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TOXICITY OF ORGANIC CHEMICALS
TO EMBRYO-LARVAL STAGES OF FISH

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Final Report

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16. ABSTRACT A continuous flow procedure was developed for evaluating effects of insoluble and volatile organics on embryo-larval stages of fish. Test compounds were selected for different combinations of solubility and volatility and included aniline, atrazine, chlorobenzene, chloroform, 2,4-dichlorophenol, 2,4-dichlorophenoxyacetic acid, dioctyl phthalate, malathion, trisodium nitrilotriacetic acid, phenol, and polychlorinated biphenyl (Capacitor 21). A closed system devoid of standing air space greatly reduced volatility as a test variable. Mechanical homogenization proved highly effective in suspending hydrophobic compounds in influent water. Continuous agitation in the test chamber and regulation of detention time further precluded the need for carrier solvents. Test results indicated good reproducibility of exposure concentrations. The most toxic compounds included Capacitor 21, chlorobenzene, 2,4-dichlorophenol, and phenol. Chlorobenzene at 90 µg/l produced complete lethality of trout eggs. The three other compounds gave log probit LC_{50}'s of 2 to 70 µg/l when trout stages were exposed in hard water, and LC_1's were 0.3, 1.0, and 1.7 µg/l for phenol, Capacitor 21, and 2,4-dichlorophenol. Chloroform also was highly toxic to trout stages and LC_1's ranged from 4.9 to 6.2 µg/l. When bass and goldfish stages were exposed to chlorobenzene, LC_1's ranged from 8 to 33 µg/l. Compared to other species, trout developmental stages generally exhibited the greatest sensitivity. The LC_1 values determined in embryo-larval tests compared closely with maximum acceptable toxicant concentrations developed in life-cycle studies. Most compounds produced appreciable frequencies of teratic larvae.

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ABSTRACT

A continuous flow procedure was developed for evaluating effects of insoluble and volatile organics on embryo-larval stages of fish. A closed system devoid of standing air space was used to minimize volatility as a test variable. Insoluble compounds were suspended in influent water by mechanical homogenization, without the use of carrier solvents. Tests were performed on aniline, atrazine, chlorobenzene, chloroform, 2,4-dichlorophenol, 2,4-dichlorophenoxyacetic acid (2,4-D), dioctyl phthalate (DOP), malathion, trisodium nitrilotriacetic acid (NTA), phenol, and polychlorinated biphenyl (Capacitor 21). Maintaining water hardness at 50 and 200 mg/l $CaCO_3$, exposure was continuous from fertilization through 4 days posthatching for largemouth bass, bluegill sunfish, channel catfish, goldfish, rainbow trout, and redear sunfish.

Exposure levels which produced 50% (LC_{50}) and 1% (LC_1) control-adjusted impairment (lethality, teratogenesis) of test populations were calculated by log probit analysis. The LC_1's were used as a basis for estimating threshold concentrations for toxic effects. To determine reliability of LC_1 values, they were compared with maximum acceptable toxicant concentrations (MATC) developed in partial and complete life-cycle studies. Good correlations were obtained when data were adequate to permit comparisons, and the findings indicated that LC_1 values determined in embryo-larval tests carried through 4 days posthatching were useful in estimating long-term effects of aquatic pollutants.

Test results indicated good reproducibility of exposure concentrations for both volatile and insoluble toxicants. The most toxic compounds included Capacitor 21, chlorobenzene, 2,4-dichlorophenol, and phenol. Chlorobenzene at 90 µg/l produced complete lethality of trout eggs, and LC_1's ranged from 8 to 33 µg/l in tests with the largemouth bass and goldfish. The three other compounds gave log probit LC_{50}'s of 2 to 70 µg/l when trout stages were exposed in hard water, and LC_1's were 0.3, 1.0, and 1.7 µg/l for phenol, Capacitor 21, and 2,4-dichlorophenol. Phenol was less toxic to developmental stages of the goldfish and bluegill. When tests were conducted in hard water, the LC_{50}'s were 0.34 and 1.69 mg/l and the LC_1's varied from 2.0 to 8.8 µg/l. Depending on water hardness, LC_1's determined in µg/l with the rainbow trout ranged from 4.9 to 6.2 for chloroform, 21.9 to 32.5 for 2,4-D, and 29.0 to 77.2 for atrazine. Though not tested on the trout, LC_1's determined with the goldfish ranged from 143.2 to 215.0 µg/l for aniline and 141.1 to 439.6 µg/l for malathion. The organics least toxic to the trout included NTA and DOP, and the LC_{50}'s varied from 90.5 to 114.0 and 139.5 to 149.2 mg/l, respectively. Compared to the other species, trout developmental stages generally exhibited the greatest overall sensitivity. Though water hardness did not substantially alter toxicity of the selected organic compounds, phenol was somewhat more toxic in hard water. All compounds produced appreciable frequencies of teratic larvae.

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INTRODUCTION

The toxicological characterization of organic pollutants is frequently complicated by physical and chemical properties of the test compounds (Schoor, 1975; Veith and Comstock, 1975). Volatility or low water solubility may preclude adequate regulation of exposure concentrations in aquatic test systems, especially when open test chambers are used. Though emulsifiers or carrier solvents may be of some aid in testing hydrophobic organics, they generally introduce undesirable variables. The initial objective of this investigation was to develop a continuous flow system designed for testing volatile and insoluble organic compounds which are difficult to stabilize with conventional techniques. Using fish embryo-larval stages as test organisms, a closed flow-through test chamber devoid of an air-water interface was used to minimize evaporative loss of volatile organics. Insoluble compounds were suspended in influent water by mechanical homogenization, and maintained by continuous agitation in the exposure chamber and regulation of detention time. Fish embryos were selected as test organisms because of their simple culture requirements, suitability for use in a closed test system, and high sensitivity to aquatic contaminants. Reconstituted water, with physicochemical characteristics representative of natural freshwater, was formulated to provide stable, reproducible test conditions and to minimize problems with background contaminants. In the process of developing the new procedures, tests were performed with eleven organic compounds, selected for varying degrees of volatility and water solubility (Table 1).

Table 1. Organic compounds used in toxicity tests.

Compound	Formula	Company
Aniline	$CHCHCHCHCHCNH_2$	J.T. Baker Chemical Co. Phillipsburg, N.J.
Atrazine	$NCClNC(NHC_2H_5)NCNH(n\text{-}C_3H_7)$	ICN Pharmaceuticals, Inc. Plainview, N.Y.
Capacitor$_{TM}$ 21[1]	CXCXCXCXCXCCCXCXCXCXCX X = Cl or H	Monsanto Co. St. Louis, Mo.
Chlorobenzene	CHCHCHCHCHCCl	J.T. Baker Chemical Co. Phillipsburg, N.J.
Chloroform	$CHCl_3$	Fisher Scientific Co. Fair Lawn, N.J.
2,4-Dichlorophenol	CClCHCClCHCHCOH	Aldrich Chemical Co. Milwaukee, Ws.
2,4-Dichloro- phenoxyacetic acid	$C(OCH_2COOH)CClCHCClCHCH$	Aldrich Chemical Co. Milwaukee, Ws.
Dioctyl phthalate	$C(COOC_8H_{17})C(COOC_8H_{17})CHCHCHCH$	U.S. Steel Chemicals Div. Pittsburgh, Pa.
Malathion	$(CH_3O)_2PSSCH(COOC_2H_5)CH_2(COOC_2H_5)$	American Cyanamid Co. Princeton, N.J.
Trisodium nitrilo- triacetic acid	$N(CH_2CO_2Na)_3$	ICN Pharmaceuticals, Inc. Plainview, N.Y.
Phenol	CHCHCHCHCHCOH	J.T. Baker Chemical Co. Phillipsburg, N.J.

[1]Trademark of the Monsanto Co., St. Louis, Missouri.

CONCLUSIONS

A continuous flow system was developed for testing insoluble and volatile organic compounds on embryo-larval stages of fish. Use of a closed test system, devoid of an air-water interface greatly reduced volatility as a test variable. Fluctuations in exposure concentrations were no greater for chloroform than for non-volatile compounds. Mechanical homogenization proved highly effective in suspending hydrophobic compounds in influent water. Continuous, moderate agitation in the test chamber and regulation of detention time further precluded the need for carrier solvents. Fish eggs and larvae were easily maintained in the closed system, and there was no evidence that this procedure altered test responses.

Numerous classes of organic compounds were found to be highly toxic and teratogenic to developmental stages of fish. Of eleven compounds tested, those which proved most toxic to eggs, embryos, and early larvae included chlorobenzene, 2,4-dichlorophenol, phenol, and polychlorinated biphenyl (Capacitor 21). Chlorobenzene at 90 µg/l produced complete lethality of trout eggs, and LC_1's ranged from 8 to 33 µg/l in tests with the largemouth bass and goldfish. The three other compounds gave log probit LC_{50}'s of 2 to 70 µg/l when trout stages were exposed in hard water, and LC_1's were 0.3, 1.0, and 1.7 µg/l for phenol, Capacitor 21, and 2,4-dichlorophenol. Phenol was less toxic to developmental stages of the goldfish and bluegill. The LC_{50}'s were 0.34 and 1.69 mg/l when tests were conducted in hard water, and the LC_1's varied from 2.0 to 8.8 µg/l. Depending on water hardness, LC_1's (µg/l) determined with the rainbow trout ranged from 4.9 to 6.2 for chloroform, 21.9 to 32.5 for 2,4-D, and 29.0 to 77.2 for atrazine. Though not tested on the trout, LC_1's determined with the goldfish ranged from 143.2 to 215.0 µg/l for aniline and 141.1 to 439.6 µg/l for malathion. The least toxic compounds included NTA and DOP. In tests with trout developmental stages, the LC_{50}'s varied from 90.5 to 114.0 and 139.5 to 149.2 mg/l, respectively. Though phenol was somewhat more toxic in hard water, hardness was not an appreciable factor in most tests.

On the basis of these data, it was evident that chlorinated aromatic hydrocarbons were among the most toxic compounds. These findings are consistent with other studies which also have shown that numerous chlorinated aromatic hydrocarbons exert marked effects on fish reproduction, often accumulating to high levels in eggs and tissues (Birge, et al., 1979b). Only chloroform and phenol exhibited comparable effects on fish embryo-larval stages. Chloroform, a solvent of high lipid solubility, is a narcotizing agent, and phenol, a widely used germicide, is an effective protein denaturant. Of three monosubstituted benzene compounds tested (i.e., aniline, chlorobenzene, phenol), toxicity varied with the different substitution groups, generally increasing in the order NH_2, OH, and Cl.

It was further concluded that log probit analysis could be successfully applied to dose-response data to determine threshold concentrations (LC_1) at which organic compounds become lethal or teratogenic to embryo-larval stages. In addition, when exposure was maintained from fertilization through 4 days posthatching and responses for lethality and teratogenesis were combined, LC_1's provided a close approximation to maximum acceptable toxicant concentrations (MATC) determined in partial and complete life-cycle tests (Table 20).

Developmental stages of the different fish species usually exhibited differential sensitivity to the various organic toxicants. Though the order of species sensitivity varied somewhat with different compounds, trout embryos and alevins generally exhibited the least tolerance. Differences between LC_1 values determined for the trout and other species frequently exceeded one and sometimes two orders of magnitude. Less variation occurred among the five remaining species, and the goldfish often was the most tolerant.

RECOMMENDATIONS

Consideration should be given to revising the protocol for embryo-larval tests, to provide technological improvements which will ensure 1) more adequate regulation of exposure concentrations of volatile toxicants, and 2) testing of hydrophobic compounds under conditions which minimize or preclude the need for carrier solvents. Additional study is recommended to modify the new procedure described herein to accommodate 1) testing of organics which exist in the gaseous state at ambient temperatures, and 2) use of a wider variety of test organisms (*e.g.*, *Daphnia*, juvenile fish). Several halomethanes included among the 129 priority toxicants listed by EPA have boiling points which range from -29 to +4.5^{o}C (*e.g.*, methyl chloride, methyl bromide, dichlorodifluoromethane). Consequently, such compounds cannot be stabilized adequately in conventional open aquatic test systems. However, the closed flow-through procedure described in the present study could be further adapted to facilitate such testing. Gases would be dispersed in influent water using the mixing assembly, and test water would be perfused continuously through the closed exposure chamber. Moderate agitation in the exposure chamber and regulation of detention time would further augment homogeneous dispersal of toxicant in test water. The new procedure also could be effectively modified to permit use of *Daphnia* and other aquatic species in tests with volatile and hydrophobic compounds which are difficult to stabilize using conventional procedures.

Convincing evidence has been presented that fish embryo-larval tests which extend beyond hatching by 30 days or more yield responses comparable to those produced in chronic life-cycle studies (McKim, 1977). However, considering the many contaminants which actually or potentially may enter aquatic ecosystems, still shorter and more cost-feasible tests are needed for environmental assessments. In the present investigation, when embryo-larval tests were carried through 4 days posthatching and frequencies of lethality and teratogenesis were combined, log probit LC_1's were in good agreement with MATC's developed in chronic life-cycle studies. These data indicated that embryo-larval tests of shorter duration than those presently recommended (U.S. EPA, 1978) may prove valuable in estimating long-term effects of aquatic toxicants. Accordingly, it may be appropriate to consider revising exposure periods specified in the present protocols for embryo-larval testing (U.S. EPA, 1978).

In addition, further attention should be given to use of LC_1 values in determining water quality criteria. Unlike the MATC which generally is expressed as the range between the lowest toxic and highest no-effect concentrations, the LC_1 represents a discrete value for which reliable confidence limits can be established. Furthermore, it may prove useful to calculate LC_{10}'s which could be used in conjunction with LC_1 values to characterize slope of the threshold end of the dose-response curve and to provide an additional reference point for establishing regulatory criteria.

Provided that the number of exposure concentrations is sufficient to delineate an adequate dose-response curve, LC_1 values can be calculated with present log probit programs. However, as existing probit methods were designed primarily for calculating LC_{50} values (Finney, 1971; Stephan, 1977), attention should be given to the development of new regression procedures formulated especially to delineate near-threshold concentrations (e.g., LC_{10}, LC_1).

