8-1984

Toxicological Studies on Aquatic Contaminants Originating from Coal Production and Utilization: The Induction of Tolerance to Silver in Laboratory Populations of Fish and the Chronic Toxicity of Nickel to Fish Early Life Stages

Digital Object Identifier: https://doi.org/10.13023/kwrri.rr.151

Wesley J. Birge
University of Kentucky

Jeffrey A. Black
University of Kentucky

James F. Hobson
University of Kentucky

Albert G. Westerman
University of Kentucky

Research Report No. 151

TOXICOLOGICAL STUDIES ON AQUATIC CONTAMINANTS ORIGINATING FROM COAL PRODUCTION AND UTILIZATION: THE INDUCTION OF TOLERANCE TO SILVER IN LABORATORY POPULATIONS OF FISH AND THE CHRONIC TOXICITY OF NICKEL TO FISH EARLY LIFE STAGES

By

Wesley J. Birge
Principal Investigator

Jeffrey A. Black
Co-Investigator

James F. Hobson
Graduate Assistant

Albert G. Westerman
Terry M. Short
Postdoctoral Research Associates

Project Number: A-090-KY (Completion Report)

Agreement Number: G-844-02 (FY 1983)

Period of Project: July 1983 - August 1984

Water Resources Research Institute
University of Kentucky
Lexington, Kentucky

The research on which this report is based was financed in part by the U.S. Department of the Interior, as authorized by the Water Research and Development Act of 1978 (P.L. 95-467).

August 1984

DISCLAIMER

Contents of this publication do not necessarily reflect the views and policies of the U.S. Department of the Interior, nor does mention of trade or commercial products constitute their endorsement by the U.S. Government.

ABSTRACT

Aquatic toxicity studies were performed on two important coal-derived contaminants, silver and nickel. Silver was investigated with regard to metal-induced tolerance in laboratory populations of the fathead minnow (Pimephales promelas). Fish were exposed to acute silver concentrations following acclimation to sublethal exposures of this metal. Based on median lethal times (LT50), animals which had received 14 days prior exposure to 1.5 and 15 µg Ag/L were three to four times more resistant to silver than were previously unexposed organisms. This metal-induced resistance was not a sustained response. After organisms which had been acclimated to 15 µg/L had been transferred to clean water for two weeks, LT50 values determined with these animals were statistically indistinguishable from those calculated with non-acclimated control fish. With respect to nickel, a 32-day continuous-flow test was performed with the fathead minnow. Nickel was administered in duplicate at six exposure concentrations ranging from 0.038 to 0.733 mg/L in medium-hard water (100 mg $CaCO_3$/L). Animal test responses included mortality, teratogenesis, and growth. Based on frequencies of mortality after 32 days of continuous exposure, significant effects were recorded at a nickel concentration of 0.120 mg/L, and the no observed effect concentration (NOEC) was determined to be 0.057 mg Ni/L.

DESCRIPTORS: Aquatic Environment; Aquatic Populations; Toxins; Fish; Fish Toxins; Mortality; Silver; Nickel

IDENTIFIERS: Teratogenesis; Coal-Derived Contaminants

ACKNOWLEDGMENTS

The authors would like to thank personnel from the EPA Newtown Fish Toxicology Laboratory, Cincinnati, Ohio, for their cooperation in providing test organisms for this study. We also extend appreciation to Scott Taylor for his very capable technical assistance. We gratefully thank Connie Birdsong for preparation of the manuscript. This study represents a portion of a dissertation research project to be submitted in partial fulfillment of the degree of Doctor of Philosophy by James F. Hobson.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

As one consequence of industrialization, numerous and varied environmental problems have been created by the release of heavy metals to aquatic resources. The continued occurrence of acid deposition (1) and the widespread atmospheric contamination by heavy metals (2, 3) indicate that future problems will involve diffuse sources. Propects for further contamination of water resources stem from our increased dependence on coal and other fossil fuels as national energy sources. Freshwater systems constitute the major repositories for most coal-derived compounds, which include upwards of 60 inorganic aquatic pollutants (4). Unlike certain petroleum hydrocarbons that are subject to biodegradation, metals are persistent in the environment. The present project was undertaken to investigate the aquatic toxicity of two heavy metals (i.e., Ag, Ni), characterized as important environmental contaminants which result in part from increased and more diversified utilization of coal. In addition, both metals have appeared on the U.S. Environmental Protection Agency's list of priority pollutants (5).

Initial studies were performed using silver and focused on two principal objectives. The first was to determine if and to what extent fish adapt to chronic metal exposure, and the second was to ascertain the degree to which animals maintain acclimation-induced changes in metal tolerance. To achieve the first objective, populations of fathead minnows (Pimephales promelas) were acclimated to silver at two sublethal exposure concentrations and subsequently subjected to 96-h toxicity testing with the same metal. The level of acute toxicity observed with these animals was compared to that obtained with fish maintained in uncontaminated control water. To accomplish the second objective, fish which had been exposed to sublethal silver concentrations were deacclimated in control water and then used in acute toxicity tests with silver. This work was an important extension of similarly-oriented research performed earlier in our laboratory on other metallic contaminants (i.e., Cd, Cu, Zn; 6). The extent to which fish can acclimate

to chronic exposure to heavy metals has not been fully investigated. These studies are deemed essential, as resulting changes in tolerance may be of considerable importance in hazard assessment, particularly when applied to energy technologies and the development of site-specific criteria for aquatic life.

The second phase of this project involved determining a no observed effect concentration (NOEC) for nickel administered to sensitive life-cycle stages of fish. To accomplish this objective, an extensive 32-day continuous-flow test was performed on eggs and early larvae of the fathead minnow. Test responses included mortality, teratogenesis, and growth of newly hatched organisms. This study was conducted to aid in establishing a numerical criterion for nickel with respect to chronic effects on aquatic biota.

