar to those given above, except that the ODS-18 column was eluted with a 70:30 methanol:water mixture. The limit of detection for this compound was 10 µg/L.

Phenol was determined using the 4-aminoantipyrine procedure with chloroform extraction, as outlined in Standard Methods (29). Samples were quantified spectrophotometrically at 460 nm, and the detection limit was 1.0 µg/L.

Figure 1

Embryo-Larval Test System



Dilution water and toxicant were supplied to the mixing chamber using peristaltic and syringe pumps. After blending, the water-toxicant mixture was perfused through the test chamber (egg compartment) under positive pressure. A magnetic stirrer was used to insure homogeneous distribution of toxicant.

Figure 2

Flow-Through Bioassay System for Embryo-Larval Stages

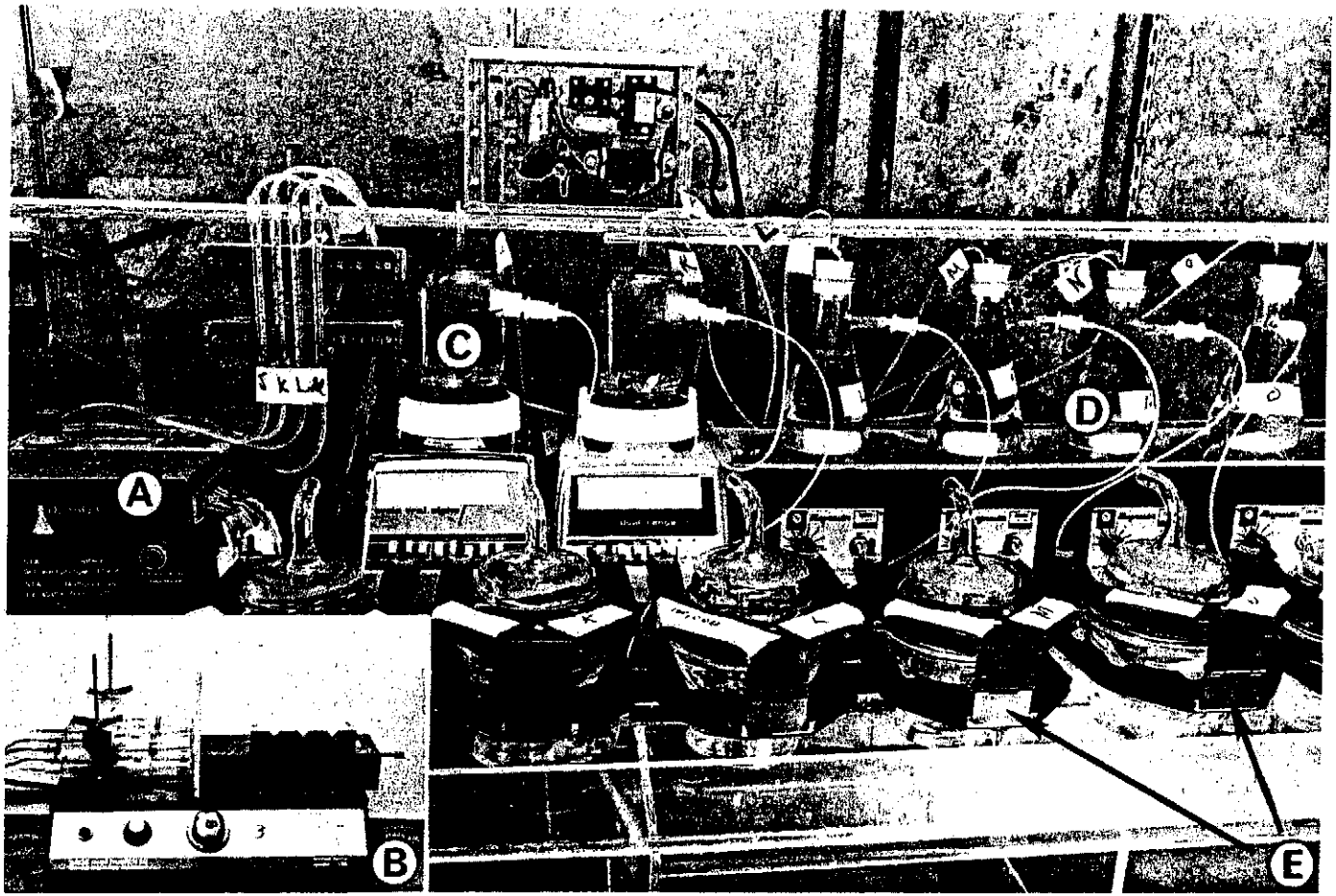
Peristaltic pumps (A) and syringe pumps (B) were used to supply dilution water and toxicant to mixing chambers (C and D). Water and toxicant were blended with homogenizers (C) or magnetic stirrers (D) and delivered under positive pressure to test chambers (E). The multichannel system was maintained in an environmental room and syringe pumps were mounted on the outside wall to avoid effects of high humidity on operation.

Figure 3

Exposure Chamber

3.1 Disassembled chamber, including cover (A), egg compartment (B), stirring compartment (C), screen support (D), and O-ring with inlet and outlet screens (E).

3.2 Assembled test chamber, showing outlet from egg compartment (A), locking clamp (B), and stirring compartment inlet (C).


A
B
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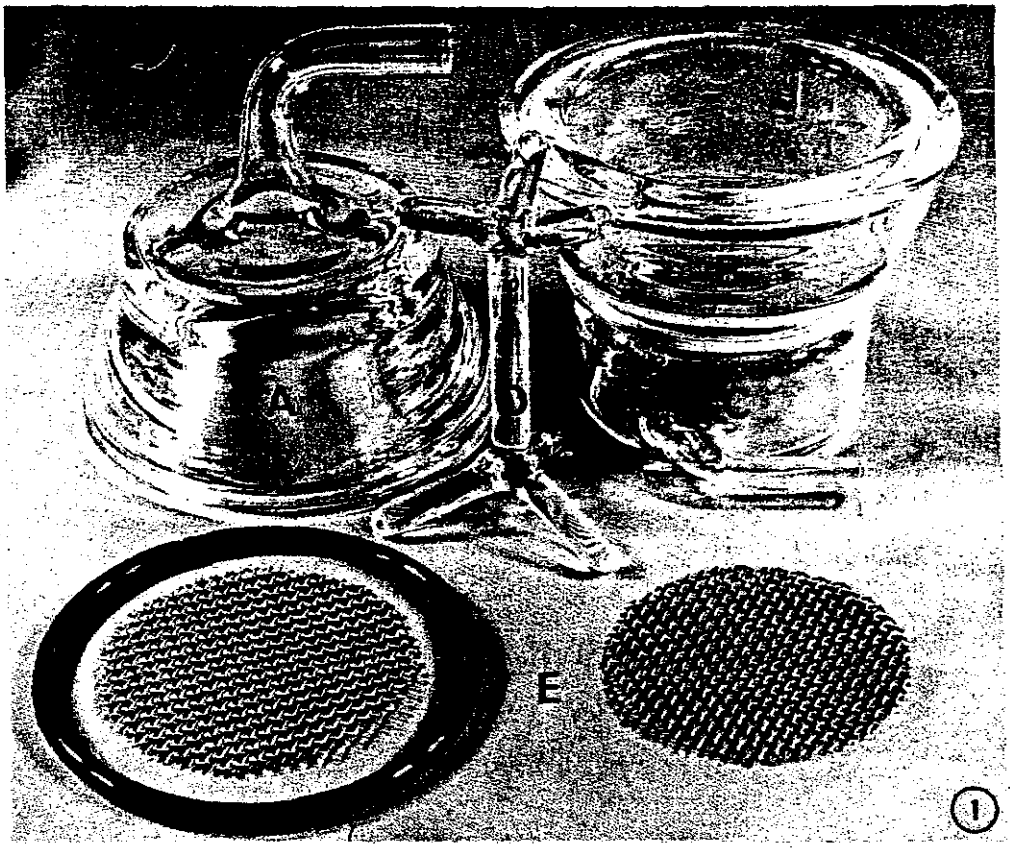
E


