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ABSTRACT

The effects of zinc on embryos and larvae of the fathead minnow (Pimephales promelas) were studied using standard 8-day embryo-larval bioassay techniques. The objective was to determine if there was a period in embryonic development which would be the most sensitive to the toxic and teratogenic effects of zinc. Five developmental stages were exposed to 0.5, 1.0 and 3.0 mg/L. After 96 hr, all animals exposed to 3.0 mg Zn/L were dead. The hatching stage was the most affected by 1.0 mg/L, with only 59% surviving after 96 hr, while the tailbud stage showed essentially control-level survival. However, virtually all larvae at all stages were abnormal at 1.0 mg Zn/L, displaying edema and curvature of the vertebral axis. Although LC_{50} values for the five developmental stages ranged from 0.13 to 0.62 mg/L, there was not a significantly most sensitive stage based upon this criterion. Findings during the posthatch stage proved interesting. When larvae were exposed to 1.0 mg Zn/L only during the 96 hr after hatching, all animals were anomalous. However, if zinc exposure occurred only during the prehatch period and larvae were placed in control water posthatch, only 5% of the larvae were abnormal. These results indicate that at sublethal concentrations perhaps the chorion imparts a protective mechanism to the teratogenic effects of zinc, but that this protection is lost after hatching.

Descriptors: Bioassay
Embryonic Growth Stage
Larval Growth Stage

Identifiers: Embryo-Larval Bioassays
Pimephales promelas
Teratogenesis
Zinc

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CHAPTER I

INTRODUCTION

Assessments of the impact of environmental contaminants on aquatic biota have been concerned largely with determining concentrations which produce mortality in aquatic plants and animals. When toxicants are present in low, subacute concentrations over a broad range of the aquatic environment, fish and amphibians may be stressed such that their growth and development, as well as their physiology and behavior, may be affected. One major sublethal effect that has not been extensively investigated is that of the occurrence of birth defects (terata). Abnormal animals do not usually survive in natural populations and, therefore, the presence of a teratogen can prove important in the maintenance of the integrity of an aquatic ecosystem.

The specific objectives of this study included 1) identifying representative chemicals which demonstrate teratogenic potential, 2) examining teratic responses found in representatives from two aquatic vertebrate classes (i.e., Osteichthyes, Amphibia), 3) determining the stage or stages in embryonic development when the organisms are most susceptible to teratogenic action, and 4) initiating a study of the specific defect found in newly-hatched organisms.

A literature survey was undertaken to identify chemicals which were known teratogens to aquatic vertebrate species. Among the heavy metals

which have produced abnormalities in embryos and larvae of fish and amphibians were cadmium, copper, and zinc. Zinc is present in most aquatic systems, usually adsorbed to sediments (1). The potential for release of sediment-bound zinc increases with increasing acidification and the toxicity of zinc varies with water hardness. As a result, comparisons of zinc toxicity from various studies are difficult. Pickering and Vigor (2) and Benoit and Holcombe (3) reported the effects of zinc on embryos and larvae of the fathead minnow. Although malformations and abnormal development were noted in these studies, no measure of the type or severity was reported. Brungs (4) reported that hatchability and survival of fathead minnow eggs was dependent upon the concentration of zinc and that posthatch mortality was high at levels of 1.3 mg/L and above. Based upon these early reports and the more recent use of terata in evaluating toxic effects (5-7), zinc was selected as the toxicant to examine. A study by Dawson, et al. (7) confirmed this selection when it was reported that the frequency of teratic larvae was a valid criterion to use in evaluating the toxicity of metal-contaminated sediments to fathead minnows and frogs. Their results indicated that zinc was a teratogen to both species.

The second objective of this project was undertaken by exposing eggs of the fathead minnow (Pimephales promelas) and African clawed frog (Xenopus laevis) to a broad range of zinc concentrations. Preliminary results indicated that the fathead minnow was substantially more sensitive to zinc than was the frog. Therefore, this study concentrated on the effects of zinc on embryos and larvae of the fathead minnow. Since not all exposure concentrations produced large numbers of abnormal animals, the range of

exposures was decreased to three to include the highest levels which produced the maximum number of terata.

To determine the stage at which fathead minnows were most susceptible to the toxic and teratogenic action of zinc (Objective 3 above), embryos were exposed at five developmental stages. The stages were selected to examine the effect of zinc on major developmental events, including gastrulation, somite expansion, onset of circulation, expansion of circulation and further refinement of the central and peripheral nervous systems, and the period of hatching (8, 9). The results of these bioassays were evaluated after 96 hr of zinc exposure for each stage and at the end of 4 days after hatching. Data collected from the period of hatching through 4 days posthatching posed some interesting questions about the toxic and teratogenic effects of zinc. Therefore, additional experiments were conducted to evaluate further the effect of zinc on prehatch and posthatch organisms.

The embryos and larvae from all bioassays were preserved for histological examination. An initial evaluation of the chondrofication of the skeletal system was undertaken in light of the large number of terata with axial bends and other curvatures of the axial column. The preservation of these specimens will allow future evaluation of any histological deformities caused by zinc.

CHAPTER II

RESEARCH PROCEDURES

Selection of Animal Test Species

One species of warm water fish was used in this study. Eggs of the fathead minnow (Pimephales promelas) were obtained from the Aquatic Biology Section, Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Newtown, Ohio. The selection of species was made in part on the basis of economic importance, seasonal availability, ease of propagation in the laboratory, the data base available for comparison of test results, and general acceptance as test organisms (10-12). Eggs of the fathead minnow were collected by U.S. EPA personnel, transported to the laboratory in insulated coolers, and immediately used in bioassays.

