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Jeffrey A. Black
University of Kentucky

Wesley J. Birge
University of Kentucky

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AN AVOIDANCE RESPONSE BIOASSAY FOR AQUATIC POLLUTANTS

By

Jeffrey A. Black
Wesley J. Birge

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University of Kentucky
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ABSTRACT

Avoidance response bioassays were conducted with eight aquatic contaminants, including cadmium, copper, mercury, zinc, chloroform, dioctyl phthalate (DOP), trisodium nitrilotriacetic acid (NTA), and phenol. Tests were performed in a dual-channel fluviarium system, and the toxicant injection procedure used provided good regulation of exposure concentrations. Juvenile stages of largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), and rainbow trout (*Salmo gairdneri*), and tadpoles of the American toad (*Bufo americanus*) proved to be suitable animals for evaluating avoidance or attraction responses. The trout was the most sensitive species tested.

Avoidance was significant in tests with cadmium, phenol, and zinc, and significant attraction resulted from exposures to chloroform, DOP, and mercury. Animals generally avoided lower concentrations of copper but were attracted to higher exposure levels. NTA produced variable responses. In tests with trout, threshold concentrations for avoidance or attraction were estimated to fall at 0.0002 mg/l mercury, 0.047 mg/l zinc, 0.052 mg/l cadmium, 0.074 mg/l copper, 11.9 mg/l chloroform, and 56.6 mg/l NTA. The threshold for phenol, determined using bluegill, was 39.0 mg/l. Results from fish embryo-larval toxicity tests were used to gauge sensitivity of the avoidance response bioassay. Toxicant concentrations which produced embryo-larval lethality or teratogenesis at frequencies of 10% (LC_{10}) and 1% (LC_1) were compared to behavioral threshold concentrations. The avoidance test was observed to be a less sensitive procedure for evaluating the effects of all the selected toxicants, except zinc. However, the behavioral test provided valuable information which was not obtainable using other bioassay methodologies.

Descriptors:	Identifiers:
Avoidance Responses*	Attraction*
Behavior*	Juvenile Fish*
Fishes	Embryo-Larval Tests
Amphibia	NTA
Water Quality	
Cadmium	
Chloroform	
Copper	
Mercury	
Phenol	
Zinc	

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CHAPTER I

INTRODUCTION

Present testing methods developed for aquatic hazard assessment focus largely on determining toxicant concentrations which produce lethality or impair the health of aquatic species (NAS-NAE Committee, 1973; Cairns, *et al.*, 1978). However, it is becoming increasingly apparent that trace levels of aquatic pollutants which do not directly impair health may limit distribution, migration, or reproductive behavior of fish and other species (Sprague, *et al.*, 1965; Sprague, 1968; Weir and Hine, 1970; Birge, *et al.*, 1974). For example, in a correlated field and laboratory study involving effects of copper on the green sunfish (*Lepomis cyanellus*), chronic laboratory bioassays clearly were not adequate for assessing environmental hazards (Geckler, *et al.*, 1976). Trace levels of copper which were not toxic in bioassays affected the in-stream distribution of sunfish. Field studies showed that sunfish populations were restricted to aquatic compartments which were most copper free, severely limiting suitable feeding and spawning habitats. Zinc and other toxicants have also been shown to disrupt distribution and migration of fish species (Sprague, *et al.*, 1965; Anderson, 1971; Kleerekoper, 1976). Such pollution-induced behavioral effects are based largely on avoidance responses and are seldom considered in establishing protective guidelines for freshwater ecosystems. Accordingly, to detect and preclude such forms of ecological degradation, it is important to develop a suitable and well-defined avoidance response bioassay.

Avoidance and attraction in fish recently have been reviewed by McCauley (1977), Kleerekoper (1976), Geckler, *et al.* (1976), Cherry, *et al.* (1976), Sprague (1968), and others (Hasler, 1957; Sprague, *et al.*, 1965; Anderson, 1971; Todd, 1971). These behavioral patterns generally are initiated by olfactory or gustatory reception, though avoidance may be induced by less specific irritability responses mediated through general exteroception. Avoidance behavior in salmon and trout is first observed

at 5 weeks of age (Bishai, 1962). As noted by Hasler (1957), olfactory sacs open at about this stage (21 mm), and removal of olfactory tissue is known to extinguish avoidance response to certain toxicants (Hoglund, 1961). In trout, acuity of the avoidance response increases rapidly with growth, and high sensitivity to zinc is exhibited by juveniles of 30 to 60 mm (Bishai, 1962; Sprague, 1968).

Avoidance thresholds vary significantly for different toxicants, and may be affected by physicochemical water characteristics such as pH and temperature (Bishai, 1962; Sprague, *et al.*, 1965; Hoglund and Hardig, 1969; Weir and Hine, 1970; Cherry, *et al.*, 1976; Reynolds, 1977). Frequency of avoidance generally is quantitatively correlated with toxicant concentration. This dose-response relationship was well established by Sprague (1968) in zinc avoidance studies with rainbow trout. He determined a zinc avoidance threshold of 5.6 µg/l, with 100% avoidance occurring at 100 µg/l. The avoidance threshold was only 0.01 of the lethal threshold for adult trout. Numerous other investigators have shown avoidance thresholds for other toxicants (*e.g.*, As, Hg, Pb, Se, DDT) to fall well below lethal concentrations for adult fish (Weir and Hine, 1970; Dill and Saunders, 1974; Drummond, *et al.*, 1974). However, such comparisons have been based largely on lethal exposure levels determined in short-term acute bioassays, often conducted under static conditions. Of greater importance, no in-depth studies have been attempted to correlate results from avoidance tests with sensitive embryo-larval or chronic life-cycle bioassays. Consequently, for most aquatic contaminants, there are no present means by which avoidance response thresholds can be adequately compared with or indexed to exposure concentrations which produce irreversible disabilities (*e.g.*, lethality, teratogenesis).

The principal objectives of the present study were 1) to use avoidance behavior in fish to develop a sensitive aquatic bioassay system for detecting and evaluating inorganic and organic contaminants; 2) to determine avoidance response patterns and threshold concentrations for four metals (*i.e.*, Cd, Cu, Hg, Zn) and four organic compounds (*i.e.*, chloroform, DOP, NTA, phenol); 3) to evaluate juvenile stages of the channel catfish, bluegill sunfish, largemouth bass, and rainbow trout and tadpoles of the

American toad for suitability as test animals; and 4) to compare the avoidance test to embryo-larval toxicity tests for economy, reliability, sensitivity, and value as a predictive tool.

CHAPTER II

RESEARCH PROCEDURES

Selection of animal species. Animals used in this study included the largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*), bluegill sunfish (*Lepomis macrochirus*), rainbow trout (*Salmo gairdneri*), and the American toad (*Bufo americanus*). This choice was based in part on economic importance, seasonal availability, and good handling characteristics for laboratory investigations. In addition, these species differ significantly in ecological requirements, habitat, and behavior and were selected to provide a sufficiently broad basis for evaluating contaminant-induced avoidance responses.

Rainbow trout were provided by the Erwin National Fish Hatchery, Erwin, Tennessee. Bass, bluegill, catfish, and American toads were obtained from the Frankfort National Fish Hatchery, Frankfort, Kentucky.

Selection of test compounds. Tests were conducted with cadmium ($CdCl_2$), copper ($CuSO_4$), mercury ($HgCl_2$), zinc ($ZnCl_2$), chloroform, dioctyl phthalate (DOP), trisodium nitrilotriacetic acid (NTA), and phenol. All tests were performed with analytical, reagent, or spectrophotometric grade compounds. Data were expressed as concentrations (mg/l) of the metal cation for the inorganic toxicants and as concentrations of the pure compound for the organic toxicants. These compounds are known to affect important waterways in the eastern U.S. (Stephenson, 1975; Shackelford and Keith, 1976; U.S. Environmental Protection Agency, 1976), and all except NTA appear on the initial list of 65 priority toxicants identified by EPA (U.S. Environmental Protection Agency, 1978).

Test system. Avoidance responses were evaluated using a fluviarium test system similar to those described by Hoglund (1961) and Wilson (1973). The avoidance test chamber is illustrated in Figure 1. The chamber was constructed with one-quarter inch plexiglass and had dimensions of 60 cm X 30 cm X 6.5 cm, with an overall capacity of 10 liters. The size and shape

of the test chamber easily accommodated the test animals, which ranged from 30 to 100 mm in length.