CHAPTER II

RESEARCH PROCEDURES

Selection of animal species

All toxicity tests described in this study were performed using the fathead minnow, Pimephales promelas. This species is an important component in aquatic food chains (7) and has a wide distribution throughout the central and northeastern United States (8). In addition, the fathead minnow has been used extensively in aquatic toxicity testing (9). Juvenile fish for the silver acclimation studies and freshly fertilized embryos for the early life stage tests on nickel were obtained from the EPA Aquatic Toxicology Laboratory, Newtown, Ohio.

Selection of toxicants

Nickel and silver were selected for study in this investigation due to their occurrence in coal and flyash effluents (Table 1) and for their importance as environmental contaminants. Both metals have been designated by the U.S. Environmental Protection Agency as priority pollutants (5). Analytical standards and toxicant stock solutions used for testing were based on metal content and prepared from reagent-grade nickelous chloride ($NiCl_2 \cdot 6H_2O$) or certified ACS grade silver nitrate ($AgNO_3$). These compounds were obtained from the J.T. Baker Chemical Company and from the Fisher Scientific Company, respectively.

Acclimation experiments with silver on juvenile stages of the minnow

Studies were conducted to investigate the differing degrees of silver tolerance exhibited by juvenile stages of fathead minnows following acclimation to sublethal silver concentrations for varying periods of time. Fish were held in charcoal-filtered tap water for a minimum of two weeks prior to silver exposure. General characteristics for this water, given as mean values $\pm$ standard deviations, were 7.6 $\pm$ 0.2 for pH, 7.6 $\pm$ 0.3 mg/L for dissolved oxygen, 342 $\pm$ 82 µmhos/cm for conductivity, 141 $\pm$ 13 mg $CaCO_3$/L

for hardness, and 85 ± 10 mg $CaCO_3$/L for alkalinity. The above analyses were performed at 2- to 3-day intervals during acclimation, using a Corning pH meter (model 110), YSI oxygen meter (model 51A), Radiometer Conductivity meter (model DCM 2E), the EDTA titrimetric procedure (10), and the methyl orange titrimetric procedure (10), respectively.

Following the holding period, separate populations of fathead minnows were exposed to 0, 1.5 and 15 µg Ag/L for 14 days in 200-liter fiberglass tanks. A continuous flow of charcoal-filtered tap water was supplied to each tank. Silver solutions were prepared in deionized water, acidified to pH of 2, and delivered to acclimation tanks via Sage syringe pumps (Model 355) fitted with double-ground glass syringes. Exposure levels were regulated by varying the concentration of toxicant delivered from the syringe pump. Dilution water flow rates were monitored using Gilmont flow meters and timed volumetric measurements. The flow rate was set at 9 L/h, giving one volume addition every 24 hr. The loading capacity of the acclimation tanks was under the limit of 1 g fish/L water as recommended by ASTM (11). Fish were fed during acclimation and maintained on a 16-h light/8-h dark photoperiod. Water temperature, as measured by a mercury thermometer, ranged between 20.5 and 23.0°C.

After 0, 7, and 14 days, fish were removed from each acclimation and control tank and used in static 96-hr toxicity tests with silver. Five toxicant concentrations (i.e., 15, 25, 30, 40, 50 µg Ag/L) and a control were used in each acute test. The acute test chamber consisted of a 20-liter glass aquarium divided into two subchambers by a chemically inert, perforated plastic screen. This chamber was designed to allow the acute administration of identical silver exposure concentrations to two separate groups of animals from different acclimation conditions. Moderate aeration was provided to each chamber, and temperature was maintained at 22 ± 2°C. Silver was added to each test chamber at the initiation of the 96-h experiment, and exposure concentrations for subsequent analysis of dose-response data were based on measurements taken at this time. The stability of silver in the dilution water was monitored throughout all acute toxicity tests. Based on analyses performed on a Perkin-Elmer atomic absorption spectrophotometer (model 503) equipped with a graphite furnace (model HGA 2100), exposure levels were within 5% of the calculated concentrations at time 0 and decreased by about 50% after 96h.

Fish not included in the acute toxicity tests, but which had been acclimated to sublethal silver concentrations for 14 days, were then exposed to clean dilution water only. Seven and 14 days after termination of silver exposure, these organisms were used in acute tests with silver in order to determine if any loss of tolerance occurred with time. Mortality data from the 96-h acute tests were used to calculate median lethal concentrations (LC50) and median lethal times (LT50), following the methods of Finney (12) and Litchfield (13), respectively. Statistical comparisons between the LC50 or LT50 values were accomplished using the procedures of Sprague and Fogels (14).

Fathead minnow early life stage test with nickel

A 32-day early life stage test with the fathead minnow was performed on nickel, closely following the procedures outlined by ASTM (15), Mount (16), and Stephan (17). Further details of these methods are given below.
Test system. Tests were performed using a continuous-flow system similar to that described previously (18, 19). The experiment was conducted in a temperature-regulated environmental room, and photoperiod was maintained at 16-light and 8-dark h/day. Graduated flow from a syringe pump (Sage model 355) was used to administer nickel to mixing chambers situated ahead of the exposure chambers. Reconstituted dilution water, as described below, was delivered from aerated Nalgene carboys to the mixing chambers by regulated flow from a peristaltic pump (Brinkmann model IP 12). Solutions from the syringe and peristaltic pump channels were mixed by magnetic stirrers and delivered from the mixing units to the test chambers at a rate of 200 mL/h under moderate positive pressure. Exposure levels were regulated by varying concentrations of toxicant delivered from the syringe pump. Flow rates from both syringe and peristaltic pumps were monitored using timed volumetric measurements.
Test conditions. Tests were performed using six nickel exposure concentrations, with controls. All tests were performed in duplicate. Initially, 35 eggs were placed in a 500-mL Pyrex exposure chamber. Construction and design of this test chamber have been described in earlier publications (18, 19). The eggs were examined daily and dead organisms removed accordingly. Hatching of most animals began 4 days after initiation of the test and was completed by the fifth day. One-day-old larvae were then transferred from

each egg exposure chamber to a corresponding one-liter Pyrex beaker. A glass inlet tube was positioned approximately 4 cm above the bottom of each container, and an effluent siphon was situated 3 cm below the top. At the time of fish transfer, the feeding regime was initiated. For the first 9 days posthatching, fish in each container were fed live, newly-hatched brine shrimp 4 times per day at 4-h intervals. Beginning on the tenth day, the frequency of feeding was reduced to 3 times daily. Feeding was terminated 24 h prior to the end of the experiment. The chambers were cleaned of accumulated debris by suction every second day. The experiment was terminated 32 days after the introduction of fertilized eggs (i.e., ~28 days posthatching). Wet weight and standard length were determined on each live fish at the end of the experiment.