Dilution Water

Dilution water used in all phases of this study was a synthetic fresh water of moderate hardness (i.e., 80-100 mg/L as $CaCO_3$) recommended by the U.S. Environmental Protection Agency (11, 12). Distilled, deionized water was reconstituted by the addition of appropriate quantities of reagent-grade sodium, potassium, magnesium, and calcium salts. This synthetic water was

used as the dilution water for the embryo-larval bioassays. Physicochemical characteristics of the dilution water are presented in Table 1.

Selection and Analysis of Toxicant

Zinc acetate ($Zn(C_2H_3O_2)_3 \cdot 2H_2O$) was chosen as the toxicant, and was obtained from Fisher Scientific Company (Lot #743126). A stock solution of 10 g Zn/L was prepared in distilled, deionized water and this stock solution was diluted to the appropriate exposure concentration for all bioassays. Actual zinc concentrations were determined daily for each exposure using a Perkin Elmer Atomic Absorption Spectrophotometer (model 3030).

Procedures for Conducting Embryo-Larval Bioassays

Embryo-larval test system. To determine the toxic and teratogenic effects of zinc on embryonic stages, embryo-larval bioassays were conducted following static-renewal procedures described by Horning and Weber (11) and Birge, et al. (13). Fertilized fathead minnow eggs were placed in deep Pyrex petri dishes (400 mL capacity), with a sample size of approximately 100 eggs per dish for both experimental and control populations. Test and control water were changed at regular 24-hr intervals. Aeration was supplied directly to each test chamber. Water quality parameters, including pH, temperature, dissolved oxygen, and conductivity, were determined daily utilizing a Corning pH meter (model 10), a mercury bulb thermometer, YSI oxygen meter (model 54), and Markson conductivity meter (model 10).

Measurements on alkalinity and hardness were accomplished using the methyl orange and EDTA titrimetric procedures, respectively, as described in Standard Methods (14). Test organisms were monitored daily to gauge extent of development and to remove dead specimens.

Exposure concentrations and duration of test. The effect of zinc was tested initially in a range-finder bioassay at five exposure concentrations ranging from 0.01 to 5.0 mg/L, using at least two replicates. Based upon both mortality and the frequency of terata, the range-finder allowed the number of exposure concentrations in subsequent bioassays to be narrowed to three, (i.e., 0.5, 1.0, 3.0 mg/L). Bioassays were initiated soon after fertilization (approximately 14 hr) and continued through 4 days posthatching. Average hatching time was approximately 4-5 days for fathead minnows at an overall mean temperature of 23°C.

Although all tests were carried through 4 days posthatching, daily examination of the eggs and embryos allowed determination of numbers of mortalities and terata after 96 hr of zinc exposure. In order to determine the developmental stage which was most sensitive to zinc, exposures were initiated at various developmental ages. The stage of embryonic development was confirmed upon consultation with a staging guide prepared by Devlin (8). Zinc exposure was initiated at 14 hr of age or less (gastrula), 24 hr (tailbud, post neurula stage), 48 hr (onset of circulation), 72 hr (expansion of circulation stage), and 96 hr (beginning of hatching period). In order to evaluate the effects of zinc on fathead minnow embryos when exposed only prior to hatching, embryos were treated with 0.5 and 1.0 mg Zn/L for 96 hr and the unhatched eggs were then transferred to dilution

water without zinc. Hatching embryos were then observed for 4 days posthatching, noting mortality and teratogenic effects.

Test responses and analysis of data. Test responses in embryo-larval bioassays were limited to mortality and teratogenesis. Percent survival and teratogenesis were determined after 96 hr of zinc exposure and at 4 days posthatching. Frequencies of terata were expressed as the percentage of abnormal animals in hatched populations. Teratogenic defects were identified as those survivors affected by gross, debilitating anomalies (5, 15). Counting teratic larvae as dead organisms, median lethal concentrations (LC_{50}) were calculated using the Trimmed Spearman-Karber method (16) and no observed effect concentrations (NOEC) were determined using the methods of Dunnett (17) and Chi-square/Fisher's exact test (18). The lowest observed effect concentration (LOEC) was the lowest zinc concentration which showed statistically significant response. The median effective concentration for malformations (EC_{50}) also was determined using the Spearman-Karber method (16).

Histological examination. All animals, both normal and abnormal, were preserved appropriately for further histological examination. Initial examinations were made on whole animals by a specialized technique which is designed to stain both bone and cartilage in the intact organism. Both normal and abnormal newly-hatched animals were fixed with 10% buffered formalin, stained for cartilage with alcian blue and for bone with alizarin red S, and stored in glycerin (19-22). Observations on cartilage and bone deposition in normal and teratic organisms were made using an AO dissecting microscope. Those animals not prepared for cartilage/bone staining were fixed in Bouin's fixative and embedded in paraffin (23). These specimens

will be examined subsequently for developmental defects at the tissue and cellular level.

In addition, bioassays were initiated at the same five developmental stages described above and representative samples of 25 organisms were collected every 12 hr and preserved in Bouin's fixative. These specimens will be examined to evaluate the time-course of the impact of zinc on organ development.

Table 1. Physicochemical characteristics of synthetic fresh water used as dilution water in embryo-larval bioassays with zinc.