Overall design of the avoidance test system is shown in Figure 2. The test chamber was operated on a flow-through principle, receiving carbon-filtered tap water from a constant head box. An in-line pressure regulator was used for coarse adjustments of water input to the head box. Adjustable standpipes were used to regulate flow rates for control and experimental channels of the test chamber. Flow rates were monitored with high resolution flow meters (Gilmont, Inc.). Water administered to the control channel and diluent supplied to the test channel were of identical physicochemical composition, originating from the same source (head box). For all test compounds except DOP, a variable speed peristaltic pump (Brinkmann model 131900) was used to inject toxicant into a mixing chamber placed immediately ahead of the test channel (Figure 2). Diluent and toxicant were thoroughly blended in the mixing chamber (*i.e.*, 125 ml side-arm flask) by use of a magnetic stirrer. The supply of toxicant to the test chamber was regulated by adjusting concentrations in the toxicant reservoir (Figure 2). For DOP, the only insoluble compound tested, toxicant was injected via a syringe pump (Sage model 355) to a 400-ml mixing chamber situated on top of a high speed blender (Osterizer Pulsematic). This precluded the need for carrier solvents (*e.g.*, acetone) which may have interfered with test responses.

Avoidance response test. Prior to testing, stocks of juvenile fish were maintained in temperature-regulated fiberglass tanks (200 liter) provided with a continuous water flow (15 l/hr) from the same carbon-filtered source as used for avoidance studies. Test water was monitored at regular intervals for temperature, dissolved oxygen, water hardness, pH, and specific conductivity, using a YSI tele-thermometer with thermocouple (model 42SC), YSI oxygen meter (model 51A), Orion divalent cation electrode (model 93-92), Corning digital pH meter (model 110), and a Radiometer conductivity meter (model DCM 2e). Mean water temperatures (± S.E.) were 17.8 ± 0.2°C, 19.1 ± 0.2°C, 23.0 ± 0.1°C, and 23.2 ± 0.2°C during experiments with the trout, toad, bluegill, and bass, respectively. Dissolved oxygen was maintained at 85 to 90% of saturation, using continuous

aeration in the constant head box. Other monitoring data for general water quality characteristics are given in Table 1.

As noted above, juvenile stages of fish and amphibian species were used as test animals. The standard length for the fish species ranged as follows: 35 to 60 mm for bluegill; 35 to 40 mm for bass; 75 to 100 mm for catfish; and 30 to 50 mm for trout. Tadpoles of the American toad varied in length from 12 to 17 mm.

Avoidance tests were performed using a sample size of 10 fish per exposure concentration, as follows (Figure 1).

1. Withholding toxicant, water flow was initiated in control and test channels. A flow rate of 250 ml/min was used, giving a detention time of 20 minutes for each of the two 5-liter channels.
2. Fish were introduced into the decision area and maintained there for 20 minutes to allow for acclimation.
3. The screen enclosing the decision area was removed, and distribution of fish was recorded at 30 second intervals for 10 minutes to ascertain any significant non-random channel preferences.
4. Fish were relocated in the decision area and toxicant was administered to the test channel for 20 minutes (one dilution interval) to permit stabilization of toxicant exposure level.
5. The screen was again removed from the decision area, and distribution of animals was recorded at 30 second intervals for 20 minutes. Initial dye tests and subsequent toxicant analyses showed that the interface between the control and test channels was maintained in the decision area. Therefore, fish residing in the decision area during testing periods also were scored as selecting either control or toxicant channels. Water samples were collected from each channel at the beginning and end of each 20 minute test period and analyzed for toxicant concentration (Analytical procedures).
6. The control channel and test channel were reversed and steps 1 through 5 were replicated for each toxicant concentration.

Expression and analysis of avoidance data. Using mean scores for control (C) and test (T) channel distributions, percent avoidance (A) was

Table 1. Physicochemical charactersitics of dilution water used for avoidance tests.

Test Condition	Mean	Standard Deviation
pH	7.6	0.3
Alkalinity (mg/l $CaCO_3$)	63.0	5.0
Hardness (mg/l $CaCO_3$)	112.4	30.8
Conductivity (μmhos/cm)	136.0	20.2

determined for each toxicant concentration as $A = (C - T)/(C + T) \times 100\%$. Negative avoidance was defined as attraction. Similarly, percent avoidance (A) was also determined for each complementary 10 minute control experiment, conducted to detect a right or left preference (*i.e.*, no toxicant administration; Step 3 above). Channels for the latter were designated as either test (T) or control (C) to correspond with the subsequent 20 minute toxicant run. For each toxicant concentration, scores for distribution of animals were pooled for both replicates (*i.e.*, two 20 minute runs) and statistically compared to data pooled for both corresponding control replicates (*i.e.*, two 10 minute runs) using the Student's *t* test. Particular attention was given to determining the lowest toxicant concentration (threshold level) which produced significant responses for avoidance or attraction ($P < 0.01$). These data were then compared to results of embryo-larval bioassays obtained in earlier studies. Procedures for the latter are summarized below.

Embryo-larval toxicity tests. To provide a baseline for evaluating sensitivity and reliability of the avoidance tests, embryo-larval toxicity tests were completed on the selected inorganic and organic compounds. Flow-through and 12-hr static-renewal bioassays were performed using procedures previously described (Birge and Black, 1979; Birge, *et al.*, 1979b, c). Administration of toxicant was initiated subsequent to fertilization and continued through 4 days posthatching, giving total treatment times of 28, 9, 7, and 7 days for rainbow trout, largemouth bass, bluegill sunfish, and American toad, respectively. Toxicity tests were performed in temperature-regulated environmental rooms. Eggs and larvae were examined daily to gauge extent of development and to remove dead specimens. Control eggs were cultured simultaneously with experimentals and under identical conditions, except for omission of the toxicant. Percent survival was determined as frequency in experimental populations/controls and was determined at 4 days posthatching for a minimum of five exposure concentrations. In all instances, survival frequencies were based on accumulative test responses incurred from onset of treatment. Teratogenesis was determined at hatching and was based on survivors affected by gross debilitating anomalies (Birge and Black, 1977). Counting teratic larvae as lethals,

log probit analysis (Finney, 1971) was used to calculate control-adjusted LC_{50}, LC_{10}, and LC_1 values. The LC_1's and LC_{10}'s were used to estimate toxicant concentrations which produced 1% and 10% impairment of test populations.

Analytical procedures. Exposure concentrations for all toxicants were confirmed by actual analysis of test water. In embryo-larval bioassays, test water was sampled at regular daily intervals. In avoidance tests, water samples were taken from the control channel and the test channel prior to and immediately following the 20-minute exposure period.

Aqueous metal concentrations were determined by atomic absorption spectrophotometry, using a Perkin-Elmer AAS (model 503) equipped with a mercury analysis system and a graphite furnace (model HGA 2100). Mercury determinations were performed using the cold vapor technique originally proposed by Hatch and Ott (1968), giving a detection limit of 0.1 µg/l. Cadmium, zinc, and copper were analyzed using an air-acetylene flame, providing detection limits of 1.0, 1.0, and 50 µg/l, respectively. Lower concentrations of copper were quantified with the graphite furnace. Using the latter, the detection limit was 1.0 µg/l.

Chloroform was analyzed directly from 1 to 15 ml aliquots of test water, using a Hewlett Packard gas chromatograph (model 5838A) equipped with a Purge and Trap system (model 7675A). Each sample was purged with dry, pre-purified nitrogen at 10 ml/min for 10 minutes. Chloroform was adsorbed on a Tenex 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 20 M on 80/100 Anakrom U, and the detector temperature was 250°C. Nitrogen was used as the carrier gas, with a flow rate of 19 ml/min. The detection limit was 1 µg/l.

Dioctyl phthalate (DOP) was extracted from 0.5 liter aliquots of test water using reagent grade chloroform. The chloroform extracts were dried with anhydrous sodium sulfate and concentrated to several ml with an air stream. DOP was then quantitatively reconstituted in ethyl acetate. Concentrations of DOP were determined on a Packard gas chromatograph

(model 7400) equipped with a flame ionization detector (model 881) and a glass column (46 cm X 2 mm I.D.). The stationary phase was 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W HP (Supelco, Inc.). The oven, inlet, and detector temperatures were 235°C, 250°C, and 260°C, respectively. The detection limit for DOP was 25 µg/l.