A
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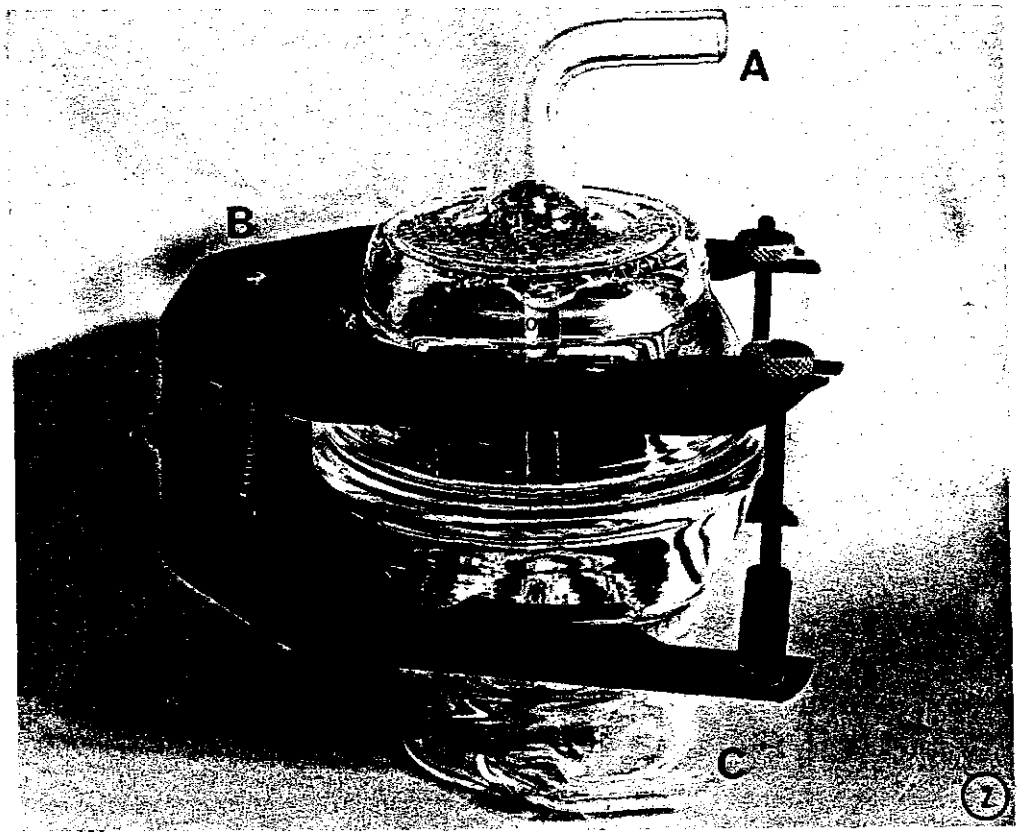
1


A
B
C
2

CHAPTER III

RESULTS AND DISCUSSION

Toxicity tests with embryo-larval stages were performed on 11 organic compounds, using medium hard water (i.e., 100 mg/L as $CaCO_3$). As noted above, survival data were adjusted to a control baseline which ranged from 84% to 99% at 4 days posthatching. Dose-response data for the selected organics are given in Tables 3 through 13, and log-probit values are presented in Tables 14 and 15. Toxicant monitoring data indicated good reproducibility of exposure concentrations.

Toxicity tests with benzene were performed on embryo-larval stages of the rainbow trout, A. gracile, and R. pipiens (Table 3). Rana pipiens exhibited the greatest sensitivity. Teratic organisms occurred at a frequency of 16% at an exposure level of 5.07 mg/L. Survival of normal larvae at 4 days posthatching was 95%, 90%, 79%, 62% and 32% at benzene concentrations of 0.016, 0.048, 0.61, 2.99, and 5.07 mg/L, respectively. Concerning the three species tested, the order of increasing tolerance was R. pipiens, A. gracile, and rainbow trout, and LC_{50} values at 4 days posthatching were 3.66, 5.21, and 8.25 mg/L, respectively (Table 14). Based on data in Table 3, benzene was not considered to be a highly teratogenic agent to any of the test animals.

Carbon tetrachloride was administered to embryo-larval stages of the fathead minnow, rainbow trout, A. gracile, R. pipiens, R. temporaria, and X. laevis (Table 4). Rana temporaria was the most sensitive species, and complete mortality was observed at 41.2 mg/L. Terata occurred at frequencies of 3% to 67% over a concentration range of 0.67 to 41.2 mg/L. Survival at 4 days posthatching was 94%, 52%, and 19% at exposure levels of 0.01, 0.67, 24.0 mg/L, respectively. Including the six species tested in this study and two additional amphibian species evaluated in an earlier investigation (11), the order of increasing

tolerance was R. temporaria, R. pipiens, rainbow trout, A. gracile, R. palustris, B. fowleri, fathead minnow, and X. laevis. This ranking was based on LC_{50} values of 1.16, 1.64, 1.97, 1.98, 2.37, 2.83, 4.00, and 22.42 mg/L, respectively (Table 14). While teratogenesis was observed with all test species, this response generally was not significant at the lower end of the carbon tetrachloride exposure ranges.

Chlorobenzene was tested on developmental stages of the rainbow trout, A. gracile, and R. pipiens (Table 5). The least tolerant species was the trout, suffering nearly complete mortality at 0.52 mg/L. Both mortality and teratogenesis were detected at exposure levels as low as 0.013 mg/L. Teratic organisms were observed at frequencies of 4%, 14%, and 60% at concentrations of 0.062, 0.20, and 0.52 mg/L, respectively. Survival of normal larvae was 90% at 0.013 mg/L, 27% at 0.20 mg/L, and 0% at 9.79 mg/L. The order of increasing tolerance for the test species was rainbow trout, A. gracile, R. pipiens, and LC_{50} values were 0.11, 1.15, and 1.20 mg/L, respectively (Table 14). Teratogenesis was not considered to be a highly significant test response for the two more tolerant species.

Aquatic toxicity tests with chloroform were performed on eggs and larvae of the fathead minnow, A. gracile, R. temporaria, and X. laevis (Table 6). Of this group, R. temporaria was the most sensitive. Control-adjusted survival varied from 51% to 102% at a concentration range of 9.90 to 0.001 mg/L and decreased to 35% at 64.1 mg/L. Anomalous larvae were observed at frequencies of 3%, 9%, and 21% at toxicant exposure levels of 6.05, 9.90, and 64.1 mg/L, respectively. Frequencies of teratogenesis were similar for the other species. The order of increasing tolerance to chloroform, including results from earlier investigations (11, 22), was rainbow trout, R. pipiens, R. temporaria, R. palustris, A. gracile,

B. fowleri, fathead minnow, and X. laevis, for which LC_{50}'s at 4 days post-hatching were 2.03, 4.16, 16.95, 20.55, 21.58, 35.14, >58, and >68 mg/L (Table 14).

Toxicity tests with 1,2-dichlorobenzene were conducted on developmental stages of the rainbow trout and R. pipiens (Table 7). The trout was the more sensitive species, and complete mortality occurred at a concentration of 13.2 mg/L. Survival of normal larvae at 4 days posthatching was 89%, 82%, and 71% at 0.007, 0.29, and 2.31 mg/L, respectively, and anomalous larvae were observed at frequencies of 6% to 16% in this toxicant exposure range. Teratogenesis was somewhat less a factor in studies with R. pipiens. The LC_{50}'s were calculated to be 3.01 and 5.56 mg/L in tests with the trout and R. pipiens, respectively (Table 14).

Embryo-larval stages of the rainbow trout, A. gracile, and R. pipiens were given continuous exposure to 1,2-dichloroethane, and dose-response data are presented in Table 8. Comparing the three species, A. gracile was the most sensitive to this compound. Survival frequencies varied from 96% to 67% over a concentration range of 0.002 to 0.99 mg/L and decreased to 24% at 21.4 mg/L. Although teratogenesis was observed in tests with all three species, it was not a significant response at the lower exposure concentrations. The order of increasing species tolerance was A. gracile, R. pipiens, and rainbow trout, based on LC_{50} values of 2.54, 4.40, and ~34 mg/L, respectively (Table 14).

Methylene chloride was administered to embryo-larval stages of six fish and amphibian species, including the fathead minnow, rainbow trout, A. gracile, R. pipiens, R. temporaria, and X. laevis (Table 9). Although the trout was the least tolerant of this group, R. temporaria and A. gracile were only slightly less sensitive. Survival of normal trout larvae at 4 days posthatching was 100%, 85%, 44%, and 9% at exposure levels of 0.008, 0.41, 23.1, and 36.5 mg/L, re-

spectively. Teratic larvae were observed only at exposure concentrations of 5.55 mg/L or greater. A similar trend was noted for all other species tested. Methylene chloride proved to be one of the least toxic compounds to embryo-larval stages. The order of increasing species tolerance, including data for two amphibians from a previous study (11), was rainbow trout, R. temporaria, A. gracile, fathead minnow, X. laevis, R. palustris, B. fowleri, and R. pipiens. Respective methylene chloride LC_{50} values were 13.16, 16.93, 17.82, ~34, >29, >32, >32, and >48 mg/L (Table 14). The differences in species sensitivity were not as great with methylene chloride as they were in tests with the more toxic organic compounds (e.g., phenol).