DEVELOPMENT OF TEST SYSTEM AND PROCEDURES

Materials and Methods

Selection of animal species. Fish used in this study included the bluegill sunfish (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), goldfish (*Carassius auratus*), largemouth bass (*Micropterus salmoides*), rainbow trout (*Salmo gairdneri*), and redear sunfish (*Lepomis microlophus*). Species were chosen for economic importance, seasonal availability, suitable egg production, and for variations in ecological and geographic distribution, including warm and cold water habitats. This selection also included species with different patterns of reproduction, involving a number of developmental variables which may respond differentially to organic toxicants (*e.g.*, yolk quantity, hatching time, spawning habits).

Gravid rainbow trout were provided by the Erwin National Fish Hatchery, Erwin, Tennessee. Eggs and sperm were obtained by artificial spawning and milking procedures of Leitritz and Lewis (1976). Fertilization was accomplished by mixing eggs and milt for 20 min. Freshly fertilized eggs from bass, bluegill, goldfish, and redear sunfish were collected locally from the Frankfort National Fish Hatchery, Frankfort, Kentucky. Channel catfish spawn was obtained from either the Frankfort Hatchery or the Senecaville National Fish Hatchery, Senecaville, Ohio.

Selection of organic toxicants. Toxicity tests were conducted with aniline, atrazine, Capacitor 21, chlorobenzene, chloroform, 2,4-dichlorophenol, 2,4-dichlorophenoxyacetic acid, dioctyl phthalate, malathion, trisodium nitrilotriacetic acid, and phenol. All analytical and toxicity data were expressed as concentrations of the pure compounds, except for atrazine which was reported as the wettable powder (80% pure). These compounds were selected to provide varying combinations of volatility and water solubility and included aromatic hydrocarbons, aromatic amines, chlorinated hydrocarbons, organophosphates, and phthalates. This choice permitted adequate evaluation of aquatic test procedures for organic compounds and provided fish embryo-larval toxicity data for a number of important classes of organic trace contaminants. Chemical formulae and

Table 2. Toxicity tests performed on embryo-larval stages of fish.

Organic Compound	Fish Species
Aniline	Largemouth Bass, Channel Catfish, Goldfish
Atrazine	Channel Catfish, Rainbow Trout
Capacitor 21	Largemouth Bass, Redear Sunfish, Rainbow Trout
Chlorobenzene	Largemouth Bass, Goldfish, Rainbow Trout
Chloroform	Rainbow Trout
2,4-Dichlorophenol	Channel Catfish, Goldfish, Rainbow Trout
2,4-Dichloro-phenoxyacetic acid	Largemouth Bass, Goldfish, Rainbow Trout
Dioctyl phthalate	Largemouth Bass, Goldfish, Rainbow Trout
Malathion	Goldfish
Trisodium nitrilo-triacetic acid	Channel Catfish, Goldfish, Rainbow Trout
Phenol	Bluegill Sunfish, Goldfish, Rainbow Trout

sources of the selected organic compounds are given in Table 1. A summary of the toxicity tests performed in this investigation is presented in Table 2.

Test conditions and expression of data. Each organic compound was tested using four or more concentrations at each of two water hardness levels (50 and 200 mg/l $CaCO_3$). Exposure was initiated 20 min after fertilization in trout, 1 to 2 hr postspawning for bass, bluegill, goldfish, and redear sunfish, and 2 to 12 hr after spawning for channel catfish. Average hatching times were 23, 4.5, 4, 3.5, 3.5, and 2.5 days for trout, catfish, goldfish, redear, bass, and bluegill, respectively. Toxicity tests were performed in temperature-regulated environmental rooms. Test water was monitored at regular intervals for temperature, dissolved oxygen, specific conductivity, water hardness, and pH, using a YSI tele-thermometer with thermocouple (model 42SC), YSI oxygen meter (model 51A), Radiometer conductivity meter (model DCM 2e), Orion divalent cation electrode (model 93-32), and a Corning digital pH meter (model 110). Flow rates from peristaltic and syringe pumps were monitored twice daily. Temperature varied from 12.5 to 14.5°C for trout, 25.9 to 29.6°C for catfish, and 18.2 to 25.8°C for the remaining species. Dissolved oxygen levels at the above temperature ranges were 9.1 to 10.5, 5.8 to 6.8, and 6.5 to 8.9 mg/l, respectively. Monitoring data for pH, hardness, conductivity, and flow rates are summarized in Table 3. Although routine assays were not conducted for suspended solids, sample measurements ranged from 4.0 to 15.0 mg/l (American Society for Testing and Materials, 1977).

Control eggs were cultured simultaneously with experimentals and under identical conditions, except for omission of the toxicants. Eggs were examined daily to gauge extent of development and to remove dead specimens. Sample size ranged from 100 to 150 eggs per exposure concentration. Percent survival, expressed as the frequency in experimental populations/controls, was determined at hatching and 4 days after hatching. In all instances, survival frequencies were based on accumulative test responses incurred from onset of treatment. Although about 50% of the tests were extended through 8 days posthatching, larval lethality usually was insignificant after the first 4 days. Significant lethality occurred after 4 days only in tests with aniline and 2,4-D, and these results are discussed in the text. Hatchability included all embryos which survived to complete the hatching process. Teratogenesis was determined at hatching and expressed as the percent of survivors affected by gross, debilitating abnormalities likely to result in eventual lethality (Birge and Black, 1977a). Normal survivors were defined as those animals free from teratic defects. Teratic organisms were seldom encountered in control populations and never exceeded 1%. Counting teratic larvae as lethals, log probit analysis (Finney, 1971) was used to compute control-adjusted LC_{50} and LC_1 values with 95% confidence limits. The LC_1's were used to estimate toxicant concentrations which produced 1% impairment of test populations. All probability (P) levels were determined using analysis of variance.

Test water. Considerable attention was given to the development of a reconstituted water suitable for toxicity testing. Reconstituted water usually provides more stable test conditions than natural water, as the latter may be subject to substantial seasonal fluctuations in composition (e.g., total dissolved solids, hardness, pH). Also, problems with background contaminants generally are minimized when prepared water is used. However, it is essential to use a formulation which gives chemical and physical characteristics similar to natural water. The test water described below has been used extensively during the past four years, and has given toxicity responses with metals (e.g., Cd, Cu, Hg, Zn) that compare closely with results obtained using natural water of high quality. Considering access, quality control, and other factors, use of reconstituted water did not increase cost.

Reconstituted water 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 4. Concentrations of cations and anions were within ranges published for freshwater resources in Arizona (Dutt and McCreary, 1970), Kentucky (U.S. Geological Survey, 1970), and other areas of the U.S. (McKee and Wolf, 1963; Mount, 1968). 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 (Hart, et al., 1945). Specific conductivity compared favorably with values of 150-500 μmhos/cm recommended for fish propagation (McKee and Wolf, 1963), and osmolarity was well under the maximum limit of 50 mOsm/Kg water suggested for U.S. freshwaters (National Technical Advisory Committee, 1968). Total alkalinity and pH also

Table 3. Water quality characteristics observed during toxicity tests with organic compounds.

Embryo-Larval Bioassays		Observed Test Parameters (Mean ± Standard Error)			
Compound	Designated Water Hardness (mg/l $CaCO_3$)	Water Hardness (mg/l $CaCO_3$)	pH	Conductivity (μmhos/cm)	Flow Rate (ml/hr)
Aniline	50	46.9 ± 3.4	7.7 ± 0.1	121.3 ± 2.0	206.7 ± 13.7
	200	195.3 ± 14.3	7.7 ± 0.1	257.7 ± 17.5	207.8 ± 7.7
Atrazine	50	51.3 ± 1.1	8.0 ± 0.1	106.2 ± 0.8	179.5 ± 10.2
	200	201.9 ± 0.2	7.9 ± 0.1	240.7 ± 4.4	178.8 ± 16.8
Capacitor 21	50	49.5 ± 0.2	7.8 ± 0.1	109.9 ± 1.0	200.4 ± 11.0
	200	204.0 ± 1.7	7.6 ± 0.1	240.8 ± 4.9	204.0 ± 5.6
Chlorobenzene	50	51.2 ± 1.2	7.6 ± 0.0	104.2 ± 1.9	216.2 ± 4.0
	200	203.4 ± 3.3	7.6 ± 0.1	260.3 ± 13.9	213.3 ± 5.9
Chloroform	50	48.8 ± 0.7	7.3 ± 0.0	91.8 ± 1.4	191.8 ± 1.8
	200	210.2 ± 1.2	7.3 ± 0.0	223.4 ± 1.1	190.7 ± 1.5
2,4-Dichlorophenol	50	50.0 ± 0.9	7.8 ± 0.1	124.2 ± 13.0	193.9 ± 2.3
	200	200.0 ± 2.2	7.8 ± 0.1	263.1 ± 19.6	191.8 ± 4.2
2,4-Dichloro-phenoxyacetic acid	50	50.8 ± 1.5	7.8 ± 0.2	122.4 ± 1.3	185.4 ± 6.2
	200	200.1 ± 2.9	7.7 ± 0.1	259.2 ± 4.4	170.0 ± 11.0
Dioctyl phthalate	50	53.3 ± 0.9	7.5 ± 0.2	101.6 ± 2.1	186.1 ± 3.7
	200	205.2 ± 2.7	7.4 ± 0.1	235.5 ± 4.1	187.1 ± 6.6
Malathion	50	54.3 ± 1.3	7.7 ± 0.1	106.6 ± 0.7	181.6 ± 2.0
	200	196.6 ± 3.6	7.6 ± 0.1	220.0 ± 5.0	179.6 ± 1.0
Trisodium nitrilo-triacetic acid	50	51.8 ± 3.1	8.1 ± 0.1	140.0 ± 4.1	195.6 ± 1.2
	200	187.1 ± 18.6	7.9 ± 0.1	290.0 ± 5.1	194.9 ± 0.1
Phenol	50	50.9 ± 1.2	7.9 ± 0.1	123.3 ± 11.9	193.5 ± 2.5
	200	199.2 ± 1.0	7.8 ± 0.1	258.5 ± 15.6	194.3 ± 4.2

were within optimum ranges for aquatic habitat (Baas Becking, et al., 1960; McKee and Wolf, 1963; NTAC, 1968). As maintained in the test system described below, dissolved oxygen ranged from 9.1 to 10.5 mg/l at temperatures of 12.5 to 14.5°C used for trout embryos. A minimum of 7 mg/l has been recommended for trout and salmon spawning waters (NTAC, 1968).

Embryo-larval test system. Toxicity tests were conducted using the flow-through system illustrated in Figures 1 and 2. Using graduated flow from a syringe pump, toxicant was administered to a mixing chamber which was situated ahead of each egg exposure chamber. Test 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 micro and no. 12 liquid flow meters, respectively. Flow rate was set at 200 ml/hr for 500-ml test chambers, giving a detention time of 2.5 hr. The flow-through system was operated using Brinkmann (model 131900) and Gilson (model HP8) multichannel peristaltic pumps and Sage syringe pumps (model 355). Sage pumps were fitted with modified syringe holders, as noted previously by Birge, et al. (1979a), and each unit was operated using up to six double-ground glass syringes. Syringe capacity varied from 1 μl to 100 ml, depending upon the toxicant.

To preclude loss of organic toxicants of high volatility, a closed exposure chamber devoid of an air-water interface was designed for use with fish embryo-larval stages. Test chambers were constructed from 3" Pyrex pipe joints, provided with clamp-locking O-ring seals. Using standard glass-blowing techniques, the pipe was cut and sealed to give a capacity of 0.5 liter (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 positioned 3 cm above the bottom of the dish, dividing the chamber into an upper egg compartment and a lower stirring compartment. Fish eggs were supported on the inlet screen, and a Teflon-coated magnetic stirring bar was used in the lower compartment to

Table 4. Reconstituted test water.

Components and Characteristics	Hardness (mg/l $CaCO_3$)	
	50	200
Dissolved Salts[1], mg/l		
$CaCl_2$	37.5	150
$MgSO_4 \cdot 7H_2O$	37.5	150
$NaHCO_3$	100	100
KCl	5	5
Chemical Composition, mg/l		
Ca	13.6	54.2
Mg	3.7	14.8
Na	27.4	27.4
K	2.6	2.6
Cl	26.3	98.2
HCO_3	72.6	72.6
SO_4	14.6	58.5
Physicochemical Characteristics[2]		
Hardness, as mg/l $CaCO_3$	53.3 ± 1.3	197.5 ± 5.8
pH	7.84 ± 0.02	7.78 ± 0.02
Total alkalinity, as mg/l $CaCO_3$	66.7 ± 0.4	65.3 ± 0.6
Conductivity, μmhos/cm	133.6 ± 1.4	282.0 ± 1.9
Osmolarity, mOsm/Kg H_2O	8.9 ± 0.2	12.7 ± 0.4
Total dissolved solids, mg/l	121.4 ± 4.4	336.7 ± 7.8
Dissolved oxygen, mg/l at 13.5°C	9.9 ± 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.