Trisodium nitrilotriacetic acid (NTA) was analyzed by the zinc-zincon method (U.S. Environmental Protection Agency, 1974), with a detection limit of 0.5 mg/l. Absorbance was measured at 620 nm, using a model 635 Varian-Techtron spectrophotometer. To preclude interference from calcium and magnesium ions, NTA test water samples were batch-treated with an ion exchange resin (Dowex 50W-X8, 50-100 mesh).

Phenol concentrations were determined using the chloroform extraction, 4-aminoantipyrine procedure, as outlined in Standard Methods (American Public Health Association, 1975). Prepared samples were quantified spectrophotometrically at a wavelength of 460 nm, and the detection limit was 2.0 µg/l.

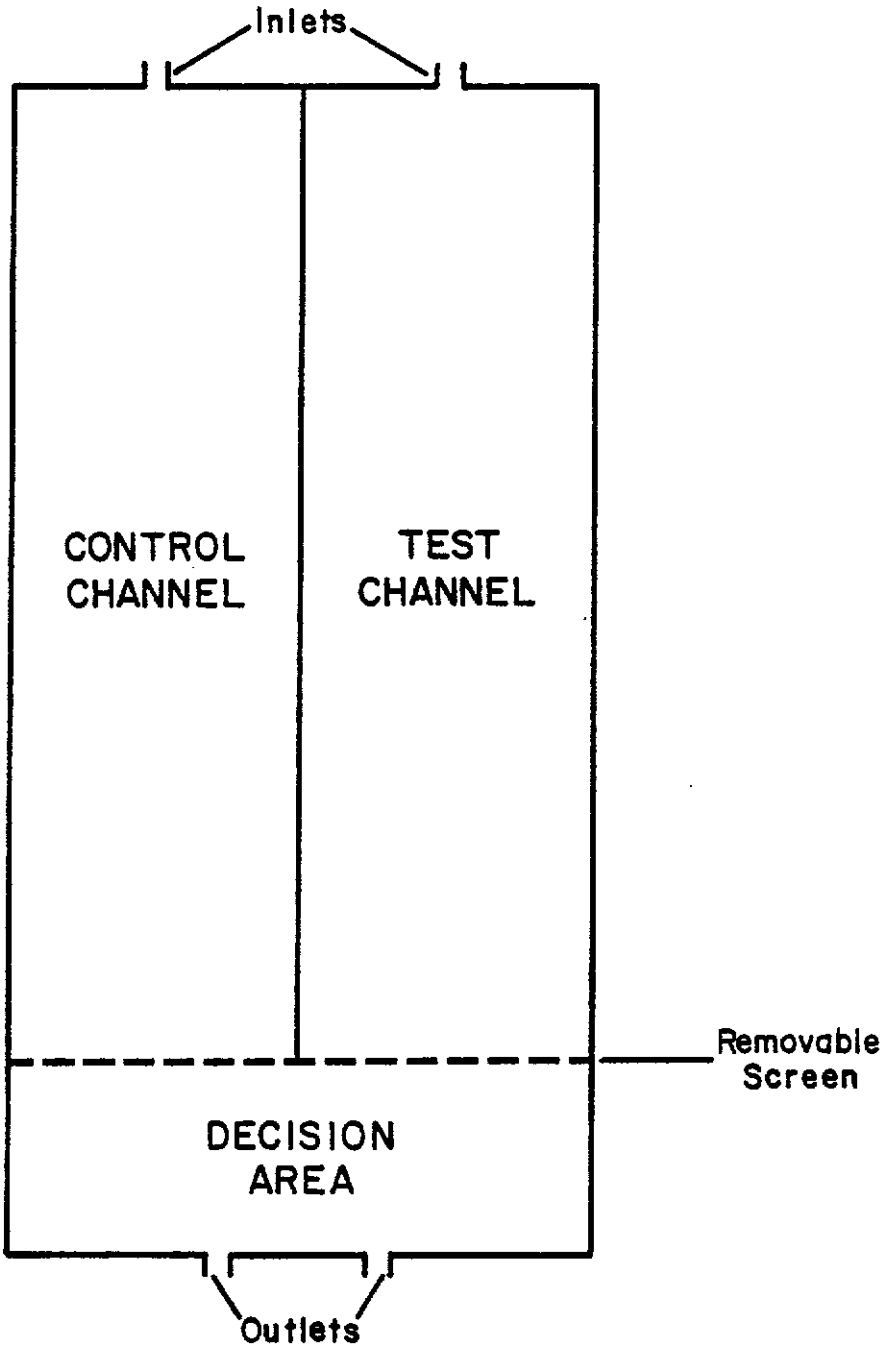

Figure 1. Avoidance test chamber (fluviarium). Design involved a plexiglass chamber with overall dimensions of 60 cm X 30 cm X 6.5 cm and a capacity of 10 liters. During acclimation, test animals were restricted to the decision area by a removable screen.

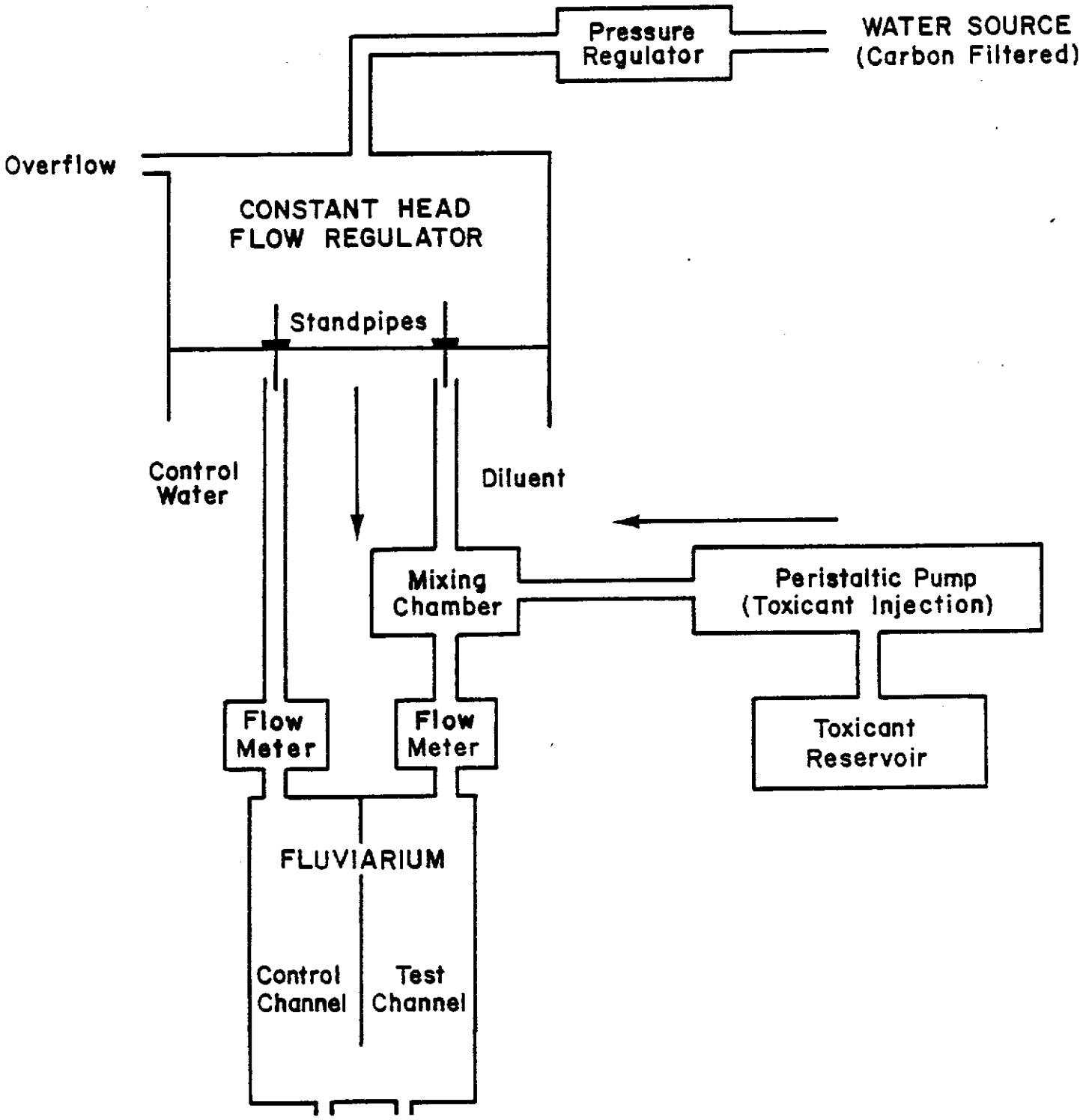

Figure 2. Avoidance test system. Flow rates for control and diluent water were regulated by use of a constant head box equipped with adjustable standpipes. Toxicant and diluent were blended in a mixing chamber situated ahead of the test channel. Arrows indicate direction of flow.