	Mean[1]	Standard Error
Temperature (°C)	23.6	0.3
Hardness (mg/L as $CaCO_3$)	87.7	1.9
Alkalinity (mg/L as $CaCO_3$)	64.4	1.0
pH	7.73	0.06
Conductivity (umhos/cm)	321.2	0.9
Dissolved Oxygen (mg/L)	8.35	0.11

[1]Means determined from analyses of control water used in each bioassay.

CHAPTER III

DATA AND RESULTS

Water quality characteristics. The physicochemical characteristics of the water used in each bioassay are presented in Table 2. All parameters were within limits established by the U.S. EPA for water of moderate softness (11, 12). Mean hardness values ranged from 84.7 to 90.1 mg/L as $CaCO_3$, while pH and alkalinity varied from 7.46 to 7.69 and 63.3 to 67.1 mg/L as $CaCO_3$, respectively. The overall variation in mean exposure temperature was minimal, ranging from 22.7 to 23.5°C. Dissolved oxygen was maintained at saturation and conductivity varied from 320.2 to 323.4 μmhos/cm.

As seen in Table 3, the measured zinc concentrations at each exposure in each bioassay were in good agreement with nominal levels. Therefore, all bioassays were comparable and all further discussion will refer to nominal zinc concentrations.

Sensitivity of various developmental stages to zinc. Two experimental end points were selected for analyzing toxicity data. One end point, a total of 96 hr exposure to zinc, was selected in order to evaluate an equal exposure period to the toxicant. The second end point reflected the full 8-day study, with data examined at 4 days posthatching. These data were less useful in determining LC_{50} values, but did provide insight into the overall effects of zinc in the posthatch period.

When zinc exposure was initiated at five different developmental stages, 0.5 mg/L did not significantly reduce survival after 96 hr of exposure (Table 4, Figure 1). However, exposure to 1.0 mg/L resulted in reductions in survival that ranged from 41% at an initial exposure of 96 hr to essentially no mortality compared to controls at an initial exposure of 24 hr (Table 4, Figure 2). At 3.0 mg Zn/L, no survival was observed in any population after 96 hr of exposure. Mortality in the controls ranged from 0% to 8%.

The frequency of abnormal animals in the posthatched populations after 96 hr of exposure was relatively low at 0.5 mg/L, ranging from 0% in the 24-hr population to 20% in the larvae exposed at 48 hr (Table 5, Figure 1). An exposure concentration of 1.0 mg/L resulted in virtually all survivors showing some degree of teratic defect (Table 5, Figure 2).

The most frequent anomaly at both 0.5 and 1.0 mg/L was a mild though seemingly permanent bend in the vertebral axis. This bending usually resulted in animals that swam in circles or did not move about in a normal manner. Varying degrees of peritoneal and pericardial edema were found in a few larvae and at 1.0 mg/L severe hemorrhaging of the cardiac region and cardiac anomalies were observed. Premature hatching occurred at 3.0 mg/L and all animals were dead by 96 hr of exposure. The populations exposed to 3.0 mg/L were all abnormal prior to death and especially displayed severe cardiac and opthalmic edema, as well as foreshortening of the entire body.

Based solely upon survival, the LC_{50} values for zinc after 96 hr of exposure ranged from 0.34 mg/L at initial exposure age of 14 hr (gastrula) to 1.49 mg/L at initial exposure age of 24 hr (tailbud stage) (Table 6).

Thus, on the basis of LC_{50} values for mortality alone, the period of gastrulation appears to be somewhat more sensitive to zinc than do most embryonic stages, with an LC_{50} of 0.34 mg/L, while the tailbud stage is the most tolerant, at 1.49 mg/L. These data were somewhat lower than the LC_{50} of 3.6 mg/L reported by Dawson, et al. (7) for 6-day exposure of fathead minnows eggs to zinc sulfate. When abnormal animals were considered as mortalities, all LC_{50} values were lowered (Table 6). The LC_{50}'s at the gastrula (14 hr) and onset of circulation (48 hr) stages were 0.16 and 0.13 mg/L, respectively, while at the tailbud stage (24 hr) the LC_{50} was 0.62 mg/L (Table 6). However, 95% confidence limits could not be calculated at the 24-hr exposure age and this LC_{50} value is somewhat in question. Because the LC_{50} values were not significantly different from each other, it appears that zinc is not substantially more toxic to any particular embryonic stage than any other in the development of the fathead minnow.

The lowest observed effect concentrations (LOEC's) for zinc also were calculated. When mortalities alone were considered, the LOEC's were 0.52, 2.82, 0.94, 0.84, and 0.89 mg Zn/L at initial exposure ages of 14, 24, 48, 72, and 96 hr, respectively (Table 6). Calculation of the 96-hr LOEC, counting terata as mortalities, indicated that, except at 24 hr of age, exposure to 0.5 mg Zn/L resulted in a significant response (Table 6). This level was in complete agreement with Dawson, et al. (7), who reported a 6-day LOEC of 0.43 mg Zn/L.

When the median effective concentrations (EC_{50}'s) for abnormalities in the 96-hr populations were determined, levels ranged from 0.16 mg/L at 48 hr to 0.63 mg/L at 24 hr (Table 7). Although these values were lower than those reported by Dawson, et al. (7), they were in relative close agreement.