Nitrobenzene was tested on embryos and larvae of the rainbow trout and R. pipiens. Trout was decidedly the more sensitive species in terms of both mortality and teratogenesis (Table 10). Survival of normal organisms at 4 days posthatching was 62% and 3% at exposure levels of 0.001 mg/L and 0.12 mg/L, respectively. The LC_{50} for nitrobenzene was 0.002 mg/L, a value approximately 300 times greater than that of 0.64 mg/L determined with R. pipiens (Table 14).

Aquatic toxicity tests with phenol were performed on embryo-larval stages of the fathead minnow, A. gracile, R. temporaria, and X. laevis (Table 11). Of these four species, R. temporaria was the most sensitive. A concentration of 14.0 mg/L produced complete mortality by 4 days posthatching, and phenol at 0.002, 0.01, 0.72, and 1.45 mg/L reduced survival of normal larvae to 90%, 82%, 36%, and 6%, respectively. Anomalous R. temporaria larvae were detected at frequencies of 0% to 56% in this exposure range. Tests with phenol have been conducted in our laboratory with four additional animal species (11, 31), and the order of increasing tolerance for all eight species was R. pipiens, rainbow trout, R. temporaria, A. gracile, B. fowleri, X. laevis, R. palustris,

and the fathead minnow. Respective LC_{50} values were 0.04, 0.15, 0.27, 0.38, 2.45, 7.68, 9.87, and ~32 mg/L (Table 14).

Toluene was administered to eggs and larvae of three species, including the rainbow trout, A. gracile, and R. pipiens (Table 12). The trout exhibited the greatest sensitivity to this compound, and 100% mortality was observed at a concentration of 11.3 mg/L. Survival of normal trout larvae was 84%, 49%, 29%, and 6% at toluene exposure levels of 0.004, 0.026, 0.081, and 0.16 mg/L, respectively. Teratogenesis reached frequencies as high as 31% in this range of concentrations. Although anomalous larvae were observed with the other two species, teratogenesis was not appreciable except at the higher exposure levels. Increasing tolerance for the three species was in the order trout, R. pipiens, and A. gracile, based on LC_{50} values of 0.02, 0.39, and 0.85 mg/L, respectively (Table 14).

Aquatic toxicity tests were conducted on m-xylene, using embryo-larval stages of the rainbow trout and R. pipiens (Table 13). The two species were about equally sensitive to this compound, as reflected by the LC_{50} values of 3.53 for R. pipiens and 3.77 mg/L for trout (Table 14). However, it should be noted that trout embryos were more susceptible to teratogenesis throughout the entire xylene exposure range. For example, at concentrations of 0.005, 0.16, 8.41, and 21.8 mg/L, the frequencies of anomalous trout larvae were 9%, 5%, 31%, and 44%, respectively. By comparison, teratic larvae occurred sporadically with R. pipiens and did not exceed 6% at concentrations below 33.6 mg/L.

In most tests, fish and amphibian embryos proved to be considerably more sensitive than larvae to the selected organic compounds. Survival usually did not decrease substantially during the posthatched period, and except in several instances, LC_{50} values calculated at 4 days posthatching were not decidedly lower than those determined at hatching (Table 14). Examples of high larval mortality

included tests in which carbon tetrachloride was administered to the fathead minnow, A. gracile, and R. pipiens (Table 4) and dichlorobenzene and nitrobenzene were administered to R. pipiens (Tables 7, 10).

To provide a toxicological ranking for the 11 organic compounds, all were used in tests with embryo-larval stages of the rainbow trout and R. pipiens. Based on median lethal concentrations determined at 4 days posthatching with the trout (Table 14), the order of decreasing toxicity was as follows: nitrobenzene (0.002 mg/L), toluene (0.02 mg/L), chlorobenzene (0.11 mg/L), phenol (0.15 mg/L), carbon tetrachloride (1.97 mg/L), chloroform (2.03 mg/L), 1,2-dichlorobenzene (3.01 mg/L), m-xylene (3.77 mg/L), benzene (8.25 mg/L), methylene chloride (13.16 mg/L), and 1,2-dichloroethane (~34 mg/L). In tests with R. pipiens (Table 14), the toxicological ranking was phenol (0.04 mg/L), toluene (0.39 mg/L), nitrobenzene (0.64 mg/L), chlorobenzene (1.20 mg/L), carbon tetrachloride (1.64 mg/L), m-xylene (3.53 mg/L), benzene (3.66 mg/L), chloroform (4.16 mg/L), 1,2-dichloroethane (4.40 mg/L), 1,2-dichlorobenzene (5.56 mg/L), and methylene chloride (>48 mg/L). Though the order of toxicity varied somewhat for the two species, several trends were evident. The most toxic compounds were nitrobenzene, toluene, chlorobenzene, and phenol, and the least toxic compounds included 1,2-dichloroethane and methylene chloride. A particularly interesting relationship was observed for the three chlorinated alkanes (i.e., carbon tetrachloride, chloroform, methylene chloride). Toxicity to embryo-larval stages of these species increased with the degree of chlorination. With the trout, the LC_{50} values were 13.16, 2.03, and 1.97 mg/L for methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), and carbon tetrachloride (CCl_4), respectively. In tests with R. pipiens, respective LC_{50}'s for these compounds were >48, 4.16, and 1.64 mg/L. Because of this relationship, six additional animal species were used to evaluate the toxicity of these compounds.

In all eight comparisons, carbon tetrachloride proved to be the most toxic of the three chlorinated alkanes. For about half the species, methylene chloride was the least toxic compound and in the other cases, chloroform and methylene chloride produced similar dose-response effects. Although the correlation was less definitive for certain animal species, it was generally concluded that increasing chlorination resulted in increased toxicity for these compounds. This is consistent with results obtained in an earlier investigation with polychlorinated biphenyls, in which toxicity to fish and amphibian embryo-larval stages increased with percent chlorination (30).

Another structure-activity relationship was apparent from this study. Benzene always was found to be less toxic than its monosubstituted analogs. For example, in tests with trout, the median lethal concentration was 8.25 mg/L for benzene (C_6H_6), compared to LC_{50} values of 0.15 mg/L for phenol (C_6H_5OH), 0.11 mg/L for chlorobenzene (C_6H_5Cl), 0.02 mg/L for toluene ($C_6H_5CH_3$), and 0.002 mg/L for nitrobenzene ($C_6H_5NO_2$). Tests with R. pipiens resulted in LC_{50}'s of 3.66, 1.20, 0.64, 0.39, and 0.04 mg/L for benzene, chlorobenzene, nitrobenzene, toluene, and phenol, respectively. Although the data are inconclusive at this time, the monosubstituted benzenes appeared to be more toxic than corresponding disubstituted benzenes. When trout embryo-larval stages were exposed to chlorobenzene (C_6H_5Cl) and dichlorobenzene ($C_6H_4Cl_2$), respective LC_{50} values were 0.11 and 3.01 mg/L. Using the same species, the LC_{50} for toluene ($C_6H_5CH_3$) was 0.02 mg/L compared to a value of 3.77 mg/L for m-xylene ($C_6H_4(CH_3)_2$). Similar patterns of toxicity were observed in tests with R. pipiens.

Results of the present study, in conjunction with selected data taken from several previous investigations (11, 22, 31), were used to compare the relative sensitivities of certain fish and amphibian species to organic compounds. Tests

with eight species were performed on carbon tetrachloride, chloroform, methylene chloride, and phenol (Table 14). In studies with chloroform, the order of decreasing species sensitivity was rainbow trout, R. pipiens, R. temporaria, R. palustris, A. gracile, B. fowleri, fathead minnow, and X. laevis, based on LC_{50} values at 4 days posthatching of 2.03, 4.16, 16.95, 20.55, 21.58, 35.14, >58, and >68 mg/L, respectively. In tests conducted with phenol, species sensitivity decreased in the order R. pipiens (0.04 mg/L), rainbow trout (0.15 mg/L), R. temporaria (0.27 mg/L), A. gracile (0.38 mg/L), B. fowleri (2.45 mg/L), X. laevis (7.68 mg/L), R. palustris (9.87 mg/L), and fathead minnow (~32 mg/L). For carbon tetrachloride, the order was R. temporaria (1.16 mg/L), R. pipiens (1.64 mg/L), rainbow trout (1.97 mg/L), A. gracile (1.98 mg/L), R. palustris (2.37 mg/L), B. fowleri (2.83 mg/L), fathead minnow (4.00 mg/L), and X. laevis (22.42 mg/L). Species sensitivity to methylene chloride decreased in the series rainbow trout, R. temporaria, A. gracile, fathead minnow, X. laevis, R. palustris, B. fowleri, and R. pipiens, based on LC_{50} values of 13.16, 16.93, 17.82, ~34, >29, >32, >32, and >48 mg/L, respectively. The most sensitive species included rainbow trout, R. pipiens, and R. temporaria, while the fathead minnow, B. fowleri, R. palustris, and X. laevis were the most tolerant. For the most part, A. gracile reflected a sensitivity intermediate to those displayed by the other groups.

