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ACUTE TOXICITY OF SELECTED TOXICANTS TO SIX SPECIES OF FISH

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ACUTE TOXICITY OF SELECTED TOXICANTS TO SIX SPECIES OF FISH

Environmental Research Laboratory
Office of Research and Development
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Toxicity curves relating median lethal concentration to exposure time were of three types. One curve, resembling a rectangular hyperbola, characterized the toxicity of sodium cyanide, while another curve, sigmoid in shape, characterized the toxicity of selenium dioxide. Both types of curves were observed in toxicity tests with sodium pentachlorophenate, sodium arsenite and beryllium sulfate. Linear toxicity curves were recorded for some fish species exposed to selenium dioxide, sodium arsenite and beryllium sulfate, but these were usually encountered when exposure times were less than 96 hr.

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ABSTRACT

The relationship between median lethal concentration and exposure time was determined for five chemicals and up to six species of freshwater fish in a flow-through system. The lowest median lethal concentrations found were 0.114 mg/ℓ for sodium cyanide, 0.118 mg/ℓ for sodium pentachlorophenate, 2.9 mg/ℓ for selenium dioxide, 18.0 mg/ℓ for sodium arsenite, 25.4 mg/ℓ for beryllium sulfate, and greater than 100 mg/ℓ for lead chloride.

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SECTION I
CONCLUSIONS

1. Sodium pentachlorophenate was the most toxic chemical studied, producing some mortality at concentrations less than 100 $\mu g/\ell$, while lead chloride, even at concentrations well above the solubility limit (~ 2 mg/ℓ), was not toxic in the moderately hard, somewhat alkaline diluent water. The order of toxicity was sodium pentachlorophenate, sodium cyanide, selenium dioxide, sodium arsenite, beryllium sulfate, and lead chloride.

2. The order of sensitivity of the test species varied with each toxicant. Brook trout and fathead minnow were generally the most sensitive and goldfish and bluegill the least sensitive. Channel catfish and flagfish were of intermediate sensitivity.

3. Upon prolonged exposure of the various fish species, two main types of toxicity curves, hyperbolic and sigmoid, were observed. The toxicity curve resembling a rectangular hyperbola was found exclusively in toxicity tests of sodium cyanide, but infrequently in toxicity tests of the other compounds. It denoted that acute toxicity ceased at certain toxicant concentrations and exposure times, and was believed to reflect one basic mode of toxicant action. The sigmoid toxicity curve predominated in toxicity tests of all compounds except cyanide and was observed exclusively in tests of selenium dioxide conducted for 336 hr. All species of fish exhibited both types of toxicity curves.

4. Because fish exposed to high concentrations of beryllium produced substantial amounts of mucus, it was postulated that the specimens exposed to this chemical perished mainly from the "mucus coagulation syndrome." Enhanced mucus production was not seen in fish exposed to lead chloride, selenium dioxide, sodium arsenite, sodium cyanide or sodium pentachlorophenate at the concentrations employed.

SECTION II
RECOMMENDATIONS

1. The ultimate shapes of the toxicity curves and the mechanisms of toxic action of some of the chemicals are in doubt and should be further explored.

2. Several approaches for assessing the mechanism of toxicant action should be considered. First, the physiological condition of the test specimens should be established at the onset of toxicant exposure and monitored throughout the toxicity test. The parameters should be selected on the basis of how accurately they assess the state and functional integrity of the organism and how readily they will be accepted and used by aquatic toxicologists and water pollution biologists. Suggested parameters include hematocrit and hemoglobin for determining the functional state of the blood, specifically the presence of any anemia; total lipid, or more specifically saturated and mono-unsaturated fatty acids, for evaluating the fish's capacity to withstand a 14 day or more period of fasting; and possibly serum α- amino nitrogen, to determine the state of amino acid and protein metabolism. Although many variables could be evaluated, in order to be used widely, each must reflect the broadest range of physiological activity possible without requiring excessive investment of time and money. The results can be used to compare LC50 values from toxicity tests performed with different stocks of a fish species in order to determine whether variations in condition may have influenced the results. From the monitoring program, one could determine whether or not the fish became severely weakened in the later stages of exposure as a result of the stresses arising from fasting, handling and confinement.

3. For defining toxicity curves, toxicity tests should be conducted until median lethal thresholds are reached rather than be terminated after predetermined times.

4. It would be informative to determine whether transient exposures of fish to these chemicals can cause latent mortality or delayed physiological dysfunction. Furthermore, the relationship between toxicant concentration and exposure time and the severity of the delayed functional effects should be explored.

5. The relative amounts of hydrolysis or chemical degradation products arising from introduction of the parent compound into the diluent water should be determined and correlated with relative uptake by and distribution in the organisms. Median lethal concentrations should be based on the form of the chemical found to be exerting the predominant toxicity. Chemical studies should be incorporated into the toxicity testing experimental design to resolve these questions.

SECTION III
INTRODUCTION

Water quality criteria for the protection of biotic communities in aquatic environments are based primarily on bioassays or toxicity tests, which determine, as a first order approximation, the effect of a given chemical or substance on a given population of organisms. Acute toxicity tests are the first step in the experimental protocol for determining the highest concentration of a chemical or chemicals which will not affect long-term survival, growth or reproduction of a species, termed the Maximum Acceptable Toxicant Concentration or MATC (Mount and Stephan[1]). As research has accumulated on the acute effects of chemicals on aquatic organisms, it has become apparent that there is a need for standardizing the experimental conditions, for characterizing and defining the levels and states of the toxicant, the water quality variables, and the conditions of the test fish, and for providing more refined analysis of the data (Sprague[2]). As Sprague[2, 3] has noted, a thorough search of the literature or of compendiums such as McKee and Wolf[4] will often show that the toxicity of a particular compound to various aquatic organisms varies up to a thousand-fold. To partially ameliorate the problem, committees have formulated a series of standard testing procedures (Committee on Methods for Toxicity Tests with Aquatic Organisms[5, 6]), to improve on the noteworthy standard procedures developed earlier by Doudoroff et al.[7] and adopted as the standard bioassay method in the United States by the American Public Health Association[8].

Many of the experimental protocols and conditions suggested in the revised procedures have been incorporated into these studies of the acute effects of sodium pentachlorophenate, sodium cyanide, selenium dioxide, sodium arsenite, beryllium sulfate and lead chloride on six species of freshwater fish: fathead minnow (Pimephales promelas Rafinesque), goldfish [Carassius auratus

(Linnaeus)], flagfish (Jordanella floridae Goode and Bean), bluegill (Lepomis macrochirus Rafinesque), channel catfish [Ictalurus punctatus (Rafinesque)], and brook trout [Salvelinus fontinalis (Mitchill)]. Toxicity tests of these compounds have been conducted previously with at least one of the test species, but with at least two of the chemicals studied, beryllium and selenium, little was known of their effects on fish. Pomellee[9] added a beryllium sulfate-tartaric acid complex to aquaria containing goldfish, "minnows" and snails for 12 days to give a total beryllium ion (Be^{++}) concentration of 28.5 mg/ℓ and did not observe any identifiable toxic responses by the test animals. During the course of the present study, Slonim[10] and Slonim and Slonim[11] published two papers detailing principally the interaction of water hardness and beryllium sulfate toxicity to guppies (Poecilia reticulata Peters). Ninety-six hour median lethal concentrations (LC50)* ranged from 0.16 to 27.0 mg/ℓ Be^{++} depending on hardness. According to the Ohio River Sanitation Commission[12], cited by McKee and Wolf[4], 10 to 100 mg/ℓ concentrations of sodium selenite were "toxic" (nature of the toxic response undefined) to goldfish in 98 to 144 hr and 8 to 19.5 hr, respectively, in hard water. Weir and Hine[13] calculated an LC50 of 12.0 mg/ℓ elemental selenium (Se) from goldfish mortalities arising from a combination of 48 hr toxicant exposure and 168 hr (216 hr total) confinement in uncontaminated, reconstituted deionized water.

A great deal of toxicological information is available concerning the effects of arsenic, cyanide and pentachlorophenate compounds on aquatic organisms. Upon examination of the compendium of Becker and Thatcher[14], which summarizes many of the toxic limits that have been established for these compounds, the difference between the lowest and the highest LC50 values for fish exposed to cyanide was eighteen-fold, but only four-fold for invertebrates.

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*Concentration of toxicant lethal to 50% of the test specimens.

For fish exposed to sodium arsenite, the highest 48-hr LC50 estimate was 5.5-fold greater than the lowest LC50 recorded. For invertebrates in general, the magnitude of the difference between 48-hr LC50 estimates was 44-fold. Although less directly comparable data were available for pentachlorophenol, the "no effect" levels were uniformly less than 1 mg/ℓ for fish, except for one observation that 5 mg/ℓ sodium pentachlorophenate was non-toxic to the green sunfish (Lepomis cyanellus Rafinesque). Although it is apparent that toxicological information is available, it is necessary to re-evaluate the toxicity relationships in terms of the recently revised aquatic toxicological methods. Accordingly, toxicity tests were conducted using intermittent-flow proportional diluters, with water of known quality, and fish of known age and size. Toxicant concentrations were measured several times during the course of each test to determine the levels of chemical to which the fish were actually being exposed.

Another objective of the tests was to determine the relationship between concentrations found to kill 50% of the test specimens, termed the median lethal concentration or LC50, and various exposure times. Representated as toxicity curves, these relationships provide valuable information on the type of toxic action, serve to identify if and when the acute toxicity ceases (known as a median lethal threshold* [2, 15]), and permit interpolation of LC50 values for various exposure periods for comparison with other estimates.

Median lethal concentrations derived from these tests can be used in the design of long-term chronic toxicity tests and in calculation of the "application factor" (Mount and Stephan[1]). The application factor, derived by dividing the MATC by the LC50 for 48 to 336 hr exposure periods, is used to designate probable maximum acceptable toxicant concentrations for species amenable only to acute toxicity testing.

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*Concentration at which acute toxicity ceases to 50% of the test specimens.

SECTION IV
MATERIALS AND METHODS

Acute toxicity tests of selenium dioxide (SeO_2), sodium arsenite ($NaAsO_2$), sodium cyanide (NaCN), sodium pentachlorophenate (NaPCP), beryllium sulfate ($BeSO_4$), and lead chloride ($PbCl_2$) were conducted with up to six species of freshwater fish. The chemical formulae for these compounds, some of their characteristics, and the commercial suppliers are given in Table 1.

TEST FISH

The species utilized were bluegill, channel catfish, fathead minnow, brook trout, flagfish, and Ozark-strain goldfish. All fish were obtained from commercial dealers within the State of California except minnows and flagfish, which were produced in the laboratory's culture unit, brook trout, which were obtained from the California Department of Fish and Game, and goldfish.

The test specimens were juvenile fish except brook trout, which were adults. In some experiments, fathead minnows and flagfish less than 24-hr old at the onset of testing were used. The size, age and density of the fish in the test chambers in each toxicity test are given in Appendix Tables 1-5.