Reconstituted dilution water was prepared by adding reagent-grade salts (i.e., $NaHCO_3$, $CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl) to distilled deionized water. This water was formulated to meet the medium-hard water criteria (i.e., hardness, 100 mg $CaCO_3$/L), as described by Peltier and Weber (20). Water quality characteristics were monitored in all exposure chambers at 2- to 3-day intervals, employing the procedures described above, and test conditions are summarized in Table 2. Exposure concentrations for nickel were analyzed every 3 days from each chamber, using a Perkin-Elmer atomic absorption spectrophotometer equipped with a graphite furnace. Test responses (i.e., mortality, wet weight, standard length) were analyzed for statistical significance using the single-factor ANOVA/least-squares means test (21).

Table 1. Concentrations of toxic elements in coal and ash pond effluent.[1]

Element	Coal (mg/kg)	Ash Pond Effluent (mg/L)
Aluminum	4300 - 30400	1.4 - 7.2
Arsenic	0.5 - 106	0.005 - 0.038
Barium	33 - 150	0.1 - 0.3
Beryllium	0.2 - 31	<0.01 - 0.01
Cadmium	0.1 - 65	0.001 - 0.037
Chromium	0.3 - 610	0.004 - 0.067
Copper	1.8 - 185	0.01 - 0.31
Lead	4 - 218	0.01 - 0.06
Magnesium	100 - 2500	0.4 - 14
Manganese	6 - 181	0.01 - 0.58
Mercury	0.01 - 1.6	0.0002 - 0.038
Nickel	0.4 - 104	0.05 - 1.1
Selenium	0.4 - 7.7	0.002 - 0.065
Silver	0.03 - 0.19	<0.01 - 0.01
Zinc	15 - 5600	0.03 - 1.51

[1]Data taken from Chu, et al. (22), Pellizzari (23), Torrey (24).

Table 2. Test conditions observed during a continuous-flow early life stage test with fathead minnows exposed to nickel.

	Mean ± S.D.	n
Temperature (°C)	25.1 ± 0.3	476
Dissolved oxygen (mg/L)	6.7 ± 1.2	196
pH	7.4 ± 0.2	203
Alkalinity (mg/L as $CaCO_3$)	54.6 ± 8.2	203
Hardness (mg/L as $CaCO_3$)	102.6 ± 8.8	203
Conductivity (µmhos/cm)	304.0 ± 27.7	196

CHAPTER III

RESULTS AND DISCUSSION

Results are presented below for the two principal segments of this project, involving fish acclimation studies with silver and a fish early life stage experiment with nickel.

Acclimation-induced responses to silver by the fathead minnow

Studies were undertaken to investigate the extent of silver tolerance exhibited by laboratory populations of fathead minnows following 7 and 14 days of acclimation to two different sublethal silver concentrations. Survival frequencies during the entire 14-day period were 100%, 95%, and 96% for acclimation concentrations of 0, 1.5, and 15 µg Ag/L, respectively. Measured values ranged from 0.71 to 1.25 µg/L for the 1.5 µg/L nominal concentration and from 10.29 to 15.29 µg/L for the 15 µg/L nominal exposure level. However, for discussion purposes nominal concentrations will be used in describing experimental results.

As described under Research Procedures, 96-h acute toxicity tests were performed with silver on populations of fish which had had no previous exposure to silver and to fish pre-exposed to silver for 7 and 14 days. These tests were conducted to provide an indication of acclimation-induced tolerance. According to Duncan and Klaverkamp (25), tolerance is defined as the ability of an organism to survive a specific set of environmental conditions indefinitely and was determined in the present study by comparing median lethal concentrations (LC50). Resistance is described by the same investigators as the ability of an organism to survive for a limited period of time under conditions that would eventually cause death and was determined by comparing median lethal times (LT50). Pretreatment with 15 µg Ag/L resulted in a significant increase in acclimation-induced tolerance to silver. After 14 days of exposure to this acclimation concentration, the 96-h LC50 value (i.e., 46.4 µg/L) was significantly greater than that determined with previously unexposed control animals (i.e. 37.2 µg/L; Table 3). Acclimation-induced responses were even more apparent when comparisons were performed using LT50 values (Table 4). A significant increase

Table 3. Acute toxicity of silver, expressed as LC50 values, to fathead minnows acclimated and deacclimated to silver.

Calculated Acclimation Concentration (µg Ag/L)	Percent Survival of Acclimated Population	96-h LC50 (µg Ag/L) with 95% Confidence Limits Given Parenthetically[1]				
		Acclimation Period (days)			Deacclimation Period (days)	
		0	7	14	7	14
0	100	30.0 (28.4-32.4)	36.6 (31.3-44.3)	37.2 (23.3-43.4)	18.3 (11.2-22.9)	36.0 (29.5-39.9)
1.5	95	(--)	42.6 (35.4-63.8)	41.3 (25.8-50.1)	24.5 (22.8-37.1)	(--)
15	96	(--)	45.6 (34.8-59.8)	46.4* (12.5-53.6)	29.1* (9.5-30.6)	31.0 (--)

[1]Asterisk denotes values that are statistically different ($p<0.05$) from controls, as determined by Sprague and Fogels (14).

Table 4. Acute toxicity of silver, expressed as LT50 values, to fathead minnows acclimated and deacclimated to silver.