With regard to the two fish species tested, embryo-larval stages of the rainbow trout were consistently more sensitive than those of the fathead minnow. This relationship was in agreement with results from previous studies (32). Furthermore, embryos and larvae of a number of warmwater fish species, such as the largemouth bass, bluegill sunfish, and goldfish, have been found to be more tolerant than early-life stages of the trout (22, 31, 33, 34). One possible explanation for the increased sensitivity of the trout relates to its longer

period of embryonic development (23 days at 13°C), compared to that for warm-water species (3 to 4 days at 20-22°C).

In earlier investigations in which developmental stages of several species of amphibians were treated with metallic toxicants, a correlation was observed between species sensitivity and particular ecological and reproductive adaptations. Results indicated that anuran species which were more narrowly adapted for ecological requirements and/or mode of reproduction generally were more susceptible to pollution stress. Conversely, greater tolerance was observed for more broadly adapted anurans, particularly those capable of withstanding greater latitudes of natural environmental stress. Initial support for this concept was presented in studies with copper (34), and embryo-larval bioassay data for mercury provided an even closer relationship between tolerance and species adaptability (33).

Ecological requirements and reproductive characteristics of the amphibian species included in this study have been reviewed by Birge and Black (34), Birge, et al. (33), Bishop (35), Conant (36), Leutscher (37), Vial (38), and Wright and Wright (39). Those species observed to be more sensitive to organic pollutants (i.e., R. pipiens, R. temporaria) usually are restricted to aquatic or moist terrestrial habitats. With the exception of X. laevis which is principally an aquatic organism, those species found to have greater tolerance (i.e., B. fowleri, R. palustris) are more broadly adapted ecologically. They include semi-aquatic and terrestrial species which generally can frequent a wide variety of habitats. These and other considerations further support the view that more broadly adapted anuran species usually exhibit greater tolerance to pollution stress. As noted above, an intermediate level of sensitivity was exhibited by A. gracile, the northwestern salamander. As this was the only urodele included for study with

the five anuran species, no firm conclusions can be reached concerning differences between the two major groups of amphibians.

In order to estimate toxicity thresholds for early life-cycle stages of fish and amphibians, log probit analyses were used to calculate concentrations of organic contaminants which produced 10% (LC_{10}) and 1% (LC_1) control-adjusted impairment of test populations. These determinations were based on combined frequencies of mortality and teratogenesis observed for embryo-larval stages and were calculated using dose-response data taken at 4 days posthatching. In most cases, LC_1 and LC_{10} values were calculated for the two most sensitive fish and/or amphibian species tested (Table 15). The selection of sensitive species was based on comparisons of median lethal concentrations (LC_{50}) determined for the different organic toxicants. It should be noted that in determining the LC_1 or LC_{10}, it is important to achieve a good delineation of test responses. Sharp truncations of or internal discontinuities within the dose-response curve may skew or preclude calculation of these lower LC values. In this investigation, the characterization of the dose response generally was adequate for reliable determinations. However, the toxicity data from tests with 1,2-dichlorobenzene and m-xylene were not sufficient to allow calculation of reliable LC_1 and LC_{10} values. For the same reason, data on only one species is presented for 1,2-dichloroethane and methylene chloride.

In fish embryo-larval tests with both inorganic and organic aquatic contaminants (22, 23), it was established that probit LC_1 values taken at 4 days posthatching generally compared closely with maximum acceptable toxicant concentrations (MATC) determined in chronic life-cycle studies. McKim (40) has provided additional support for using embryo-larval tests to estimate MATC's for freshwater biota. As few chronic life-cycle studies have been conducted on

the 11 compounds evaluated in this investigation, LC_1 values determined in these embryo-larval tests provided a basis for estimating the threshold tolerance of fish and amphibian species to organic toxicants (30, 33). In addition, LC_{10} values were used to delineate the concentrations at which toxicants began to produce appreciable reproductive impairment.

In toxicity tests performed on benzene, the two most sensitive species of the three tested were R. pipiens and A. gracile. Probit LC_1 and LC_{10} values ranged from 3.2 to 75.6 µg/L and 68.2 to 478.1 µg/L, respectively (Table 15). No chronic toxicity data on benzene have been reported for freshwater vertebrates. However, based on the above results, it would appear that reproduction of sensitive amphibian species may be appreciably impaired at benzene concentrations above 100 µg/L.

Carbon tetrachloride was administered to eight fish and amphibian species, and the two most sensitive were R. temporaria and R. pipiens. The LC_1 and LC_{10} values calculated at 4 days posthatching were 1.1 and 25.0 µg/L in tests with R. temporaria and 1.4 and 33.9 µg/L in tests with R. pipiens (Table 15). As with benzene, no chronic toxicity tests on carbon tetrachloride have been reported for freshwater species. Using data from the present study, it was estimated that concentrations of 30 µg/L or above could adversely affect certain sensitive amphibian species.

Embryo-larval stages of three species were exposed to chlorobenzene. The rainbow trout and A. gracile exhibited the greatest sensitivity, and the LC_1 values were 14.3 and 9.7 µg/L, respectively. The corresponding LC_{10}'s were 36.1 and 82.7 µg/L (Table 15). In previous investigations studying the effects of tri- and tetrachlorobenzenes on embryo-larval stages of the fathead minnow, concentrations of 286 to 750 µg/L were reported to produce chronic

effects (41). This range is approximately an order or magnitude greater than the LC_{10} values given above. However, as indicated earlier, the fathead minnow has proved to be consistently more tolerant than the trout or sensitive amphibian species.

Chloroform was administered to eight different fish and amphibian species, and embryo-larval stages of the rainbow trout and R. pipiens were the least tolerant. The LC_1 values ranged from 6.2 to 54.9 µg/L and LC_{10}'s varied from 83.2 to 383.4 µg/L (Table 15). No data on the chronic toxicity of chloroform were available for freshwater vertebrates. However, considering results of this study and those previously reported for a highly sensitive amphibian species (i.e., Hyla crucifer, 11), critical life stages may be affected by concentrations of about 5 µg/L, and embryopathic effects may become appreciable in the range of 20 to 400 µg/L.

Developmental stages of three fish and amphibian species were given continuous exposure to 1,2-dichloroethane. In tests with R. pipiens, the LC_1 was 13.7 µg/L and the LC_{10} was 183.2 µg/L (Table 15). A chronic value of 20 mg/L has been reported for this compound in a previous test with the fathead minnow (42). However, as noted above, fathead minnow stages were found to be substantially more tolerant than sensitive amphibian species to several organic toxicants. In addition, data given in Tables 8 and 14 for A. gracile, R. pipiens and rainbow trout indicated that 1,2-dichloroethane may be selectively more toxic to amphibians than to fish. Based on results from the tests with R. pipiens, concentrations exceeding 200 µg/L could prove deleterious to the reproductive success of sensitive amphibian species.

Methylene chloride was administered to embryo-larval stages of eight vertebrate species. Rana temporaria was one of the most sensitive species tested,

and the LC_1 and LC_{10} values for this compound were 69.9 and 822.4 µg/L, respectively (Table 15). These concentrations were close to those of 92.5 and 981.0 µg/L reported earlier for the bullfrog, R. catesbeiana (11). No chronic studies on methylene chloride have been performed with freshwater organisms. However, it appeared from the above data that developmental stages of certain amphibian species may be affected by concentrations at approximately 100 µg/L and that concentrations at or above 1 mg/L may produce substantial reproductive impairment.