Fish obtained from commercial dealers were quarantined for control and elimination of pathogens and acclimated to laboratory conditions for at least one month subsequent to their arrival. With the exception of the disease-free stock of fathead minnows and flagfish produced by the culture unit, all species apparently harbored aeromonad bacteria since low grade infections of bacterial hemorrhagic septicemia were occasionally observed within a week after the fish's arrival or as

Table 1. CHEMICALS USED IN ACUTE TOXICITY TESTS

Chemical common name	Source	Purity	Formula	Water solubility, g/100 mℓ	Probable major hydrolysis product
Sodium arsenite	J. T. Baker	A.R.[a]	$NaAsO_2$	Soluble[b]	H_3AsO_3
Beryllium sulfate	Research Organic/ Inorganic Chem. Corp.	99.5%	$BeSO_4 \cdot 4H_2O$	$43.78^{30°C}$	$(BeOH)_n^{n+} + nH^+$
Lead chloride	J. T. Baker	A.R.	$PbCl_2$	$0.99^{20°C}$	$Pb(CO_3)_2 \cdot Pb(OH)_2$
Selenium dioxide	Research Organic/ Inorganic Chem. Corp.	99.9%	SeO_2	$38.4^{14°C}$	$HSeO_3^-$
Sodium cyanide	J. T. Baker	A.R	NaCN	$48.0^{10°C}$	HCN
Pentachlorophenol	Aldrich Chemical Co.	99 + % (Gold Label)	C_6Cl_5OH	Insoluble[c]	$C_6Cl_5O^-$

[a]Analytical reagent

[b]Solubility at least 100 g/100 mℓ distilled water

[c]Soluble as the sodium salt.

the water temperatures were being increased to the acclimation temperature of 25°C (15° C for brook trout). The disease was treated by adding sufficient oxytetracycline - HCl ("TM-50" Pfizer) to the food to give a concentration of 0.44% active ingredient (A.I.), or 75 mg A.I./kg fish/day. Since immune fish can act as carriers and transmit the disease to susceptible individuals at a later date (Snieszko and Bullock[16]), oxytetracycline treatment was continued for several weeks after evidence of disease had abated. According to Patterson[17], rainbow trout (Salmo gairdneri Richardson) force-fed sufficient "TM-50" in tablets to give doses over 1,000 mg/kg fish did not incur mortality or external pathology in a ten day post-treatment period. Depuration of TM-50" from renal and somatic tissues of rainbow trout, and brown trout (Salmo trutta Linnaeus) was usually complete in 14 days and from hepatic tissues within 28 days. Herman[18] also concluded that rainbow trout could be fed more than 1,000 mg oxytetracycline/kg in a single dose without apparent ill effects. In reality, fish will not usually consume TM-50-treated food dosed above approximately 75 mg A.I./kg fish/day. Because the aeromonad bacteria are free-living facultative pathogens which usually cause pathogenesis in fish when they have been stressed or held under unsanitary conditions (also a stresser), the holding tanks were treated weekly with 2 mg/ℓ "Roccal" (National Laboratories, Montvale, New Jersey) to reduce bacterial buildup.

Other diseases positively identified and for which therapy was instituted included bacterial gill disease and external protozoan infections. Upon arrival at the laboratory, brook trout had bacterial gill disease, which was eliminated over a period of two weeks with daily, one hour baths of 10 mg A.I./ℓ soluble oxytetracycline - HCl. The two-hr LC50 for the soluble form of oxytetracycline HCl is greater than 500 ppm (Herman[19]). Upon arrival, channel catfish carried the protozoans, Trichodina sp. and Ichthyopthirius multifilus, and the external

monogenetic trematode, Gyrodactylus sp. All parasites were controlled over a three week period with daily one-hour baths of 50 to 100 mg/ℓ formalin (17 to 33 mg/ℓ formaldehyde) accompanied with artificial aeration. The maximum formalin concentration of 100 mg/ℓ was selected from results of a static acute toxicity test conducted with the newly-arrived fish. The tanks were also artificially aerated during formalin treatment since it was observed that the 35 to 50 mm fish became stressed when treated without aeration. Maintenance of high dissolved oxygen concentrations during static formalin treatments is important since formaldehyde lowers the oxygen uptake of fish (Wedemeyer[20]).

During acclimation to a standard photoperiod of 12-hr light, 12-hr dark, appropriate water temperatures, and laboratory conditions in general, brook trout, goldfish, bluegill and channel catfish were fed twice daily with a dry pelleted ration (Moore-Clark Company, Salt Lake City, Utah) at a rate of 2% of their body weight/day (trout) or ad libitum (goldfish, bluegill and catfish). Juvenile fathead minnows and flagfish were fed twice daily a combination of brine shrimp nauplii (Artemia salina) and dry trout starter (Moore-Clark).

TOXICITY TESTING CONDITIONS

All test species were acclimated to the above conditions for at least one month prior to introduction into the test chambers. For specific acclimation to toxicity test conditions, fish were introduced into the chambers 72 hr prior to toxicant introduction. They were fasted during this period as well as during toxicant exposure, which lasted up to 336 hr (408 hr total). The order of introduction of test specimens and the position of the test containers were selected with random number tables. Ten specimens were tested in each container except for brook trout, where the large size of the fish necessitated use of five individuals per container.

All toxicity tests were conducted with an intermittent-flow test system consisting of a two-liter proportional diluter (Mount and Brungs[21]) and twelve glass test chambers (30.5 x 30.5 x 30.5 cm) containing 20ℓ of test solution in the toxicity tests of adult trout and 16.5ℓ in tests of the other species. Light intensity from fluorescent lamps (Sylvania "Gro-Lux" and Durotest "Optima") averaged 1019 lux (94 fc). Toxicity tests were conducted with two replicates, each including one control and five toxicant concentrations. For all toxicants except sodium cyanide, the diluter delivered sufficient water to replace six tank volumes per day, assuring 90% molecular displacement in nine hours. Because test concentrations of sodium cyanide were observed to undergo a temporal decline, the rate of toxicant introduction was doubled to compensate for the loss. Although water flow into the tanks maintained dissolved oxygen concentrations above 70% of air saturation in most toxicity tests, it was not adequate in those utilizing brook trout, necessitating artificial aeration. Toxicant concentrations were successively diluted by a factor of 0.75.

As mortalities occurred, and at the end of the toxicity test, fish were measured for total length to the nearest millimeter and for wet body weight to the nearest gram or milligram, depending on size, after excess moisture had been removed with toweling. All fish were measured from each tank of one of the replicates. The data were later pooled if visual examination of the data indicated no size differences between treatments. Length and weight measurements were not taken prior to toxicity testing since it was believed that the stresses associated with handling and anesthesia (Houston, et al.[22, 23] Wedemeyer[24]) would be far more significant in terms of their influence on the LC50 than the changes in body weights during the course of the test.

Water quality in the test chambers was measured 24 hr prior to, and at least twice during toxicant addition for comparison of the effects of the toxicant's presence on water quality, for checking the water's suitability for uncompromised fish survival, and for estimating water quality variation. Seven variables were determined using standard methods recommended by the Environmental Protection Agency[25] or the American Public Health Association[8]. Except for rare instances, measurements were made on samples collected less than 4 to 6 hr earlier. Water temperatures were monitored with thermistors connected to a 12-channel recorder (Honeywell, Inc., Philadelphia, Pa.). Dissolved oxygen (D.O.) concentrations were determined with the azide modification of the Winkler method or with an oxygen meter (Model 54, Yellow Springs Instrument Company, Yellow Springs, Ohio), pre-calibrated with the Winkler method. In toxicity tests of sodium arsenite, dissolved oxygen was measured with the meter because the toxicant interfered with the Winkler method. Percentage saturation of the water was estimated with a nomograph using the known water temperatures and D.O. concentrations. A glass electrode was used to determine pH, while total alkalinity was determined by electrometric titration with 0.02 N reagent sulfuric acid to a pH of 4.5. Acidity was also determined electrometrically by titration with carbon dioxide-free 0.05 N sodium hydroxide to a pH of 8.3. Measurement of total hardness was derived by titration with disodium ethylenediaminetetraacetic acid using Eriochrome Black T indicator. Specific conductance was measured with a conductivity bridge (Beckman Instruments, Inc., Cedar Grove, New Jersey) using a cell with a constant of 1.0.

A number of ions were also determined by a commercial laboratory to gain a more specific description of the water's composition (Table 2). Calcium, magnesium, potassium, sodium, chloride and sulfate ions were determined every four months over the preceding year, while ammonia was measured biannually. Other substances were determined once.

Table 2. REPRESENTATIVE QUALITY OF SOURCE WATER

Variable	Unit	Concentration	Variable	Unit	Concentration
Calcium	mg/ℓ	31.1	Ammonia	mg/ℓ NH_3-N	0.16
Magnesium	mg/ℓ	13.1	Phenol	mg/ℓ	0.001
Potassium	mg/ℓ	2.0	Fluoride	mg/ℓ	0.96
Sodium	mg/ℓ	15.4	Cyanide	mg/ℓ	0.0005
Chloride	mg/ℓ	11.3	Iron	mg/ℓ	0.001
Sulfate	mg/ℓ	8.6	Copper	mg/ℓ	0.005
Sulfide	mg/ℓ	< 0.002	Zinc	mg/ℓ	0.001
Nitrate	mg/ℓ	4.65	Cadmium	mg/ℓ	0.010
Nitrite	mg/ℓ	0.005	Chromium	mg/ℓ	0.025

TOXICANT ANALYSIS

During each toxicity test, samples of water were collected for toxicant analysis an average of three different times to determine the levels of chemical to which the fish were exposed and to define variation in the toxicant concentrations during the course of the toxicity test. Water samples were collected at mid-depth in the container, filtered through Whatman No. 1 paper and stored at approximately 5° C until analysis. Prerequisite to these determinations, analyses were made to describe the accuracy and reproducibility of the methods applied.

All metals, namely arsenic, beryllium, lead and selenium, were assayed on an atomic absorption spectrophotometer (Model 303, Perkin-Elmer Corp.) using sample preparation and analytical methods specified by EPA[25] or the Perkin-Elmer Corporation[26]. To verify the accuracy and reproducibility of the methods, known amounts of the metals were spiked into laboratory water which had been filtered through Whatman No. 1 paper. The results showed the methods employed were highly reproducible and that samples spiked into water taken from tanks containing control fish had concentrations in agreement with standard curves. The coefficients of variation for selenium, arsenic, beryllium, and lead were 1.1, 5.3, 0.6, and 1.1%, respectively (Appendix Tables 6, 7, 8 and 9).

Sodium cyanide was measured colorimetrically using a colorimeter (Spectronic 20, Bausch and Lomb) and the pyridine-pyrazalone method (EPA[25]). The analytical method was checked for reproducibility and accuracy using the same protocol as described for the metals. The coefficient of variation, 3.1%, indicated low error of measurement (Appendix Table 10).

Insoluble pentachlorophenol was converted to water soluble sodium pentachlorophenate by dissolving the former in one molar sodium hydroxide for toxicity testing. Concentrations of sodium pentachlorophenate were subsequently analyzed by gas-liquid chromatography using the acetylation procedure of Rudling[27]. The reproducibility and accuracy of the method was checked with the aforementioned protocol and showed a low coefficient of variation of 5.7% for five replicates spiked into filtered water taken from tanks containing control fish (Appendix Table 11).

Stock toxicant solutions were prepared in deionized water as needed and dispensed from Mariotte bottles using a funnel dosing apparatus developed by Mount and Brungs[21]. Usually 2 mℓ of stock solution were dispensed per cycle of the proportional diluter, although larger volumes were required for administering lead chloride, which had a solubility in distilled water of approximately 9.9 mg/ℓ at 20° C (Weast and Selby[28]). Stock solutions of sodium cyanide were prepared daily because it was found that concentrations declined in the stock solutions, presumedly due to volatilization of hydrocyanic acid formed by hydrolysis ($CN^- + H_2O \rightleftharpoons HCN + OH^-$). In the tests with cyanide, it was also necessary to augment the flow of water through the test chambers, decreasing the 90% molecular displacement time to approximately five hours, to compensate for cyanide loss through volatilization of HCN.

Concentrations of the chemicals measured in the chambers during the course of the toxicity tests are given in Appendix Tables 12 through 16 for NaPCP, NaCN, SeO_2, $NaAsO_2$ and $BeSO_4$.