Calculated Acclimation Concentration (µg Ag/L)	Percent Survival of Acclimated Population	96-h LC50 (µg Ag/L) with 95% Confidence Limits Given Parenthetically[1]				
		Acclimation Period (days)			Deacclimation Period (days)	
		0	7	14[2]	7	14
0	100	18.0	16.0	4.5	8.3	14.0
		(--)	(13.3-19.3)	(3.0-6.8)	(6.8-10.8)	(11.4-17.2)
1.5	95	(--)	18.5	15.1*	9.4	(--)
			(11.6-29.4)	(8.5-26.7)	(4.5-19.8)	
15	96	(--)	24.5*	17.5*	16.0*	12.0
			(21.7-27.7)	(14.1-21.7)	(11.7-21.9)	(8.9-17.5)

[1]Asterisk denotes values that are statistically different ($p<0.05$) from controls, as determined by Sprague and Fogels (14).

[2]Calculation of LT50's were based on a nominal exposure concentration of 50 µg Ag/L at this time and on 40 µg Ag/L at other time periods.

in fish resistance to silver was observed for the acclimation concentration of 1.5 µg/L after 14 days and for the 15 µg/L exposure level after both 7 and 14 days. For the latter, the LT50 value was approximately 4 times greater than that observed for control animals.

The acclimation-induced tolerance/resistance to silver did not appear to be a sustained response. For example, when fish which had been exposed to silver at 15 µg/L for 14 days were returned to control water for 2 weeks, both LC50 and LT50 values were no longer significantly greater than those calculated with controls (Tables 3, 4). Moreover, at the lower acclimation concentration of 1.5 µg/L, resistance of fish to silver was lost after only one week in control water (Table 4).

The trends in increasing tolerance/resistance with the period of sublethal exposure to silver are illustrated in Figures 1 and 2. As given in these figures, a ratio was established between the LC50 or LT50 values determined in tests with acclimated animals and LC50's or LT50's calculated with organisms having no previous exposure to silver. At both the 1.5 µg/L (Figure 1) and 15.0 µg/L (Figure 2) exposure concentrations, acclimation time correlated inversely with acute toxicity. Furthermore, significantly less acute toxicity was observed for organisms exposed to the higher of the two acclimation concentrations. However, as noted above, acclimation-induced tolerance/resistance generally decreased rapidly after the discontinuation of sublethal exposure. In earlier acclimation studies on cadmium and copper with the same species of fish, similar results were observed (6, 26).

There is some evidence that genetic factors rather than sublethal toxicant exposures may be responsible for organismal tolerance/resistance to environmental contaminants. For example, laboratory populations of fathead minnows demonstrated a greater toxic response to hydrogen sulfide than did natural populations, although neither group had any previous exposure to this compound (27). The relaxation of normal selection pressures in cultured populations and resultant declines in vigor may be attributed to the greater susceptibility of laboratory populations to toxicant stress. Nevertheless, in the present investigation, acclimation-induced tolerance or resistance by fathead minnows to silver was demonstrated under laboratory conditions. In addition, fish lost their acquired tolerance to silver after transfer to control water. This may indicate that there is a biological mechanism in the fish which functions to mitigate the toxic effects of metals and that a