CHAPTER III

RESULTS AND DISCUSSION

Avoidance bioassays were performed in duplicate on the eight selected toxicants, using juvenile stages of fish and tadpoles of the American toad. Prior to actual testing, experiments were conducted on each of the potential animal test species to determine their suitability for use in avoidance bioassays. With no toxicant in the avoidance chamber, largemouth bass, bluegill sunfish, rainbow trout, and American toad swam about randomly and independently after being released from the decision area. However, channel catfish remained in the decision area and swam back and forth in a compact group. Because of their apparent "schooling" behavior, catfish were not used in the avoidance studies. While most behavioral tests were conducted with animals which had been raised in uncontaminated water, several tests were performed using juvenile trout which had been exposed as embryos to arsenic, boron, iron, and nickel. Avoidance test results indicated that pre-exposure had no apparent effect on behavioral responses.

Analytical monitoring data indicated good reproducibility of exposure concentrations for the eight toxicants. These values, as well as biological response data for the avoidance bioassays, are summarized in Table 2. Results were averaged from plicate experiments conducted at each exposure concentration. Using scores for control (C) and test (T) channel distributions, percent avoidance (A) was determined for each toxicant concentration as $A = (C - T)/(C + T) \times 100\%$. Negative avoidance was defined as attraction. These values are presented in Table 2 under the column for percent gross response. The latter were then control-adjusted using data obtained in experiments conducted without toxicant to determine non-random channel preferences (Research Procedures; Avoidance response test). These control-adjusted values are presented under the column for percent net response. Responses found to be statistically

significant (Student's t test, $P < 0.01$) were indicated by an asterisk. The threshold concentration for avoidance or attraction was defined as the lowest exposure level producing a significant response.

Avoidance tests with cadmium were performed on juvenile stages of three fish species, including the bluegill sunfish, largemouth bass, and rainbow trout (Table 2, Figure 3). Cadmium was administered at concentrations of 0.09, 0.90, and 8.8 mg/l to the bass and at 0.8, 8.3, and 41.1 mg/l to the bluegill. No significant responses were observed for either species. In tests with the trout, cadmium at 0.01, 0.05, 0.10, and 1.0 mg/l produced net avoidance at frequencies of 37.3%, 51.7%, 55.3%, and 72.3%, respectively. The last three values were found to be significant, giving an avoidance threshold concentration of 0.05 mg/l.

Copper was tested on juvenile stages of the bluegill and trout and on tadpoles of the American toad (Table 2). Rainbow trout was the most sensitive species to copper treatment, exhibiting significant avoidance at 0.07 mg/l, the lowest concentration tested. This value was taken as the copper threshold for behavioral response. However, as concentrations were elevated to 4.6 and 7.6 mg/l, trout showed significant attraction to copper. Similarly, American toad tadpoles avoided a copper concentration of 0.10 mg/l but were attracted to copper at 0.93 mg/l. Copper was the only toxicant tested which produced this pattern of response with at least two animal species (Figure 4). When tests were conducted with the bluegill, copper levels of 8.5 and 43.2 mg/l resulted in significant attraction. It appeared that while certain aquatic animals may avoid copper when it is present in relatively low amounts, these same species likely are attracted to higher concentrations.

Avoidance response tests were conducted with mercury, using juvenile stages of the rainbow trout (Table 2). Mercury was administered at 0.0002 and 0.0074 mg/l and elicited net attraction at frequencies of 24.5% and 18.0%, respectively. The latter were both found to be statistically significant, giving a threshold value for mercury of 0.2 µg/l. This was the lowest exposure concentration at which any of the selected toxicants produced significant responses.

Zinc was administered to three fish species, including the bluegill

sunfish, largemouth bass, and rainbow trout (Table 2). Trout was the most sensitive species to zinc exposure. At concentrations of 0.01, 0.05, 0.10, and 1.1 mg/l, trout juveniles avoided zinc at net frequencies of 11.0%, 94.5%, 95.0%, and 99.8%, respectively. The last three values were statistically significant, indicating an avoidance threshold for zinc of 0.5 mg/l. Juvenile stages of the largemouth bass also were affected by zinc exposure and avoided concentrations of 7.0 to 39.2 mg/l at net frequencies of 15.5% to 56.8%. The most tolerant species tested was the bluegill, which exhibited no significant response to levels as high as 43.7 mg/l.

Bluegill sunfish and rainbow trout were exposed to chloroform, and both species showed significant attraction to this highly volatile organic compound (Table 2, Figure 5). In bioassays with the bluegill, test animals responded at a frequency of 93.5% to a concentration of 33.2 mg/l. By comparison, trout stages were attracted at a net frequency of 21.0% to a chloroform exposure level of 11.9 mg/l. Based on these data, the attraction threshold concentrations for chloroform fell at 11.9 and 33.2 mg/l in tests with trout and bluegill, respectively.

Dioctyl phthalate (DOP) was administered to juvenile stages of the bluegill sunfish (Table 2). At a concentration of 112.4 mg/l, test animals were attracted to this compound at a net frequency of 41.1%. Because only one concentration was tested, a threshold level for DOP could not be estimated. It should be noted that the homogenized suspension of this compound produced cloudiness in the test channel, and the latter may have affected distribution of animals.

Trisodium nitrilotriacetic acid (NTA) was administered to juvenile stages of two fish species, including the bluegill sunfish and rainbow trout (Table 2). No significant attraction or avoidance was observed for the bluegill, as net responses were +4.5%, -5.8%, and +2.8% at NTA concentrations of 0.23, 6.65, and 39.8 mg/l. In tests with the trout, NTA at a level of 56.6 mg/l produced 23.8% avoidance. However, when the concentration was increased to 101.3 mg/l, test animals were attracted to this compound at a frequency of 24.5%. Although the responses to NTA were somewhat variable, the avoidance threshold was estimated to fall at 56.6 mg/l.

Avoidance tests with phenol were conducted on juvenile stages of the bluegill sunfish (Table 2). At concentrations of 0.76, 6.76, and 39.0 mg/l, test animals responded at net frequencies of -2.8%, +2.9%, and +75.3%, respectively. Avoidance was significant only at the highest concentration. Based on these data, the threshold concentration for phenol was set at 39.0 mg/l.

In summary of the data presented above, avoidance was significant in tests with cadmium, zinc, and phenol, and significant attraction resulted from exposure to mercury, chloroform, and dioctyl phthalate. In bioassays with copper, animals generally avoided lower concentrations but were attracted to higher exposure levels (Figure 4). NTA produced variable responses. Patterns of avoidance and attraction responses observed in tests with the bluegill sunfish and rainbow trout are illustrated in Figures 6 and 7.

Juvenile stages of the bluegill sunfish, largemouth bass, and rainbow trout, as well as tadpoles of the American toad, all appeared to be suitable test animals for use in avoidance response bioassays. Each of these species exhibited the capacity to discriminate between control and toxicant exposures in tests with one or more compounds during the course of this study (Table 2). The most sensitive species was the rainbow trout which responded to exposure concentrations as low as 0.0002 mg/l mercury, 0.047 mg/l zinc, 0.052 mg/l cadmium, 0.074 mg/l copper, and 11.9 mg/l chloroform (Figure 7). This high relative sensitivity of the rainbow trout was consistent with results from earlier embryo-larval toxicity tests. In the latter, trout developmental stages generally suffered higher rates of lethality and teratogenesis than did other fish species (Birge, *et al.*, 1978, 1979a, d).