STATISTICAL ANALYSIS

Simple summary statistics were utilized to analyze length-weight, water quality, and toxicant concentration data. Except were noted, results are represented as means $\pm$ one standard deviation.

Concentration-percent mortality data generated from the toxicity tests were analyzed with logarithmic-probability (log-probit) methods using either the manual procedure of Litchfield and Wilcoxon[29] or the computer program of Dixon[30]. The log-probit method was selected because it is a more objective approach than the graphical interpolation method, offers a test of the regression line's goodness-of-fit, and provides the statistics necessary for calculating 95% confidence limits for median lethal concentrations (LC50) and for comparing differences between two LC50 values. The LC50 is the concentration of toxicant killing 50% of the test specimens in a specified period of time. In most cases, concentration-percent mortality data from toxicity tests which were conducted in duplicate were pooled for statistical analysis. Data were not pooled from tests of sodium cyanide using brook trout since the tests were performed at different times under slightly different water quality conditions and toxicant concentrations.

For homogeneous data, upper and lower 95% confidence limits for LC50 values determined from the computer program were calculated as (LC50) (f) and LC50/f, respectively, where f = antilogarithm of the $1.96\ \hat{\sigma}\ (N'/2)^{-\frac{1}{2}}$, $\hat{\sigma}$ is the standard deviation of the logarithm of the population tolerance frequency distribution, and N' is the number of test animals expected to have perished within the percent mortality interval of 16 to 84% (Litchfield and Wilcoxon[29]). An equivalent equation is $\pm$ 1.96 S.E. log LC50 (Bliss[31]). For heterogeneous data, i.e., where Chi-square analysis of the fitted line indicated lack of goodness-of-fit, the equation f = (Student's t-value) $(\hat{\sigma})\ (\chi^2/n)^{-\frac{1}{2}}$ was employed (Bliss[31]). The logarithms of the median lethal concentrations were plotted against the logarithms of the exposure times to give a toxicity curve (Sprague[2]).

The accuracy of LC50 estimates and their 95% confidence limits generated by the Litchfield and Wilcoxon[29] and computer program (Dixon[30]) methods were compared with similar calculations made by eight other aquatic toxicology laboratories using standard concentration-percent response data (Appendix Table 17) supplied by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Our LC50 estimates were in agreement with the average LC50 computed by the other laboratories (Appendix Table 18) using both methods of analysis. In our laboratory, 95% confidence limits were usually narrower with the computer method than with that of Litchfield and Wilcoxon[29].

Median lethal times for measured toxicant concentrations were calculated in some cases. Data were analyzed in the same manner as for the calculation of LC50 values and were plotted on the same toxicity curve, with the exception that 95% confidence limits were determined for the independent variable, time, rather than the LC50.

Control mortality occurred in less than five percent of the toxicity tests and was less than ten percent in all cases. Median lethal concentrations were corrected for control mortality, where applicable, using Dixon's[30] computer program.

In this program, it was considered more important to define the relationship between median lethal toxicant concentration and exposure time, i.e., define the shape of the toxicity curve, than it was to determine LC50 values for specified exposure times (e.g., 24, 48, 96 hr). Since many workers in aquatic toxicology base their toxicity data on predetermined exposure times, such values were estimated using simple linear regression (Steel and Torrie[32]) where applicable.

All statistics were initially calculated for the undissociated or parent compound used in the toxicity tests. Although it was known that several of the parent chemicals were capable of hydrolysis or other chemical reactions upon their introduction into the diluent water (Table 1), determination of the exact forms of the chemicals, particularly those which were biologically significant in terms of their relative toxicity, was beyond the scope of this project. However, as Lee[33] and Black, et al[34] have recently emphasized, such knowledge is essential for assessing the toxicity of chemicals to aquatic organisms. Since it is well known (Doudoroff, Leduc and Schneider[35]) that within the pH range of 6 to 8 cyanide toxicity is due mainly to hydrocyanic acid (HCN), which was measured in the pyridine-pyrazalone method along with cyanide ion as total cyanide, LC50 estimates and 95% confidence limits for this chemical only were also calculated as cyanide ion (CN^-).

SECTION V
RESULTS

TOXICITY TESTS OF SODIUM PENTACHLOROPHENATE

Water Quality

The sodium salt of pentachlorophenol was soluble at all levels employed for toxicity testing and did not change any of the seven water quality variables monitored. In general, mean dissolved oxygen concentrations ranged from 6.1 $\pm$ 0.5 to 7.4 $\pm$ 0.3 mg/ℓ and were greater than 70% of air saturation in all toxicity tests. Mean pH ranged from 7.59 $\pm$ 0.08 to 7.94 $\pm$ 0.16; total alkalinity, from 153 $\pm$ 4 mg/ℓ to 167 $\pm$ 2 mg/ℓ $CaCO_3$; acidity, from 5.9 $\pm$ 0.9 to 7.5 $\pm$ 6.0 mg/ℓ $CaCO_3$; total hardness, from 145 $\pm$ 6 to 156 $\pm$ 11 mg/ℓ $CaCO_3$; and specific conductance, from 367 $\pm$ 14 to 379 $\pm$ 21 μmhos/cm (Table 3).

Toxicity

Sodium pentachlorophenate (NaPCP) produced total mortality of all fish at concentrations greater than 1.0 mg/ℓ. The descending order of species sensitivity to NaPCP was brook trout, fathead minnow, goldfish and bluegill.

Toxicity curves relating median lethal concentration of sodium pentachlorophenate to exposure time for the four species indicated that the halogenated phenol had a fairly narrow range of acute lethality and had a toxic action which varied with respect to species (Figure 1). Two types of toxicity curves were obtained: for brook trout, acute lethality ceased at a NaPCP concentration of 0.118 mg/ℓ and an exposure time of 220 hr; but for fathead minnow, goldfish and bluegill, mortalities did not cease at test concentrations as low as 0.08 mg/ℓ and toxicant exposure times as long as 406 hr. The changes in the slopes of the toxicity curves for fathead minnows, goldfish and bluegill occurred

Table 3. WATER QUALITY DURING TOXICITY TESTS OF SODIUM PENTACHLOROPHENATE

Species	Water temperature, °C	Dissolved oxygen, mg/ℓ	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/ℓ $CaCO_3$	Total hardness, mg/ℓ $CaCO_3$	Specific conductance, μmhos/cm
Fathead minnow[b]	24.9[a] ±0.3	7.4 ±0.3	88.1 ±3.1	7.83 ±0.05	162 ± 4	156 ±11	379 ±21
Bluegill	24.9 ±0.3	6.7 ±0.3	80.2 ±3.2	7.94 ±0.16	153 ± 4	145 ± 6	367 ±14
Brook trout[b]	15.4 ±0.6	8.4 ±0.9	83.1 ±9.4	7.89 ±0.06	161 ± 4	147 ± 8	377 ±13
Goldfish	25.1 ±0.2	6.1 ±0.5	72.8 ±6.6	7.59 ±0.08	167 ± 2	148 ± 4	367 ± 8

[a] Means ± 1 standard deviation are given

[b] Acidity averaged 5.9 ± 0.9, 7.5 ± 6.0, and 6.4 ± 2.0 mg/ℓ $CaCO_3$ in toxicity tests using fathead minnow, bluegill and brook trout, respectively.

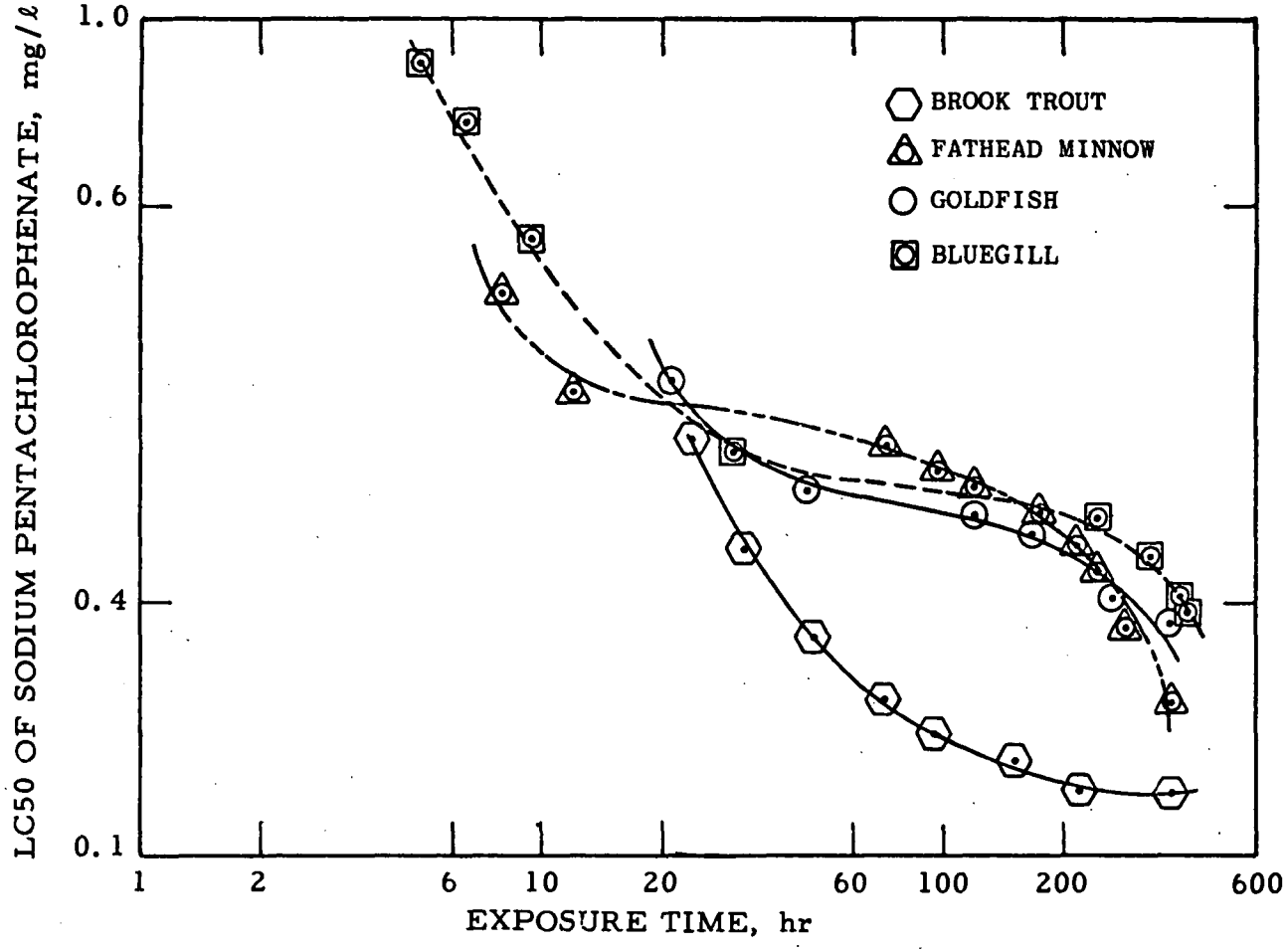

Fig. 1. Relationship between median lethal concentration (LC50) of sodium pentachlorophenate and exposure time for four species of freshwater fish.

after approximately 96 hr, suggesting a bimodal type of toxicity since fish died at a faster rate upon prolonged exposure to low concentrations than at lesser exposures to intermediate concentrations. Changes in slope of the toxicity curves for very short exposures to high concentrations basically reflect the time required for a specimen to expire.