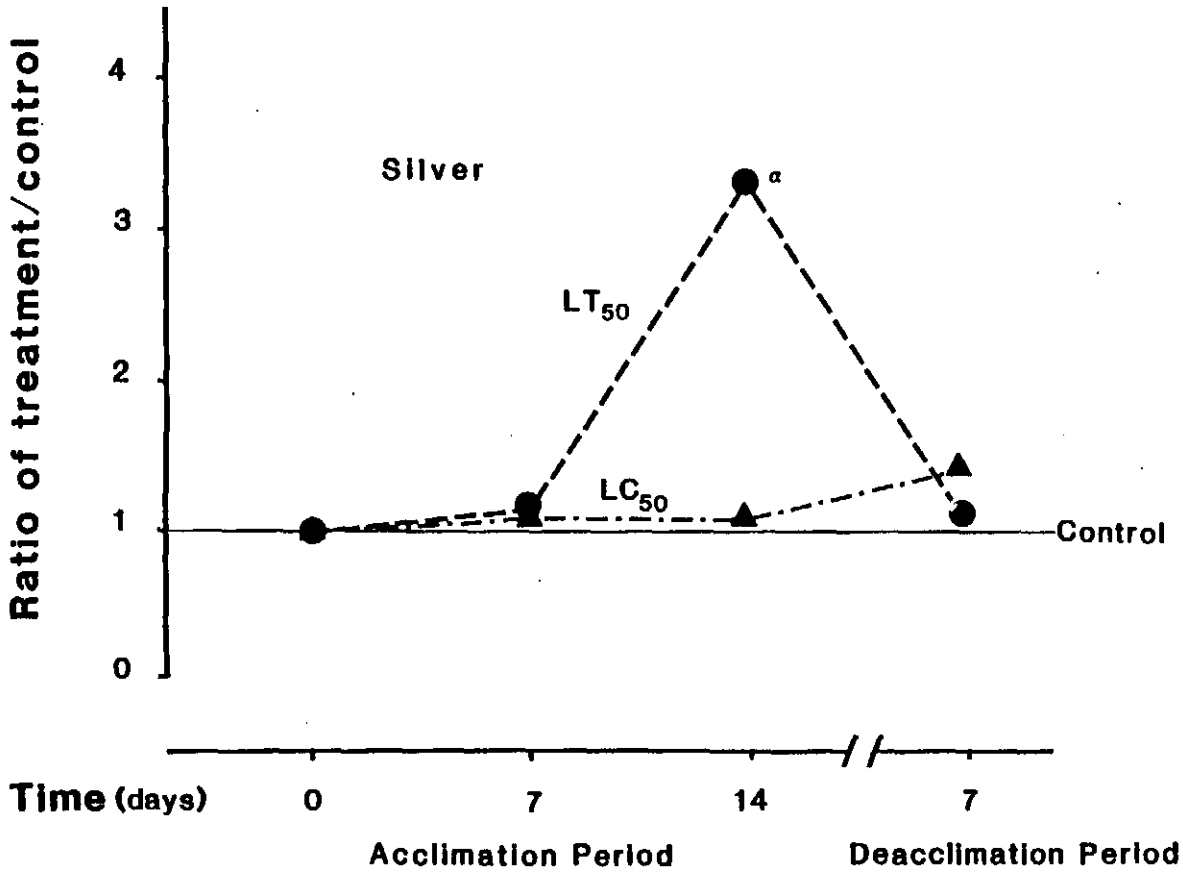

Figure 1. Acute toxicity of silver to fathead minnow populations which had been exposed to 1.5 µg Ag/L for 14 days and control water only for the subsequent 7 days. Each point on the graph represents the ratio of the LC_{50} or LT_{50} value calculated with acclimated and deacclimated fish to the LC_{50} or LT_{50} calculated with non-acclimated control animals. Significant differences from controls are indicated by the letter "a".

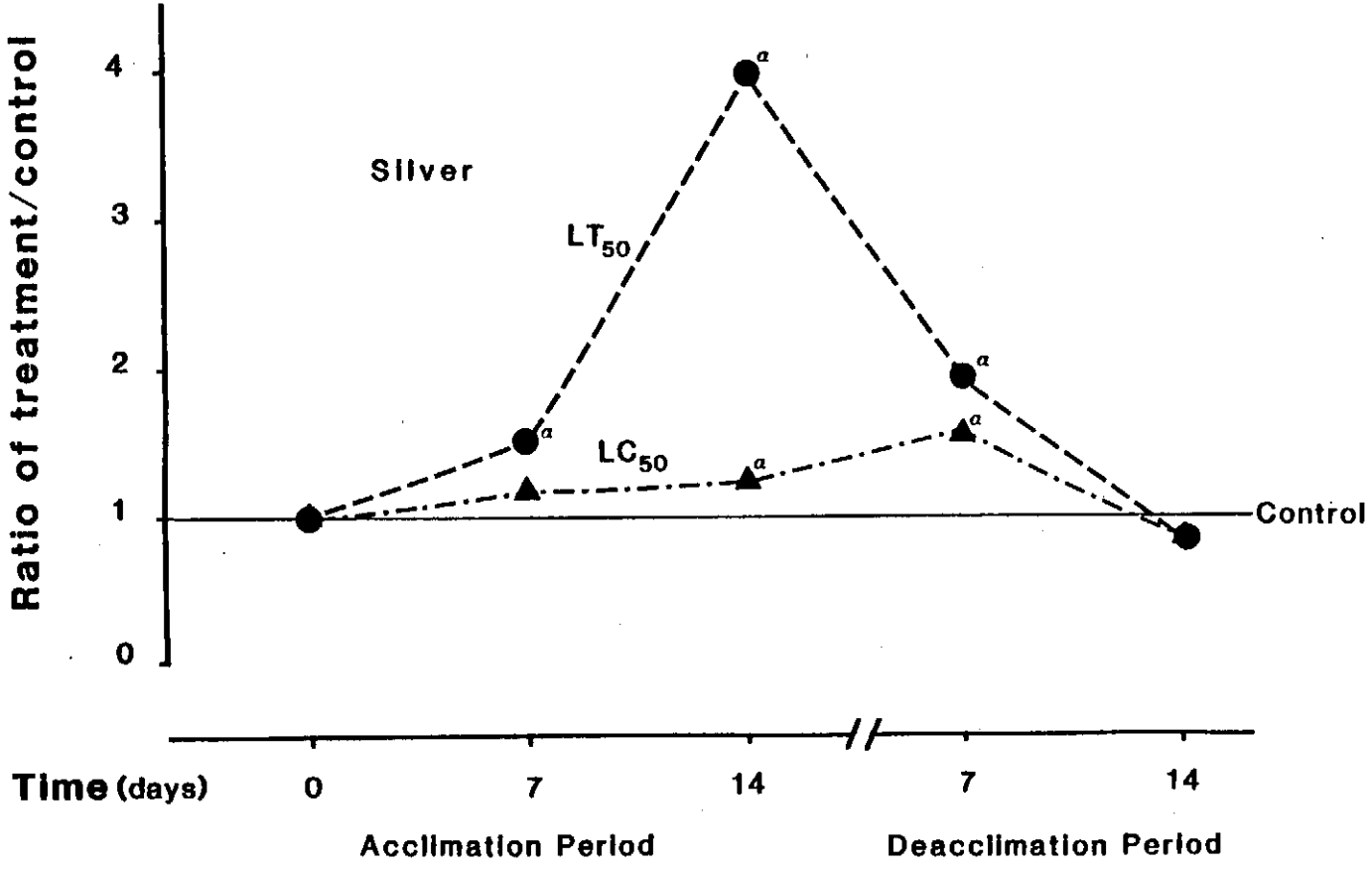

Figure 2. Acute toxicity of silver to fathead minnow populations which had been exposed to 15.0 µg Ag/L for 14 days and control water only for the subsequent 14 days. Each point on the graph represents the ratio of the LC_{50} or LT_{50} value calculated with acclimated and deacclimated fish to the LC_{50} or LT_{50} calculated with non-acclimated control animals. Significant differences from controls are indicated by the letter "a".

minimum level of metal is required to activate that mechanism. Several earlier investigations have demonstrated that exposure of fish to low concentrations of metal stimulates the synthesis of metallothionein, a protein which binds with metals to produce an inactive complex (26, 28-31).

With respect to water resource considerations, the adaptation by certain fish to heavy metals (e.g., Ag, Cd, Cu) can be viewed as beneficial. For example, in the case of an episodic discharge, biological adaptation to a contaminant or a mixture of contaminants might provide protection for some species. Conversely, the presence of tolerant organisms may create possible deleterious effects to the total environment through their potential for increased bioconcentration of toxicants (6, 26). In addition, natural variations in the assimilative capacity of organisms may favor the more tolerant species and, therefore, result in an ecological imbalance. Underestimating the impact of environmental contaminants undoubtedly will result in unacceptable and costly ecological degradation. On the other hand, overestimating potential hazards will place an undue economic burden on the implementation of new technologies. A better understanding of the concept of acclimation-induced tolerance or resistance is essential if accurate assessments on the effects of environmental pollutants are to be achieved.