As noted above, results of embryo-larval toxicity tests were used to provide a baseline for comparing the sensitivity of the avoidance response bioassays. Embryo-larval tests provide a useful means of quantifying the toxicity of many aquatic contaminants (McKim, 1977; Birge, *et al.*, 1979a, d). When care is taken to develop an adequate dose-response relationship, log probit analysis can be used to calculate LC_1 values. The

latter generally provide a reliable approximation of the threshold for toxic effects (*e.g.*, lethality, teratogenesis), and as LC_1 values are in reasonable agreement with maximum acceptable toxicant concentrations (MATC's) determined in chronic life-cycle studies, such data appear applicable to the promulgation of freshwater criteria. Furthermore, the LC_{10} can be used to provide an additional reference point for assessing toxic effects. Considering the combined effects of long-term pollution stress and natural environmental stresses, it is likely that 10% or greater impairment of reproductive potential would significantly affect population dynamics in natural communities (Gerking, 1978; Birge, *et al.*, 1979d). Results from the behavioral bioassays (*i.e.*, avoidance/attraction threshold concentrations) were compared to those from embryo-larval tests (*i.e.*, LC_1 and LC_{10} values) and are presented in Table 3. Median lethal concentrations (LC_{50}) determined in the latter were included as additional information.

As seen in Table 3, embryo-larval toxicity tests were substantially more sensitive than behavioral bioassays for evaluating the effects of cadmium, chloroform, and phenol on aquatic species. Avoidance/attraction thresholds for these toxicants were 6 to 10,000 times higher than corresponding LC_1 values. In tests on copper with the rainbow trout, the lowest concentration tested (*i.e.*, 0.074 mg/l) elicited significant avoidance, indicating that the behavioral threshold may extend somewhat below this level. However, based on the relatively low magnitude of response at 0.074 mg/l, it is unlikely that avoidance frequencies would be appreciable at concentrations approaching the embryo-larval LC_1 value of 0.003 mg/l.

Mercury produced significant attraction of rainbow trout juveniles at 0.0002 mg/l. In embryo-larval tests with the same species, a mercury concentration of 0.0001 mg/l resulted in 100% egg mortality within 8 days of treatment (Birge, *et al.*, 1979e). While the embryo-larval bioassay was considered to be the more sensitive test for mercury, both procedures reflected significant effects at very low exposure levels.

When rainbow trout juveniles were exposed to NTA, the behavioral threshold was estimated to be 56.6 mg/l. Yet at this relatively high concentration, percent avoidance was not observed to be substantial (*i.e.*,

23.8%). These results, together with the LC_1 values of 16.9 and 20.2 mg/l determined in trout embryo-larval tests, indicate the comparatively non-toxic nature of NTA to aquatic organisms.

Zinc produced significant avoidance at 0.047 and 7.03 mg/l in tests with the rainbow trout and the largemouth bass, respectively. In embryo-larval tests with the bass, the LC_1 value of 0.98 mg/l was approximately seven times lower than the avoidance threshold. However, the LC_1 of 0.216 mg/l determined with trout developmental stages was four to five times higher than the threshold for avoidance. This was the only case in which the behavioral bioassays proved to be more discriminating than the embryo-larval toxicity tests. Moreover, the +94.5% net response observed at 0.047 mg/l indicated that the avoidance threshold may be even lower. Sprague (1968) determined a zinc avoidance threshold of 5.6 µg/l in his studies with the rainbow trout.

The avoidance response test proved to be a reliable and economically feasible means for evaluating effects of aquatic contaminants on fish and amphibians. While generally not as sensitive as embryo-larval tests, the avoidance procedure provided information not obtainable using other types of bioassay methodology. For example, toxicants producing avoidance behavior, such as cadmium, zinc, and phenol, could limit distribution of aquatic animals and severely restrict their feeding and spawning habitats. As a consequence, the health and reproductive potential of many species would be jeopardized. On the other hand, chemicals producing attraction responses, such as chloroform and mercury, could also present a substantial risk to fish populations, as animals could be drawn to a pollution source for feeding and spawning activities. As a result, survival and reproduction could be appreciably curtailed. The avoidance response test shows substantial promise as a predictive tool and should be included as an integral part of testing programs designed to assess aquatic hazards.

CHAPTER IV

CONCLUSIONS

Avoidance response bioassays were conducted with eight inorganic and organic toxicants, including cadmium, copper, mercury, zinc, chloroform, dioctyl phthalate (DOP), trisodium nitrilotriacetic acid (NTA), and phenol. Tests were performed in a dual-channel fluviarium system, using carbon-filtered tap water as the dilution source. The toxicant injection procedure provided good regulation of exposure concentrations in the test channel. Juvenile stages of the largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), and rainbow trout (*Salmo gairdneri*), and tadpoles of the American toad (*Bufo americanus*) were found to be suitable animals for evaluating behavioral responses (*i.e.*, avoidance, attraction). The trout was the most sensitive species tested.

Avoidance was significant in tests with cadmium, phenol, and zinc, and significant attraction resulted from exposure to chloroform, DOP, and mercury. In bioassays with copper, animals generally avoided lower concentrations but were attracted to higher exposure levels. NTA produced variable responses. In tests with the trout, threshold concentrations for avoidance or attraction were estimated to fall at 0.0002 mg/l mercury, 0.047 mg/l zinc, 0.052 mg/l cadmium, 0.074 mg/l copper, 11.9 mg/l chloroform, and 56.6 mg/l NTA. The threshold for phenol, determined using the bluegill sunfish, was 39.0 mg/l.

Results from fish embryo-larval toxicity tests were used to gauge the sensitivity of the avoidance response bioassay. Toxicant concentrations which produced embryo-larval lethality or teratogenesis at frequencies of 10% (LC_{10}) and 1% (LC_1) were compared to behavioral threshold concentrations. Based on these data, the avoidance test was observed to be a less sensitive procedure for evaluating the effects of all the selected toxicants, except zinc. However, the avoidance test provided valuable information which was not obtainable using other bioassay methodologies. Furthermore, the system developed in this study was highly cost-feasible and provided reliable and reproducible test results.

Table 2. Avoidance/attraction responses of juvenile fish and amphibian tadpoles to aquatic contaminants.

Toxicant	Species	Toxicant Concentration Mean ± S.E. (mg/l)		Percent Gross Response[1] (Avoidance [+] or Attraction [-])	Percent Net Response[2,3] (Avoidance [+] or Attraction [-])
		Test Channel	Control Channel		
Cadmium	Bluegill Sunfish	0.79 ± 0.08	0.02 ± 0.01	+ 4.5	+ 5.0
		8.31 ± 0.41	4.02 ± 2.43	-16.8	- 9.3
		41.1 ± 3.3	1.64 ± 1.54	+30.0	+11.0
	Largemouth Bass	0.086 ± 0.004	<0.001	- 9.3	+ 5.3
		0.90 ± 0.04	0.004 ± 0.002	-10.5	-12.5
		8.83 ± 0.69	0.05 ± 0.02	- 8.0	- 1.5
	Rainbow Trout	0.012 ± 0.002	<0.001	+18.3	+37.3
		0.052 ± 0.004	<0.001	+55.3	+51.7*
		0.10 ± 0.02	<0.001	+71.8	+55.3*
		1.03 ± 0.04	0.02 ± 0.01	+89.8	+72.3*
Copper	American Toad	0.012 ± 0.001	0.008 ± 0.001	+17.8	+ 1.8
		0.10 ± 0.00	<0.001	+28.0	+39.7*
		0.93 ± 0.04	<0.001	+ 1.8	-29.8*
	Bluegill Sunfish	0.66 ± 0.07	0.03 ± 0.01	+ 5.3	- 0.3
		8.48 ± 0.42	0.29 ± 0.18	-50.3	-50.3*
		43.2 ± 2.5	0.83 ± 0.07	-61.8	-49.3*

Table 2 - continued.

Toxicant	Species	Toxicant Concentration Mean ± S.E. (mg/l)		Percent Gross Response[1] (Avoidance [+] or Attraction [-])	Percent Net Response[2,3] (Avoidance [+] or Attraction [-])
		Test Channel	Control Channel		
Copper	Rainbow Trout	0.074 ± 0.017	<0.002	+27.4	+13.2*
		0.37 ± 0.03	<0.001	-14.8	-12.8
		0.77 ± 0.06	0.02 ± 0.01	+11.6	+11.3
		4.56 ± 0.22	0.14 ± 0.14	-20.0	-37.0*
		7.56 ± 0.63	0.16 ± 0.06	-40.3	-26.8*
Mercury	Rainbow Trout	0.0002 ± 0.0000	0.0001 ± 0.0000	-25.5	-24.5*
		0.0074 ± 0.0008	0.0003 ± 0.0001	-28.5	-18.0*
Zinc	Bluegill Sunfish	11.3 ± 0.5	1.49 ± 0.63	+ 2.8	+ 7.8
		43.7 ± 1.2	6.10 ± 0.43	+ 5.0	+12.8
	Largemouth Bass	7.03 ± 0.34	0.15 ± 0.13	+50.3	+56.8*
		39.2 ± 4.9	4.11 ± 2.28	+ 4.5	+15.5*
	Rainbow Trout	0.011 ± 0.001	<0.001	-10.0	+11.0
		0.047 ± 0.005	<0.001	+95.5	+94.5*
		0.10 ± 0.01	<0.001	+96.5	+95.0*
		1.13 ± 0.12	<0.001	+99.5	+99.8*

Table 2 - continued.