As with the 96-hr study, when data were analyzed at 4 days posthatching (Table 8), it appeared that the gastrula stage was the most sensitive to zinc. It must be noted, however, that these animals received the longest exposure to zinc when the 4-day posthatching end point was selected. No matter whether the end point was after 96 hr of exposure or at the end of 4 days posthatching, 1.0 mg Zn/L proved to be the concentration producing the most abnormal larvae (Tables 5, 9). However, during the posthatch period, frequencies of anomalies increased daily at the 0.5 mg/L exposure, with the percentage of abnormal animals increasing to 51% in animals exposed at 14 hr of development (Table 9).

The presence of abnormalities in the larvae exposed to zinc at hatching (96-hr exposure population) was of special interest. These animals did not receive any extensive exposure to zinc until after hatching, yet all survivors exposed to 1.0 mg/L after 4 days were anomalous. In order to explore this observation, a bioassay was conducted in quadruplicate in which embryos at the gastrula stage (14 hr) were exposed to 0.5 and 1.0 mg Zn/L for 96 hr. Just prior to hatching, the eggs were transferred to control water (i.e., dilution water without zinc) and mortalities and abnormalities were determined after 4 days posthatching. This treatment resulted in essentially no mortalities in the posthatch populations and frequencies of terata were only slightly above control levels (Treatment C, Tables 10, 11; Figures 3, 4). Thus, the presence of zinc only in the prehatch period does not appear to induce the same numbers of abnormal larvae as it does when posthatch larvae are exposed. These results indicate that at sublethal concentrations perhaps the chorion imparts a protective mechanism to the

teratogenic effects of zinc, but that this protection is lost after hatching.

Histological examination of posthatch larvae. Preliminary histological examination of intact larvae stained for cartilage development indicated that by 4 days posthatching only the cartilagenous portions of the gill arches and brain case were developed. Somite differentiation into cartilagenous and bony elements of the vertebral column were not evident by the time of the occurrence of anomalies. Therefore, abnormalities in cartilage deposition should not be an immediate cause for the appearance of numerous animals with curves in the vertebral axis. Further histological examination should address differentiation of the somites.

Possible teratogenic action of zinc in development. The mechanisms involved in the effect of zinc on either embryos or larvae is presently unknown. Although final differentiation of many organ systems is continuing after hatching, major organ development has been completed by hatching. Thus, the effect of zinc on these posthatch animals may not only reflect a teratogenic effect but also some other physiological impact upon the animal. Zinc has been implicated in the regulation of DNA synthesis (24, 25) and Dawson, et al. (7) have speculated that disruption of DNA synthesis by zinc may result in abnormal embryonic development. Another possible mechanism, especially in light of the posthatch effects of zinc, may be related to the role zinc plays in release of neurotransmitter at the neuromuscular junction. Zinc competes with calcium ions at mammalian neuromuscular junctions, thus inhibiting proper release of transmitter, resulting in improper muscular contraction (26). Perhaps improper muscle contraction may account for the large number of kinks, bends, and curves of the vertebral

axis of the larvae. Further studies are needed to explore the possible mechanisms of zinc action in the embryos and larvae of the fathead minnow.

Table 2. Water quality characteristics observed during embryo-larval bioassays with the fathead minnow.

Initial Exposure Age (hr)	Water Quality Characteristics (Mean ± S.E.)					
	Temperature (°C)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	pH	Conductivity (µmhos/cm)	Dissolved Oxygen (mg/L)
<14	23.4 ± 0.1	84.7 ± 0.6	63.4 ± 0.9	7.69 ± 0.05	321.6 ± 2.1	8.24 ± 0.05
24	23.1 ± 0.1	88.4 ± 1.1	63.3 ± 0.9	7.54 ± 0.03	323.4 ± 1.8	8.03 ± 0.07
48	22.7 ± 0.1	87.8 ± 0.3	66.8 ± 1.4	7.48 ± 0.04	321.3 ± 0.6	8.14 ± 0.06
72	23.5 ± 0.2	90.1 ± 1.3	65.8 ± 1.2	7.67 ± 0.02	321.1 ± 1.3	8.67 ± 0.10
96	23.1 ± 0.1	86.2 ± 1.2	67.1 ± 2.6	7.46 ± 0.04	320.2 ± 0.4	7.91 ± 0.08

Table 3. Atomic absorption spectrophotometric analysis of zinc during embryo-larval bioassays with the fathead minnow.

Nominal Zn Concentration (mg/L)	Actual Zinc Concentration (mg/L)[1]				
	Exposure Age 14 hr	Exposure Age 24 hr	Exposure Age 48 hr	Exposure Age 72 hr	Exposure Age 96 hr
Control	0.02 ± 0.003	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	0.52 ± 0.01	0.47 ± 0.01	0.45 ± 0.01	0.43 ± 0.00	0.41 ± 0.02
1.0	1.02 ± 0.02	0.94 ± 0.02	0.94 ± 0.02	0.84 ± 0.00	0.89 ± 0.03
3.0	3.26 ± 0.03	2.82 ± 0.09	2.65 ± 0.02	1.93 ± 0.12	2.53 ± 0.08

[1]Expressed as mean ± standard error.

Table 4. Percent survival of developmental stages of the fathead minnow exposed to zinc for 96 hr.

Nominal Zn Concentration (mg/L)	Initial Exposure Age (hr)[1]				
	<14	24	48	72	96
Control	98	92	93	100	100
0.5	83	96	94	98	95
1.0	77	92	72	72	59
3.0	0	0	0	0	0

[1]Hatching occurred at 96 hr.