Only rainbow trout and R. pipiens were tested with nitrobenzene. The LC_1 values ranged from <0.1 to 1.9 µg/L and LC_{10}'s varied from 0.1 to 26.1 µg/L (Table 15). No chronic studies on freshwater fish have been conducted on this compound. However, results obtained in the present investigation indicated that nitrobenzene was highly toxic to embryo-larval stages of the more sensitive fish and amphibian species, as appreciable effects were detected in the range of 0.1 to 26 µg/L. Further study is required to determine if the toxicity of nitrobenzene is species-specific or if the observed effects are indicative of those expected with early life-cycle stages of other species.

Toxicity tests with phenol were performed on embryo-larval stages of eight fish and amphibian species, and rainbow trout and R. pipiens were the most sensitive. These organisms exhibited about equal sensitivity to phenol, as LC_1's were 1.1 µg/L for both species and LC_{10}'s ranged from 5.2 to 10.0 µg/L (Table 15). The LC_1's did not differ significantly from values determined earlier with goldfish and bluegill (22). Based on the above data and other considerations (14), it would appear that the maximum concentration of 0.1 mg/L originally suggested for phenol in 1973 (3) is inadequate for protection of sensitive life-cycle stages of certain fish and amphibian species. In 1976, the Environmental Protection Agency established a phenol criterion of 1 µg/L for domestic water

supplies and for protection against fish flesh tainting (14). It is of interest that the LC_1 values reviewed above are in good agreement with the organoleptic threshold, and this further supports feasibility of a freshwater criterion for phenol of 1 µg/L. It is obvious that this limiting concentration is essential not only to prevent fish flesh tainting but also to protect reproductive potential of sensitive aquatic species.

Toluene was administered to three fish and amphibian species, and rainbow trout and R. pipiens were the least tolerant. As observed with phenol, both species displayed similar sensitivities to toluene. With trout, the LC_1 and LC_{10} were 0.5 and 2.9 µg/L. Respective values of 0.5 and 10.1 µg/L were determined in tests with R. pipiens. Chronic studies on other freshwater vertebrates have not been reported for this compound. However, it is likely that concentrations of about 10 µg/L would produce appreciable effects on the more sensitive aquatic species.

Considering the limited data available from chronic life-cycle studies and the low cost feasibility of such tests, short-term embryo-larval bioassays provide a useful means of quantifying the toxicity of aquatic contaminants. When care is taken to develop an adequate dose-response relationship, log probit analysis can be used to calculate LC_{10} and LC_1 values. The latter generally provides a reliable approximation of the threshold for toxic effects (e.g., mortality, teratogenesis), and as LC_1 values usually are in reasonable agreement with MATC's determined in chronic life-cycle tests, such data appear applicable to the promulgation of freshwater criteria. Furthermore, the LC_{10} can be used to provide an additional reference point for assessing toxic effects. Considering the combined effects of long-term pollution stress and natural environmental stresses, it is likely that 10% or greater impairment of reproductive potential would signifi-

cantly affect population dynamics in natural communities (22, 23, 43). Also, the concentration intervals between LC_1 and LC_{10} values may be useful in the hazard evaluation process. As the difference between the two values decreases, accuracy in defining a regulatory criterion becomes more critical.

In addition to the toxicological evaluations described above for the 11 organic compounds, a limited number of toxicity tests were performed to determine whether embryo-larval bioassays are suitable to assess effects of transitory chemical exposures, such as those resulting from intermittent discharges or accidental spills of toxicants into water resources. Using flow-through procedures, populations of R. pipiens eggs were given one-time, 8-hr exposures to chloroform at various stages of embryonic development. Chloroform was administered to one group of organisms beginning immediately after egg fertilization; a second group of embryos was exposed during neurulation; and a third group was exposed during the hatching process. Tests on each of these groups were performed at chloroform concentrations of 2, 8, and 40 mg/L, and survival data were recorded at 4 days posthatching. Those organisms which had been exposed to toxicant during the earliest developmental stage (i.e., cleavage) displayed the greatest overall sensitivity. For example, chloroform concentrations of 2, 8, and 40 mg/L produced respective mortality frequencies at 4 days posthatching of 21%, 23%, and 28%, compared to 12% for unexposed controls. For embryos exposed during neurulation, the above concentrations produced 14%, 17%, and 21% mortality, respectively. Survival data were somewhat variable for embryos exposed during hatching, and further attention should be given to this period of development. Based on these initial experiments with chloroform, it appeared that embryo-larval toxicity tests are suitable for estimating toxicological effects produced by short-term chemical contamination of aquatic

resources. However, further study is required on additional animal species, a broader array of aquatic contaminants, and an expanded selection of exposure periods and toxicant concentrations.

CHAPTER IV

CONCLUSIONS

Aquatic toxicity tests were conducted on 11 organic compounds, including benzene, carbon tetrachloride, chlorobenzene, chloroform, 1,2-dichlorobenzene, 1,2-dichloroethane, methylene chloride, nitrobenzene, phenol, toluene, and m-xylene. The toxicity of each compound was evaluated using embryo-larval stages of up to eight fish and amphibian species. The animal test species exhibited varying degrees of sensitivity to the selected toxicants. Combined frequencies for mortality and teratogenesis at 4 days posthatching gave LC_{50} ranges of 3.66 to 8.25 mg/L for benzene, 1.16 to 22.42 mg/L for carbon tetrachloride, 0.11 to 1.20 mg/L for chlorobenzene, 2.03 to >68 mg/L for chloroform, 3.01 to 5.56 mg/L for dichlorobenzene, 2.54 to ~34 mg/L for dichloroethane, 13.16 to >48 mg/L for methylene chloride, 0.002 to 0.64 mg/L for nitrobenzene, 0.04 to ~32 mg/L for phenol, 0.02 to 0.85 mg/L for toluene, and 3.53 to 3.77 mg/L for m-xylene. In most instances, higher LC_{50} values were obtained in tests with *B. fowleri*, *R. palustris*, *X. laevis*, and the fathead minnow. The species which exhibited the greatest susceptibility to organic compounds were the rainbow trout, *R. pipiens*, and *R. temporaria*. The more sensitive amphibians generally were those which normally are restricted to aquatic or moist terrestrial habitats, whereas the more tolerant amphibians included those semi-aquatic and terrestrial species which appear to be more broadly adapted ecologically.

Of the 11 test compounds, nitrobenzene, toluene, chlorobenzene, and phenol were the most toxic. The least toxic organics included dichloroethane and methylene chloride. Consideration also was given to the relationship between

chemical structures of the selected compounds and toxicological responses produced. For example, the toxicity of three chlorinated alkanes, including methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), and carbon tetrachloride (CCl_4), generally increased with the degree of chlorination. In another comparative evaluation, benzene always was found to be less toxic than its monosubstituted analogs. For example, the median lethal concentration in tests with trout was 8.25 mg/L for benzene (C_6H_6), compared to LC_{50} values of 0.15 mg/L for phenol (C_6H_5OH), 0.11 mg/L for chlorobenzene (C_6H_5Cl), 0.02 mg/L for toluene ($C_6H_5CH_3$), and 0.002 mg/L for nitrobenzene ($C_6H_5NO_2$). These and other correlations between chemical structure and toxicity (30) indicate that predictive toxicology may prove useful in the hazard assessment of aquatic contaminants.

In order to obtain further information on the tolerance of the most sensitive fish and amphibian species tested, log probit analysis was performed to calculate toxicant concentrations which produced embryo-larval mortality and/or teratogenesis at frequencies of 10% (LC_{10}) and 1% (LC_1). The LC_1 values, used to estimate toxicity thresholds, ranged from <0.1 for nitrobenzene to 69.9 mg/L for methylene chloride. As MATC values have not been reported for the vast majority of organic contaminants, the LC_1 and LC_{10} values determined in these tests should provide valuable data concerning exposure concentrations likely to produce chronic-level effects to sensitive animal species.

In addition to the toxicological evaluations described above, a limited number of toxicity tests were performed to determine whether embryo-larval bioassays are suitable to assess effects of transitory chemical exposures, such as those resulting from intermittent discharges or accidental spills of chemicals into water resources. Results indicated that R. pipiens embryos

were sufficiently sensitive to quantify effects produced by short-term exposures of chloroform. Animals tested during the earliest embryonic stage (i.e., cleavage) appeared to be less tolerant than organisms exposed later in development (e.g., neurulation).