Toxicant	Species	Toxicant Concentration Mean ± S.E. (mg/l)		Percent Gross Response[1] (Avoidance [+] or Attraction [-])	Percent Net Response[2,3] (Avoidance [+] or Attraction [-])
		Test Channel	Control Channel		
Chloroform	Bluegill Sunfish	0.36 ± 0.10	0.08 ± 0.01	+ 0.8	+ 3.3
		3.08 ± 0.26	0.45 ± 0.16	- 1.0	- 3.5
		33.2 ± 6.8	0.98 ± 0.31	-88.0	-93.5*
	Rainbow Trout	4.18 ± 0.53	1.03 ± 0.47	+ 0.8	-10.8
		11.9 ± 1.8	0.96 ± 0.53	-13.5	-21.0*
Dioctyl phthalate	Bluegill Sunfish	112.4 ± 5.58	1.12 ± 0.51	-43.8	-41.1*
Trisodium nitrilo-triacetic acid	Bluegill Sunfish	0.23 ± 0.23	<0.5	- 6.5	+ 4.5
		6.65 ± 0.70	<0.5	+ 2.3	- 5.8
		39.8 ± 3.2	<0.5	+ 8.8	+ 2.8
	Rainbow Trout	56.6 ± 7.9	2.08 ± 2.08	+31.8	+23.8*
		101.3 ± 12.9	1.00 ± 0.58	-11.5	-24.5*

Table 2 - continued.

Toxicant	Species	Toxicant Concentration Mean ± S.E. (mg/l)		Percent Gross Response[1] (Avoidance [+] or Attraction [-])	Percent Net Response[2,3] (Avoidance [+] or Attraction [-])
		Test Channel	Control Channel		
Phenol	Bluegill Sunfish	0.76 ± 0.01	0.01 ± 0.01	- 1.5	- 2.8
		6.76 ± 0.23	<0.002	+ 2.9	+ 2.9
		39.0 ± 0.4	6.13 ± 0.83	+80.8	+75.3*

[1]Calculated as (C - T)/(C + T) X 100 where C and T represented mean distribution of animals in the control (C) and test (T) channels, respectively.

[2]Calculated as percent gross response in experimental runs minus percent response in corresponding control runs. The latter were conducted before each experimental run to determine non-toxicant induced distribution of test animals.

[3]Asterisks indicate responses which were statistically significant ($P < 0.01$, t-test).

Table 3. Comparison of threshold concentrations determined in avoidance response tests with lethal concentrations calculated in embryo-larval tests.

Toxicant	Species	Threshold Concentration[1] (Avoidance Tests) (mg/l)	Lethal Concentrations (Embryo-Larval Tests) (mg/l)		
			LC_1	LC_{10}	LC_{50}
Cadmium	Bluegill Sunfish	>41.1	0.006	0.029	0.20
	Largemouth Bass	>8.83	0.182	0.399	1.04
	Rainbow Trout	0.052 (Av)	0.008	0.029	0.14[2]
Copper	American Toad	0.10 (Av)	-	-	-
	Bluegill Sunfish	8.48 (At)	-	-	-
	Rainbow Trout	0.074 (Av)	0.003	0.016	0.110[2]
Mercury	Rainbow Trout	0.0002 (At)	<<0.0001[3]	-	-
Zinc	Bluegill Sunfish	>43.7	-	-	-
	Largemouth Bass	7.03 (Av)	0.975	2.06	5.18
	Rainbow Trout	0.047 (Av)	0.216	0.451	1.12[2]
Chloroform	Bluegill Sunfish	33.2 (At)	-	-	-
	Rainbow Trout	11.9 (At)	0.006 0.005	0.099 0.086	2.03[4] 1.24

Table 3 - continued.

Toxicant	Species	Threshold Concentration[1] (Avoidance Tests) (mg/l)	Lethal Concentrations (Embryo-Larval Tests) (mg/l)		
			LC_1	LC_{10}	LC_{50}
Trisodium nitrilo-triacetic acid	Bluegill Sunfish	>39.8	-	-	-
	Rainbow Trout	56.6 (Av)	16.9	35.9	90.5[4]
			20.2	43.9	114.0
Phenol	Bluegill Sunfish	39.0 (Av)	0.004	0.067	2.42[4]
			0.002	0.038	1.69

[1]Concentrations which produced significant responses are designated (Av) for avoidance or (At) for attraction.

[2]Lethal concentrations taken from Birge, *et al.*, 1979d.

[3]Data taken from Birge, *et al.*, 1979e.

[4]Lethal concentrations computed from data presented in Birge, *et al.*, 1979a. The two sets of LC values were calculated from tests performed using soft (50 mg/l $CaCO_3$) and hard (200 mg/l $CaCO_3$) water, respectively.

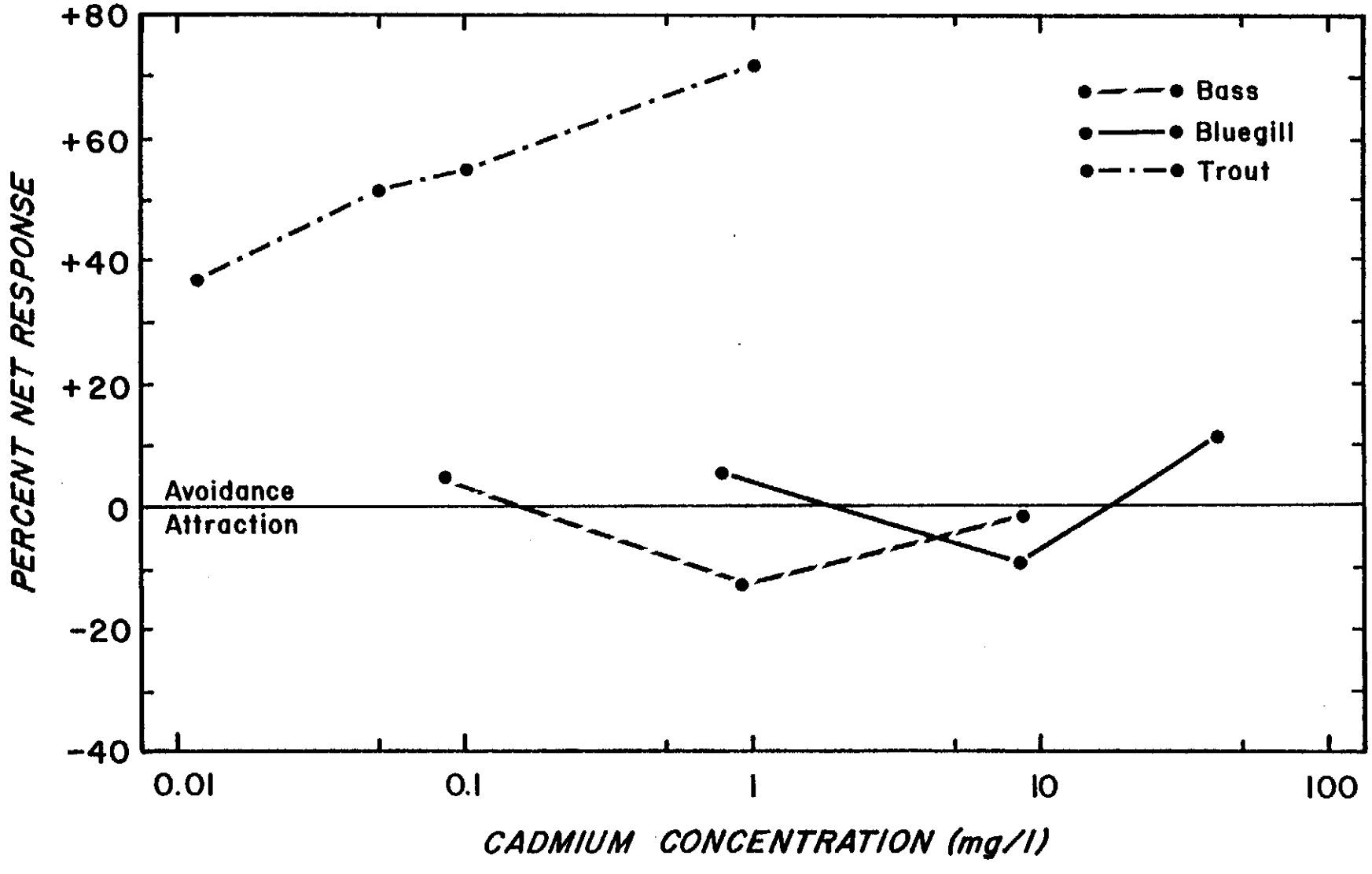

Figure 3. Avoidance/attraction responses of juvenile fish to cadmium.