Table 5. Percent of fathead minnow populations anomalous after 96 hr of zinc exposure.

Nominal Zn Concentration (mg/L)	Initial Exposure Age (hr)[1]				
	<14	24	48	72	96
Control	2	0	0	1	1
0.5	10	0	20	12	11
1.0	100	100	100	94	100
3.0	-	-	-	-	-

[1]Hatching occurred at 96 hr.

Table 6. 96-hr LC_{50} and LOEC values for zinc determined in embryo-larval bioassays with the fathead minnow.

Initial Exposure Age (hr)	LC_{50}[1] (mg/L)	95% Confidence Limits	LOEC[1] (mg/L)	LC_{50}[2] (mg/L)	95% Confidence Limits	LOEC[2] (mg/L)
<14	0.34	0.23 - 0.51	0.52	0.16	0.10 - 0.24	0.52
24	1.49	1.43 - 1.56	2.82	0.62	-	0.94
48	0.95	0.66 - 1.35	0.94	0.13	0.07 - 0.21	0.45
72	0.92	0.80 - 1.06	0.84	0.26	0.18 - 0.39	0.43
96	0.76	0.61 - 0.95	0.89	0.23	0.16 - 0.35	0.41

[1] LC_{50} and LOEC values based on all survivors, both normal and abnormal. The LOEC is expressed as actual mean exposure concentration.
[2] LC_{50} and LOEC values based only on normal survivors. Terata counted as mortalities. The LOEC is expressed as actual mean exposure concentration.

Table 7. 96-hr EC_{50} values for abnormalities observed in embryo-larval bioassays with fathead minnows exposed to zinc.

Initial Exposure Age (hr)	EC_{50} (mg/L)	95% Confidence Limits
<14	0.41	0.28 - 0.59
24	0.63	-
48	0.16	0.11 - 0.25
72	0.47	0.29 - 0.74
96	0.30	0.21 - 0.43

Table 8. Percent survival of developmental stages of the fathead minnow exposed to zinc through 4 days posthatching.

Nominal Zn Concentration (mg/L)	Initial Exposure Age (hr)[1]				
	<14	24	48	72	96
Control	90	91	93	100	100
0.5	82	96	90	98	95
1.0	27	60	49	67	59
3.0	0	0	0	0	0

[1]Hatching occurred at 96 hr.

Table 9. Percent of fathead minnow population anomalous after exposure to zinc through 4 days posthatching.

Nominal Zn Concentration (mg/L)	Initial Exposure Age (hr)[1]				
	<14	24	48	72	96
Control	1	0	0	1	1
0.5	51	44	33	11	11
1.0	100	100	100	93	100
3.0	-	-	-	-	-

[1]Hatching occurred at 96 hr.

Table 10. Survival of fathead minnows at 4 days posthatching following various treatments with zinc.

Nominal Zn Concentration (mg/L)	Percent Survival		
	A[1]	B[2]	C[3]
Control	90	100	96
0.5	82	95	97
1.0	27	59	97

[1]A = 8 days total exposure to Zn.
[2]B = 4-day prehatch period with no Zn exposure, followed by 4-day posthatch period with Zn expousre.
[3]C = 4-day prehatch period with Zn exposure, followed by 4-day posthatch period with no Zn exposure.

Table 11. Frequencies of anomalies in fathead minnow larvae at 4 days posthatching following various treatments with zinc.

Nominal Zn Concentration (mg/L)	Percent Terata		
	A[1]	B[2]	C[3]
Control	1	1	2
0.5	51	11	4
1.0	100	100	5

[1]A = 8 days total exposure to Zn.
[2]B = 4-day prehatch period with no Zn exposure, followed by 4-day posthatch period with Zn expousre.
[3]C = 4-day prehatch period with Zn exposure, followed by 4-day posthatch period with no Zn exposure.