Table 3. Toxicity of benzene to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
Leopard Frog *(Rana pipiens)*	0.016 ± 0.003	95(0)	95	95
	0.048 ± 0.010	90(0)	90	90
	0.61 ± 0.17	81(0)	81	79
	2.99 ± 1.08	64(0)	64	62
	5.07 ± 1.84	39(16)	32	32
Northwestern Salamander *(Ambystoma gracile)*	0.016 ± 0.002	99(0)	99	99
	0.044 ± 0.009	97(0)	97	97
	0.61 ± 0.17	89(0)	89	87
	2.69 ± 0.98	75(0)	75	70
	5.43 ± 1.55	49(9)	44	44
	36.7 ± 8.9	31(27)	23	15
Rainbow Trout *(Salmo gairdneri)*	0.013 ± 0.003	90(0)	90	90
	0.021 ± 0.006	82(2)	80	80
	0.62 ± 0.16	67(6)	63	63
	5.02 ± 0.86	60(7)	56	55

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 4. Toxicity of carbon tetrachloride to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
European Common Frog *(Rana temporaria)*	0.010 ± 0.004	98(0)	98	94
	0.076 ± 0.009	92(0)	92	82
	0.67 ± 0.13	62(3)	60	52
	10.7 ± 0.5	55(12)	49	37
	24.0 ± 2.7	57(17)	47	19
	41.2 ± 4.1	23(67)	8	0
Leopard Frog *(Rana pipiens)*	0.010 ± 0.004	99(0)	99	94
	0.076 ± 0.009	94(2)	93	89
	0.67 ± 0.13	72(6)	68	55
	10.7 ± 0.5	61(11)	54	31
	24.0 ± 2.7	52(18)	42	23
	41.2 ± 4.1	33(44)	19	9
Rainbow Trout *(Salmo gairdneri)*	0.024 ± 0.006	99(1)	98	97
	0.070 ± 0.007	90(0)	90	89
	1.11 ± 0.08	65(2)	64	64
	5.61 ± 1.15	33(16)	28	27
	10.9 ± 0.5	3(86)	1	0
	45.8 ± 7.1	0(0)	0	0
Northwestern Salamander *(Ambystoma gracile)*	0.010 ± 0.004	100(0)	100	99
	0.076 ± 0.009	92(0)	92	84
	0.67 ± 0.13	72(0)	72	62
	10.6 ± 0.5	61(3)	59	47
	24.2 ± 2.9	41(10)	38	20
	41.8 ± 4.6	36(21)	28	0

Table 4 - continued.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
Fathead Minnow *(Pimephales promelas)*	0.015 ± 0.003	98(0)	98	99
	0.065 ± 0.007	95(0)	95	92
	0.72 ± 0.10	97(8)	89	67
	9.32 ± 0.84	71(12)	63	63
	24.2 ± 2.9	84(17)	70	42
	45.0 ± 3.7	38(32)	26	6
	62.8 ± 16.5	0(0)	0	0
African Clawed Frog *(Xenopus laevis)*	0.004 ± 0.003	98(0)	98	96
	0.073 ± 0.012	95(0)	95	92
	0.60 ± 0.16	90(1)	89	84
	10.5 ± 0.6	82(4)	78	73
	27.2 ± 2.6	67(10)	60	31

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 5. Toxicity of chlorobenzene to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
Rainbow Trout *(Salmo gairdneri)*	0.013 ± 0.001	92(1)	91	90
	0.062 ± 0.005	78(4)	77	74
	0.20 ± 0.01	33(14)	29	27
	0.52 ± 0.02	7(60)	3	3
	9.79 ± 4.22	0(0)	0	0
Northwestern Salamander *(Ambystoma gracile)*	0.013 ± 0.002	100(0)	100	98
	0.044 ± 0.008	94(0)	94	90
	0.28 ± 0.04	83(0)	83	78
	0.63 ± 0.09	76(7)	71	66
	18.4 ± 5.8	23(27)	17	12
	34.5 ± 15.5	0(0)	0	0
Leopard Frog *(Rana pipiens)*	0.011 ± 0.002	95(0)	95	94
	0.045 ± 0.012	96(0)	96	94
	0.31 ± 0.04	74(0)	74	68
	0.63 ± 0.09	71(4)	68	64
	14.6 ± 5.6	36(38)	22	22
	34.5 ± 15.5	0(0)	0	0

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 6. Toxicity of chloroform to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
European Common Frog *(Rana temporaria)*	0.001 ± 0.001	102(0)	102	102
	0.11 ± 0.02	100(0)	100	100
	1.04 ± 0.06	96(0)	96	94
	6.05 ± 0.74	68(3)	67	65
	9.90 ± 1.58	62(9)	57	51
	64.1 ± 6.2	46(21)	37	35
Northwestern Salamander *(Ambystoma gracile)*	0.001 ± 0.001	105(0)	105	105
	0.11 ± 0.02	103(0)	103	103
	1.04 ± 0.06	97(0)	97	93
	5.89 ± 0.83	71(3)	69	69
	9.12 ± 1.63	66(9)	60	58
	63.3 ± 7.0	58(14)	50	38
Fathead Minnow *(Pimephales promelas)*	0.010 ± 0.003	95(0)	95	94
	0.12 ± 0.01	90(0)	90	88
	0.97 ± 0.16	82(2)	80	74
	5.88 ± 0.45	81(5)	77	59
	11.5 ± 0.8	84(7)	78	59
	58.3 ± 2.0	69(19)	56	56
African Clawed Frog *(Xenopus laevis)*	0.001 ± 0.001	100(1)	100	99
	0.08 ± 0.02	100(0)	100	100
	1.02 ± 0.06	98(3)	99	95
	6.15 ± 1.17	92(2)	91	91
	8.40 ± 2.37	93(3)	87	83
	67.8 ± 6.5	82(15)	70	70

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 7. Toxicity of 1,2-dichlorobenzene to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
Rainbow Trout *(Salmo gairdneri)*	0.007 ± 0.001	94(6)	89	89
	0.013 ± 0.001	100(4)	97	96
	0.29 ± 0.03	86(6)	82	82
	2.31 ± 0.14	84(16)	71	71
	13.2 ± 1.8	0(0)	0	0
Leopard Frog *(Rana pipiens)*	0.001 ± 0.0005	99(1)	98	97
	0.012 ± 0.001	97(2)	96	96
	0.15 ± 0.01	90(6)	85	77
	1.64 ± 0.12	91(5)	87	83
	7.43 ± 2.55	64(6)	61	54
	12.3 ± 1.2	61(10)	55	20
	47.5 ± 3.3	0(0)	0	0

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 8. Toxicity of 1,2-dichloroethane to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
Northwestern Salamander *(Ambystoma gracile)*	0.002 ± 0.001	96(0)	96	96
	0.009 ± 0.002	93(0)	93	89
	0.20 ± 0.02	85(0)	85	83
	0.99 ± 0.14	77(0)	77	67
	2.58 ± 0.52	55(5)	53	45
	21.4 ± 2.6	41(16)	35	24
Leopard Frog *(Rana pipiens)*	0.002 ± 0.001	98(0)	98	96
	0.009 ± 0.002	93(0)	93	91
	0.19 ± 0.02	90(0)	90	86
	1.07 ± 0.13	76(3)	74	74
	2.69 ± 0.58	59(6)	55	54
	21.9 ± 2.9	31(25)	24	24
Rainbow Trout *(Salmo gairdneri)*	0.002 ± 0.0004	98(0)	98	98
	0.026 ± 0.003	97(2)	96	96
	0.20 ± 0.01	91(2)	89	89
	1.57 ± 0.11	85(4)	82	82
	3.49 ± 0.20	76(8)	70	70
	34.4 ± 3.2	61(15)	52	52

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 9. Toxicity of methylene chloride to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration[1] Mean ± S.E. (mg/L)	Percent Hatchability[2]	Percent Survival Normal Organisms[3]	
			Hatching	4 Days Posthatching
Rainbow Trout *(Salmo gairdneri)*	0.008 ± 0.001	100(0)	100	100
	0.042 ± 0.004	93(0)	93	92
	0.41 ± 0.04	86(0)	86	85
	5.55 ± 1.06	73(2)	72	70
	23.1 ± 1.7	48(9)	44	44
	36.5 ± 2.8	18(49)	9	9
European Common Frog *(Rana temporaria)*	0.004 ± 0.002	98(0)	98	98
	0.18 ± 0.03	98(0)	98	95
	0.65 ± 0.09	91(0)	91	89
	8.05 ± 0.70	88(0)	88	81
	18.9 ± 1.8	59(3)	57	49
	30.8 ± 2.2	41(13)	36	28
Northwestern Salamander *(Ambystoma gracile)*	0.004 ± 0.002	100(0)	100	100
	0.18 ± 0.03	100(0)	100	97
	0.65 ± 0.09	97(0)	97	91
	7.83 ± 0.75	88(0)	88	82
	18.6 ± 2.0	68(0)	68	55
	29.4 ± 1.9	38(5)	35	20
Fathead Minnow *(Pimephales promelas)*	0.003 ± 0.001	99(0)	99	94
	0.11 ± 0.02	97(0)	97	90
	0.80 ± 0.07	95(6)	90	81
	6.77 ± 1.01	95(0)	95	78
	21.3 ± 3.8	87(7)	81	56
	34.3 ± 1.8	69(22)	54	49

Table 9 - continued.