The range at which sodium pentachlorophenate was acutely lethal was much narrower than that characterizing the four metals tested. For brook trout, the 24-hr LC50 was 0.315 mg/ℓ, while the median lethal threshold(219-hr LC50) was 0.118 mg/ℓ. The difference between the 24 and 219-hr median lethal concentrations was only 63% (Appendix Table 19). A similar relationship existed for the three other species, where the differences between 24 and 336 hr LC50 estimates were approximately 67, 49 and 41% for fathead minnow, goldfish and bluegill, respectively. The differences in sensitivity between minnow, goldfish and bluegill to sodium pentachlorophenate were slight, as indicated by the fact that the 336-hr LC50 estimates were 0.153 mg/ℓ for fathead minnows, 0.189 mg/ℓ for goldfish, and 0.215 mg/ℓ for bluegill (Appendix Tables 20 to 22 and Figure 1).

TOXICITY TESTS OF SODIUM CYANIDE

Water Quality

Sodium cyanide was soluble in all proportions used for toxicity testing and did not alter the water quality variables measured. Dissolved oxygen concentrations were above 78% of air saturation in all tests, ranging from 6.6 ± 0.2 to 8.5 ± 0.3 mg/ℓ. The diluent water was moderately alkaline, as shown by pH readings of 7.64 ± 0.08 to 7.85 ± 0.10 mg/ℓ and total alkalinities of 162 ± 3 to 170 ± 6 mg/ℓ $CaCO_3$, and of intermediate hardness (148 ± 2 to 155 ± 10 mg/ℓ $CaCO_3$) and conductivity (376 ± 28 to 391 ± 15 µmhos/cm) (Table 4).

Table 4. WATER QUALITY DURING TOXICITY TESTS OF SODIUM CYANIDE

Species	Water temperature, °C	Dissolved oxygen, mg/ℓ	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/ℓ $CaCO_3$	Total hardness, mg/ℓ $CaCO_3$	Specific conductance, μmhos/cm
Fathead minnow	25.3 [a] ±0.2	6.9 ±0.1	82.5 ±1.7	7.64 ±0.08	169 ± 6	154 ±13	391 ±15
Goldfish	25.0 ±0.1	6.6 ±0.2	78.4 ±1.8	7.74 ±0.08	168 ± 5	152 ± 4	382 ±11
Brook trout	15.4 ±0.5	8.5 ±0.3	83.7 ±2.2	7.83 ±0.03	170 ± 5	155 ±10	376 ±28
Bluegill	25.4 ±0.2	6.7 ±0.2	80.8 ± 2.1	7.68 ±0.13	170 ± 6	155 ± 16	384 ± 14
Channel catfish	25.2 ±0.2	7.2 ±0.3	86.4 ±3.6	7.85 ±0.10	162 ± 3	148 ± 2	384 ± 3

[a] Means ± 1 standard deviation are given.

Toxicity

Sodium cyanide was the second most toxic chemical to the fish species studied, being almost as acutely lethal as sodium pentachlorophenate. The order of species sensitivity was somewhat different than that generally found for the other chemicals, notably in the substantial sensitivity of bluegill. Fathead minnows were the most sensitive, followed by bluegill, brook trout, channel catfish and goldfish.

For all species, sodium cyanide toxicity ostensibly ceased after approximately 100 hr toxicant exposure, indicating a rapid type of toxicant action. In one of the brook trout toxicity tests, but not in the other, there was a slight shift in the slope of the toxicity curve after 200 hr. The significance of the shift is difficult to assess owing to the proximity of the last three LC50 estimates (Figure 2). A true median lethal threshold was approached but not reached in the toxicity test of goldfish.

The lowest concentration of sodium cyanide found to produce acute lethality was in the toxicity test using fathead minnows, where a median lethal threshold of 0.114 mg/ℓ was obtained in 192 hr (Appendix Table 23). Median lethal thresholds of 0.116 mg/ℓ and 0.126 mg/ℓ CN^- were obtained for bluegill and brook trout exposed for 168 and 288 hr, respectively (Appendix Tables 24 and 25). Toxicant exposures were not sufficiently long to facilitate estimation of a median lethal threshold for channel catfish, although one was being approached in the region of 0.16 mg/ℓ CN^- after 30 hr (Appendix Table 26). A similar pattern was evident for the toxicity test using goldfish, where exposure for 336 hr to sodium cyanide concentrations as low as 0.156 mg/ℓ CN^- did not result in definition of the median lethal threshold. The 336-hr LC50 was 0.261 mg/ℓ CN^- (Appendix Table 27).

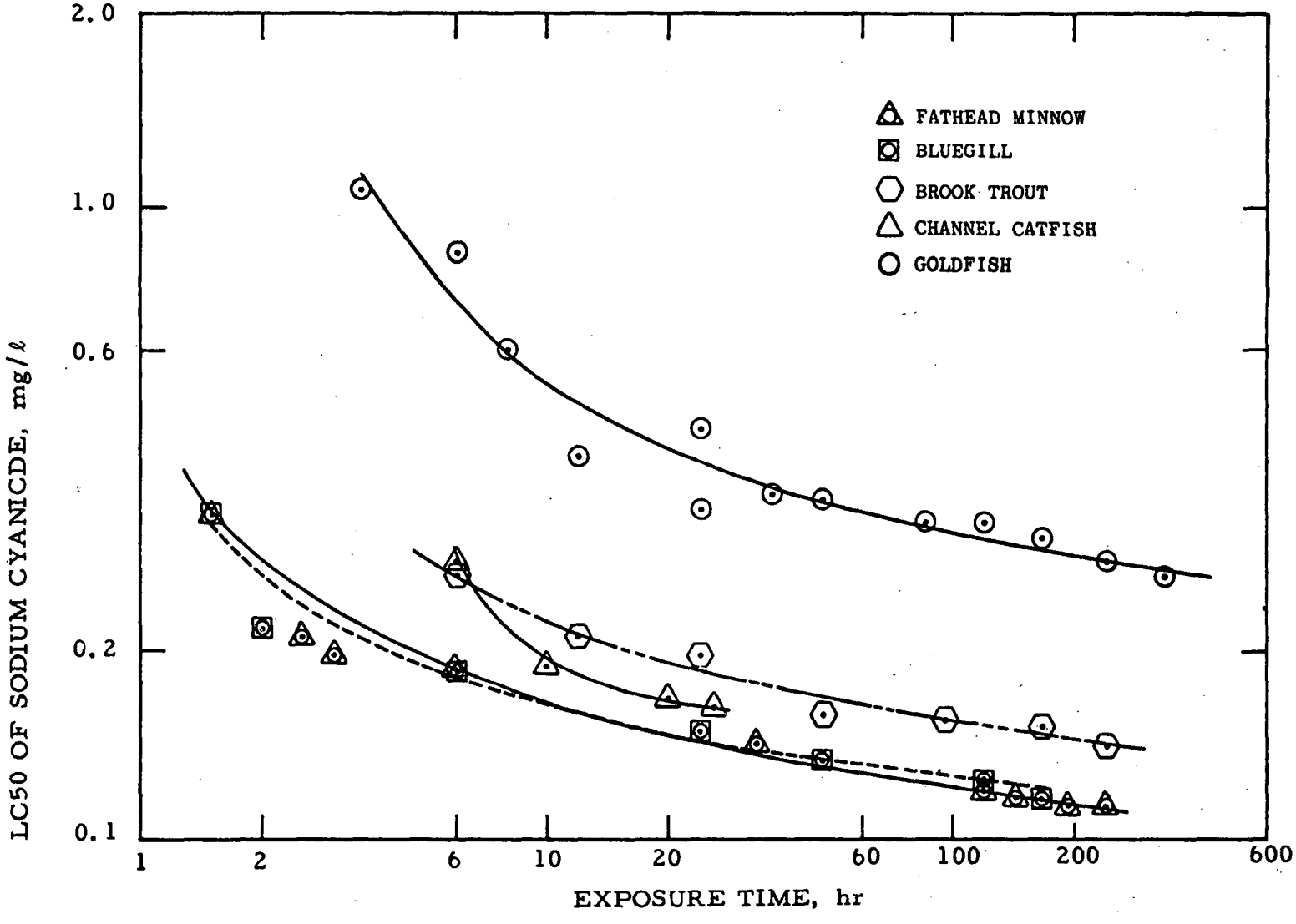

Fig. 2. Relationship between median lethal concentration (LC50) of sodium cyanide as CN^- and exposure time for five species of freshwater fish.

As was also observed in the toxicity tests of sodium pentachlorophenate, the range within which sodium cyanide produced acute mortality was very narrow. For the most sensitive species, the fathead minnow, the difference between the 24-hr LC50 estimate and the median lethal threshold was only 20%. Although goldfish were much less sensitive than minnows, the difference was almost equivalent to that for minnows, 21%.

TOXICITY TESTS OF SELENIUM DIOXIDE

Water Quality

Selenium dioxide was soluble in water at all concentrations tested. The presence of the chemical, however, was found to affect water quality, reducing pH and total alkalinity while increasing acidity, probably because of conversion of selenium dioxide to biselenite ion and selenous acid ($SeO_2 + H_2O \rightleftharpoons H^+ + HSeO_3^-$). The greatest alteration in water quality occurred in one test using goldfish, where pH and total alkalinity were reduced to 6.58 and 97 mg/ℓ $CaCO_3$ respectively, at a measured concentration of 114 mg/ℓ SeO_2 (Table 5). In all cases, the changes in water quality were greater at higher toxicant concentrations. Water quality for the tests of each species is given in Table 6. Because of the toxicant's influence on pH, alkalinity, and acidity, means and standard deviations for these three variables were derived from measurements made in chambers containing control fish, while summary statistics for the other variables, i.e., dissolved oxygen, total hardness, and specific conductance, were derived from measurements made in all treatments, including controls.

Table 5. LOWEST LEVELS OF pH AND TOTAL ALKALINITY ENCOUNTERED IN TOXICITY TESTS OF SELENIUM DIOXIDE (SeO_2)

Species	Measured concentration of SeO_2, mg/ℓ	Minimum level observed	
		pH	Total alkalinity, mg/ℓ $CaCO_3$
Fathead minnow	54	7.43	143
Channel catfish	63	7.53	120
Brook trout	102	7.45	112
Flagfish	38	7.57	149
Goldfish	114	6.58	97
Bluegill	133	6.80	110

Table 6. WATER QUALITY DURING TOXICITY TESTS OF SELENIUM DIOXIDE

Species	Water temperature °C	Dissolved oxygen, mg/ℓ	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/ℓ $CaCO_3$	Total hardness, mg/ℓ $CaCO_3$	Specific conductance μmhos/cm
Fathead minnow	24.7[a] ±0.4	7.1 ±0.6	83.9 ±7.8	7.80 ±0.09	167 ± 4	151 ± 9	351 ±16
Flagfish[b]	24.5 ±0.4	7.8 ±0.3	92.6 ±3.6	7.90 ±0.04	164 ± 4	152 13	374 ± 5
Bluegill	24.9 ±0.4	6.8 ±0.4	81.0 ±5.2	7.75 ±0.05	169 ± 4	150 ± 6	357 ±23
Channel[b] catfish	24.9 ±0.3	6.7 ±0.9	80.3 ±10.6	7.93 ±0.09	140 ± 3	140 ± 3	---
Goldfish	25.4 ±0.3	6.8 ±0.5	81.3 ±5.4	7.63 ±0.10	159 ± 5	148 ± 8	375 ±18
Brook[b] trout	15.5 ±0.6	8.1 ±0.4	79.9 ±3.8	7.80 ±0.07	157 ±11	148 ±10	383 ±20

[a] Means ± 1 standard deviation are given.

[b] Acidity averaged 3.7 ± 2.4, 4.6 ± 0.9, and 7.5 ± 3.1 mg/ℓ Ca CO_3 in toxicity tests of flagfish, catfish and trout, respectively.