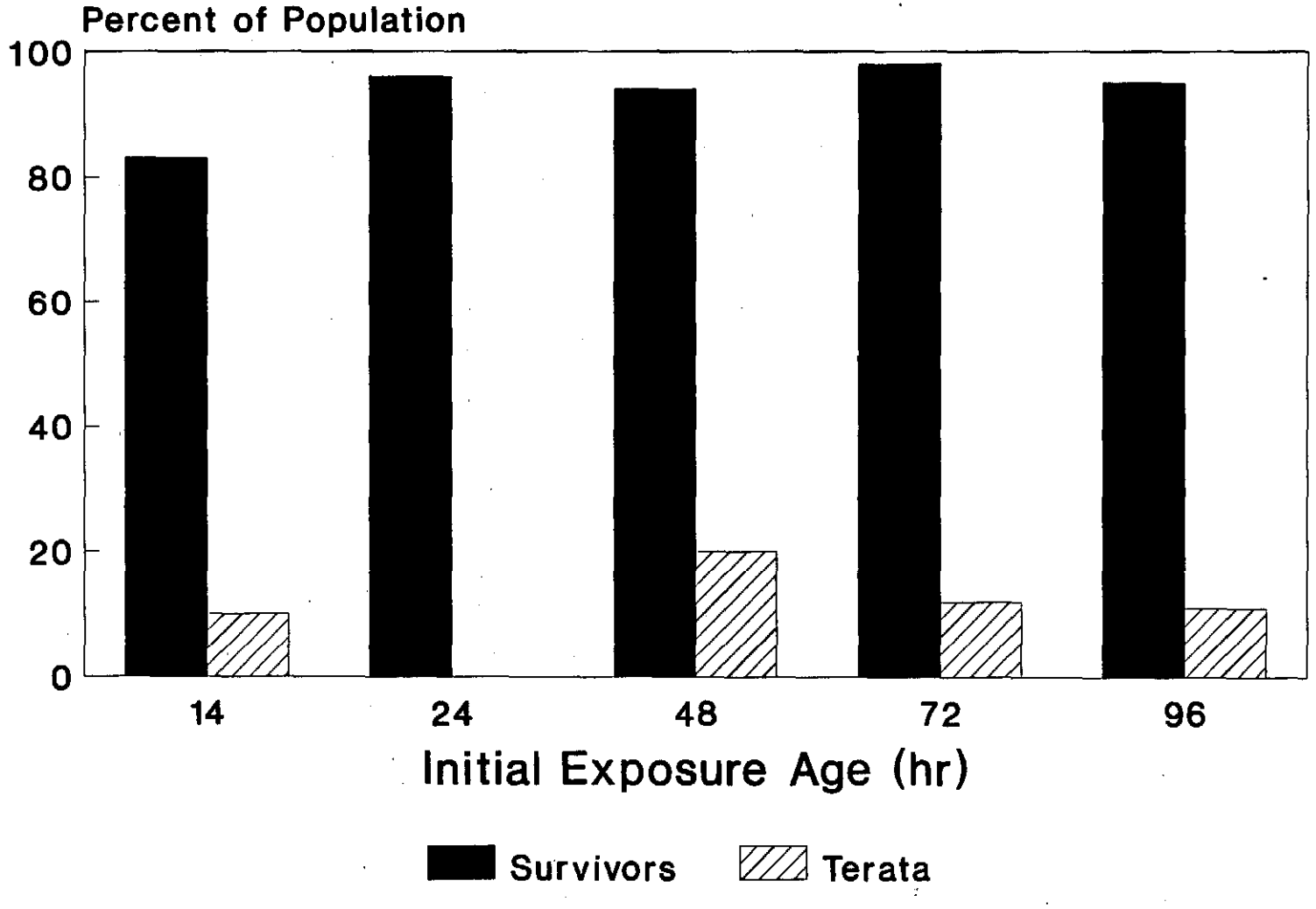

Figure 1. Teratogenic effects of 0.5 mg Zn/L to fathead minnow larvae after 96 hr of exposure.