Effects of nickel on early life stages of the fathead minnow

Eggs and larvae of the fathead minnow were exposed to nickel in a 32-day continuous-flow test to estimate a no-effect concentration for this metal to fish early life stages. This experiment was performed in duplicate at six exposure concentrations selected to produce a geometric series in which any given concentration was at least 50% of the next higher concentration. The continuous-flow system provided good reproducibility and accuracy of nickel exposure concentrations. With only one exception, actual mean nickel concentrations averaged for both replicates were within 5 to 10% of the calculated values (Table 5).

Mortality of fathead minnow embryos determined at hatching for both replicates ranged from 4% to 17% at nickel concentrations of 0.038 to 0.733 mg/L (Table 6). Although populations were examined daily for dead larvae, mortality data were reported here at 11, 18, 25, and 32 days after the

Table 5. Mean exposure concentrations of nickel in a continuous-flow early life stage test with fathead minnows.

Replicate	Calculated Concentration (mg/l)	Actual Concentration[1] (mg/l)	S.D.	n
I	0.04	0.037	0.018	11
	0.07	0.044	0.012	11
	0.13	0.124	0.016	11
	0.22	0.233	0.023	11
	0.40	0.456	0.045	11
	0.71	0.789	0.089	11
II	0.04	0.039	0.009	11
	0.07	0.069	0.011	11
	0.13	0.116	0.025	11
	0.22	0.239	0.026	11
	0.40	0.390	0.083	11
	0.71	0.676	0.061	11
I & II	0.04	0.038	0.014	22
	0.07	0.057	0.012	22
	0.13	0.120	0.021	22
	0.22	0.236	0.025	22
	0.40	0.423	0.064	22
	0.71	0.733	0.075	22

[1]Control chambers were sampled at regular intervals and nickel concentrations were always below the detection limit of 0.005 mg/l.

Table 6. Toxicity of nickel to fathead minnows in a continuous-flow early life stage test.

Replicate	Actual Concentration (mg/l)	Percentage of Dead Organisms					
		Day 0	Day 4-5[1]	Day 11	Day 18	Day 25	Day 32
I	Control	0	3	6	9	9	11
	0.037	0	6	11	11	11	11
	0.044	0	6	9	9	9	9
	0.124	0	6	11	14	17	20
	0.233	0	23	54	54	54	60
	0.456	0	14	46	60	60	66
	0.789	0	20	57	80	89	89
II	Control	0	3	6	9	9	9
	0.039	0	3	6	9	9	9
	0.069	0	3	6	6	9	9
	0.116	0	3	3	11	14	17
	0.239	0	9	31	34	43	46
	0.390	0	9	31	57	66	66
	0.676	0	14	40	66	80	89
I & II	Control	0	3	6	9	9	10
	0.038	0	4	9	10	10	10
	0.057	0	4	7	7	9	9
	0.120	0	4	7	13	16	19
	0.236	0	16	43	44	49	53
	0.423	0	11	39	59	63	66
	0.733	0	17	49	73	84	89

[1]Survival was calculated at hatching which began on Day 4 and was completed by Day 5.

initiation of the test, following procedures of Mount (16) and Stephan (17). Based on the sample size of 70 for both replicates at each concentration, mortality frequencies after 32 days were 10, 19, 53, 66, and 89% at 0.038, 0.120, 0.236, 0.423, and 0.733 mg Ni/L, respectively (Table 6).

Data from each duplicate test were combined and statistically analyzed to determine the lowest nickel concentrations which produced significant frequencies of mortality at hatching and after 32 days of continuous exposure. In addition, analyses were performed on the frequencies of mortality plus surviving terata at both time periods (Table 7). Terata were defined as organisms affected by grossly anomalous defects likely to result in eventual mortality (19). By comparing the frequencies at 32 days for mortality with data for mortality plus terata, it is evident that most anomalous organisms had died by the end of the test (Table 7). Significant effect levels ($p<0.05$) were determined to be 0.236 and 0.120 mg Ni/L at hatching and 32 days, respectively.

In addition to the above responses, the effects of nickel on growth of fathead minnow larvae were examined. Both wet weight and standard length measurements were determined on all live organisms after 32 days of exposure (Tables 8, 9). Taking combined replicates, mean wet weights ranged from 0.1216 g for control organisms to 0.0948 g for fish subjected to 0.733 mg Ni/L (Table 8). No significant reductions in weight were observed at any exposure concentration ($p<0.05$). Mean standard length values varied from 19.4 mm for controls to 17.6 mm for fish exposed to 0.733 mg Ni/L, and the latter concentration was found to produce a statistically significant effect ($p<0.01$).

In summary, the highest no observed effect concentration (NOEC) for nickel was 0.057 mg/L, when based on frequencies of mortality plus live terata after 32 days of continuous exposure to early life stages of the fathead minnow. This value compared closely to the lower end of the maximum acceptable toxicant concentration range (0.068-0.132 mg Ni/L) estimated for fathead minnows exposed in water having a hardness of 20 mg $CaCO_3$/L (32, 33). Using the same species, Lind, _et al._ in U.S. EPA (34) determined a chronic value for nickel of 0.109 mg/L in soft water (_i.e._, 45 mg $CaCO_3$/L). Thus, it appears from the above data that nickel concentrations of 0.050 to 0.100 mg/L may not significantly impair the reproduction of warmwater fish species residing in soft to medium-hard waters.

Coal ash effluents, some of which contain nickel in excess of 1.0 mg/L (Table 1), could pose a potential threat to the propagation of certain aquatic biota (34). Whether or not fish populations can acquire a tolerance to nickel similar to that observed for silver and other heavy metals (6, 26) remains uncertain. Because of its importance as an aquatic contaminant in water resources (34, 35), nickel appears to be a prime candidate for further studies designed to investigate acclimation-induced responses in fish.