As in the tests of the other compounds, dissolved oxygen concentrations were greater than 70% of air saturation, and there was little variation in pH or hardness of the diluent water entering the system. Average pH readings from one test to the next ranged from 7.63 $\pm$ 0.1 to 7.93 $\pm$ 0.9, while total alkalinity varied from 140 $\pm$ 3 mg/ℓ $CaCO_3$ in the toxicity test of channel catfish to 169 $\pm$ 4 mg/ℓ $CaCO_3$ in that of bluegill. Acidity was less than 10 mg/ℓ $CaCO_3$. Variation in mean total hardness and specific conductivities in all tests was less than ten percent (Table 6).

External Pathology Produced in Fish

Exposure to acutely toxic concentrations of selenium dioxide produced external hemorrhaging or lesions or both within 48 hr of the fish's exposure to the toxicant in four of the species tested: brook trout, channel catfish, goldfish and fathead minnow. Similar signs may have occurred in flagfish juveniles, but they would have been obscure because of the small size of the specimens. The incidence of cutaneous lesions appeared to increase at higher concentrations of toxicant. Control fish did not exhibit external evidence of pathology or behavior which appeared to deviate from the state judged to be normal. Channel catfish, goldfish, and fathead minnows had hemorrhages only, whereas brook trout had marked discoloration and lesions throughout the body, and necrosis and sloughing of epithelial tissue in the cranial region, particularly in the snout. Tissue destruction was far more severe and dramatic in brook trout. Although it is possible that a disease organism was responsible for these observations, the rapidity of the onset of pathological signs, the graded nature of the response, and the occurrence of the pathology in fathead minnows, a species which had no previous history of disease in our laboratory, contradicts a microbial etiology, although the possibility of one cannot be excluded.

Toxicity

Selenium dioxide proved to be less toxic than sodium pentachlorophenate and sodium cyanide, but more toxic than sodium arsenite, beryllium sulfate and lead chloride. The order of descending sensitivity was fathead minnow, brook trout, channel catfish, flagfish, goldfish and bluegill.

No acute median lethal thresholds were manifested for any of the test species, even for those exposed for 336 hr. In fact, substantial mortality resulted when fish had been exposed for more than 96 to 120 hr to low concentrations of selenium dioxide. Inspection of the toxicity curve indicated that the rate of mortality was higher and that the slope had changed (Figure 3). At shorter exposures, the relationship between LC50 and time was linear and the slopes of the toxicity curves were similar for all species except flagfish, where the slope of the line was less. None of the concentrations tested was high enough to demonstrate the point at which the toxicity curve became asymptotic to the LC50 coordinate (ordinate). The median lethal concentrations calculated for various exposure periods, their 95% confidence limits, and statistical information on the nature of the concentration-percent mortality curves for given exposure periods, are detailed in Appendix Tables 28 to 33 for fathead minnow, brook trout, channel catfish, flagfish, goldfish and bluegill.

The range in which selenium dioxide was acutely lethal to fish was uniformly broad. For example, the 24-hr LC50 of 77.3 mg/ℓ SeO_2 determined for juvenile bluegill, the least sensitive of the test species, was more than four times its 336-hr LC50 of 17.6 mg/ℓ SeO_2. Similarly, the 24.5-hr LC50 of 24.3 mg/ℓ SeO_2 for juvenile fathead minnow, the most sensitive species, was more than eight times its 168-hr LC50 of 2.9 mg/ℓ SeO_2.

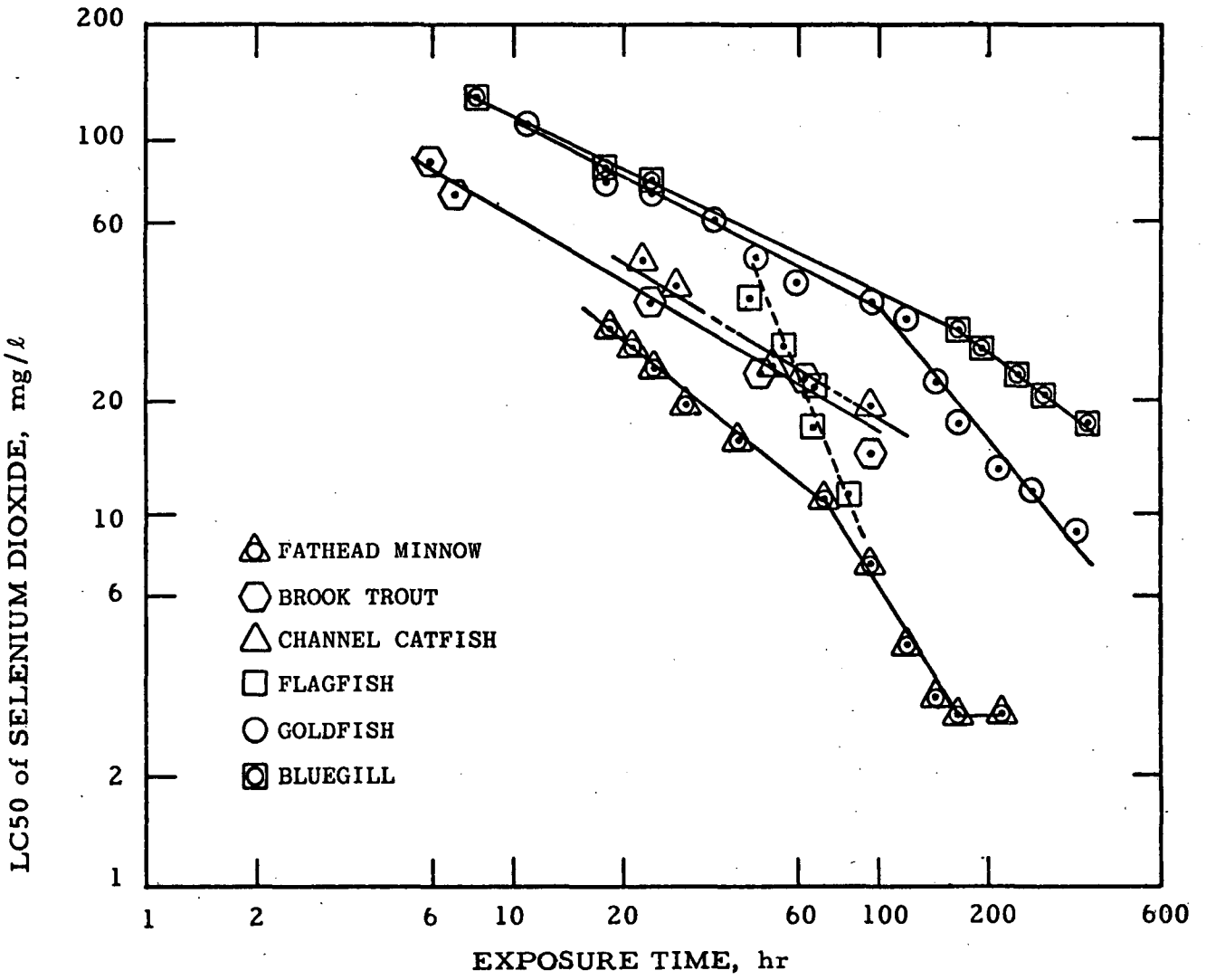

Fig. 3. Relationship between median lethal concentration(LC50) of selenium dioxide and exposure time for six species of freshwater fish.

Similar patterns were evident for the other species. Sensitivity to selenium dioxide was also affected by size of the test fish. The 96-hr LC50 of 2.9 mg/ℓ SeO_2 estimated for 5 mm fathead minnow fry less than 24 hr old was 40% that of the 96-hr LC50 of 7.3 mg/ℓ SeO_2 determined for fathead minnows two to three months older and four to five times longer.

TOXICITY TESTS OF SODIUM ARSENITE

Water Quality

Sodium arsenite was soluble in the laboratory water at all concentrations tested. The presence of the chemical affected water quality, but in a manner opposite that observed for selenium dioxide. Because of hydrolysis of sodium arsenite to the very weakly acidic arsenous acid, with concomitant liberation of hydroxyl ions (e.g. $AsO_2^- + 2H_2O \rightleftharpoons H_3AsO_3 + OH^-$), there were increases in pH and total alkalinity and reductions in acidity on addition of sodium arsenite. As shown in Table 7, the highest levels of pH and total alkalinity encountered were approximately 8.9 and 256 mg/ℓ $CaCO_3$, respectively, at a concentration of 272 mg/ℓ $NaAsO_2$ in one test of goldfish. However, lesser toxicant concentrations raised the levels of these variables, particularly pH, to similar extents. Average water quality measured in the various tests is summarized in Table 8. Because of the toxicant's effect on pH, total alkalinity, and acidity, values for these three variables were necessarily derived from measurements made in uncontaminated (control) water. Water temperatures were maintained at 15° C for tests using brook trout and at 25° C for the remaining species. Dissolved oxygen concentrations were greater than 70% of air saturation in all toxicity tests. Average pH readings ranged from 7.61 $\pm$ 0.08 in the tests using goldfish to 7.98 $\pm$ 0.21 in those using channel catfish, while the range in mean total alkalinity was 140 $\pm$ 8 mg/ℓ $CaCO_3$ in the tests of channel catfish to 168 $\pm$ 4 mg/ℓ $CaCO_3$ in those employing brook trout. Variation in total hardness was low (range of means 140 to 152 mg/ℓ $CaCO_3$), as was that for specific conductance (range of means 367 to 388 μmhos/cm).

Table 7. HIGHEST LEVELS OF pH AND TOTAL ALKALINITY ENCOUNTERED IN TOXICITY TESTS OF SODIUM ARSENITE ($NaAsO_2$)

Species	Measured $NaAsO_2$ conc., mg/ℓ	pH	Total alkalinity, mg/ℓ $CaCO_3$
Bluegill	67.9	8.30	185
Goldfish	272.0	8.90	256
Channel catfish	72.4	8.92	169
Fathead minnow	82.1	8.80	206
Brook trout	84.7	8.70	215

Table 8. WATER QUALITY DURING TOXICITY TESTS OF SODIUM ARSENITE

Species	Water temperature, °C	Dissolved oxygen, mg/ℓ	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/ℓ $CaCO_3$	Total hardness, mg/ℓ $CaCO_3$	Specific conductance, μ mhos/cm
Goldfish	25.1[a] ±0.4	6.7 ±0.5	80.3 ±5.8	7.61 ±0.08	159 ±5	148 ±7	373 ±17
Fathead minnow[b]	25.0 ±0.3	6.9 ±0.3	82.1 ±3.0	7.77 ±0.13	166 ±5	149 ±9	379 ±24
Brook trout[c]	15.1 ±0.6	7.5 ±0.2	73.2 ±3.5	7.75 ±0.10	168 ± 4	152 ±3	388 ±15
Bluegill	24.9 ±0.1	6.8 ±0.2	81.1 ±1.8	7.82 ±0.06	166 ±4	147 ±15	378 ±23
Channel catfish[d]	24.9 ±0.3	6.5 ±0.4	77.1 ±5.1	7.98 ±0.21	140 ±8	140 ±4	367 ±9

[a] Means ± 1 standard deviation are given

[b] Acidity averaged 5.4 ± 0.4 mg/ℓ

[c] Acidity averaged 5.5 ± 2.1 mg/ℓ

[d] Acidity averaged 5.4 ± 3.4 mg/ℓ

External Pathology

Although evidence of cutaneous hemorrhaging was evident in certain individuals of each species exposed to acutely toxic concentrations of sodium arsenite, none of the species except brook trout developed hemorrhages and lesions on the scale noted for selenium dioxide. Essentially, all brook trout developed patches of marked discoloration or a mottled appearance within 12 to 24 hr after toxicant introduction. Trout exposed for longer periods developed extensive tissue destruction in the cranial regions, although lesions of the skin were also evident in posterior areas.