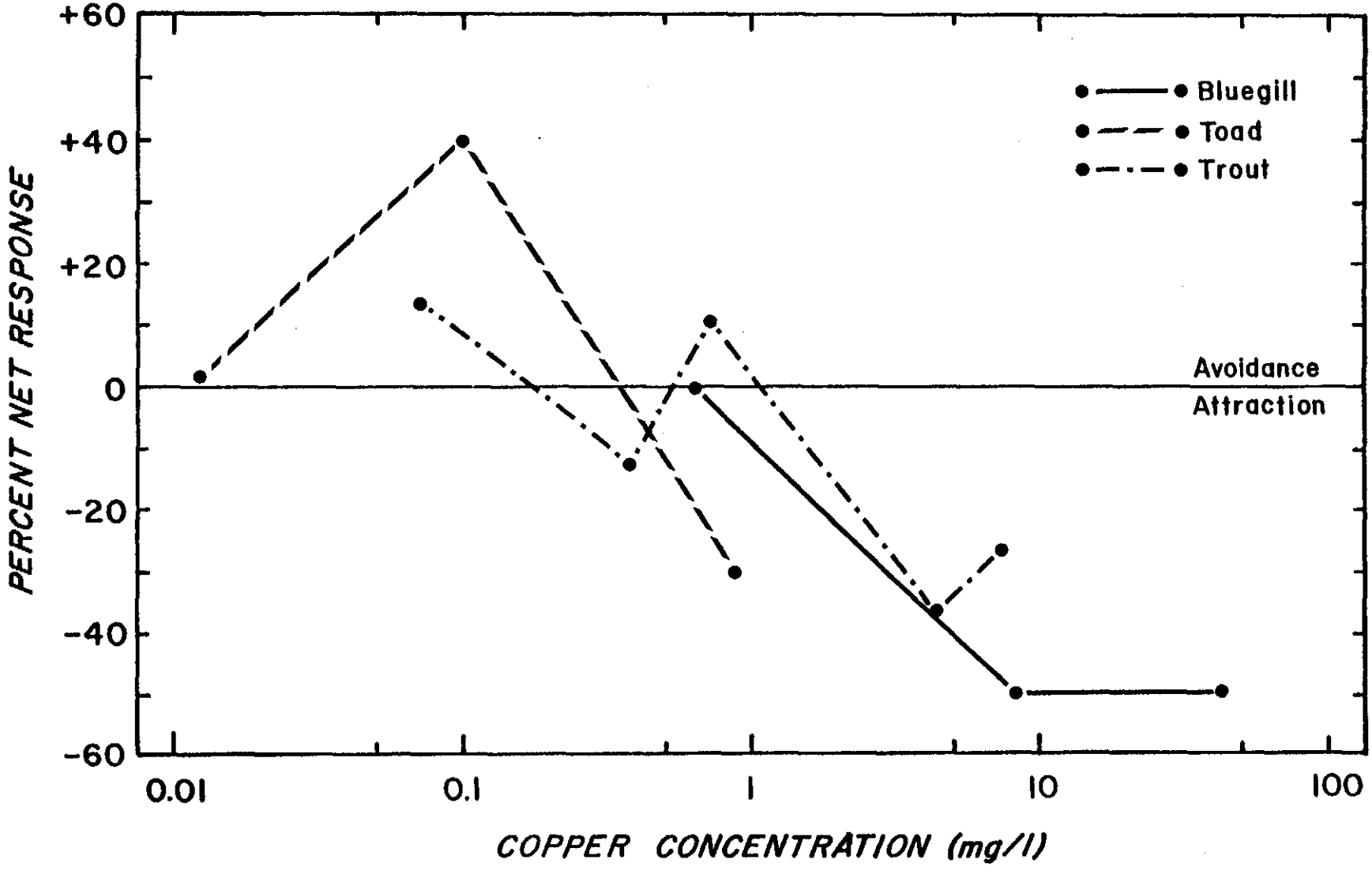

Figure 4. Copper-induced avoidance/attraction responses of juvenile fish and amphibian tadpoles.

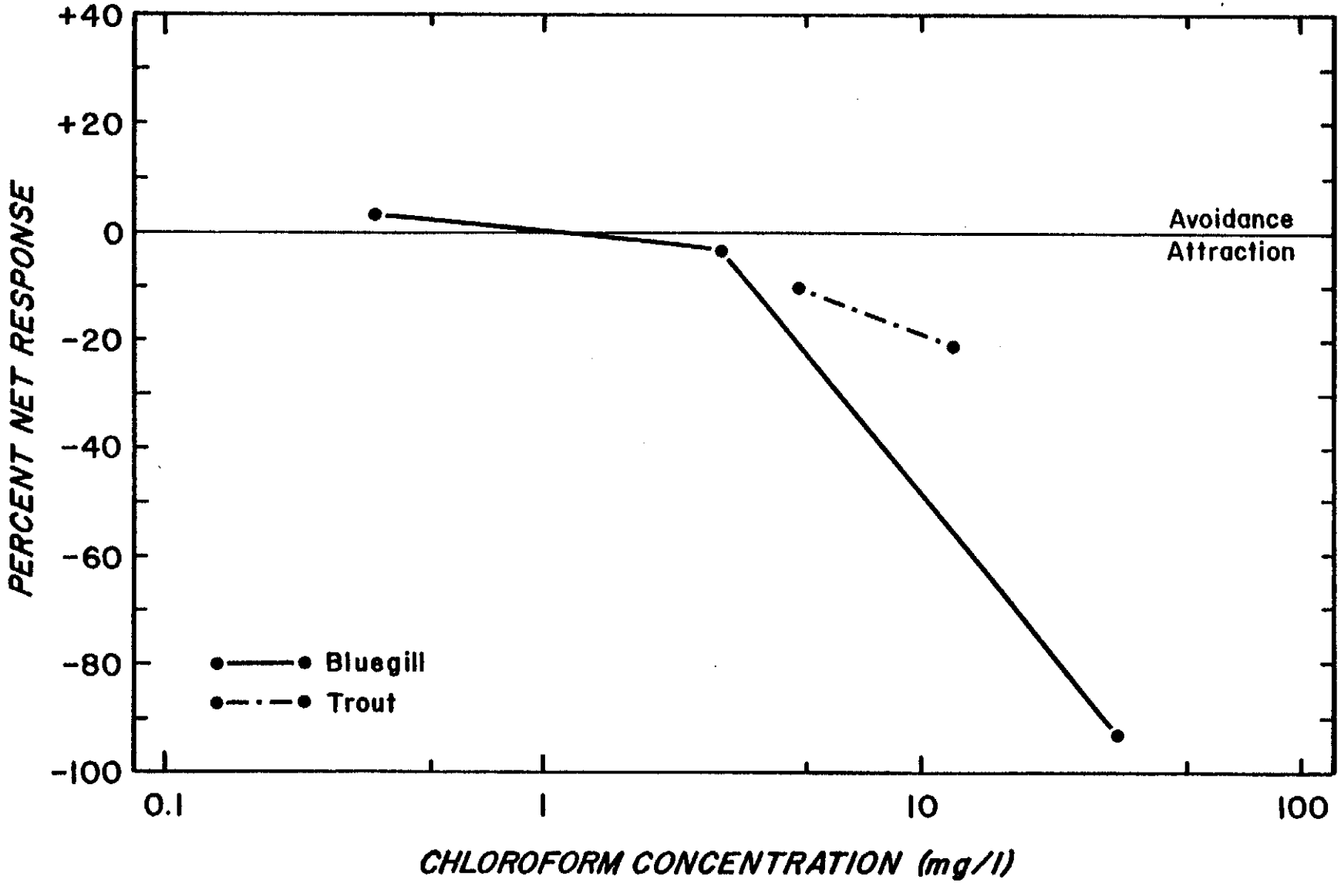

Figure 5. Avoidance/attraction responses of juvenile fish to chloroform.