Species	Toxicant Concentration[1] Mean ± S.E. (mg/L)	Percent Hatchability[2]	Percent Survival Normal Organisms[3]	
			Hatching	4 Days Posthatching
African Clawed Frog *(Xenopus laevis)*	0.003 ± 0.002	100(0)	100	99
	0.18 ± 0.04	97(0)	97	96
	0.65 ± 0.09	94(0)	94	91
	7.61 ± 0.83	95(0)	95	92
	18.6 ± 2.3	95(2)	94	89
	29.3 ± 2.2	69(3)	67	56
Leopard Frog *(Rana pipiens)*	0.010	98(0)	98	93
	0.077 ± 0.009	98(0)	98	98
	1.17 ± 0.14	94(0)	94	91
	28.7 ± 2.1	92(3)	90	86
	47.8 ± 2.5	84(10)	76	75

[1]Concentration without standard error is a nominal value.

[2]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[3]Normal organisms were defined as those free of gross teratic defects.

Table 10. Toxicity of nitrobenzene to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration[1] Mean ± S.E. (mg/L)	Percent Hatchability[2]	Percent Survival, Normal Organisms[3]	
			Hatching	4 Days Posthatching
Rainbow Trout *(Salmo gairdneri)*	0.001	64(3)	62	62
	0.010	24(13)	21	21
	0.12 ± 0.04	5(33)	3	3
	0.36 ± 0.06	0(0)	0	0
	0.91 ± 0.06	0(0)	0	0
	11.9 ± 0.3	0(0)	0	0
Leopard Frog *(Rana pipiens)*	0.001	99(0)	99	94
	0.010	97(0)	97	87
	0.05	94(0)	94	85
	0.10 ± 0.02	90(2)	89	77
	0.41 ± 0.07	81(0)	81	55
	1.27 ± 0.07	66(4)	63	36

[1]Concentrations without standard errors are nominal values.

[2]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[3]Normal organisms were defined as those free of gross teratic defects.

Table 11. Toxicity of phenol to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
European Common Frog *(Rana temporaria)*	0.002 ± 0.001	92(0)	92	90
	0.010 ± 0.001	86(0)	86	82
	0.12 ± 0.01	69(2)	68	63
	0.72 ± 0.08	45(6)	42	36
	1.45 ± 0.06	13(56)	6	6
	14.0 ± 0.6	0(0)	0	0
	26.4 ± 2.7	0(0)	0	0
Northwestern Salamander *(Ambystoma gracile)*	0.002 ± 0.001	96(0)	96	96
	0.010 ± 0.001	91(0)	91	89
	0.12 ± 0.01	79(0)	79	75
	0.72 ± 0.08	44(0)	44	38
	1.45 ± 0.06	10(0)	10	8
	14.0 ± 0.6	0(0)	0	0
	26.4 ± 2.7	0(0)	0	0
African Clawed Frog *(Xenopus laevis)*	0.002 ± 0.001	98(0)	98	98
	0.010 ± 0.001	98(0)	98	97
	0.12 ± 0.01	96(0)	96	95
	0.72 ± 0.06	91(0)	91	89
	1.45 ± 0.07	80(1)	79	75
	14.0 ± 0.6	53(8)	49	44
	26.4 ± 3.0	43(16)	36	23

Table 11 - continued.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
Fathead Minnow *(Pimephales promelas)*	0.003 ± 0.001	100(0)	100	98
	0.013 ± 0.002	98(0)	98	96
	0.16 ± 0.01	98(0)	98	94
	0.78 ± 0.04	97(0)	97	93
	1.85 ± 0.17	89(2)	88	80
	11.9 ± 1.4	77(8)	71	58
	31.9 ± 4.8	72(16)	60	54

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 12. Toxicity of toluene to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
Rainbow Trout *(Salmo gairdneri)*	0.004 ± 0.001	87(0)	87	84
	0.026 ± 0.003	52(4)	49	49
	0.081 ± 0.010	39(10)	35	29
	0.16 ± 0.02	13(31)	9	6
	11.3 ± 3.7	0(0)	0	0
	31.7 ± 8.4	0(0)	0	0
Leopard Frog *(Rana pipiens)*	0.015 ± 0.006	94(0)	94	91
	0.023 ± 0.005	86(5)	82	78
	0.24 ± 0.03	61(0)	61	46
	0.41 ± 0.05	58(3)	56	54
	22.6 ± 6.3	27(50)	14	14
	35.3 ± 9.9	4(100)	0	0
Northwestern Salamander *(Ambystoma gracile)*	0.012 ± 0.004	97(0)	97	97
	0.025 ± 0.005	92(0)	92	90
	0.21 ± 0.03	84(0)	84	79
	0.40 ± 0.04	68(10)	61	61
	25.4 ± 5.2	25(36)	16	11
	41.5 ± 9.4	4(100)	0	0

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 13. Toxicity of m-Xylene to embryo-larval stages of fish and amphibians.

Species	Toxicant Concentration Mean ± S.E. (mg/L)	Percent Hatchability[1]	Percent Survival Normal Organisms[2]	
			Hatching	4 Days Posthatching
Leopard Frog *(Rana pipiens)*	0.001 ± 0.0002	97(2)	95	93
	0.034 ± 0.009	95(0)	95	91
	0.12 ± 0.01	94(2)	92	92
	1.43 ± 0.13	88(6)	83	77
	3.16 ± 0.48	80(0)	80	74
	33.6 ± 5.0	9(100)	0	0
Rainbow Trout *(Salmo gairdneri)*	0.002 ± 0.0003	92(4)	89	87
	0.005 ± 0.0003	96(9)	88	88
	0.16 ± 0.02	92(5)	88	87
	2.68 ± 0.22	86(13)	76	76
	8.41 ± 0.69	53(31)	36	2
	21.8 ± 2.6	12(44)	7	0

[1]Egg hatchability was based on all animals, normal and aberrant, which completed hatching. Frequencies of teratic survivors appearing in hatched populations were expressed parenthetically.

[2]Normal organisms were defined as those free of gross teratic defects.

Table 14. Log probit LC_{50} values for organic compounds administered to embryo-larval stages of fish and amphibians.[1]

Compound	Species[2]	Exposure Days Beyond Hatching	LC_{50} (mg/L)	95% Confidence Limits
Benzene	Leopard Frog *(Rana pipiens)*	0	4.03	2.18 - 10.08
		4	3.66	2.00 - 8.77
	Northwestern Salamander *(Ambystoma gracile)*	0	6.68	3.97 - 12.12
		4	5.21	3.44 - 7.93
	Rainbow Trout *(Salmo gairdneri)*	0	8.64	3.93 - 26.29
		4	8.25	3.80 - 24.53
Carbon tetrachloride	European Common Frog *(Rana temporaria)*	0	4.56	2.54 - 8.13
		4	1.16	0.65 - 1.96
	Leopard Frog *(Rana pipiens)*	0	6.77	4.09 - 11.20
		4	1.64	0.99 - 2.60
	Rainbow Trout *(Salmo gairdneri)*	0	2.02	1.66 - 2.38
		4	1.97	1.62 - 2.32
	Northwestern Salamander *(Ambystoma gracile)*	0	9.01	4.78 - 17.25
		4	1.98	1.07 - 3.34
	Pickerel Frog* *(Rana palustris)*	0	3.62	2.68 - 4.97
		4	2.37	1.74 - 3.21
	Fowler's Toad* *(Bufo fowleri)*	0	>92	-
		4	2.83	1.95 - 4.05
	Fathead Minnow *(Pimephales promelas)*	0	16.25	11.45 - 21.25
		4	4.00	2.53 - 5.90
	African Clawed Frog *(Xenopus laevis)*	0	>27	-
		4	22.42	11.11 - 59.02

Table 14 - continued.