Toxicity

Sodium arsenite was the fourth most toxic chemical to the freshwater fish tested, ranking just behind selenium dioxide. The order of species sensitivity was very similar to that observed for selenium dioxide except that adult brook trout were more sensitive than juvenile fathead minnows. Bluegills were the least sensitive species. The minimum concentration of sodium arsenite found to be acutely lethal was in the tests of adult brook trout, where the LC50 for 262 hr exposure was 18.0 mg/ℓ $NaAsO_2$ or 10.4 mg/ℓ elemental arsenic.

Toxicity curves relating median lethal concentration to exposure time are given in Figure 4 for the six test species. Two types of curves were evident. For brook trout and goldfish, median lethal thresholds were reached, indicating a unimodal type of sodium arsenite acute toxicity. In contrast, median lethal thresholds were not evident for fathead minnows and bluegill, and probably not for channel catfish, and two modes of toxicity were suggested by the curve. In the latter three species, prolonged (i.e. >96 hr) exposure to the lower concentrations produced substantial mortality and a shift in the slope of the toxicity curve. There were insufficient observations to distinguish whether the toxicity curve for flagfish fry followed the pattern of minnows and bluegill.

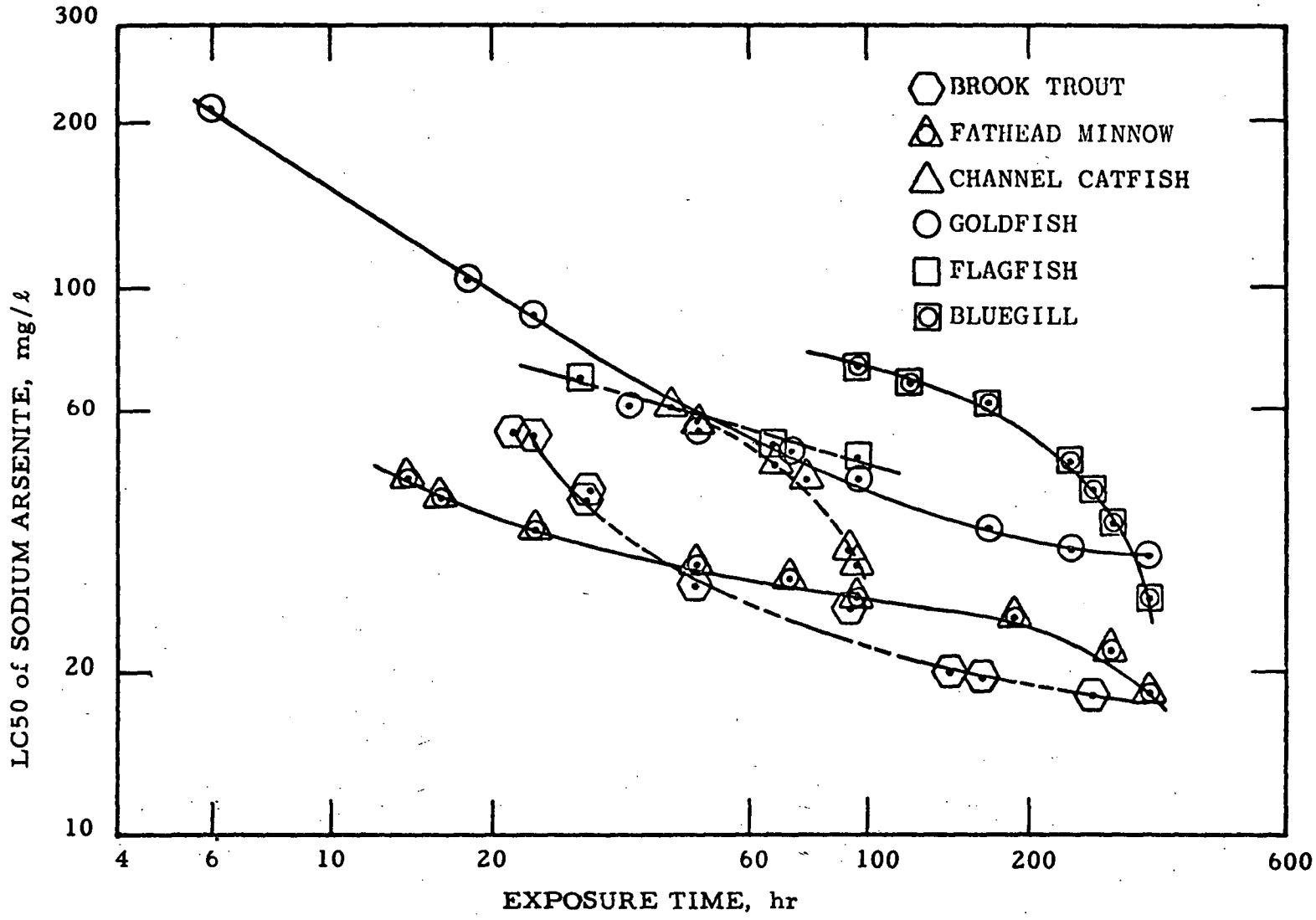

Fig. 4. Relationship between median lethal concentration (LC50) of sodium arsenite and exposure time for six species of freshwater fish

Median lethal concentrations, their 95% confidence limits, and statistical information for the concentration-percent response curves for given exposure times are detailed in Appendix Tables 34 to 39 for all six test species. The 96-hr LC50 estimates were 72.0 mg/ℓ for bluegill, an average of 48.5 mg/ℓ for flagfish, 44.9 mg/ℓ for goldfish, 31.2 mg/ℓ for channel catfish, 27.0 mg/ℓ for fathead minnow, and 25.8 mg/ℓ $NaAsO_2$ for brook trout. A median lethal threshold of 32.0 mg/ℓ was manifested in 336 hr with goldfish and one of 18.0 mg/ℓ developed after 262 hr with brook trout. In both cases, median lethal thresholds were approximately 30% lower than the 96-hr LC50. The sensitivities of flagfish fry, juvenile goldfish and channel catfish were very similar, particularly in the concentration range of 45 to 70 mg/ℓ $NaAsO_2$.

TOXICITY OF BERYLLIUM SULFATE TETRAHYDRATE

Water Quality

Beryllium sulfate precipitated when introduced into the test containers, and large amounts accumulated at the bottom during the course of the test. Beryllium sulfate was probably reacting in the ambient water to form beryllium hydroxides of low solubility. A likely hydrolysis product is $Be(OH)_2$, which has a solubility of 2 ppm (Lange[36]). The probable reaction is $nBe^{++} + nH_2O \rightleftharpoons (BeOH)^{n+} + nH^+$ (Everest[37]), which proceeds strongly to the right at the average experimental pH of 7.7 to 7.8. Since all tests were conducted at concentrations exceeding the theoretical solubility of beryllium sulfate or beryllium hydroxide, mortality of the test specimens could be expected to be essentially equivalent between concentrations. Because graded, concentration-dependent mortality was observed, the lethal effects of beryllium may have been caused either before or during its precipitation or by the precipitated material or both. In order to determine the rate of what appeared to be a slow precipitation reaction, a chamber

similar to those used for testing was filled with 10 ℓ of filtered water (Whatman No. 1 filter paper), spiked with sufficient $BeSO_4 \bullet 4H_2O$ to provide an initial concentration of 150 mg/ℓ (89 mg/ℓ $BeSO_4$) and sampled serially thereafter for five days. Upon collection, samples were filtered through a fine (4.0 - 5.0 μ) fritted disc into a test tube containing one milliliter of concentrated hydrochloric acid. In trial runs, precipitated beryllium was not observed to be passed through this filter. After mixing, the sample was measured for elemental beryllium on an atomic absorption spectrophotometer. As shown in Figure 5, there was a rapid decline in dissolved beryllium (as $BeSO_4$) to 26.7% of nominal within one minute. Thereafter, concentrations of dissolved beryllium remained relatively stable for 30 to 60 min, then gradually declined to 2% of nominal one day after introduction. This experiment indicated that precipitation of beryllium sulfate proceeded slowly, confirming laboratory observations and the findings of Everest[37].

The presence of beryllium sulfate or its probable hydrolysis product, beryllium hydroxide, in the laboratory water reduced pH, total alkalinity, and acidity in a manner similar to that described for selenium dioxide; however, the dimunitions in pH and total alkalinity were less. The minimum pH (6.60) was observed at a measured concentration of 59.0 mg/ℓ $BeSO_4$, while the lowest total alkalinity, 107 mg/ℓ, was observed at a measured concentration of 64.0 mg/ℓ in the test using brook trout (Table 9). Hardness, specific conductance and dissolved oxygen concentrations were not observed to be affected by the addition of beryllium sulfate.

Average values for eight water quality variables monitored during the toxicity tests of beryllium sulfate are given in Table 10. Because of the effect of the chemical on pH, total alkalinity and acidity, values cited represent measurements in uncontaminated (control) water only. Water temperatures varied within $\pm$ 0.5° C of the specified temperatures of 15°C for brook trout and 25°C for the remaining species. Dissolved

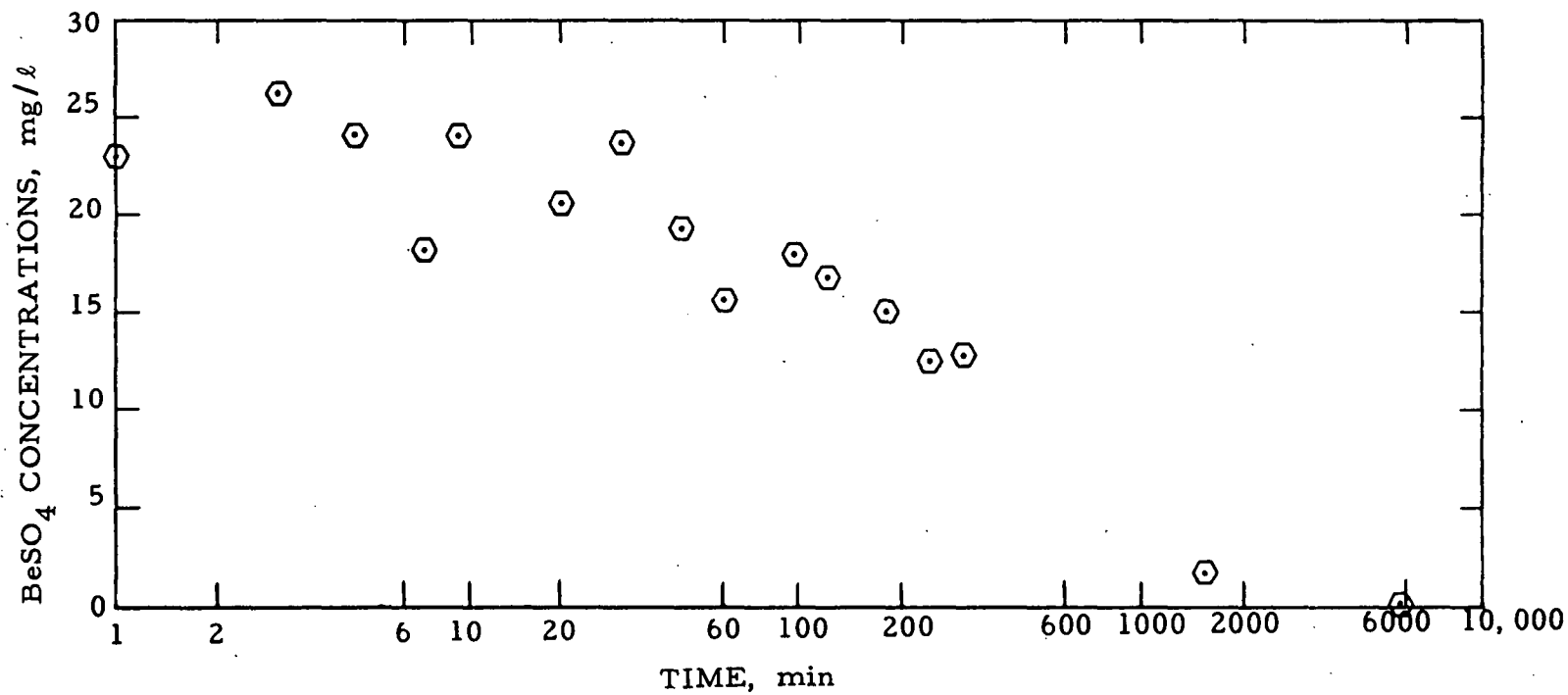

Fig. 5. Changes in the concentration of dissolved beryllium (as $BeSO_4$) spiked into 10 ℓ of diluent water at a level of 89 mg/ℓ $BeSO_4$