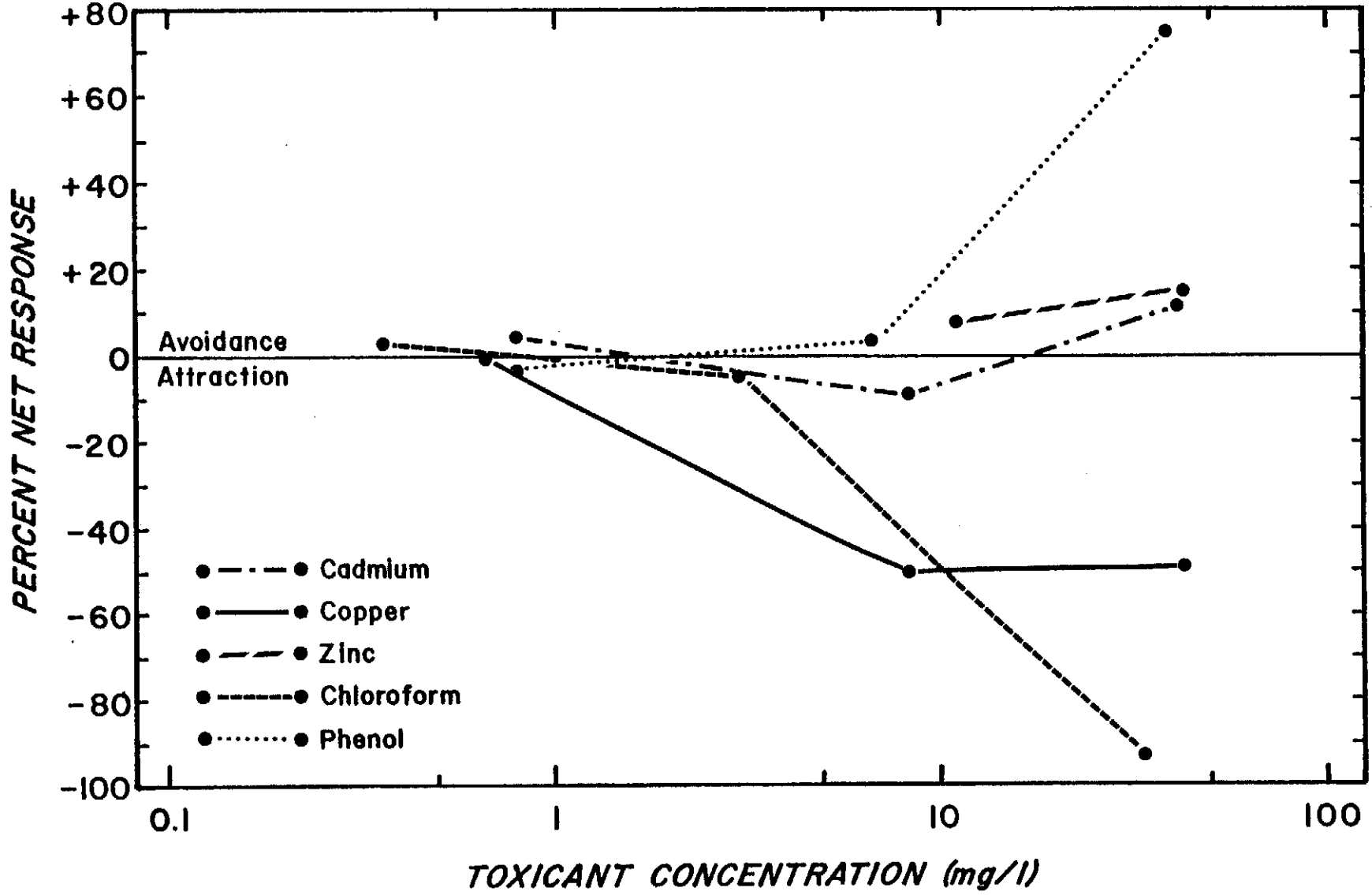

Figure 6. Avoidance/attraction responses of juvenile bluegill sunfish to aquatic contaminants.

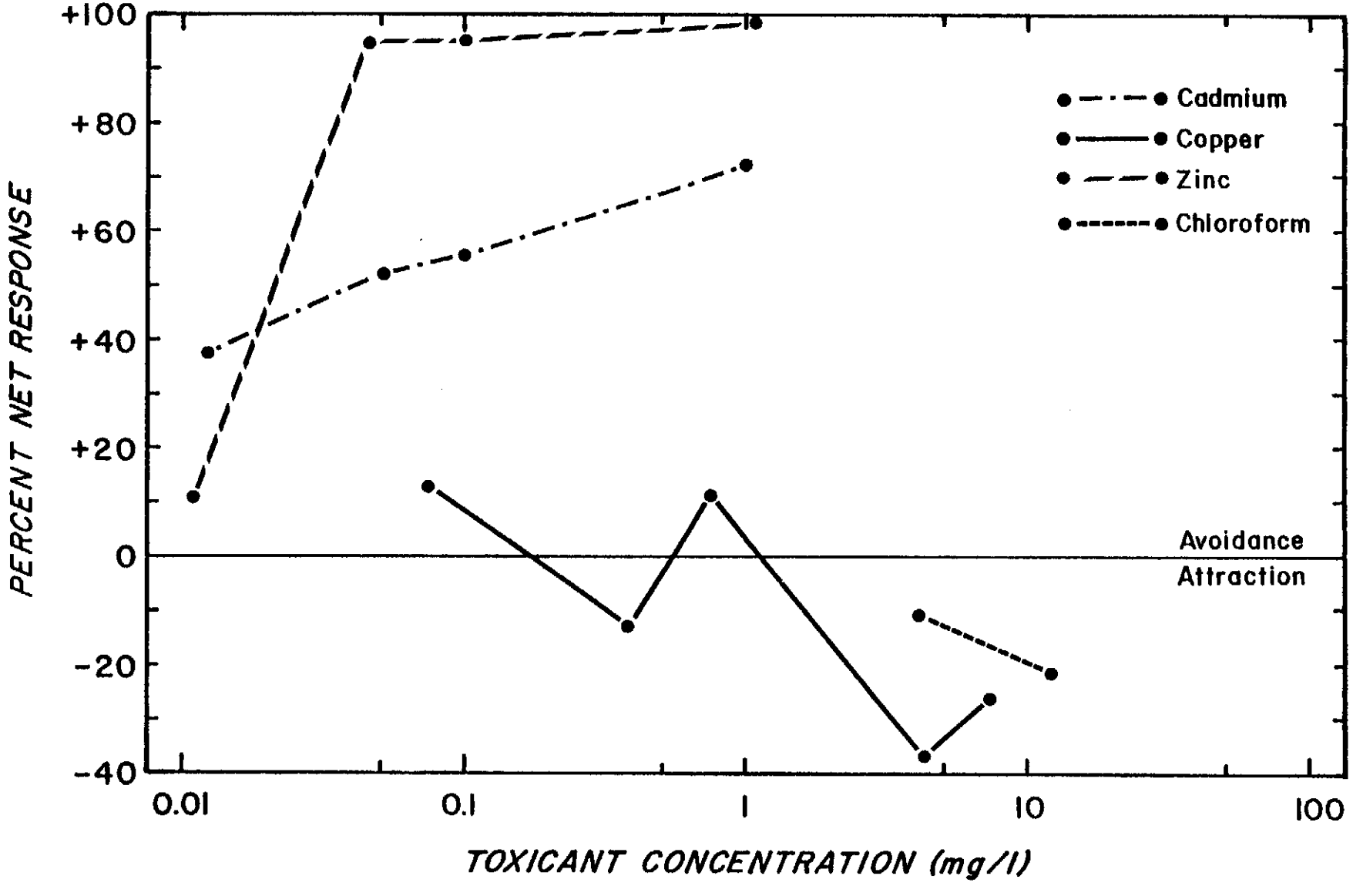

Figure 7. Avoidance/attraction responses of juvenile rainbow trout to aquatic contaminants.

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