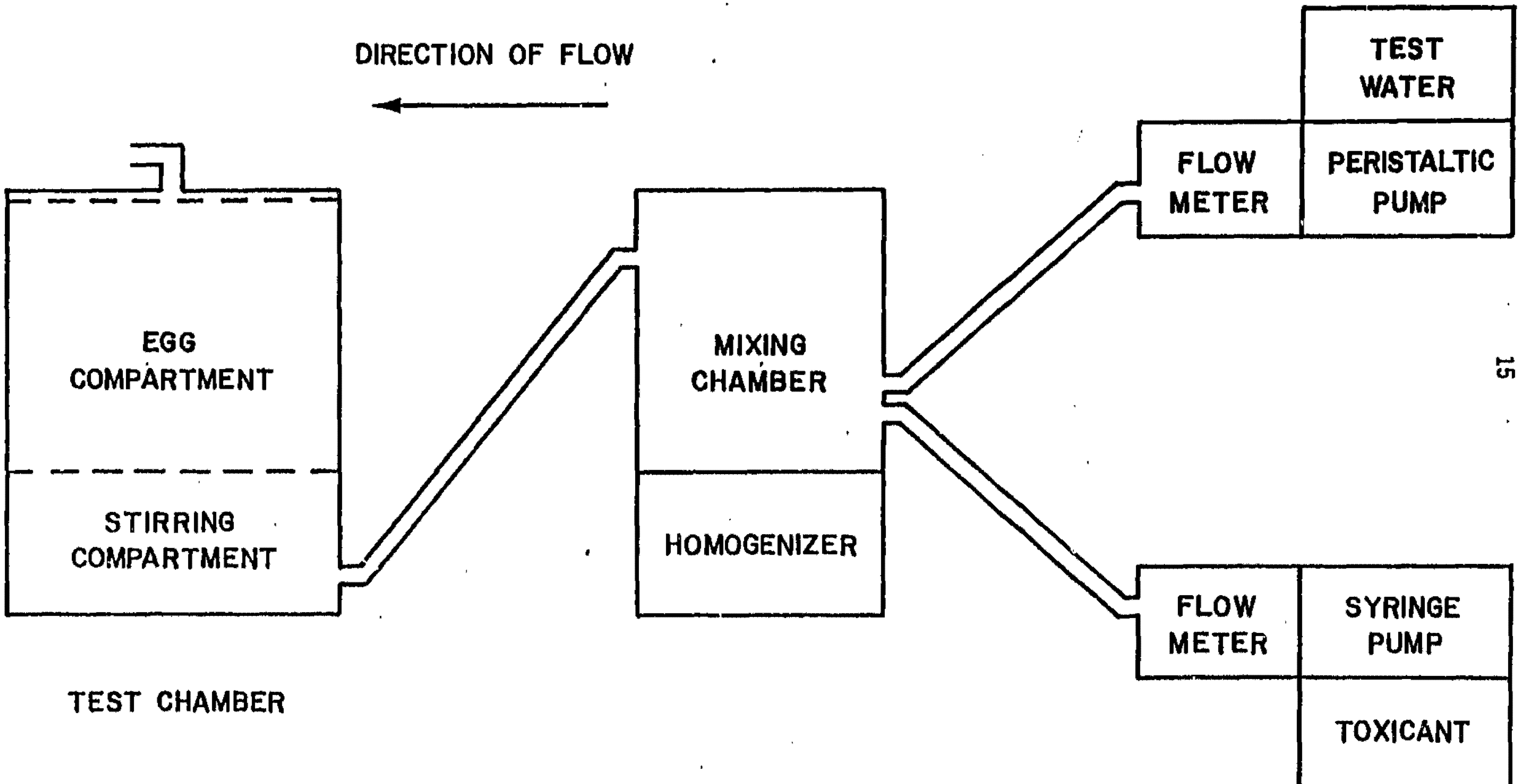
provide moderate, continuous agitation of test water. An upper outlet screen was used to retain test organisms. The outlet screen was held in place by a Pyrex pedestal, and the inlet screen was supported on the constricted upper wall of the stirring compartment (Figure 3). Access to test organisms was 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 test water were blended by either mechanical mixing or homogenization, using mixing chambers. A stoppered 250-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 hydrophobic organics in test water. This was accomplished with an Oster homogenizer, equipped with a 400-ml glass container. The latter was provided with terminal inlets for syringe and peristaltic pump lines and a side outlet for supply 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 organics from partitioning out of test water.

Analytical procedures. Exposure concentrations for all organic toxicants were confirmed by daily analyses of test water, using either gas chromatography (GLC) or spectrophotometric methods. Aniline, chlorobenzene, dioctyl phthalate, and malathion were analyzed on a Packard gas chromatograph (model 7400) with a flame ionization detector (FID). Capacitor 21 was analyzed with the same instrument, using an electron capture detector (unmodified tritium foil). Quantification of 2,4-dichlorophenoxyacetic acid was accomplished with an FID, using a Hewlett Packard gas chromatograph (model 5838A). Chloroform concentrations were determined by direct sampling, using the Hewlett Packard GLC equipped

Figure 1

Embryo-Larval Test System



Test water and toxicant were supplied to the mixing chamber using peristaltic and syringe pumps. Insoluble toxicants were suspended in test water by mechanical homogenization and a magnetic stirrer was used to provide additional agitation in the stirring compartment of the test chamber.

with a Purge and Trap system (model 7675A) and a flame ionization detector. Pre-purified nitrogen served as the carrier gas for all GLC determinations and as the purge gas for the chloroform analyses. Column packings were obtained from Supelco, Inc., except for 10% Carbowax 20M on 80/100 Anakrom U which was prepared in our laboratory. External standards were used for quantification unless otherwise indicated. Atrazine, 2,4-dichlorophenol, trisodium nitrilotriacetic acid, and phenol were analyzed on a Varian-Techtron 635 spectrophotometer. Standard curves were prepared from authentic samples of toxicants in the appropriate solvents.

Aniline was extracted from 0.25 to 1.0 liter water samples with reagent-grade benzene. The extracts were dried with anhydrous sodium sulfate and concentrated using an air stream. Aniline concentrations were determined using a glass column (2 m X 2 mm I.D.). The stationary phase was 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W HP. Oven, inlet, and detector temperatures were 75°, 190°, and 200°C, respectively, and the nitrogen flow rate was 40 ml/min. The detection limit for aniline in water was 40 μg/l. Aniline standard solutions were prepared in distilled water, extracted, and analyzed in the same manner as test water samples.

Atrazine was determined employing a modification of a previously reported procedure (White, et al., 1967). A 100-ml test water sample was extracted with chloroform. Carbon tetrachloride (5 ml) and 50% sulfuric acid (2 ml) were added to the chloroform layer, and this mixture was shaken for 30 sec at 15-min intervals over a 2-hr period. The solution was transferred to a 125-ml erlenmeyer flask, mechanically mixed for 15 min with 20 ml of water, and allowed to stand for 2 hr. Atrazine in the water layer was analyzed spectrophotometrically at 225, 240, and 255 nm, and the detection limit was 10 μg/l.

Capacitor 21 was extracted from 0.5 to 2.0 liter test water samples, using multiple aliquots of reagent-grade chloroform. The combined extracts were dried with anhydrous sodium sulfate, concentrated to near dryness with an air stream, and quantitatively reconstituted in ethyl acetate. Capacitor 21 concentrations were determined on a 2 m X 2 mm I.D. glass column. The stationary phase was 3% Dexsil 300 GC on 80/100 Chromosorb W HP. Oven, inlet, and detector temperatures were 230°, 250°, and 260°C, respectively, and the carrier gas flow rate was 55 ml/min. The 4-chlorobiphenyl component of Capacitor 21 was used as

Figure 2

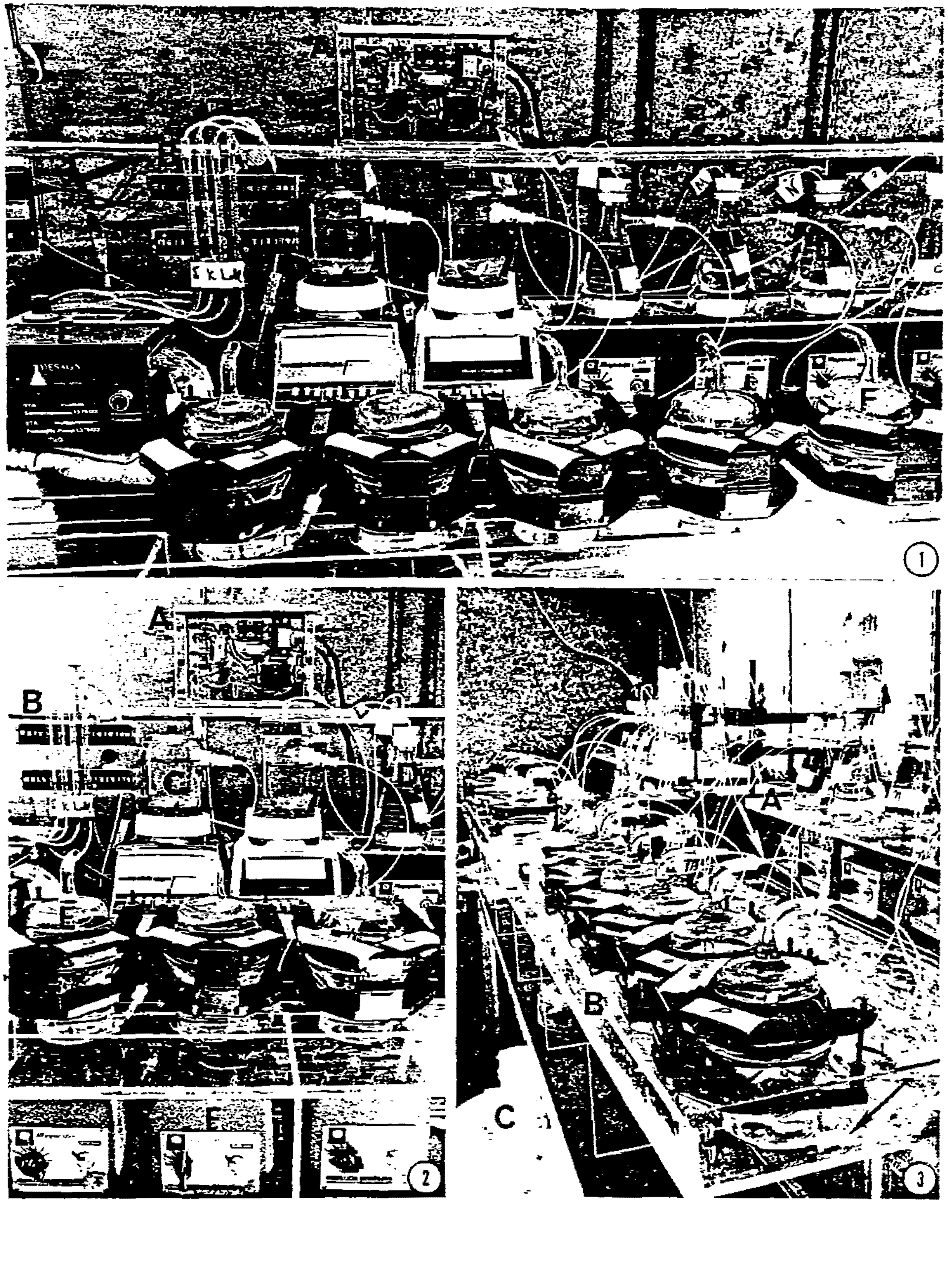
Multichannel Assembly of Toxicity Test Units

2.1 Components included an electronic timer (A), liquid flow meters (B), mixing chambers used for insoluble (C) and soluble (D) toxicants, peristaltic pump (E), and exposure chambers (F). Syringe pumps were mounted outside the environmental room to avoid effects of low temperature and high humidity on operation.

2.2 View of magnetic stirrers (F) situated beneath the drainboard used to support exposure chambers (E).

2.3 A bank of 10 exposure chambers housed in a 6' X 10' environmental room. Inlet lines from mixing chambers (A) were attached with rapid disconnects (black arrow). The watertight drainboard (B) contained spillage. Test chamber outlet lines (white arrow) were connected to waste receptacles (C).

17(a)



a quantitative "marker" for this multi-component toxicant. Capacitor 21 in reagent-grade ethyl acetate was used to prepare standards for quantification, and the detection limit was 0.1 μg/l.

Chlorobenzene was extracted from 0.1 to 1.0 liter water samples using ether or chloroform. The extracts were dried with anhydrous sodium sulfate and concentrated with an air stream. Chlorobenzene was analyzed on the same column used for aniline determinations. Oven, inlet, and detector temperatures were 80°, 115°, and 230°C, and the carrier gas flow rate was 37 ml/min. Chlorobenzene in benzene or ethyl acetate was used to prepare the standard curve, and the detection limit was 5.0 μg/l.

Chloroform was analyzed directly from 1 to 15 ml samples of test water, using the purge and trap system described above. Each sample was purged with dry, pre-purified nitrogen (10 ml/min). Chloroform was adsorbed on a Tenax GC trap at ambient temperature, desorbed at 200°C, and analyzed at programmed temperatures of 70 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. The carrier gas flow rate was 19 ml/min, and the detection limit was 0.1 μg/l.

Samples of 2,4-dichlorophenol were analyzed using a modification of the phenol analysis procedures (American Public Health Association, 1975). Added to each 0.25 liter sample of test water were 2 ml of ammonium chloride (50 g/l), 5 ml of 0.5 N ammonium hydroxide, 2 ml of 4 aminoantipyrine (12 g/l), and 2 ml of potassium ferricyanide (48 g/l). After standing 0.5 to 1.0 hr, the solution was extracted with chloroform and dried over anhydrous sodium sulfate. The samples were quantified at 470 nm, and the detection limit was 1.0 μg/l.

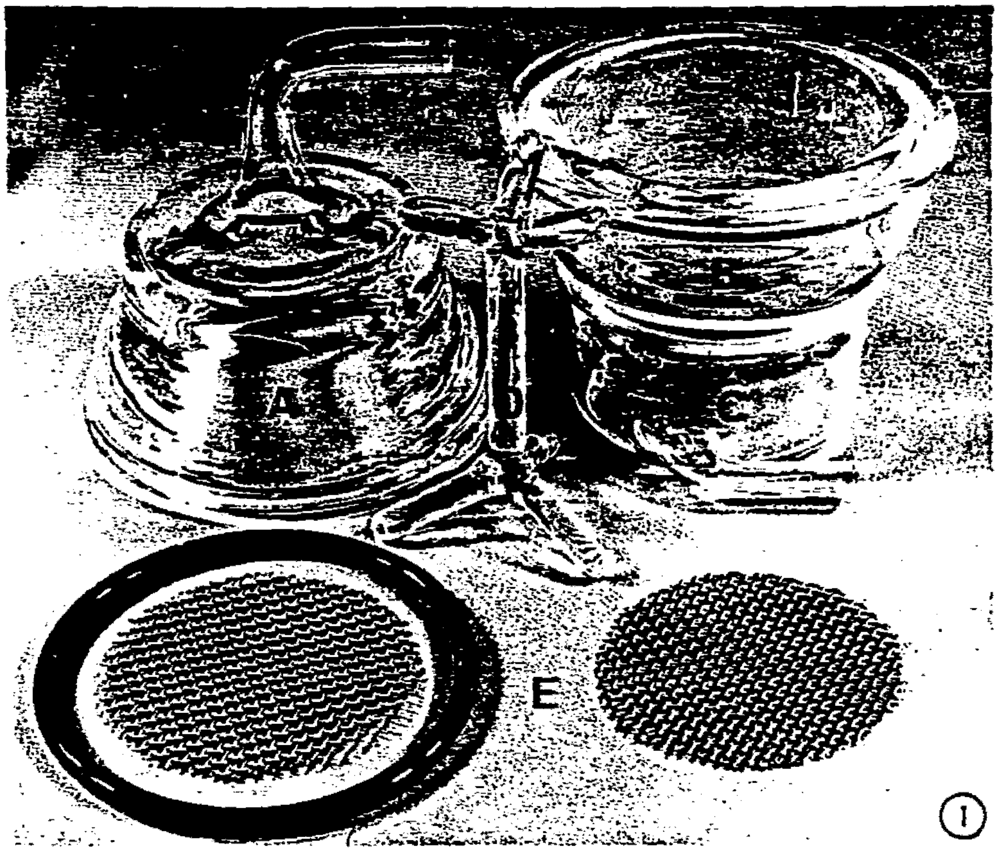
Test water samples of 2,4-dichlorophenoxyacetic acid (2,4-D as the potassium salt) were collected in 0.05 to 0.5 liter volumes, diluted to 0.5 l with distilled water where necessary, and acidified with 5 ml of concentrated hydrochloric acid. The 2,4-D was extracted with multiple aliquots of reagent-grade chloroform. The extracts were evaporated to dryness with a stream of air and reacted at 60°C with diazomethane in ether for 10 min. Several ml of ethyl acetate were added, and the subsequent mixture was concentrated by evaporation with an air stream. Samples of the 2,4-D methyl ester were analyzed on a 2 m X 2 mm I.D. glass column at programmed temperatures of 160 to 240°C. The

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).