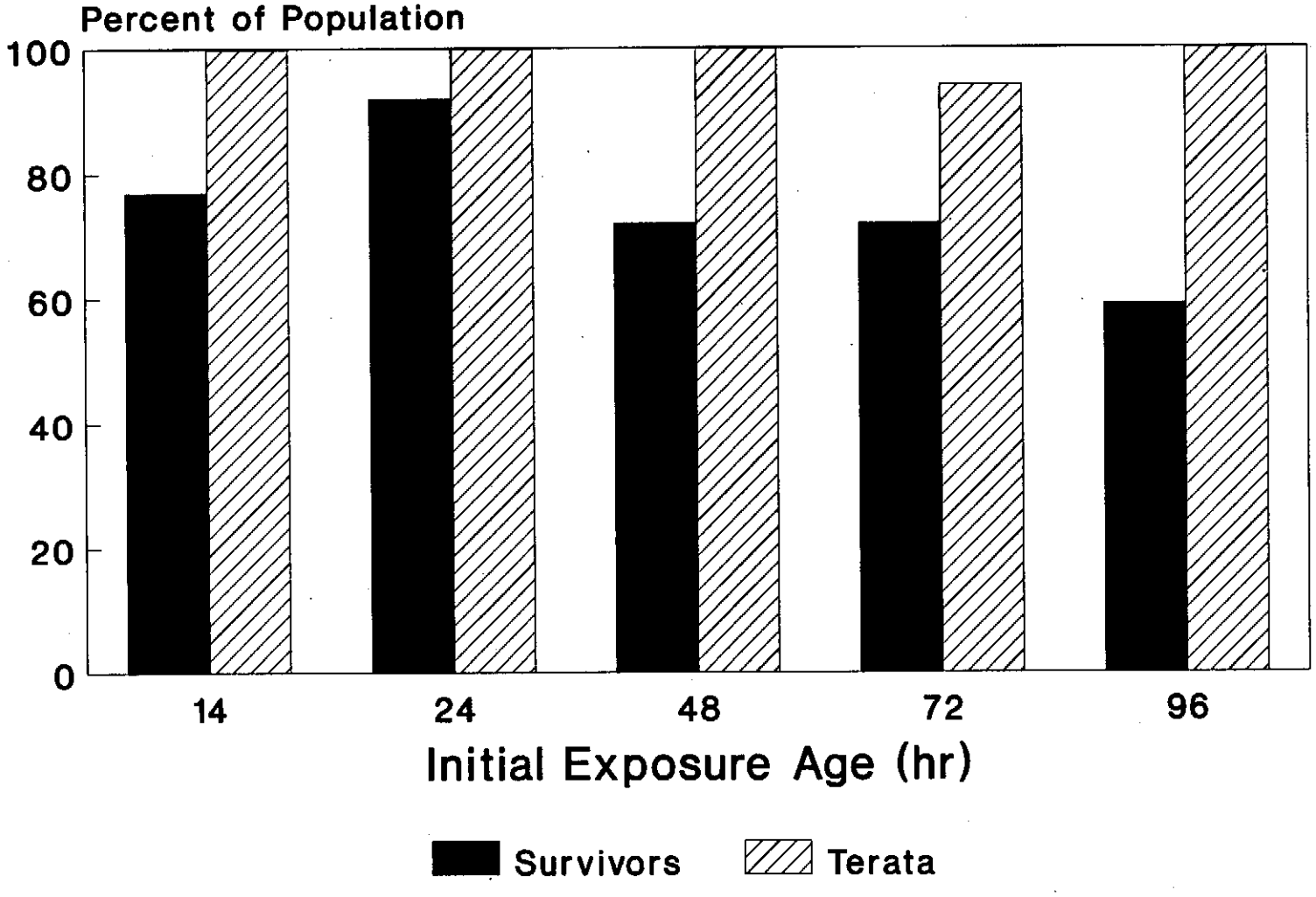

Figure 2. Teratogenic effects of 1.0 mg Zn/L to fathead minnow larvae after 96 hr of exposure.

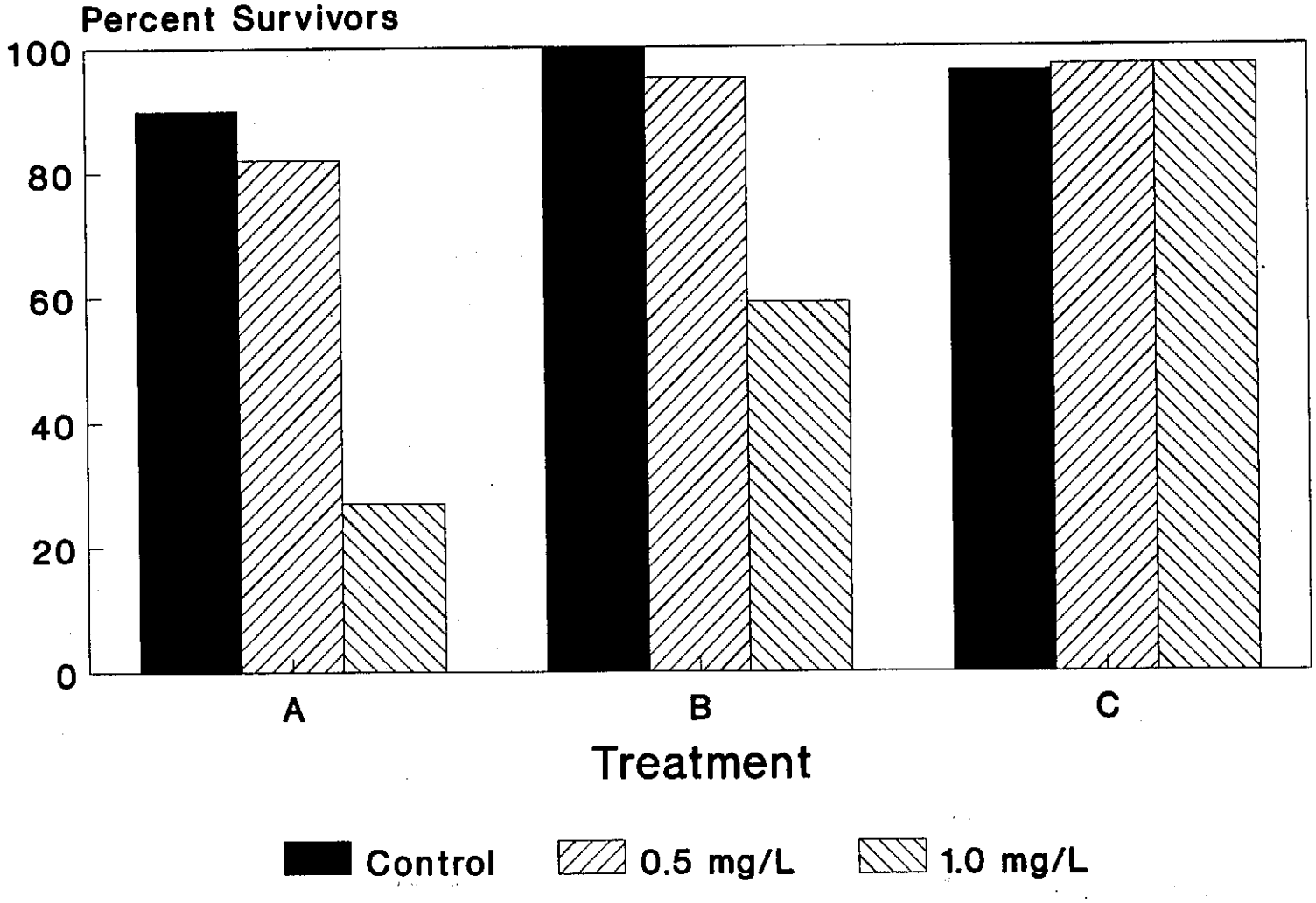

Figure 3. Survival of fathead minnow larvae following 8-day (A), 4-day posthatch (B), and 4-day prehatch (C) exposure to zinc. (See footnote, Table 10)