Table 9. MINIMUM LEVELS OF pH AND TOTAL ALKALINITY OBSERVED IN TOXICITY TESTS OF BERYLLIUM SULFATE ($BeSO_4$)

Species	Measured concentration of $BeSO_4$, mg/ℓ	pH	Total alkalinity, mg/ℓ $CaCO_3$
Fathead minnows	47.8	6.95	114
Goldfish	59.0	6.60	124
Channel catfish	47.8	6.95	114
Brook trout	64.0	7.46	107

Table 10. WATER QUALITY DURING TOXICITY TESTS OF BERYLLIUM SULFATE

Species	Water temperature, °C	Dissolved oxygen, mg/ℓ	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/ℓ $CaCO_3$	Acidity, mg/ℓ $CaCO_3$	Total hardness, mg/ℓ $CaCO_3$	Specific conductance, μmhos/cm
Fathead[a] minnow	24.8[b] ± 0.4	7.4 ±0.2	88.5 ± 2.5	8.04 ±0.17	146 ± 6	4.6 ±2.5	140 ± 4	372 ± 28
Goldfish	25.2 ± 0.4	6.5 ±0.3	76.9 ± 4.2	7.57 ±0.09	161 ± 6	-[c]	147 ± 8	392 ± 13
Channel catfish	24.9 ± 0.4	7.3 ±0.3	87.5 ± 3.0	8.04 ±0.17	146 ± 6	4.6 ± 2.5	140 ± 3	372 ± 28
Brook trout	15.2 ± 0.3	7.1 ±0.6	70.0 ± 6.0	7.90 ±0.30	144 ± 5	6.6 ±2.9	137 ± 4	364 -

[a] Tests using one day-old flagfish fry were conducted concurrently in the same containers.

[b] Means ± 1 standard deviation are given.

[c] No observation

oxygen concentrations were 70% of air saturation in all tests. The range of mean pH was 7.57 ± 0.09 to 8.04 ± 0.17; that of total alkalinity, 144 ± 5 to 161 ± 6 mg/ℓ $CaCO_3$; and that of acidity, 4.6 ± 2.5 to 6.6 ± 2.9 mg/ℓ $CaCO_3$. Mean total hardness varied from 137 ± 4 to 147 ± 8 mg/ℓ $CaCO_3$ and specific conductance from 364 to 392 μ mhos/cm.

Toxicity

Beryllium sulfate had a relatively low toxicity to the freshwater fish tested, being more toxic than lead chloride, but less toxic than the pentachlorophenol, cyanide, selenium and arsenic compounds. The descending order of toxicity was fathead minnow, flagfish fry, goldfish, brook trout, and channel catfish.

Toxicity curves encompassing exposures for up to 336 hr were obtained for juvenile fathead minnows, flagfish fry and juvenile goldfish. Juvenile channel catfish and adult brook trout were exposed for 96 hr to 59.3 mg/ℓ $BeSO_4$ without mortality. Ten percent mortality in brook trout after 120 hr exposure accounted for their placement ahead of channel catfish in terms of sensitivity. Two types of curves may have characterized the toxicity of beryllium sulfate to the freshwater fish studied (Figure 6). A median lethal threshold of 25.4 mg/ℓ $BeSO_4$ was reached in 336 hr in the toxicity test using fathead minnows. Linear toxicity curves portrayed the relationship between LC50 estimates and toxicant exposure times for juvenile goldfish and flagfish fry, although averaging of the concentration-percent response data and an insufficient number of observations precluded anything but a tentative conclusion for flagfish. Although the toxicity curve for goldfish suggested linearity, the 216-hr and 240-hr LC50 estimates for goldfish may have indicated increased mortality rates after exposure for these periods, similar to those observed for selenium dioxide.

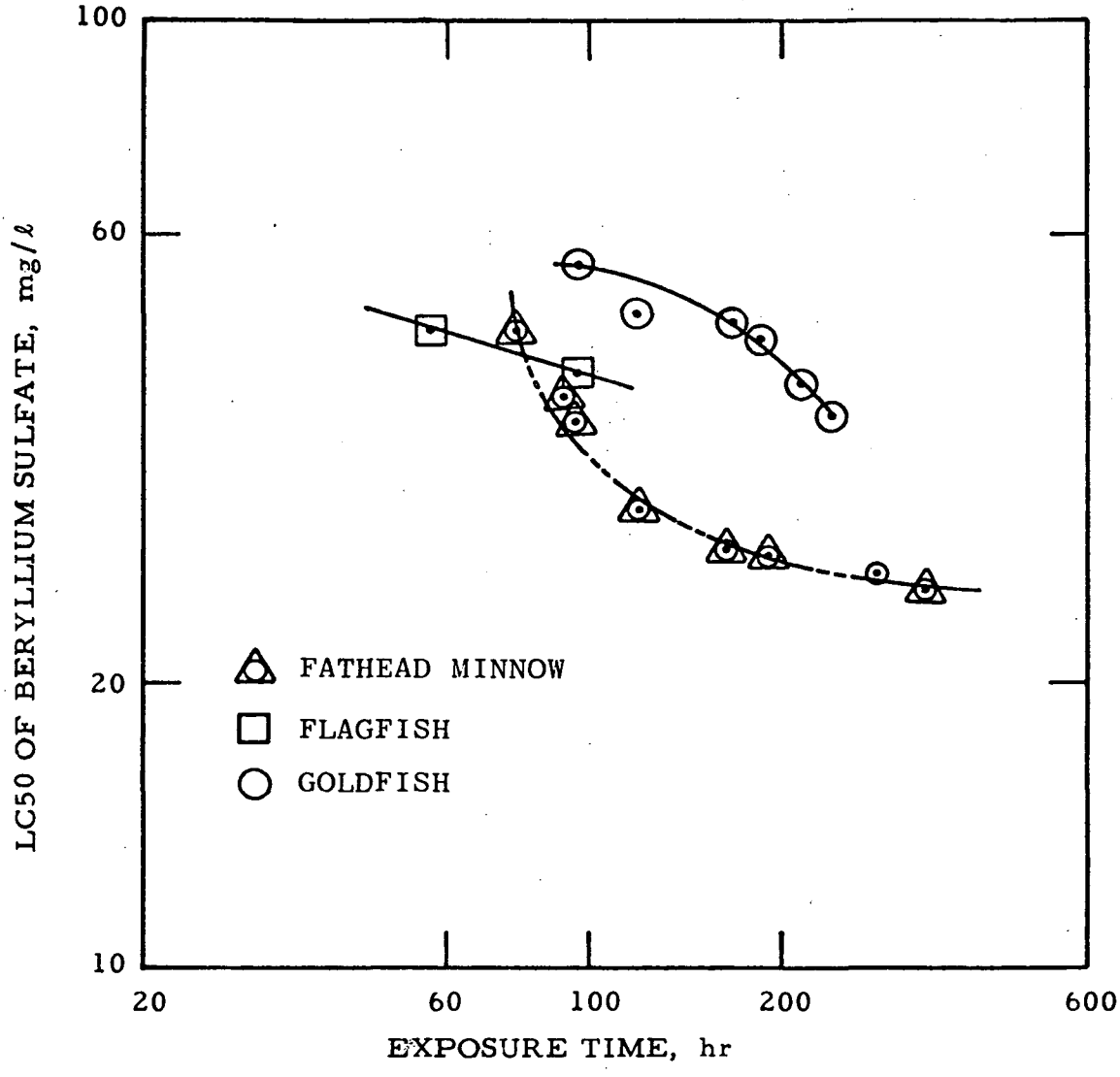

Fig. 6. Relationship between median lethal concentration (LC50) of beryllium sulfate and exposure time for three species of freshwater fish.

For the species tested, beryllium sulfate was acutely lethal down to 25.4 mg/ℓ which was the 336-hr LC50 for fathead minnows and about twelve times the theoretical solubility limit of beryllium hydroxide in distilled water. The 336-hr LC50, also designated as the median lethal threshold, was only 33% lower than the 96-hr LC50 of 37.9 mg/ℓ (Appendix Table 40). Ninety-six hour LC50 estimates of 46.3, 41.1 and 41.1 mg/ℓ $BeSO_4$ were determined for three groups of flagfish initially exposed within 24 hr of their hatching (Appendix Table 41). These estimates, which were pooled and averaged (42.8 mg/ℓ $BeSO_4$) for graphical presentation, were only slightly higher than the 96 hr estimate for minnows. The 96 hr LC50 for juvenile goldfish was 55.9 mg/ℓ, 1.5 times that for minnows. The lowest LC50 estimate calculated for goldfish was 38.4 mg/ℓ $BeSO_4$ for 240 hr exposure (Appendix Table 42 and Figure 6).

TOXICITY OF LEAD CHLORIDE

Lead chloride was not found to be acutely toxic to juvenile fathead minnows or adult brook trout at concentrations up to 100 mg/ℓ $PbCl_2$ and exposures up to 14 days. Preliminary static tests utilizing up to 500 mg/ℓ $PbCl_2$ indicated no acute lethality to fathead minnows in four day periods. Upon mixing with the diluent water, the toxicant precipitated immediately and rapidly settled out in the diluter apparatus and in the test containers. Under the test conditions, which were essentially the same as described for the other chemicals, there was little evidence that coagulation of the fish's mucus occurred.

SECTION VI
DISCUSSION

TOXICITY OF SODIUM PENTACHLOROPHENATE

The acute toxicity of the sodium salt of pentachlorophenol (NaPCP) was characterized by two types of toxicity curves, one resembling a rectangular hyperbola and the other a sigmoid shape. Only in the tests of brook trout was a rectangular hyperbola designating an acute median lethal threshold observed. Sigmoid-shaped curves characterized the responses of fathead minnows, goldfish and bluegill to the chemical. A change in slope of the toxicity curve commonly is interpreted to indicate a change in the mechanism or transmission of toxicity or a change in the resistance of the test specimens (Sprague[2]). In light of the known functional effects of NaPCP on fish, detailed below, it is likely that the fish's resistance was diminished through a progressive deterioration in physiological condition which ultimately resulted in their mortality.

One of the salient physiological consequences of sodium pentachlorophenate intoxication is an increase in oxygen consumption (Crandall and Goodnight[38]), which is due to the uncoupling of oxidative phosphorylation. In studies of the effects of sublethal concentrations of sodium pentachlorophenate (0.1 mg/ℓ) on selected enzymes of glycolysis, the pentose-phosphate shunt, the Embden-Meyerhof pathway, the tricarboxylic acid cycle, and the cytochromes in Atlantic eel (Anguilla anguilla), Boström and Johansson[39] found that the activity of enzymes associated with anaerobic catabolism of carbohydrates (glycolysis) was reduced, but that enzymes associated with the aerobic pathways (i.e. pentose phosphate shunt, TCA cycle, and the cytochromes) were enhanced. The augmented, NaPCP-induced energy demands of fasting fish are supplied initially by fatty acids (e.g. palmitic, stearic and oleic acid) from triglycerides (Hanes, et al[40, 41]). In many cases, extensive diminution or exhaustion of saturated and monounsaturated fatty acids

is followed by utilization of proteins and long-chain polyunsaturated fatty acids to fulfill the energy demands (Saddler, Koski and Cardwell[42]). Utilization of proteins and phospholipids may be regarded as a lethal physiological manifestation since the structural integrity of the animal is being severely compromised.