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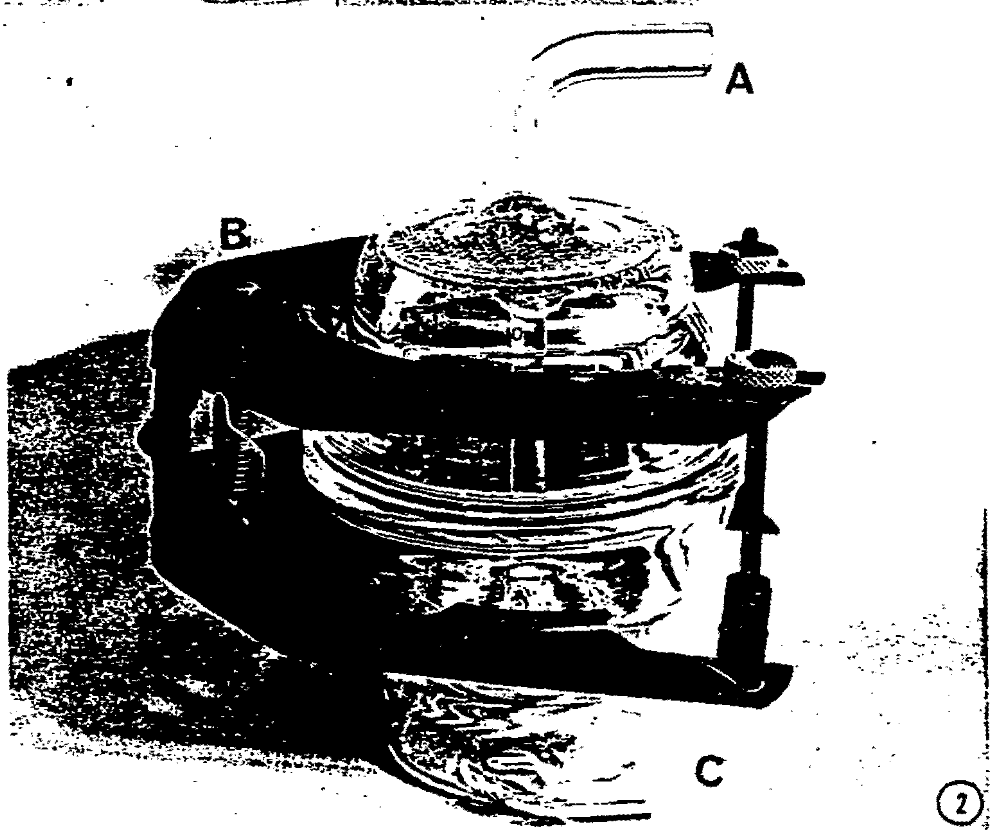

2

stationary phase was 10% Carbowax 20M on 50/60 Anakrom U. Inlet and detector temperatures were 250° and 265°C, respectively. Standards for 2,4-D were prepared in ethyl acetate, and the detection limit was 50 μg/l.

Dioctyl phthalate (DOP) was extracted from 0.1 to 1.0 liter test water samples with multiple aliquots of reagent-grade chloroform. The combined extracts were dried with anhydrous sodium sulfate and concentrated to near dryness with an air stream. DOP was reconstituted in ethyl acetate and quantified using a 0.5 m X 2 mm I.D. glass column. The stationary phase was 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W HP. Oven, inlet, and detector temperatures were 235°, 250°, and 260°C, respectively, and the carrier gas flow rate was 50 ml/min. DOP in reagent-grade ethyl acetate was used to prepare the standard curve, and the detection limit was 25 μg/l.

Malathion was extracted from 0.1 to 2.0 liter test water samples with several aliquots of chloroform. The combined extracts were dried with anhydrous sodium sulfate and evaporated to near dryness with air. Malathion, reconstituted in ethyl acetate, was quantified with the same column used for analyses of dioctyl phthalate. Oven, inlet, and detector temperatures were 210°, 230°, and 250°C, respectively. The carrier gas flow rate was 45 ml/min. Malathion standards were prepared in ethyl acetate, and the detection limit was 50 μg/l.

Trisodium nitrilotriacetic acid (NTA) was analyzed by the zinc-zincon method (U.S. EPA, 1974). To prevent interference with calcium and magnesium ions, NTA samples were batch-treated with ion exchange resin (Dowex 50W-X8, 50-100 mesh). Prepared samples were quantified at 620 nm, and the detection limit was 0.5 mg/l.

Phenol concentrations were determined using the 4-aminoantipyrine procedure with chloroform extraction as described in Standard Methods (American Public Health Association, 1975). Samples were quantified at 460 nm, and the detection limit was 1.0 μg/l.

Initial Performance Evaluation

Sudan IV dye in chlorobenzene (10 g/l) was injected into the test system at rates calculated to give dye concentrations which ranged from

0.75 to 1.84 mg/l, using peristaltic/syringe pump dilution ratios of 13,300 to 5,440, respectively. The dye-chlorobenzene was quantified at a wavelength of 540 nm, using a Model 635 Varian-Techtron spectrophotometer. Actual dye concentrations, determined on samples of test water pipetted at random from the egg compartment, were in close agreement with calculated values (Table 5). The flow rate for test water was 200 ml/hr and collection intervals varied from 5 to 60 min for operating periods of 0.5 to 5.0 hr. Visual inspection of the flow pattern also revealed highly uniform distribution of the insoluble Sudan IV-chlorobenzene.

Subsequent to this initial evaluation, the system continued to provide good reproducibility of exposure concentrations in actual toxicity tests. Results summarized in Table 6 include analytical data for chlorobenzene and dioctyl phthalate, two compounds of low water solubility, and chloroform, a highly volatile organic. Variations in exposure levels were no greater than for soluble compounds of low volatility, such as NTA (Table 18) and phenol (Table 19). In addition, toxicant concentrations were regulated with precision down to 1 µg/l or less (Tables 11, 19). For example, using a calculated concentration of 1 µg/l phenol, actual concentrations (mean ± standard error) in four tests were 0.7 ± 0.2, 1.2 ± 0.3, 1.3 ± 0.3, and 1.5 ± 0.3.

Table 5. Regulation of Sudan IV-Chlorobenzene in continuous flow tests.

Dye Dilution Factor	Concentration (mg/l)[1]		Operating Time (minutes)	Sampling Interval (minutes)
	Calculated	Actual, Mean ± S.E.		
13300	0.75	0.73 ± 0.03	150	30
8980	1.11	1.10 ± 0.02	60	5
7900	1.27	1.36 ± 0.04	60	5
6670	1.50	1.60 ± 0.03	300	60
6670	1.50	1.58 ± 0.03	150	30
6000	1.67	1.64 ± 0.09	30	5
6000	1.67	1.72 ± 0.06	30	5
5830	1.72	1.77 ± 0.04	30	5
5630	1.78	1.73 ± 0.04	60	5
5440	1.84	1.83 ± 0.04	60	5

[1] Sudan IV dye in chlorobenzene (10 g/l) was delivered to exposure chambers by a Sage syringe pump (model 355), and the flow of dilution water was regulated with a multichannel Gilson peristaltic pump (model HP8).

Table 6. Regulation of organic compounds in continuous flow toxicity tests with fish embryo-larval stages.

Compound (Species)	Water Hardness (mg/l $CaCO_3$)	Actual Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Chlorobenzene (Largemouth Bass)	50	0.013 ± 0.002	91(2)	89	80
		0.038 ± 0.003	86	86	60
		0.16 ± 0.01	75(11)	67	24
		2.55 ± 0.28	27(55)	12	0
		27.3 ± 1.4	4(100)	0	0
	200	0.009 ± 0.001	100	100	93
		0.040 ± 0.006	93(2)	91	64
		0.15 ± 0.02	72(13)	63	14
		3.10 ± 0.34	25(42)	15	0
		23.2 ± 1.8	4(100)	0	0
Chloroform (Rainbow Trout)	50	0.004 ± 0.001	95	95	95
		0.008 ± 0.001	92	92	92
		0.059 ± 0.006	89(3)	86	86
		0.69 ± 0.03	73(4)	70	70
		10.1 ± 0.7	36(37)	23	23
	200	0.003 ± 0.001	106	106	106
		0.010 ± 0.001	88(1)	87	87
		0.056 ± 0.004	83(3)	80	80
		0.63 ± 0.02	72(3)	70	70
		10.6 ± 0.4	23(40)	14	14
Dioctyl phthalate (Largemouth Bass)	50	0.055 ± 0.006	97	97	95
		0.30 ± 0.03	93	93	91
		46.3 ± 4.0	74	74	67
		66.9 ± 3.3	39(1)	39	26
		149 ± 15	13(4)	12	2
	200	0.065 ± 0.012	97	97	96
		0.30 ± 0.03	91(1)	90	90
		35.5 ± 3.1	71	71	64
		60.6 ± 4.3	39(1)	39	30
		146 ± 16	16(3)	16	7

[1]Frequency determined as survival in experimental population/control.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

APPLICATION OF TEST SYSTEM

Embryo-Larval Toxicity Tests

Toxicity tests were performed on the eleven organic compounds listed in Table 2, using two levels of water hardness (50 and 200 mg/l $CaCO_3$). In all cases, survival data for experimental populations were control-adjusted. Control survival ranged from 88 to 99% except in chloroform tests with trout, where it averaged 72%. Log probit values for the organic toxicants appear in Tables 7 and 8, and dose-response data are summarized in Tables 9 through 19.

Aniline. Tests were conducted on developmental stages of largemouth bass, channel catfish, and goldfish, and survival data are shown in Table 9. Using soft water, aniline LC_{50} values at hatching were 5.5, 9.3, and 32.7 mg/l for catfish, goldfish, and bass (Table 7). Embryonic sensitivity to LC_{50} and higher concentrations of aniline increased with treatment times to hatching, which were 2.5, 3.5, and 4.5 days for bass, goldfish, and catfish, respectively (Figure 4). This was in agreement with previous results for embryo-larval tests with mercury (Birge, et al., 1979c). When exposure was extended beyond hatching, the only significant change in median lethal concentrations occurred in tests with bass. The aniline LC_{50} decreased markedly to 11.8 and 5.4 mg/l at 4 and 8 days post-hatching, and these values differed significantly from those observed at hatching ($P < 0.05$). More moderate reductions to 5.5 and 5.1 mg/l were observed in tests with goldfish. In contrast, aniline LC_{50}'s for catfish showed virtually no change from hatching through 8 days posthatching. Although bass eggs exhibited the greatest tolerance to aniline, early posthatched lethality was highest for this species. It appeared that while embryonic sensitivity to aniline increased with hatching time, an inverse relationship existed between hatching time and sensitivity of early larval stages. Water hardness did not exert appreciable effects on LC_{50}'s, but low concentrations of aniline appeared somewhat more toxic in hard water. In tests with goldfish and catfish, the respective LC_1's at 4 days posthatching were 143 and 249 μg/l aniline in hard water, and 215 and 648 μg/l in soft water (Table 8). Aniline produced substantial teratogenic impairment only at the higher exposure concentrations, and