Compound	Species[2]	Exposure Days Beyond Hatching	LC_{50} (mg/L)	95% Confidence Limits
Chlorobenzene	Rainbow Trout	0	0.11	0.10 - 0.13
	(Salmo gairdneri)	4	0.11	0.09 - 0.13
	Northwestern Salamander	0	1.65	1.04 - 2.59
	(Ambystoma gracile)	4	1.15	0.72 - 1.83
	Leopard Frog*	0	1.53	1.02 - 2.31
	(Rana pipiens)	4	1.20	0.80 - 1.84
Chloroform	Rainbow Trout*	0	2.03	0.95 - 3.75
	(Salmo gairdneri)	4	2.03	0.95 - 3.75
	Leopard Frog*	0	4.56	2.20 - 7.67
	(Rana pipiens)	4	4.16	1.96 - 7.06
	European Common Frog	0	20.66	13.39 - 36.45
	(Rana temporaria)	4	16.95	11.05 - 28.91
	Pickerel Frog*	0	28.17	15.57 - 64.43
	(Rana palustris)	4	20.55	11.53 - 43.83
	Northwestern Salamander	0	34.54	19.28 - 87.27
	(Ambystoma gracile)	4	21.58	13.25 - 41.77
	Fowler's Toad*	0	>40	-
	(Bufo fowleri)	4	35.14	18.37 - 92.25
	Fathead Minnow	0	>58	-
	(Pimephales promelas)	4	>58	-
	African Clawed Frog	0	>68	-
	(Xenopus laevis)	4	>68	-
1,2-Dichlorobenzene	Rainbow Trout	0	3.01	2.61 - 3.83
	(Salmo gairdneri)	4	3.01	2.61 - 3.94
	Leopard Frog	0	12.07	11.09 - 13.14
	(Rana pipiens)	4	5.56	4.85 - 6.31

Table 14 - continued.

Compound	Species[2]	Exposure Days Beyond Hatching	LC_{50} (mg/L)	95% Confidence Limits
1,2-Dichloroethane	Northwestern Salamander *(Ambystoma gracile)*	0	6.53	2.90 - 20.80
		4	2.54	1.27 - 6.07
	Leopard Frog *(Rana pipiens)*	0	4.52	2.80 - 7.93
		4	4.40	2.59 - 8.14
	Rainbow Trout *(Salmo gairdneri)*	0	~34	-
		4	~34	-
Methylene Chloride	Rainbow Trout *(Salmo gairdneri)*	0	13.51	11.32 - 15.64
		4	13.16	10.95 - 15.32
	European Common Frog *(Rana temporaria)*	0	23.03	19.04 - 29.09
		4	16.93	10.95 - 29.04
	Northwestern Salamander *(Ambystoma gracile)*	0	23.86	19.46 - 31.89
		4	17.82	14.53 - 21.51
	Fathead Minnow *(Pimephales promelas)*	0	>34	-
		4	~34	-
	African Clawed Frog *(Xenopus laevis)*	0	>29	-
		4	>29	-
	Pickerel Frog* *(Rana palustris)*	0	>32	-
		4	>32	-
	Fowler's Toad* *(Bufo fowleri)*	0	>32	-
		4	>32	-
	Leopard Frog *(Rana pipiens)*	0	>48	-
		4	>48	-

Table 14 - continued.

Compound	Species[2]	Exposure Days Beyond Hatching	LC_{50} (mg/L)	95% Confidence Limits
Nitrobenzene	Rainbow Trout	0	0.002	0.001 - 0.003
	(Salmo gairdneri)	4	0.002	0.001 - 0.003
	Leopard Frog	0	>1.27	-
	(Rana pipiens)	4	0.64	0.38 - 1.23
Phenol	Leopard Frog*	0	0.05	0.03 - 0.07
	(Rana pipiens)	4	0.04	0.03 - 0.05
	Rainbow Trout*	0	0.15	0.12 - 0.19
	(Salmo gairdneri)	4	0.15	0.12 - 0.19
	European Common Frog	0	0.35	0.24 - 0.48
	(Rana temporaria)	4	0.27	0.17 - 0.39
	Northwestern Salamander	0	0.46	0.31 - 0.62
	(Ambystoma gracile)	4	0.38	0.25 - 0.52
	Fowler's Toad*	0	>10	-
	(Bufo fowleri)	4	2.45	1.26 - 5.61
	African Clawed Frog	0	12.71	9.07 - 18.96
	(Xenopus laevis)	4	7.68	5.73 - 10.52
	Pickerel Frog*	0	11.23	7.11 - 19.86
	(Rana palustris)	4	9.87	5.73 - 19.95
	Fathead Minnow	0	>32	-
	(Pimephales promelas)	4	~32	-
Toluene	Rainbow Trout	0	0.03	0.02 - 0.04
	(Salmo gairdneri)	4	0.02	0.02 - 0.03
	Leopard Frog	0	0.51	0.32 - 0.83
	(Rana pipiens)	4	0.39	0.24 - 0.65
	Northwestern Salamander	0	1.09	0.65 - 1.84
	(Ambystoma gracile)	4	0.85	0.51 - 1.42

Table 14 - continued.

Compound	Species[2]	Exposure Days Beyond Hatching	LC_{50} (mg/L)	95% Confidence Limits
m-Xylene	Leopard Frog *(Rana pipiens)*	0	4.06	3.47 - 4.87
		4	3.53	3.03 - 4.20
	Rainbow Trout *(Salmo gairdneri)*	0	5.95	4.79 - 7.17
		4	3.77	3.30 - 4.30

[1]Grossly teratic larvae were counted as lethals.

[2]Data for species marked with an asterisk were taken from previous investigations conducted in our laboratory (11, 22, 31). All experiments were performed using a water hardness of 100 mg/L as $CaCO_3$ except for the chloroform test with trout which was performed at a hardness level of 50 mg/L as $CaCO_3$.

Table 15. Log probit LC_{10} and LC_1 values determined at 4 days posthatching for organic compounds administered to embryo-larval stages of fish and amphibians.[1]

Compound	Species[2]	LC_{10} (µg/L)	95% Confidence Limits	LC_1 (µg/L)	95% Confidence Limits
Benzene	Leopard Frog *(Rana pipiens)*	75.6	17.6 - 174.1	3.2	0.2 - 14.6
	Northwestern Salamander *(Ambystoma gracile)*	478.1	160.4 - 909.5	68.2	10.6 - 193.2
Carbon tetrachloride	European Common Frog *(Rana temporaria)*	25.0	7.1 - 60.9	1.1	0.2 - 4.2
	Leopard Frog *(Rana pipiens)*	33.9	11.3 - 76.2	1.4	0.3 - 4.9
Chlorobenzene	Rainbow Trout *(Salmo gairdneri)*	36.1	24.8 - 47.4	14.3	8.1 - 21.4
	Northwestern Salamander *(Ambystoma gracile)*	82.7	35.4 - 153.6	9.7	2.6 - 24.2
Chloroform	Rainbow Trout* *(Salmo gairdneri)*	83.2	9.4 - 251.4	6.2	0.2 - 34.9
	Leopard Frog *(Rana pipiens)*	383.4	60.1 - 985.0	54.9	3.1 - 225.0
1,2-Dichloroethane	Leopard Frog *(Rana pipiens)*	183.2	38.3 - 427.8	13.7	0.8 - 57.7
Methylene chloride	European Common Frog *(Rana temporaria)*	822.4	252.6 - 1643	69.9	7.9 - 232.7

Table 15 - continued.

Compound	Species[2]	LC_{10} (µg/L)	95% Confidence Limits	LC_1 (µg/L)	95% Confidence Limits
Nitrobenzene	Rainbow Trout *(Salmo gairdneri)*	0.1	0.1 - 0.3	<0.1	-
	Leopard Frog *(Rana pipiens)*	26.1	4.7 - 61.6	1.9	0.1 - 26.1
Phenol	Leopard Frog* *(Rana pipiens)*	5.2	2.8 - 8.1	1.1	0.4 - 2.1
	Rainbow Trout* *(Salmo gairdneri)*	10.0	5.8 - 15.6	1.1	0.5 - 2.1
Toluene	Rainbow Trout *(Salmo gairdneri)*	2.9	1.5 - 4.5	0.5	0.2 - 1.1
	Leopard Frog *(Rana pipiens)*	10.1	3.8 - 20.8	0.5	0.1 - 1.6

[1]Grossly teratic larvae were counted as lethals.

[2]Data for species marked with an asterisk were taken from previous investigations conducted in our laboratory (11, 22, 31). All experiments were performed using a water hardness of 100 mg/L as $CaCO_3$ except for the chloroform test with trout which was performed at a hardness level of 50 mg/L as $CaCO_3$.

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