Table 7. Statistical analysis of response frequencies observed in nickel early-life stage toxicity tests with fathead minnows.

Observation Time	Actual Concentration (mg/l)	Percent Mortality	Statistical Significance[1]	Percent Mortality + Terata	Statistical Significance[1]
At hatching	Control	3	-	3	-
	0.038	4	ns	7	ns
	0.057	4	ns	7	ns
	0.120	4	ns	7	ns
	0.236	16	<0.05	34	<0.05
	0.423	11	ns	27	<0.05
	0.733	17	<0.05	46	<0.05
At 32 days	Control	10	-	10	-
	0.038	10	ns	11	ns
	0.057	9	ns	11	ns
	0.120	19	<0.05	19	<0.05
	0.236	53	<0.05	54	<0.05
	0.423	66	<0.05	66	<0.05
	0.733	89	<0.05	89	<0.05

[1]Statistical analyses were performed using a single-factor ANOVA/least-squares means test (21) for combined replicates.

Table 8. Statistical analysis of wet weight data calculated for fathead minnows exposed to nickel in a continuous-flow early life stage toxicity test.

Actual Concentration (mg/L)	Mean Wet Weight (g/fish)	S.D.	Statistical Significance[1]
Control	0.1216	0.0317	-
0.038	0.1179	0.0340	ns
0.057	0.1215	0.0388	ns
0.120	0.1130	0.0324	ns
0.236	0.1059	0.0197	ns
0.423	0.1044	0.0276	ns
0.733	0.0948	0.0211	ns

[1]Statistical analyses were performed using a single-factor ANOVA/least-squares means test (21) for combined replicates.

Table 9. Statistical analysis of standard length data calculated for fathead minnows exposed to nickel in a continuous-flow early life stage toxicity tests.

Actual Concentration (mg/L)	Mean Standard Length (mm/fish)	S.D.	Statistical Significance[1]
Control	19.39	1.78	-
0.038	19.12	1.96	ns
0.057	19.33	2.08	ns
0.120	18.99	1.77	ns
0.236	18.38	0.91	ns
0.423	18.25	1.42	ns
0.733	17.56	1.49	<0.01

[1]Statistical analyses were performed using a single-factor ANOVA/least-squares means test (21) for combined replicates.

CHAPTER IV

CONCLUSIONS

Aquatic toxicity studies were undertaken to observe the responses of laboratory populations of fathead minnows to acutely toxic concentrations of silver, following acclimation to two sublethal exposures of this metal (i.e., 1.5 and 15 µg/L). On the basis of 96-h LC50 values, animals which had received 14-days prior exposure to 15 µg Ag/L were significantly more tolerant to silver than were previously unexposed organisms. Based on median lethal times (LT50), populations of organisms which had been exposed to 1.5 µg Ag/L for 14 days were significantly more resistant to silver than were control fish. Following exposure to the higher acclimation concentration of 15 µg Ag/L, fish developed significant resistance after only 7 days. The increased tolerance and resistance of organisms acclimated to sublethal silver concentrations point to the induction of an adaptive mechanism sufficient to mitigate the toxic effects of exposure to subsequently higher levels of metal.

After the acclimated fish were placed in uncontaminated water for 14 days, subsequent LT50's and LC50's were not significantly different from the lethal values calculated for control organisms. Thus, the metal-induced tolerance was not a sustained response.

With respect to natural water resources, the adaptation by certain fish to heavy metals may be viewed as a beneficial, protective occurrence. However, tolerant organisms may create possible deleterious effects through their potential for increased bioconcentration of toxicants. A better understanding of the concept of acclimation-induced responses is essential to achieve accurate assessments of the effects of environmental contaminants.

The second phase of this project involved the determination of a no observed effect concentration (NOEC) for nickel administered to sensitive life-cycle stages of fish. To accomplish this, a 32-day continuous-flow early life stage test was performed with the fathead minnow in medium-hard water (100 mg $CaCO_3$/L). Nickel was administered at mean exposure concentrations of 0.038, 0.057, 0.120, 0.236, 0.423, and 0.733 mg/L, and mortality, teratogenesis, and

growth were used to assess toxic effects. The most sensitive endpoint was mortality of test organisms after 32 days of continuous exposure, and significant responses were observed at a nickel concentration of 0.120 mg/L. The NOEC was determined to be 0.057 mg/L. Based on this value and other chronic toxicity data, a nickel criterion in the range of 50 to 100 µg/L would most likely be protective in soft to medium hard water for the fathead and other piscine species of similar sensitivity. This range is in agreement with the freshwater values for nickel recommended by the U.S. EPA (34). However, the reproductive potential of other, more sensitive species such as the rainbow trout (35), certain amphibians (34), and certain invertebrates (34) may be affected at concentrations below 50 µg/L. Further studies with nickel, including those relating to acclimation-induced responses, would seem to be desirable, especially with respect to the establishment of site-specific criteria or effluent limitations for this metal.

REFERENCES

1. Haines, T.A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: A review. Trans. Am. Fish. Soc., 110: 669-707.

2. Hirao, Y. and C.C. Patterson. 1974. Lead aerosol pollution in the high Sierra overrides natural mechanisms which exclude lead from a food chain. Science, 184:989-992.

3. Van Loon, J.D. and J.R. Beamish. 1977. Heavy metal contamination by atmospheric fallout of several Flin Flon area lakes and the relation to fish populations. J. Fish. Res. Board Can., 34: 899-906.

4. Vitez, B. 1976. Trace elements in flue gasses and air quality criteria. Power Eng., 1: 55-60.

5. Keith, L.H. and W.A. Telliard. 1979. Priority pollutants I - A perspective view. Environ. Sci. Technol., 13: 416-423.

6. Birge, W.J., W.H. Benson, and J.A. Black. 1983. The Induction of tolerance to heavy metals in natural and laboratory populations of fish. Research Rept. No. 141, U.S.D.I., Water Resources Research Institute, Lexington, Kentucky. 26 p.