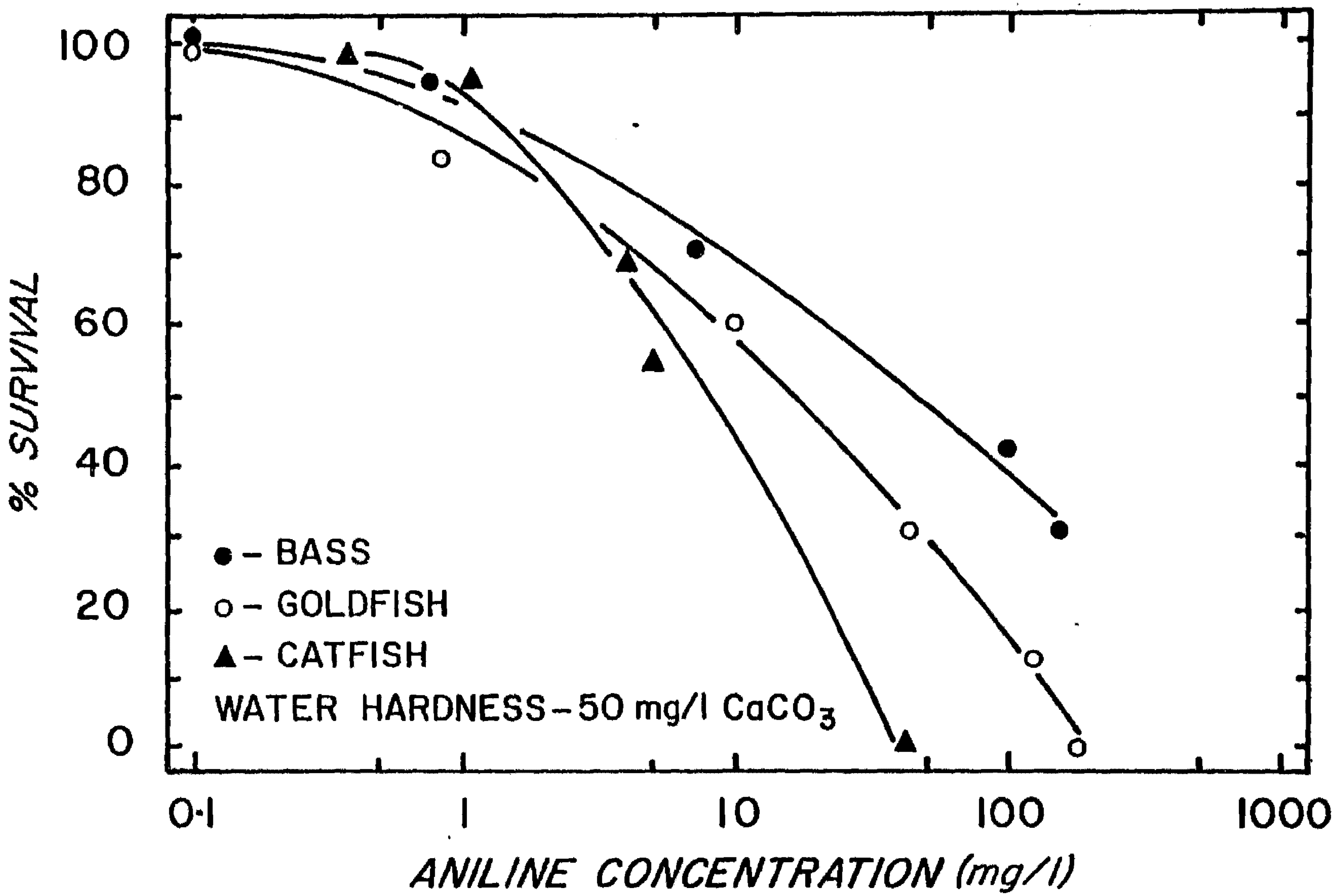

Figure 4. Toxicity of aniline to fish eggs. Aniline exposure was maintained through hatching, giving treatment times of 2.5, 3.5, and 4.5 days for bass, goldfish, and catfish.

the greatest incidence of teratic defects was observed for goldfish. At 14, 34, and 100 mg/l, aniline administered in hard water produced abnormalities in hatched populations at frequencies of 2%, 22%, and 43%.

The LC_{50} values for aniline calculated for the three fish species fell at or somewhat below the range of 10 to 100 mg/l reported in most 96-hr tests on aquatic biota (Christensen, 1976). One investigation showed aniline to be approximately ten times more toxic to trout than to goldfish (McKee and Wolf, 1963). In the present study, a 5 to 6-fold difference in sensitivity was observed between embryo-larval stages of bass and catfish when LC_{50}'s were taken at hatching. However, when treatment was extended through 8 days posthatching, no significant heterogeneity of response could be demonstrated.

Atrazine. Toxicity tests with atrazine were performed on channel catfish and rainbow trout (Table 10). As stated previously, concentrations were reported for a commercially available wettable powder which contained 80% atrazine. When trout eggs were treated in hard water, the LC_{50} at hatching and 4 days posthatching was 1.1 mg/l (Table 7). In tests with the catfish, LC_{50}'s were 0.31 and 0.24 mg/l at hatching and at 4 days. These values were 10 to 100 times lower than those reported for the fry of Coregonus fera (Gunkel and Kausch, 1976). Atrazine LC_1's calculated for the rainbow trout were 29.0 and 77.2 μg/l in soft and hard water, respectively (Table 8) and these values were in reasonable agreement with a maximum acceptable toxicant concentration (MATC) range of 65 to 120 μg/l reported in partial life-cycle studies with brook trout (Macek, et al., 1976). Catfish embryos appeared more sensitive than trout stages, but reliable LC_1 values could not be calculated. Posthatched lethality was infrequent for both trout and catfish, and water hardness did not appreciably alter toxicity. Atrazine was highly teratogenic in all tests. For example, terata occurred at frequencies of 16%, 47%, and 86% in hatched catfish populations exposed in hard water to atrazine at 0.42, 4.81, and 46.7 mg/l, respectively.

Capacitor 21. Capacitor 21, a polychlorinated biphenyl, was administered to embryos and larvae of largemouth bass, redear sunfish, and rainbow trout (Table 11). Redear sunfish and bass were exposed at both levels of water hardness and trout was tested only in hard water. Trout and bass stages were about equally sensitive to Capacitor 21, and LC_{50} values varied from 2.0 to 2.7 μg/l at hatching and from 1.5 to 2.0 μg/l at 4 days posthatching (Table 7). Developmental stages of the redear sunfish were somewhat more tolerant, and the 4-day LC_{50}'s ranged from 8.0 to 13.0 μg/l. The LC_1's calculated at 4 days varied from 1.3 to 3.5 μg/l for the redear and 0.5 to 1.0 μg/l for bass and trout (Table 8). The LC_1 values, which gave an overall range of 0.5 to 3.5 μg/l for three species of fish, were close to MATC's of 1.1 to 3.0, 1.8 to 4.6, and 2.1 to 4.0 μg/l determined in life-cycle studies with fathead minnows exposed to Aroclors 1248, 1254, and 1260, respectively (Nebeker, et al., 1974; McKim, 1977; DeFoe, et al., 1978). Similarly, Aroclor 1254 at concentrations as low as 0.48 μg/l produced 16% reproductive impairment in Daphnia magna (Nebeker and Puglisi, 1974).

Capacitor 21 proved to be the most toxic of all eleven compounds tested. Embryonic lethality was the most sensitive test response, and teratogenesis was observed only at concentrations which substantially reduced hatchability. Water hardness exerted no appreciable effects on toxicity.

Chlorobenzene. Largemouth bass, goldfish, and rainbow trout eggs were treated with chlorobenzene, and as noted previously the procedure of toxicant injection provided accurate regulation of exposure concentrations for this insoluble compound (Table 12). Trout eggs were exposed for 16 days in soft water to mean concentrations (± S.E.) of 0.09 ± 0.02, 0.31 ± 0.04, 1.60 ± 0.19, 4.27 ± 0.50, and 32.0 ± 3.7 mg/l chlorobenzene. Complete lethality occurred in all instances, and tests performed in hard water produced similar results. Embryo-larval stages of the other piscine species were more tolerant to chlorobenzene. The LC_{50}'s at 4 days posthatching ranged from 0.05 to 0.06 mg/l with bass and from 0.88 to 1.04 mg/l with goldfish (Table 7). Lethality in experimental larval populations was highly significant for bass in both hard and soft water ($P < 0.01$).

In tests conducted with goldfish, the LC_1 values at 4 days posthatching were 10 μg/l in soft water and 33 μg/l in hard water (Table 8). Frequencies of teratic bass larvae did not exceed 2% at or below an exposure concentration of 0.04 mg/l, but ranged from 11% at 0.2 mg/l to 100% at 27.3 mg/l. Fewer abnormalities occurred with the goldfish. While limited data exist concerning the effects of chlorobenzene on other aquatic species, high potentials for toxicity and bioconcentration (Metcalf, 1977; Branson, 1978) further stress the importance of developing an acceptable freshwater criterion for this hazardous compound.

Chloroform. This was the most volatile compound tested, and it was administered to embryo-larval stages of rainbow trout (Table 13). As noted above, the enclosed test chamber minimized evaporative loss of the toxicant and provided good regulation of exposure concentrations. The LC_{50} values at hatching were 2.03 and 1.24 mg/l in soft and hard water, respectively, and the larval stages were unaffected through 4 days posthatching (Table 7). Depending on water hardness, the LC_1's for chloroform ranged from 4.9 to 6.2 µg/l (Table 8). Occurrences of teratogenesis were concentration dependent, and reached 40% in hatched populations at 10.6 mg/l. Chloroform LC_{50}'s, determined in 96-hr tests on aquatic organisms, were reported by NIOSH (Christensen, 1976) to range from approximately 10 to 100 mg/l. However, these high values could be due in part to loss of this highly volatile toxicant from open exposure systems. The chronic value recently cited for freshwater invertebrates was 500 µg/l (U.S. EPA, 1979), but no details were given concerning specific test conditions. If open test chambers were employed, particularly if used with static or static-renewal procedures, variations in exposure levels of chloroform due to evaporative loss could have been appreciable.

2,4-Dichlorophenol (DCP). Aquatic toxicity tests were performed with DCP, using embryo-larval stages of channel catfish, goldfish, and rainbow trout (Table 14). When trout were exposed at the two water hardness levels, the LC_{50}'s at hatching ranged from 0.07 to 0.08 mg/l, with no further change after 4 days (Table 7). Catfish and goldfish were considerably less sensitive, as LC_{50}'s at 4 days posthatching varied from 0.26 to 1.35 mg/l. Exposure time through hatching was 24 days for trout and 4 days for catfish and goldfish, correlating well with the higher lethality observed for trout. During the posthatched period, high concentrations of DCP produced significant lethality of goldfish ($P < 0.05$). Consequently, the LC_{50} values determined with trout and goldfish tended to converge with time from hatching, and this is consistent with results for aniline. However, LC_{50}'s indicated that trout at 4 days were still about 4 to 5 times more sensitive to DCP than were goldfish. Catfish larvae, like those of trout, suffered little lethality. While DCP was not highly teratogenic, frequencies of teratic goldfish larvae ranged up to 24% and were observed at concentrations as low as 1 μg/l in tests with catfish and trout (Table 14). Variations in water hardness appeared to have no substantial effects on toxicity.

At low exposure concentrations, catfish and trout were equally susceptible to DCP, and the LC_1's calculated for these species varied between 1.6 and 2.8 μg/l (Table 8). While no freshwater criterion has been established for this compound, the LC_1's were comparable to the fish flesh tainting threshold of 0.1 to 15 μg/l determined for o-chlorophenol, p-chlorophenol, and 2,4-dichlorophenol (U.S. EPA, 1976). In embryo-larval tests with the goldfish, LC_1's were considerably higher, ranging from 39.8 to 48.1 μg/l.

2,4-Dichlorophenoxyacetic acid (2,4-D). Embryo-larval stages of largemouth bass, goldfish, and rainbow trout were exposed to 2,4-D, which was administered as the potassium salt (Table 15). With the rainbow trout, LC_{50}'s at both hatching and 4 days posthatching were 11.0 mg/l in soft water and 4.2 mg/l in hard water (Table 7). Bass eggs were less sensitive than trout, and LC_{50}'s ranged from 161 to 165 mg/l at hatching and 82 to 109 mg/l at 4 days. Goldfish eggs were extremely tolerant to 2,4-D. The LC_{50}'s at hatching exceeded the highest exposure levels administered (187-201 mg/l). However, goldfish larvae were more sensitive and posthatched lethality was significant compared to controls ($P < 0.05$). At 4 and 8 days posthatching, depending on water hardness, LC_{50}'s dropped to 119 to 133 mg/l and 58 to 68 mg/l, respectively. A similar effect on goldfish larval stages was noted in tests with DCP. The LC_{50}'s calculated with warm water species (*i.e.*, goldfish, bass) approached those reported in 96-hr tests using several species of freshwater fish (Brungs, *et al.*, 1978).

The LC_1 values for 2,4-D varied from 21.9 to 32.5 μg/l when trout stages were treated in hard and soft water (Table 8). In tests with goldfish and bass, LC_1's were considerably higher (3.2-13.1 mg/l), and approached the no-effect level of 10 mg/l for 2,4-D established in 96-hr static tests with juvenile stages of salmon (McKim, *et al.*, 1975). It is of interest to note that the LC_1 values calculated for embryo-larval stages of the three test species encompassed the MATC range of 0.3 to 1.5 mg/l determined for 2,4-D (butoxyethanol ester) in a 10-month chronic test with the fathead minnow (Mount and Stephan, 1967).

Water hardness was not a major factor concerning the toxicity of 2,4-D to goldfish and bass. However, based on LC_{50} values, trout embryo-larval stages were about 2.5 times more sensitive to 2,4-D when tests were conducted in hard water. Not only were embryonic and larval survival reduced at the higher hardness, but teratogenesis also was more frequent.

Dioctyl phthalate (DOP). DOP was tested on largemouth bass, goldfish, and rainbow trout (Table 16), and proved to be one of the least toxic compounds evaluated. Bass was the most sensitive of the three species, and 4-day LC_{50} values were 56 and 45 mg/l in soft and hard water, respectively (Table 7). When trout stages were exposed through 4 days posthatching, the LC_{50}'s were 139 mg/l in soft water and 149 mg/l in hard water. With goldfish, the high survival of embryos and larvae precluded calculation of probit LC_{50}'s. However, median lethal concentrations for DOP appeared to fall at or somewhat above 200 mg/l, based on the posthatched survival frequencies of 57 to 67% at 186 to 191 mg/l. While reliable probit LC_1's could not be determined from the dose-response data, detectable effects on fish embryo-larval stages occurred at concentrations as low as 100 to 500 µg/l, depending on test species. Frequencies of teratic larvae were low for all species and reached a maximum of only 16% when trout embryos were exposed to DOP at 148 mg/l.

The low toxicity observed with DOP was in agreement with results of a previous study in which Sugawara (1974) compared effects of three phthalate esters on eggs of the brine shrimp. While di-n-butyl phthalate (DBP) at 10.3 mg/l significantly reduced hatchability, diethyl phthalate (DEP) exerted no effects below 61.6 mg/l. Dimethyl phthalate (DMP), the least toxic of the three phthalate esters, did not reduce survival at the highest concentration tested (60 mg/l). In tests with adult fish, DBP was found to be somewhat more toxic, as 96-hr TL_{50}'s ranged from 0.7 to 6.5 mg/l (Mayer, et al., 1972). However, it should be noted that the latter investigation employed acetone at 0.7 ml/l to solubilize the toxicant, and the carrier solvent may have contributed to greater toxicity.