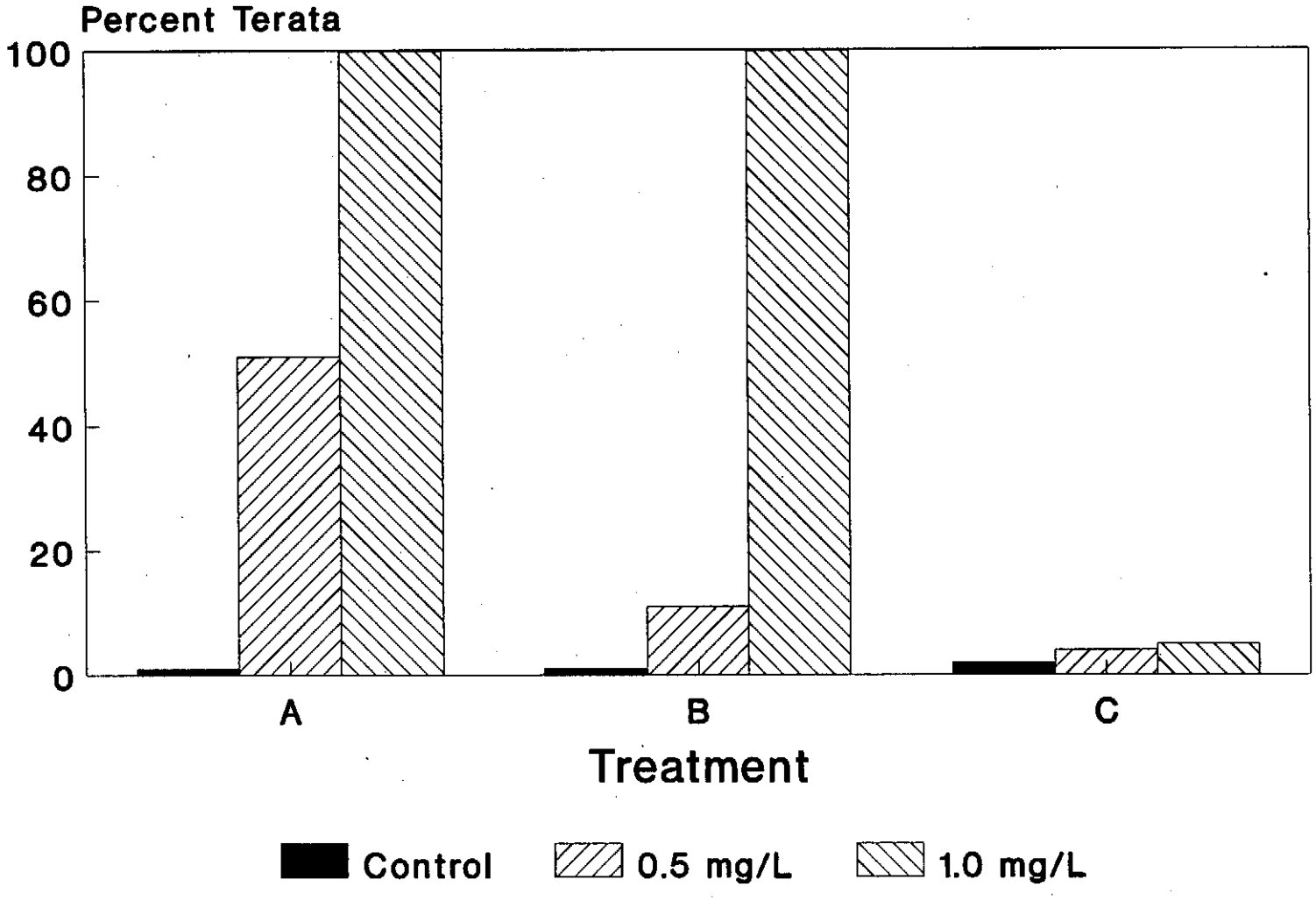

Figure 4. Frequencies of terata in fathead minnow larvae following 8-day (A), 4-day posthatch (B), and 4-day prehatch (C) exposure to zinc. (See footnote, Table 10)

CHAPTER IV

CONCLUSIONS

This study was undertaken to examine the frequency of abnormal animals in hatched populations of the fathead minnows and to evaluate the susceptibility of various developmental stages to the toxic and teratogenic actions of zinc. Abnormal animals do not usually survive in natural populations and, therefore, any information on the teratogenic action of an aquatic contaminant may prove important in the maintenance of the integrity of an aquatic ecosystem. The fathead minnow is commonly found in many freshwater streams and serves as an important member of freshwater ecosystems. Since zinc is a contaminant in many fresh waters, the effects of zinc on the developmental stages of the fathead minnow would provide additional information in maintaining waters that would be safe for the propagation of fish.

Five embryo-larval stages of the fathead minnow were exposed to a range of zinc concentrations. Although the median lethal concentrations ranged from 0.13 to 0.62 mg/L, this difference was not substantial enough to indicate that a particular stage was significantly more sensitive than any other. The sublethal effects of zinc appeared most prominently during the posthatch stage, when it was shown that surviving embryos exposed to 1.0 mg Zn/L were all anomalous. However, if embryos were exposed only prior to hatching, frequencies of abnormalities decreased to just above control

levels. Therefore, the chorion of the egg may provide a measure of protection to developing embryos, but that this protection is lost after hatching.

A report by Dyer (27) in 1982 indicates that several streams in eastern Kentucky have zinc concentrations in the range used in this study. Although these streams also have high levels of other heavy metals, it is reasonable to assume that if any fish survive in these water, they would be subject to the detrimental effects of zinc. Developing embryos and larvae of the fathead minnow would not survive in large numbers at concentrations above 0.5 mg Zn/L. Therefore, the potential for disrupting an important component of the stream ecosystem exists.

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