7. Benoit, D.A., F.A. Puglisi, and D.L. Olson. 1982. A fathead minnow (Pimephales promelas) early life stage toxicity test method evaluation and exposure to four organic chemicals. Environmental Pollution (Series A), 28: 189-197.

8. Clay, W.M. 1975. The Fishes of Kentucky. Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky. 416 p.

9. Manner, H.W. and C.M. Dewese. 1974. Early embryology of the fathead minnow (Pimephales promelas Rafinesque). The Anatomical Record, 180: 99-109.

10. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard Methods for the Examination of Water and Wastewater, 14th Ed. American Public Health Association, Washington, D.C. 1193 p.

11. American Society for Testing and Materials. 1981. Annual Book of ASTM Standards, Part 31, Water. American Society for Testing and Materials, Philadelphia, Pa. 1490 p.

12. Finney, D.J. 1971. Probit Analysis, Third Edition. Cambridge Press, New York. 333 p.

13. Litchfield, J.T., Jr. 1949. A method for rapid graphic solution of time-percent effect curves. J. Pharmacol. Exp. Ther., 94: 399-408.

14. Sprague, J.B. and A. Fogels. 1977. Watch the Y in bioassay. In Proceedings of the 3rd Aquatic Toxicity Workshop, Halifax, N.S., Nov. 2-3, 1976, Environmental Protection Service Technical Report No. EPA-5-AR-77-1, U.S. Environmental Protection Agency. pp. 107-118.

15. American Society for Testing and Materials. 1980. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. In Annual Book of ASTM Standards, Designation E 729, American Society for Testing and Materials, Philadelphia, Pa. pp. 1-25.

16. Mount, D.I. 1980. Tentative guidelines for flow-through early life stage toxicity tests with fathead minnows for use in the U.S. EPA, OTS-ORD Round Robin Test. In Proceedings of the Seminar on Biological Monitoring and Its Use in the NPDES Permit Program, EPA-600/9-80-026, U.S. Environmental Protection Agency, Cincinnati, Ohio. pp. 62-68.

17. Stephan, C.E. 1981. Draft - Proposed ASTM Standard Practice for Conducting Early-Life-Stage Tests with Fishes. Appendix E. Fathead Minnow (Pimephales promelas Rafinesque). 6 p.

18. Birge, W.J., J.A. Black, J.E. Hudson, and D.M. Bruser. 1979. Embryo-larval toxicity tests with organic compounds. In Aquatic Toxicology, L.L. Marking and R.A. Kimerle, eds., Special Technical Publication 667, American Society for Testing and Materials, Philadelphia, Pa. pp. 131-147.

19. Birge, W.J., J.A. Black, and B.A. Ramey. 1981. The reproductive toxicology of aquatic contaminants. In Hazard Assessment of Chemicals - Current Developments, J. Saxena and F. Fisher, eds., Academic Press, New York. pp. 59-115.

20. Peltier, W. and C.I. Weber. 1983. Draft. Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency. Cincinnati, Ohio. 200 p.

21. Freund, R.J. and R.C. Littell. 1981. SAS for Linear Models. SAS Institute, Inc., Cary, N.C. 231 p.

22. Chu, T.J., R.J. Ruane, and P.A. Krenkel. 1978. Characterization and reuse of ash pond effluents in coal-fired power plants. J. Water Pollut. Cont. Fed., 50: 2494-2508.

23. Pellizzari, E.D. 1978. Identification of Components of Energy-Related Wastes and Effluents. EPA-600/7-78-004, U.S. Environmental Protection Agency, Washington, D.C. 500 p.

24. Torrey, S. 1978. Trace Contaminants from Coal. Noyes Data Corp., Park Ridge, N.J. 294 p.

25. Duncan, D.A. and J.F. Klaverkamp. 1983. Tolerance and resistance to cadmium in white suckers (Catostomus commersoni) previously exposed to cadmium, mercury, zinc, or selenium. Can. J. Fish. Aquat. Sci. 40: 128-138.

26. Benson, W.H. and W.J. Birge. Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations. Environ. Tox. Chem. In press.

27. Smith, L.L., Jr., D.M. Oseid, I.R. Adelman, and S.J. Broderium. 1976. Effect of hydrogen sulfide on fish and invertebrates. Part I - Acute and chronic toxicity studies. EPA-600/3-76-062, U.S. Environmental Protection Agency.

28. Nöel-Lambot, F., Ch. Gerday, and A. Disteche. 1978. Distribution of Cd, Zn and Cu in liver and gills of the eel Anguilla anguilla with special reference to metallothioneins. Comp. Biochem. Physiol., 61C: 117-187.

29. Bouquegneau, J.M. 1979. Evidence for the protective effect of metallothioneins against inorganic mercury injuries to fish. Bull. Environm. Contam. Toxicol., 23: 218-219.

30. Takeda, H. and C. Shimizu. 1982. Existence of the metallothionein-like protein in various fish tissues. Bull. Japan. Soc. Sci. Fish., 48: 711-715.

31. Woodworth, J., A.S.A. Evans, and D. Pascoe. 1983. The production of cadmium-binding protein in three species of freshwater fish. Toxicol. Lett., 15: 289-295.

32. Pickering, Q.H. and C. Henderson. 1966. The acute toxicity of some heavy metals to different species of warmwater fishes. Air water Pollut. Int. J., 10: 453-463.

33. Pickering, Q.H. 1974. Chronic toxicity of nickel to the fathead minnow. J. Water Pollut. Control Fed., 46: 760-765.

34. U.S. Environmental Protection Agency. 1980. Ambient Water Quality Criteria for Nickel. EPA-440/5-80-060. Office of Water Regulations and Standards, U.S. EPA, Washington, D.C.

35. Birge, W.J. and J.A. Black. 1980. Aquatic toxicology of Nickel. In J.O. Nriagu, ed, Nickel in the Environment, Wiley, New York. pp. 349-366.