Malathion. Embryo-larval stages of goldfish were exposed to malathion (Table 17), and LC_{50} values at hatching were 2.61 and 3.15 mg/l in soft and hard water, respectively. Due to substantial larval lethality at high concentrations, 4-day LC_{50}'s decreased to 1.20 mg/l in soft water and 1.65 mg/l in hard water (Table 7). These values were similar to 96-hr LC_{50}'s of 1.9, 1.2, and 1.1 mg/l reported in static tests with the carp, guppy, and white perch, respectively (Rehwoldt, et al., 1977). At 4 days posthatching, LC_1's calculated for goldfish varied from 141.1 to 439.6 µg/l (Table 8). This range was close to the MATC of 200 to 580 µg/l determined for the fathead minnow exposed in a 10-month chronic test (Mount and Stephan, 1967). It appears from these values that goldfish and fathead minnow are more tolerant to malathion than several other fish species (U.S. EPA, 1976; Parrish, et al., 1977). For example, in a life-cycle reproductive study with the flagfish, the MATC was observed to fall within a range of 8.6 to 10.9 µg/l (Hermanutz, 1978). The selective toxicity of malathion for different piscine species was discussed previously in the study by Mount and Stephan (1967).

Teratogenesis was observed only at malathion concentrations which produced embryonic lethality. In soft water, abnormalities were present in 2%, 6%, and 25% of hatched populations when developmental stages were exposed to 0.6, 2.0, and 5.2 mg/l, respectively. Water hardness exerted no appreciable effects on toxicity.

Trisodium nitrilotriacetic acid (NTA). This compound was administered to channel catfish, goldfish, and rainbow trout, and was relatively non-toxic to both embryos and larvae (Table 18). At 4 days posthatching, NTA LC_{50} values were 91, 240, and 329 mg/l for trout, goldfish, and catfish stages exposed in soft water (Table 7). NTA was somewhat less toxic in hard water, with LC_{50}'s of 114, 243, and 385 mg/l. These values were comparable to the 96-hr LC_{50} of 114 mg/l calculated for the fathead minnow (Arthur, et al., 1974) and the 3-week LC_{50}'s of 145 to 650 mg/l determined in tests with Daphnia (Biesinger, et al., 1974). Much higher values were observed in static tests with eleven marine organisms, as 168-hr TL_{50}'s ranged from 1,800 to >10,000 mg/l (Eisler, et al., 1972).

In the present investigation, LC_1's varied from 17 to 138 mg/l in soft water and 20 to 131 mg/l in hard water (Table 8). These figures were in good agreement with the MATC (54-114 mg/l) reported in 8-month life-cycle studies with the fathead minnow (Arthur, et al., 1974; McKim, 1977). In embryo-larval tests with the catfish and trout, occurrences of teratogenesis were substantial throughout the NTA concentration range. However, abnormalities were far less frequent in hatched populations of goldfish.

Phenol. Toxicity tests with phenol were performed on bluegill sunfish, goldfish, and rainbow trout eggs, and survival data are summarized in Table 19. When trout stages were treated in soft water, phenol LC_{50} values were 0.33 mg/l at hatching and 0.31 mg/l at 4 days posthatching (Table 7). The LC_{50} for phenol in hard water was 0.07 mg/l at hatching, with no change at 4 days. In tests with goldfish, LC_{50}'s for phenol in soft water were 1.22 and 0.84 mg/l at hatching and 4 days posthatching. When hard water was used, the LC_{50}'s were 0.39 and 0.34 mg/l. Bluegill stages were the most tolerant, and the LC_{50}'s were 3.34 and 2.43 mg/l at hatching and 2.42 and 1.69 mg/l at 4 days posthatching in soft and hard water, respectively. The LC_1's for phenol to trout were 0.3 and 8.6 µg/l in hard and soft water (Table 8). Phenol LC_1's calculated at 4 days posthatching for bluegill and goldfish ranged from 2.4 to 4.0 µg/l in soft water and from 2.0 to 8.8 µg/l in hard water.

Frequencies of embryonic lethality increased with hatching times of the three species, which were 2.5, 3.5, and 22 days for the bluegill, goldfish, and trout, respectively (Table 19). Consistent with findings from several embryo-larval tests discussed above, trout suffered less posthatched lethality than did species with shorter developmental periods. However, considering the LC_{50}'s for phenol in hard water taken at 4 days posthatching, the trout was still approximately 5 times and 24 times more sensitive than the goldfish and bluegill, respectively. Corresponding LC_1 values also revealed phenol to be more toxic to the trout.

Compared to findings for other organics, phenol toxicity was affected more by variations in water hardness. Based on frequencies of hatchability of trout eggs exposed at concentrations of 0.01 to 10 mg/l, phenol was approximately ten times more toxic when administered in hard water (Figure 5), and hardness was even a greater factor when LC_1 values were compared at 4 days posthatching. However, median lethal concentrations for phenol were somewhat less affected by water hardness. This compound produced substantial numbers of teratic larvae in trout and goldfish populations. For example, when trout eggs were exposed in soft water, the frequencies of teratic survivors ranged as high as 73%, and teratogenesis occurred at or below the limiting concentration for lethality.

Static and flow-through tests with phenol have been reported for a number of fish species, and 48-hr and 96-hr values varied from 7 to 100 mg/l (McKee

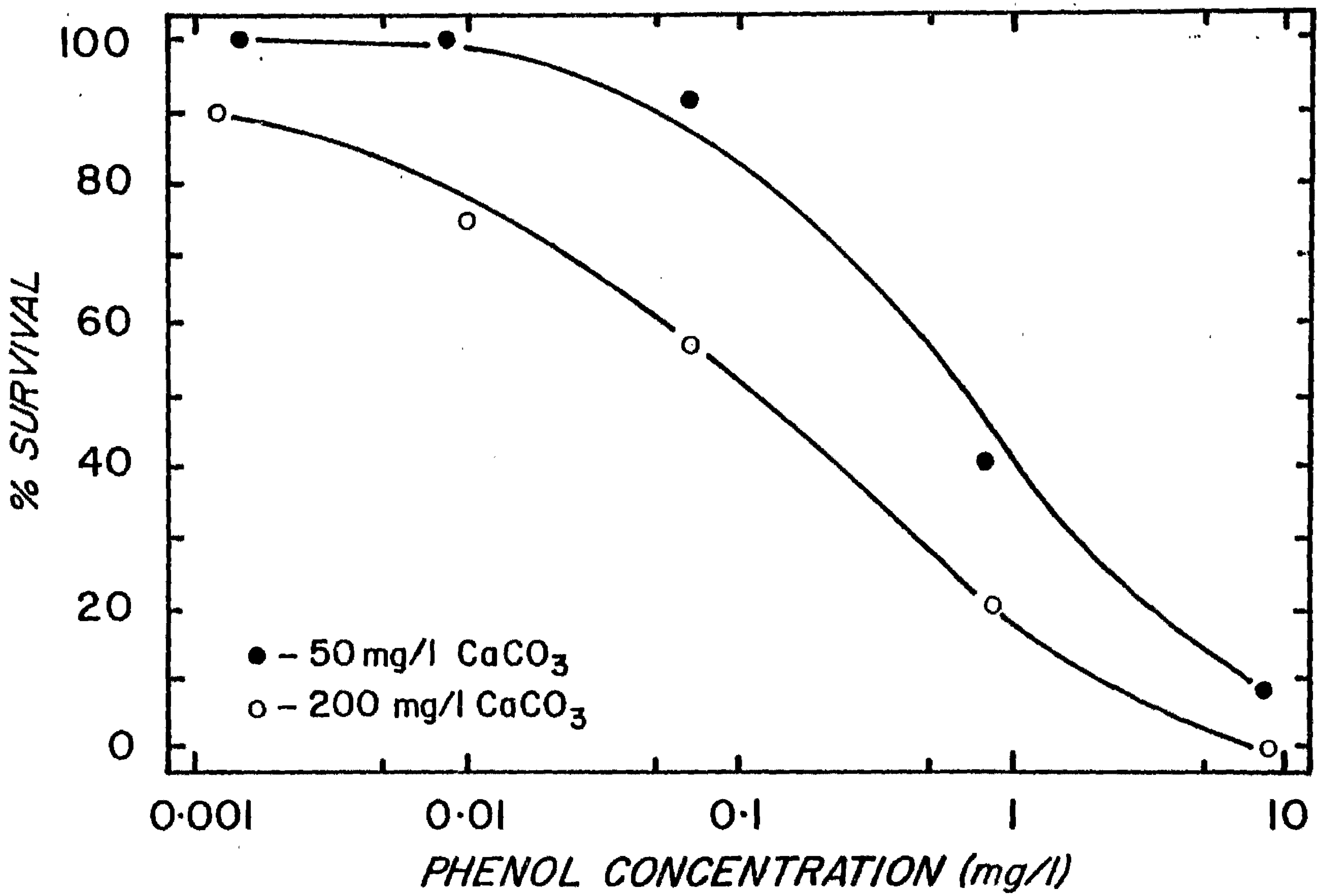

Figure 5. Effect of water hardness on phenol toxicity to trout eggs. Phenol exposure was maintained from fertilization through hatching.

and Wolf, 1963; Brown, et al., 1967; NAS-NAE Committee, 1973; Christensen, 1976; U.S. EPA, 1976; Fogels and Sprague, 1977). A 24-hr LC_{50} of 5 mg/l phenol was given for trout embryos, and 2 mg/l impaired development of oyster eggs (U.S. EPA, 1976). Although no-effect concentrations of 0.1 and 1.0 mg/l have been reported for bluegill and trout (NAS-NAE Committee, 1973), phenol at 20 to 70 µg/l has been shown to induce pathologic changes in gills and other tissues (U.S. EPA, 1976). As shown in Table 19, a concentration of 1 µg/l produced low frequencies of lethality for embryo-larval stages of goldfish and trout. For example, control-adjusted lethality averaged 10% when trout were exposed to 1 µg/l phenol administered in hard water. Considering this and the probit LC_1's of 0.3 to 8.8 µg/l determined for fish embryos and larvae, the maximum concentration of 0.1 mg/l suggested for freshwater fish (NAS-NAE Committee, 1973) may be inadequate for the protection of sensitive developmental stages. An EPA criterion of 1 µg/l has been established for domestic water supplies and for protection against fish flesh tainting (U.S. EPA, 1976). It is of interest that, as for 2,4-dichlorophenol, the LC_1's for phenol were close to the organoleptic threshold.

Good agreement was obtained when MATC's developed in partial and complete life-cycle studies were compared with LC_1 values determined in the embryo-larval tests which extended through 4 days posthatching (Table 20). Consequently, embryo-larval tests of shorter duration and higher cost feasibility than those presently recommended (U.S. EPA, 1978) may prove useful in estimating long-term biological effects of aquatic toxicants. The high sensitivity of the shorter tests was due in part to the use of combined responses for lethality and teratogenesis. The teratic larvae included in LC_1 determinations represented a significant number of test organisms which ordinarily would have become actual mortalities in longer tests. In addition, use of probit analysis gave greater discrimination of dose-response data than usually obtained with the statistical procedures (e.g., analysis of variance) traditionally applied to results of chronic studies. However, reliability of LC_1's is dependent upon adequate characterization of the dose-response. Generally, determinations are possible when treatment is administered at 4 to 6 well-selected exposure levels, but additional exposure concentrations may be required in certain instances. Truncations of or significant internal discontinuities within the survival curves may skew LC_1 values or affect determinations of reliable confidence intervals. Such problems were most apparent in tests with DOP (Table 16).

Teratogenesis in Fish Embryos

The debilitating effects of organic toxicants on fish development were not limited to embryonic and larval lethality, as most test compounds produced appreciable numbers of teratic larvae. The occurrence of embryonic anomalies ranged from 0% to 12% at near-threshold concentrations and usually increased with toxicant exposure level. Compounds producing the greatest frequencies of terata included atrazine, chlorobenzene, and NTA. There were several instances in which teratogenesis occurred at or below the limiting concentration for lethality (e.g., phenol to trout). However, considering overall effects of the eleven compounds, embryonic lethality usually was a more sensitive test response.

The teratic defects observed in hatched populations did not differ substantially from those described previously for boron and certain heavy metals (Birge and Black, 1977a, b). Defects of the vertebral column were the only teratogenic responses observed in all toxicity tests. The most common types of spinal defects included acute lordosis, scoliosis, kyphosis, and extreme rigid coiling of the vertebral column. In the order named, overall frequencies for these abnormalities in trout were 16%, 32%, 25%, and 17%, amounting to 90% of the gross teratogenic impairments observed for this species. Among other defects, there were less frequent occurrences of dwarfed bodies, microcephaly, partial twinning, absent or reduced eyes and fins, amphiarthrodic jaws, and pericardial edema.

SUMMARY

Tests were conducted on eleven compounds selected to represent various degrees and combinations of volatility and water solubility. The principal objective was to develop a test system especially designed to accommodate compounds difficult to stabilize using conventional procedures. The flow-through test system which was developed provided adequate regulation of toxicant injection, giving good reproducibility of exposure concentrations for volatile and hydrophobic organics. Aeration supplied to peristaltic pump reservoirs was adequate to meet oxygen demands of test organisms, precluding any significant air flow through the exposure chamber. Lack of direct aeration and elimination of standing air space from the test chamber effectively minimized volatility, and fish eggs and larvae were easily maintained in the closed test system.

Comparing effects of the eleven organics on fish embryos and larvae, the more toxic compounds included Capacitor 21, chlorobenzene, chloroform, dichlorophenol, and phenol, and the least toxic were DOP and NTA. Results supported the view that numerous aromatic and chlorinated hydrocarbons present serious hazards to developmental and juvenile stages of fish (Nebeker, *et al.*, 1974; Schimmel, *et al.*, 1974; U.S. EPA, 1976; Birge, *et al.*, 1978). For the most part, water hardness did not substantially affect the toxicity of organic compounds. However, in those instances where hardness was a factor, toxicity generally was greater when organic compounds were administered in hard water. This effect was best illustrated in testing phenol to trout and goldfish and 2,4-D to trout, and more often was due to increased effects on embryos rather than larvae.

Of the six fish species tested, developmental stages of the rainbow trout exhibited the greatest sensitivity to most organic compounds. Trout embryos proved more susceptible than trout larvae to toxicant exposure, as survival usually did not change significantly during the posthatched period. Although fish with shorter developmental times usually displayed greater overall tolerance, larval stages of the warm water species (*e.g.*, bass, goldfish) were more affected than trout alevins by certain toxicants, including chlorobenzene, 2,4-dichlorophenol, and 2,4-D.

Environmental monitoring data are largely incomplete for many organics. However, a number of compounds tested in the present study have occurred in natural waters at concentrations which could prove hazardous to embryo-larval stages of fish. Examples of such chemicals include atrazine (Richard, et al., 1975), chloroform (Metcalf, 1977), malathion (Cook, et al., 1976), PCB's (Martell, et al., 1975; Nadeau and Davis, 1976; U.S. EPA, 1976), and phenols (NAS-NAE Committee, 1973). Consequently, there is a need to delineate further the freshwater criteria for such organics.

Embryo-larval stages present a broad spectrum of target sites for trace contaminants, including mechanisms associated with fertilization, cellular differentiation, proliferation and growth, basic metabolism and systemic functions, as well as the hatching process and the initial accommodation to a free-living existence. Due to their high sensitivity and simple maintenance requirements, fish embryos and larvae are particularly suitable organisms for toxicity tests with organic compounds. When frequencies of teratogenesis were combined with embryonic and larval lethality, probit LC_1 values determined in embryo-larval tests generally provided good agreement with MATC's established in partial and complete, life-cycle studies. The present study further confirms use of embryo-larval tests in screening aquatic contaminants for toxic properties and developing freshwater criteria (McKim, 1977; Birge, et al., 1979b).

Table 7. Log probit LC_{50} values for organic compounds.

Compound	Species	Exposure Days Beyond Hatching	50 mg/l $CaCO_3$		200 mg/l $CaCO_3$	
			LC_{50} (mg/l)	95% Confidence Limits	LC_{50} (mg/l)	95% Confidence Limits
Aniline[1]	Largemouth Bass	0	32.7	24.0 - 44.1	29.9	20.8 - 42.5
		4	11.8	8.1 - 16.6	7.1	4.8 - 10.3
	Goldfish	0	9.3	7.4 - 11.6	7.6	6.0 - 9.5
		4	5.5	4.1 - 6.8	4.6	3.6 - 5.7
	Channel Catfish	0	5.5	4.8 - 6.3	6.3	5.4 - 7.3
		4	5.0	4.4 - 5.7	6.2	5.3 - 7.3
Atrazine	Rainbow Trout	0	0.92	0.67 - 1.20	1.11	0.86 - 1.39
		4	0.87	0.63 - 1.15	1.08	0.84 - 1.35
	Channel Catfish	0	0.34	0.18 - 0.59	0.31	0.20 - 0.48
		4	0.22	0.15 - 0.32	0.24	0.16 - 0.35
Capacitor 21	Redear Sunfish	0	0.019	0.016 - 0.029	0.014	0.011 - 0.080
		4	0.013	0.011 - 0.017	0.008	0.007 - 0.011
	Largemouth Bass	0	0.0023	0.0021 - 0.0027	0.0027	0.0024 - 0.0030
		4	0.0015	0.0014 - 0.0016	0.0018	0.0017 - 0.0019
	Rainbow Trout	0	-	- -	0.0020	0.0016 - 0.0021
		4	-	- -	0.0020	0.0017 - 0.0021
Chlorobenzene[1]	Goldfish	0	3.48	3.08 - 3.87	2.37	1.96 - 2.86
		4	0.88	0.67 - 1.12	1.04	0.86 - 1.25
	Largemouth Bass	0	0.34	0.22 - 0.51	0.39	0.25 - 0.58
		4	0.05	0.04 - 0.07	0.06	0.04 - 0.08
	Rainbow Trout	0	< 0.09	- -	< 0.09	- -
		4	-	- -	-	- -

Table 7 - continued.

Compound	Species	Exposure Days Beyond Hatching	50 mg/l $CaCO_3$		200 mg/l $CaCO_3$	
			LC_{50} (mg/l)	95% Confidence Limits	LC_{50} (mg/l)	95% Confidence Limits
Chloroform	Rainbow Trout	0	2.03	0.95 - 3.75	1.24	0.62 - 2.16
		4	2.03	0.95 - 3.75	1.24	0.62 - 2.16
2,4-Dichlorophenol	Goldfish	0	1.76	1.26 - 2.48	1.24	0.78 - 1.85
		4	0.39	0.29 - 0.62	0.26	0.17 - 0.97
	Channel Catfish	0	1.85	1.25 - 2.82	1.70	1.08 - 2.65
		4	1.35	0.94 - 2.02	1.07	0.68 - 1.66
	Rainbow Trout	0	0.08	0.07 - 0.10	0.07	0.05 - 0.09
		4	0.08	0.07 - 0.10	0.07	0.06 - 0.09
2,4-Dichloro-phenoxyacetic acid	Goldfish	0	> 187	- -	> 201	- -
		4	133.1	108.6 - 174.8	119.1	98.5 - 150.6
	Largemouth Bass	0	165.4	130.6 - 274.1	160.7	122.9 - 230.6
		4	108.6	92.5 - 138.4	81.6	64.8 - 103.5
	Rainbow Trout	0	11.0	7.8 - 15.1	4.2	2.8 - 5.9
		4	11.0	7.8 - 15.1	4.2	2.8 - 5.9
Dioctyl phthalate	Goldfish	0	> 186	- -	> 191	- -
		4	> 186	- -	> 191	- -
	Rainbow Trout	0	139.1	122.8 - 164.9	154.0	127.0 - 216.0
		4	139.5	123.2 - 165.2	149.2	125.8 - 203.8
	Largemouth Bass	0	65.5	59.2 - 71.9	32.1	21.5 - 46.4
		4	55.7	50.5 - 60.6	45.5	39.6 - 51.1

Table 7 - continued.

Compound	Species	Exposure Days Beyond Hatching	50 mg/l $CaCO_3$ LC50 (mg/l)	50 mg/l $CaCO_3$ 95% Confidence Limits	200 mg/l $CaCO_3$ LC50 (mg/l)	200 mg/l $CaCO_3$ 95% Confidence Limits
Malathion	Goldfish	0	2.61	2.25 - 3.08	3.15	2.81 - 3.56
		4	1.20	1.06 - 1.35	1.65	1.50 - 1.80
Trisodium nitrilotriacetic acid	Channel Catfish	0	388.3	362.7 - 415.5	393.5	345.9 - 438.2
		4	329.3	308.8 - 352.6	384.7	343.1 - 423.6
	Goldfish	0	269.6	232.3 - 309.6	257.0	221.5 - 295.5
		4	240.4	206.9 - 276.1	243.4	211.2 - 277.9
	Rainbow Trout	0	92.3	80.0 - 104.4	120.1	99.6 - 141.8
		4	90.5	78.2 - 102.6	114.0	98.6 - 129.9
Phenol[1]	Bluegill Sunfish	0	3.34	2.17 - 5.46	2.43	1.58 - 3.95
		4	2.42	1.51 - 4.06	1.69	1.05 - 2.83
	Goldfish	0	1.22	0.58 - 2.02	0.39	0.25 - 0.57
		4	0.84	0.50 - 1.32	0.34	0.23 - 0.48
	Rainbow Trout	0	0.33	0.26 - 0.40	0.07	0.04 - 0.10
		4	0.31	0.23 - 0.41	0.07	0.05 - 0.11

[1] LC_{50} values for aniline, chlorobenzene, and phenol differ somewhat from those previously published (Birge, _et al_., 1979a). In the earlier investigation, teratic larvae were not included in probit determinations.

Table 8. Log probit LC_1 values determined at 4 days posthatching for organic compounds.

Compound	Species	50 mg/l $CaCO_3$		200 mg/l $CaCO_3$	
		LC_1 (µg/l)	95% Confidence Limits	LC_1 (µg/l)	95% Confidence Limits
Aniline[1]	Largemouth Bass	-	- -	-	- -
	Goldfish	215.0	125.1 - 333.5	143.2	79.0 - 231.4
	Channel Catfish	647.6	412.6 - 892.2	249.3	158.5 - 359.6
Atrazine	Rainbow Trout	29.0	11.0 - 56.9	77.2	36.8 - 130.1
	Channel Catfish	-	- -	-	- -
Capacitor 21	Redear Sunfish	3.5	1.6 - 5.1	1.3	0.6 - 2.0
	Largemouth Bass	0.5	0.4 - 0.7	0.9	0.7 - 1.0
	Rainbow Trout	-	- -	1.0	0.6 - 1.3
Chlorobenzene[1]	Goldfish	10.0	5.0 - 17.0	33.0	18.0 - 52.0
	Largemouth Bass	-	- -	8.0	2.0 - 14.0
	Rainbow Trout	-	- -	-	- -
Chloroform	Rainbow Trout	6.2	0.2 - 34.9	4.9	0.3 - 22.5
2,4-Dichlorophenol	Goldfish	48.1	20.6 - 75.5	39.8	0.1 - 68.9
	Channel Catfish	2.8	1.0 - 5.9	1.6	0.4 - 4.8
	Rainbow Trout	2.8	1.4 - 4.8	1.7	0.7 - 3.2

Table 8 - continued.

Compound	Species	50 mg/l $CaCO_3$		200 mg/l $CaCO_3$	
		LC_1 (μg/l)	95% Confidence Limits	LC_1 (μg/l)	95% Confidence Limits
2,4-Dichloro-phenoxyacetic acid	Goldfish	8,210	2,677 - 15,027	8,851	3,835 - 14,637
	Largemouth Bass	13,102	4,418 - 21,886	3,214	1,218 - 5,989
	Rainbow Trout	32.5	8.9 - 83.6	21.9	6.2 - 55.4
Malathion	Goldfish	141.1	98.8 - 186.6	439.6	326.2 - 546.6
Trisodium nitrilo-triacetic acid	Channel Catfish	138,387	117,087 - 156,791	130,949	90,740 - 166,682
	Goldfish	28,528	17,410 - 40,835	30,142	18,925 - 42,387
	Rainbow Trout	16,902	10,864 - 23,223	20,198	12,054 - 28,566
Phenol[1]	Bluegill Sunfish	4.0	0.5 - 12.1	2.0	0.3 - 5.5
	Goldfish	2.4	0.3 - 8.7	8.8	2.5 - 20.1
	Rainbow Trout	8.6	4.2 - 14.8	0.3	0.1 - 0.8

[1]LC_1 values for aniline, chlorobenzene, and phenol differ somewhat from those previously published (Birge, et al., 1979a). In the earlier investigation, teratic larvae were not included in probit determinations.

Table 9. Toxicity of aniline to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	Aniline Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Largemouth Bass	50	0.051 ± 0.008	100	100	100
		0.75 ± 0.08	95(1)	94	83
		7.75 ± 1.95	71(4)	68	52
		105 ± 12	43(13)	37	28
		160 ± 10	31(24)	24	18
	200	0.045 ± 0.011	100(1)	99	97
		0.51 ± 0.12	89(1)	88	75
		10.2 ± 0.1	65(3)	63	44
		93.7 ± 9.1	47(10)	42	25
		169 ± 7	30(25)	22	15
Goldfish	50	≤ 0.10	100	100	100
		0.85 ± 0.08	84(1)	83	82
		10.5 ± 1.6	61(4)	59	57
		43.2 ± 8.6	31(22)	25	0
		126 ± 6	13(35)	8	0
		185 ± 35	0	0	0
	200	≤ 0.10	100	100	99
		1.13 ± 0.18	75	75	74
		13.6 ± 2.8	53(2)	52	50
		33.7 ± 3.4	29(22)	23	0
		99.5 ± 9.0	14(43)	8	0
		189 ± 39	0	0	0
Channel Catfish	50	0.38 ± 0.21	98	98	98
		1.11 ± 0.97	95(1)	94	94
		4.24 ± 0.58	69(4)	66	49
		5.06 ± 1.52	55(1)	54	54
		44.4 ± 5.4	0	0	0
		106 ± 13	0	0	0
	200	1.20 ± 0.95	98(1)	97	97
		2.27 ± 1.01	62(2)	61	58
		8.27 ± 2.46	53(13)	46	45
		27.8 ± 9.8	37(13)	32	32
		49.6 ± 2.3	0	0	0
		114 ± 11	0	0	0

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 95, 99, and 96% for largemouth bass, goldfish, and channel catfish, respectively.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 10. Toxicity of atrazine to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	Atrazine Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Rainbow Trout	50	0.019 ± 0.003	94	94	94
		0.054 ± 0.003	91(3)	88	88
		0.54 ± 0.02	73(6)	69	68
		5.02 ± 0.06	26(62)	10	10
		50.9 ± 0.4	0	0	0
	200	0.017 ± 0.002	100(2)	98	98
		0.060 ± 0.003	93(3)	90	90
		0.52 ± 0.02	78(4)	75	74
		5.02 ± 0.06	25(65)	9	9
		49.7 ± 0.6	0	0	0
Channel Catfish	50	0.028 ± 0.008	84(1)	83	83
		0.059 ± 0.008	72(4)	69	69
		0.43 ± 0.04	42(13)	37	37
		4.83 ± 0.53	43(69)	13	13
		46.7 ± 4.3	20(100)	0	0
	200	0.033 ± 0.006	90(1)	89	88
		0.054 ± 0.005	71(4)	68	67
		0.42 ± 0.03	47(16)	39	39
		4.81 ± 0.62	49(47)	26	19
		46.7 ± 3.7	23(86)	3	0

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 92 and 95% for rainbow trout and channel catfish, respectively.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 11. Toxicity of Capacitor 21 to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	Capacitor 21 Concentration Mean ± S.E. (µg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Redear Sunfish	50	1.7 ± 0.5	96	96	94
		2.1 ± 0.4	96(1)	95	94
		6.5 ± 2.7	94	94	86
		17.1 ± 3.8	63(12)	55	40
	200	1.4 ± 0.3	98	98	95
		2.2 ± 0.7	96	96	91
		4.5 ± 1.7	91(5)	86	86
		11.0 ± 1.4	64(9)	58	38
Largemouth Bass	50	0.5 ± 0.2	98	98	93
		0.8 ± 0.3	93(1)	92	86
		1.5 ± 0.5	82(2)	80	66
		2.4 ± 0.9	48(8)	44	10
	200	0.4 ± 0.1	97	97	90
		0.5 ± 0.1	98(1)	97	94
		1.5 ± 0.2	85(1)	84	69
		2.9 ± 0.7	47(9)	43	11
Rainbow Trout	200	2.2 ± 0.3	41(20)	33	32
		2.5 ± 0.7	17(8)	16	14
		4.2 ± 1.7	6(100)	0	0
		6.0 ± 1.1	0	0	0

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 97, 98, and 88% for redear sunfish, largemouth bass, and rainbow trout, respectively.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 12. Toxicity of chlorobenzene to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	Chlorobenzene Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Goldfish	50	0.007 ± 0.002	98	98	97
		0.039 ± 0.004	96(1)	95	91
		0.14 ± 0.04	98(4)	94	81
		2.93 ± 0.43	63(7)	59	34
		10.2 ± 1.1	22(78)	5	4
	200	0.010 ± 0.002	102(1)	101	98
		0.058 ± 0.009	99(1)	98	93
		0.37 ± 0.08	89(4)	85	76
		2.00 ± 0.15	66(6)	62	38
		12.2 ± 2.8	28(63)	10	0
Largemouth Bass	50	0.013 ± 0.002	91(2)	89	80
		0.038 ± 0.003	86	86	60
		0.16 ± 0.01	75(11)	67	24
		2.55 ± 0.28	27(55)	12	0
		27.3 ± 1.4	4(100)	0	0
	200	0.009 ± 0.001	100	100	93
		0.040 ± 0.006	93(2)	91	64
		0.15 ± 0.02	72(13)	63	14
		3.10 ± 0.34	25(42)	15	0
		23.2 ± 1.8	4(100)	0	0

[1] Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 99 and 93% for goldfish and largemouth bass, respectively.

[2] Frequency of teratic survivors in hatched population is given parenthetically.

Table 13. Toxicity of chloroform to embryo-larval stages of rainbow trout.

Water Hardness (mg/l $CaCO_3$)	Chloroform Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
			Hatching	4 Days Posthatching
50	0.004 ± 0.001	95	95	95
	0.008 ± 0.001	92	92	92
	0.059 ± 0.006	89(3)	86	86
	0.69 ± 0.03	73(4)	70	70
	10.1 ± 0.7	36(37)	23	23
200	0.003 ± 0.001	106	106	106
	0.010 ± 0.001	88(1)	87	87
	0.056 ± 0.004	83(3)	80	80
	0.63 ± 0.02	72(3)	70	70
	10.6 ± 0.4	23(40)	14	14

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 72%.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 14. Toxicity of 2,4-dichlorophenol (DCP) to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	DCP Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Goldfish	50	0.017 ± 0.005	96	96	96
		0.036 ± 0.004	96	96	95
		0.17 ± 0.02	87	87	82
		4.84 ± 0.74	51(14)	47	0
		27.5 ± 2.1	0	0	0
	200	0.015 ± 0.004	97	97	94
		0.025 ± 0.004	97	97	95
		0.11 ± 0.02	89	89	84
		4.24 ± 0.69	43(24)	33	0
		25.5 ± 3.3	0	0	0
Channel Catfish	50	≤ 0.001	99(2)	97	97
		0.008 ± 0.006	99(4)	95	95
		0.024 ± 0.011	101(12)	89	89
		0.24 ± 0.01	89(10)	80	79
		3.52 ± 0.18	72(8)	66	55
		25.9 ± 3.1	0	0	0
	200	≤ 0.001	98(1)	97	97
		0.008 ± 0.002	96(6)	90	92
		0.016 ± 0.006	93(6)	87	86
		0.082 ± 0.015	92(10)	82	80
		0.10 ± 0.03	88(9)	80	79
		3.05 ± 0.36	68(6)	62	51
		24.6 ± 3.6	0	0	0
Rainbow Trout	50	0.026 ± 0.004	83(1)	82	82
		0.052 ± 0.002	62(1)	61	61
		0.072 ± 0.007	65(1)	64	64
		0.47 ± 0.01	22(9)	20	20
		0.86 ± 0.08	0	0	0
		4.64 ± 0.59	0	0	0
		27.4 ± 4.7	0	0	0
	200	0.024 ± 0.004	85	85	85
		0.048 ± 0.002	51	51	51
		0.071 ± 0.006	61(1)	60	60
		0.51 ± 0.01	24(9)	22	20
		0.81 ± 0.07	0	0	0
		6.35 ± 0.97	0	0	0
		34.4 ± 5.0	0	0	0

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 96, 96, and 92% for goldfish, channel catfish, and rainbow trout, respectively.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 15. Toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D) to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	2,4-D Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Goldfish	50	0.20 ± 0.03	100(1)	99	96
		5.12 ± 0.56	97(1)	96	91
		37.5 ± 3.8	93(4)	89	83
		78.0 ± 6.0	91(7)	85	71
		187 ± 4	76(11)	68	34
	200	0.28 ± 0.08	100	100	97
		4.95 ± 0.28	97	97	93
		37.1 ± 4.4	93(3)	90	84
		61.3 ± 7.4	91(10)	82	72
		201 ± 8	73(12)	64	30
Largemouth Bass	50	≤ 1.00	100	99	99
		2.59 ± 0.39	98	98	97
		51.0 ± 3.0	89	89	79
		119 ± 22	65(3)	63	47
	200	≤ 1.00	100(1)	99	98
		5.07 ± 0.84	97	97	94
		35.6 ± 5.0	88(1)	87	78
		178 ± 61	49(15)	42	30
Rainbow Trout	50	< 0.05	96(1)	95	95
		0.32 ± 0.04	86	86	86
		5.66 ± 0.54	69(2)	68	68
		36.0 ± 2.3	45(4)	43	43
		82.2 ± 10.4	30(15)	26	26
		144 ± 21	0	0	0
	200	< 0.05	91	91	91
		0.63 ± 0.07	72(2)	71	71
		9.54 ± 0.94	55(4)	53	53
		47.9 ± 0.3	33(24)	25	24
		78.5 ± 8.5	6(100)	0	0
		134 ± 12	0	0	0

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 98, 99, and 89% for goldfish, largemouth bass, and rainbow trout, respectively.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 16. Toxicity of dioctyl phthalate (DOP) to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	DOP Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Goldfish	50	0.040 ± 0.004	100	100	101
		0.099 ± 0.014	101(2)	99	99
		0.52 ± 0.10	101(2)	99	99
		28.1 ± 2.3	97(3)	94	86
		64.4 ± 2.9	96(3)	93	80
		186 ± 15	89(6)	84	67
	200	0.030 ± 0.004	99	99	98
		0.080 ± 0.011	99(1)	98	93
		0.44 ± 0.05	100(1)	99	94
		40.0 ± 4.1	96(3)	93	79
		80.7 ± 5.1	88(5)	84	64
		191 ± 14	77(11)	69	57
Rainbow Trout	50	0.040 ± 0.004	101	101	101
		0.10 ± 0.01	99(1)	98	98
		0.48 ± 0.03	95	95	95
		55.3 ± 3.1	94(3)	91	91
		71.9 ± 3.2	89(5)	85	85
		148 ± 10	53(16)	45	45
	200	0.045 ± 0.006	99(1)	98	96
		0.10 ± 0.01	97(1)	96	96
		0.50 ± 0.03	92(2)	90	90
		48.9 ± 4.0	87(3)	84	84
		88.8 ± 8.0	82(7)	76	76
		142 ± 12	54(12)	48	47
Largemouth Bass	50	0.055 ± 0.006	97	97	95
		0.30 ± 0.03	93	93	91
		46.3 ± 4.0	74	74	67
		66.9 ± 3.3	39(1)	39	26
		149 ± 15	13(4)	12	2
	200	0.065 ± 0.012	97	97	96
		0.30 ± 0.03	91(1)	90	90
		35.5 ± 3.1	71	71	64
		60.6 ± 4.3	39(1)	39	30
		146 ± 16	16(3)	16	7

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 95, 94, and 98% for goldfish, rainbow trout, and largemouth bass, respectively.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 17. Toxicity of malathion to embryo-larval stages of goldfish.

Water Hardness (mg/l $CaCO_3$)	Malathion Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
			Hatching	4 Days Posthatching
50	≤ 0.05	99	99	98
	0.28 ± 0.12	97(1)	96	92
	0.60 ± 0.15	87(2)	85	74
	1.99 ± 0.33	73(6)	69	41
	5.24 ± 0.65	31(25)	23	0
200	≤ 0.05	98	98	96
	0.11 ± 0.07	96(1)	95	89
	1.02 ± 0.08	90(1)	89	73
	2.16 ± 0.21	68(4)	65	37
	5.50 ± 0.70	33(20)	26	0

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 98%.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 18. Toxicity of trisodium nitrilotriacetic acid (NTA) to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	NTA Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Channel Catfish	50	1.05 ± 0.23	100	100	100
		9.30 ± 0.81	98(3)	95	94
		48.9 ± 5.5	99(3)	96	94
		96.7 ± 5.6	92(2)	90	89
		241 ± 23	91(9)	83	74
		512 ± 36	47(19)	38	16
		741 ± 15	10(55)	5	0
		918 ± 63	0	0	0
		1100 ± 200	0	0	0
	200	1.21 ± 0.26	100	100	100
		8.65 ± 0.45	100	100	98
		47.0 ± 2.0	98(1)	97	96
		95.1 ± 6.3	91	91	91
		226 ± 26	84(5)	80	79
		501 ± 15	58(15)	49	42
		736 ± 41	23(35)	15	0
		954 ± 49	21(50)	11	0
		1100 ± 200	0	0	0
Goldfish	50	10.6 ± 0.5	98	98	97
		48.0 ± 4.1	92	92	90
		110 ± 6	83(3)	81	76
		216 ± 15	65(6)	61	56
		503 ± 85	39(3)	38	28
		910 ± 30	0	0	0
	200	8.73 ± 0.66	98	98	98
		50.9 ± 4.1	90(1)	89	87
		109 ± 11	82	82	80
		205 ± 12	60(4)	58	54
		498 ± 78	42(4)	40	30
		825 ± 55	0	0	0

Table 18 - continued.

Species	Water Hardness (mg/l $CaCO_3$)	NTA Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Rainbow Trout	50	1.21 ± 0.10	100	100	100
		9.94 ± 0.55	85(4)	82	82
		48.2 ± 2.2	82(9)	75	74
		92.0 ± 2.6	59(19)	48	48
		193 ± 9	42(48)	22	22
		317 ± 17	4(75)	1	1
		455 ± 18	0	0	0
		1000 ± 82	0	0	0
	200	1.05 ± 0.11	97(2)	95	95
		8.94 ± 1.59	88(2)	86	86
		47.6 ± 2.3	85(6)	80	80
		94.4 ± 3.7	65(14)	56	56
		177 ± 5	53(38)	33	33
		299 ± 20	25(23)	19	19
		461 ± 21	12(91)	1	1
		1135 ± 126	0	0	0

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 97, 96, and 91% for channel catfish, goldfish, and rainbow trout, respectively.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 19. Toxicity of phenol to embryo-larval stages of fish.

Species	Water Hardness (mg/l $CaCO_3$)	Phenol Concentration Mean ± S.E. (mg/l)	Percent Hatchability[1,2]	Percent Normal Survival[1]	
				Hatching	4 Days Posthatching
Bluegill Sunfish	50	0.004 ± 0.001	99	99	99
		0.010 ± 0.001	97	97	95
		0.091 ± 0.005	94	94	90
		1.07 ± 0.05	66(2)	65	60
		10.2 ± 0.3	39(15)	33	31
	200	0.006 ± 0.001	99	99	98
		0.009 ± 0.001	96	96	94
		0.090 ± 0.005	93	93	87
		0.95 ± 0.02	64(3)	62	57
		9.88 ± 0.42	35(14)	30	27
Goldfish	50	0.0013 ± 0.0003	99	99	98
		0.009 ± 0.002	93	93	90
		0.072 ± 0.014	88(8)	81	80
		0.99 ± 0.11	64(15)	54	54
		10.0 ± 0.3	29(38)	18	12
	200	0.0007 ± 0.0002	99	99	98
		0.008 ± 0.001	99	99	96
		0.079 ± 0.010	80(3)	78	78
		0.88 ± 0.11	43(21)	34	34
		9.58 ± 0.61	18(72)	5	4
Rainbow Trout	50	0.0015 ± 0.0003	100	100	100
		0.009 ± 0.001	100(2)	98	96
		0.068 ± 0.009	92(6)	86	86
		0.84 ± 0.07	41(32)	28	27
		8.79 ± 0.40	9(73)	2	0
	200	0.0012 ± 0.0003	90	90	90
		0.010 ± 0.001	75(1)	74	74
		0.070 ± 0.009	58(4)	56	56
		0.91 ± 0.07	21(22)	16	15
		9.33 ± 1.23	0	0	0

[1]Frequency determined as survival in experimental population/control. Control survival at 4 days posthatching averaged 98, 91, and 89% for bluegill sunfish, goldfish, and rainbow trout, respectively.

[2]Frequency of teratic survivors in hatched population is given parenthetically.

Table 20. Comparison of LC_1's determined in embryo-larval tests with MATC's derived from life-cycle studies.

Organic Compound	LC_1	Embryo-Larval Test Species	MATC	Life-Cycle Test Species	Type of Life-Cycle Test[1]	References
Atrazine (μg/l)	29.0-77.2	Rainbow Trout	65-120	Brook Trout	P	Macek, *et al.* (1976)
2,4-D[2] (mg/l)	0.02-0.03 3.2-13.1 8.2-8.9	Rainbow Trout Largemouth Bass Goldfish	0.3-1.5	Fathead Minnow	P	Mount & Stephan (1967)
Malathion (μg/l)	141.1-439.6	Goldfish	200-580	Fathead Minnow	P	Mount & Stephan (1967)
NTA (mg/l)	16.9-20.2 28.5-30.1 130.9-138.4	Rainbow Trout Goldfish Channel Catfish	54.0-114.0	Fathead Minnow	P	Arthur, *et al.* (1974)
PCB (μg/l) (Capacitor 21)	0.5-0.9 1.0 1.3-3.5	Largemouth Bass Rainbow Trout Redear Sunfish	1.8-4.6 (PCB 1254) 5.4-15.0 (PCB 1242) 1.1-3.0 (PCB 1248) 2.1-4.0 (PCB 1260)	Fathead Minnow	C	Nebeker, *et al.* (1974) DeFoe, *et al.* (1978)

[1] LC_1 values were compared with MATC's from partial (P) and complete (C) life-cycle tests.

[2] 2,4-D was administered as the potassium salt and the butoxyethanol ester in embryo-larval and life-cycle tests, respectively.

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