Thus, in the present studies, the heightened energy demands of pentachlorophenate-exposed fish, coupled with those associated with starvation over a cumulative 17 day period, could have resulted in the exhaustion of the energy stores of the fish and led to the fatal utilization of other substrates essential for the animal's biochemical integrity. If the minnows, bluegill, and goldfish had proportionally less depot fat than the adult brook trout, they may have exhausted their supplies earlier and subsequently perished. Growth of guppies (Poecilia reticulata Peters) (Crandall and Goodnight[38]) and of underyearling sockeye salmon [Oncorhynchus nerka (Walbaum)](Webb and Brett[43]) exposed for 45 to 90 days to sublethal concentrations of sodium pentachlorophenate has been found to have been depressed even though the fish were fed during the period. Webb and Brett[43] also found conversion efficiency to be reduced.

Curves resembling that obtained for brook trout have been obtained by others. A rectangular hyperbola described the acute toxicity of sodium pentachlorophenate to yearling sockeye salmon which were exposed for 504 hr in a flow-through system (Webb and Brett[43]). However, close inspection of the curve revealed what may have been a slight shift in the curve's slope between 100 and 200 hr, similar to what we observed for bluegill, minnow, and goldfish. No such change was observed for underyearling sockeye, which had been found to have a sensitivity equal to that of the older fish. Norup[44] also described a rectangular hyperbola toxicity curve for guppies exposed for 168 hr to NaPCP.

The acute toxicity tests conducted by our laboratory with an intermittent-flow bioassay system indicated that the median lethal threshold for the four species tested was at least 0.11 mg/ℓ NaPCP. This figure is one-half or less of those stated in much of the early literature, but is somewhat higher than the median lethal threshold of 0.057 mg/ℓ NaPCP and the levels of 0.00174 to 0.0018 mg/ℓ affecting growth and food conversion efficiency in 50% of the underyearling sockeye salmon exposed in a continuous-flow system (Webb and Brett[43]). As noted earlier, Boström and Johansson[39] observed significant alterations in the activities of certain enzymes in Atlantic eels exposed for 96 hr to 0.1 mg/ℓ NaPCP in a static system. One of the highest lethal thresholds is that of 0.75 mg/ℓ PCP, given by Bandt and Nehring[45] for rainbow trout. The lethal threshold for 72 hr exposure for five species of minnows (cyprinidae) is between 0.2 and 0.4 mg/ℓ NaPCP (Goodnight[46]). More recently, Norup[44] determined that the median lethal threshold for guppies was of the order of 2 mg/ℓ. He also presented a toxicity curve representing 25 fish species which indicated a composite lethal threshold of less than 0.1 mg/ℓ NaPCP for fish in general.

For shorter periods of exposure, comparison of LC50 estimates derived from the present work with those derived by others indicates closer agreement regardless of whether the tests were conducted in static or continuous-flow systems. For the bluntnose minnow [Pimephales notatus (Rafinesque)], a close relative of the fathead minnow, Goodnight[46] calculated that 0.4 mg/ℓ NaPCP would produce 100% mortality in 7 to 45 hr. This was surprisingly close to the LC50 value of 0.47 mg/ℓ NaPCP we calculated for the same period for fathead minnows. Crandall and Goodnight[47] calculated a 96-hr LC50 of 0.35 mg/ℓ NaPCP for 50 mm fathead minnows. This figure is only slightly higher than the interpolated estimate of 0.29 mg/ℓ determined in this laboratory for the same species. However, the 24-hr LC50 for rainbow trout and brown trout has been estimated to be around 0.01 mg/ℓ (Alabaster[48]), a much lower figure than that found for brook trout.

The disparity in tolerance between rainbow and brown trout and that of brook trout is so great that some of the difference is probably ascribable to variations in the characteristics of the experimental fish, test conditions and water quality.

The toxicity of sodium pentachlorophenate is known to increase with a decline in experimental pH. Goodnight[46] noted that fish survived longer at pH 7.6 than at 6.6. Later studies by Crandall and Goodnight[47] confirmed the earlier observation. They found that the median lethal time for fathead minnows exposed to 1.0 mg/ℓ NaPCP at a pH of 5.9 to 6.0 averaged 21 to 38 min, but increased to 72 to 93 min at a pH of 7.5 and 7.6, and to at least 1440 min at a pH of 8.9 to 9.0. According to these investigators, earlier studies by Blackman et al.,[49] which indicated that the physiological effects of substituted phenols was greatest when the pH of the water was closest to the pK of pentachlorophenol (4.8), confirmed their observations. At pH 4.8 pentachlorophenol is present in equal quantities of free acid and conjugate base. It thus appears that the free acid is more toxic than the phenoxide ion (Crandall and Goodnight[47]). However, the change in NaPCP toxicity may not be as great as indicated by their work since the minnows were not acclimated to the test temperatures but rather introduced abruptly. Nevertheless, the median lethal times for exposure to 1.0 mg/ℓ NaPCP of 225 to 281 min indicated much longer survival at 10°C than at 18° C (LT50 = 72 to 93 min) or 26° C (LT50 = 35 to 57 min).

TOXICITY OF SODIUM CYANIDE

Toxicity curves for the five species of freshwater fish indicated that acute mortality essentially ceased after approximately 100 hr. That sodium cyanide had a median lethal threshold is consistent with its well known physiologic action of causing tissue hypoxia and ultimately anoxia through competitive inhibition of enzymes (e.g. cytochrome oxidase) participating in oxidative phosphorylation.

Since the effect is based on competition for active sites on enzymes, the magnitude of hypoxia depends on the relative mole fractions of cyanide ion and the competing molecules. Several other investigators have also found that sodium cyanide has a median lethal threshold for fish. Wuhrmann[50] calculated that thresholds of 0.06, 0.08, 0.10, and 0.30 mg/ℓ prussic acid (hydrocyanic acid, HCN) existed for minnows (Phoxinus laevis), perch (Perca sp.), tench (Tinca tinca), and chub (Squalius cephalus), respectively, with the threshold for P. laevis being reached in 22 hr. The data of Doudoroff[51] indicate that a median lethal threshold of 0.23 mg/ℓ cyanide ion (CN^-) was reached in a maximum of 96 hr in static toxicity tests using 50 mm juvenile fathead minnows. In general, although the cyanide concentrations at which the median lethal thresholds were observed were of the same order as those found in our studies, they manifested themselves earlier. The difference can be probably traced to the fact that cyanide concentrations decline temporarily as a result of the hydrolysis of cyanide to hydrocyanic acid (i.e., $NaCN + H_2O \rightleftharpoons HCN + OH^- + Na^+$) and concomitant loss of hydrocyanic acid from the system because of volatilization. Doudoroff[51] was aware of the loss of HCN, the most toxic form of cyanide, noting that minnows perished within 14 hr when introduced into fresh cyanide solutions (e.g., 0.32 mg/ℓ CN^-), but incurred only 20% mortality within an equivalent interval when introduced into a solution which had been allowed to stand for 24 hr after preparation. The net effect of hydrocyanic acid loss is to produce an apparent median lethal threshold in a toxicity test, unless additional cyanide is added to stabilize toxicant levels. In our intermittent-flow toxicity tests, temporal monitoring of the total cyanide concentrations facilitated maintenance of toxicant concentrations at prescribed levels through manipulation of toxicant renewal rates and daily preparation of fresh stock toxicant solutions. The range of acute lethality of sodium cyanide was also shown to be slightly broader than found by others using static test conditions, again probably ascribable to the stable toxicant concentrations.

Much of the data describing the toxicity of cyanides to freshwater fish have been derived from tests conducted under static conditions, with or without renewal or measurement of the toxicant. Although the more sophisticated methods and toxicity testing equipment developed within the last decade and utilized in the present studies were designed to provide more accurate results than the earlier methods, comparison of LC50 values determined in this laboratory with those obtained by earlier workers indicates good agreement. For example, the 96-hr LC50 for fathead minnows exposed to sodium cyanide has been variously determined to be 0.23 mg/ℓ CN^- (Doudoroff[51]), 0.19 mg/ℓ CN^- in hardwater (Henderson, Pickering, and Lemke[52]) and 0.12 mg/ℓ CN^- in soft water (Henderson, et al.[52]). In one of the few tests where sodium cyanide concentrations were continually renewed, the 96-hr LC50 for 50 mm bluegill was approximately 0.15 mg/ℓ HCN, equivalent to the median lethal threshold (Doudoroff et al.[35]). We determined that the 96-hr LC50 for fathead minnows was less than half this value (0.065 mg/ℓ CN^-).

Further search of the literature also indicated fairly good agreement of toxicity data for bluegill exposed to sodium cyanide under static and intermittent-flow conditions. Henderson, et al.[52] calculated the 96 hr LC50 to be 0.08 mg/ℓ for bluegill exposed in hardwater. This value is only slightly higher than the interpolated LC50 value of 0.068 mg/ℓ CN^- determined here. Ninety-six hour median lethal concentrations for bluegill exposed to potassium cyanide by Cairns[53] and Cairns and Scheier[54,55,56] were essentially the same as estimates based on sodium cyanide, regardless of whether the diluent water was hard (range 0.14 to 0.17 mg/ℓ CN^-) or soft (0.13 mg/ℓ CN^-).

The small variation in the median lethal concentrations of the simple cyanide salts (NaCN and KCN) for fresh water fish indicates a uniformity in toxicity which is not significantly affected by water quality, the type of bioassay system employed, or by the size, age or condition of the test fish, even though the bioassays were conducted by investigators using different water qualities, stocks of test fish, and methods. Sprague[2] has commented that because of this variation, lethal levels for toxicants in general may vary a thousand-fold. Variation in toxicity of simple cyanides is obviously much lower.

Although pH is known to dramatically affect the toxicity of metal cyanide complexes, it does not have much of an affect on the toxicity of sodium or potassium cyanide in waters of approximately neutral pH, since cyanide is present predominately as the free acid (HCN). The influence of pH may be notable when the cyanide is present in a complex ion. An increase in the hydrogen ion concentration will cause liberation of HCN from the complex. These relationships have been examined extensively by Doudoroff[51] and Doudoroff, et al.[35]

TOXICITY OF SELENIUM

Selenium is a relatively common element which comprises approximately 6×10^{-5} percent of the earth's crust. In some areas of the United States, particularly in the midwest, seleniferous deposits are extensive and are major sources of selenium pollution of some fresh waters. In Nebraska, for example, Engberg[57] found that over 33% of the wells and 25% of the surface waters contained elemental selenium in excess of the 10 $\mu g/\ell$ upper limit established by the U.S. Public Health Service for drinking water. Selenium is commonly produced from the anode muds of electrolytic copper refineries. Industrially, the various allotropic forms of selenium are used in a variety of processes and components, including photocells, solar cells, and semiconductors. It is also employed in the dye and pigment industry and, because of its biological potency, as an insecticide in certain applications.

Although selenium is similar chemically to sulfur and tellurium, its toxicity is most similar to that of arsenic. The toxicology of selenium to mammals is much better known than that to fish because it is responsible for the "Alkali Disease" and "Blind Staggers" syndromes of grazing animals. These maladies result when animals graze on certain types of vegetation in soils containing high concentrations of selenium. When the element is abundant, certain plants accumulate large concentrations and store it in various organic forms. The pathologies associated with the two syndromes differ from that observed for inorganic selenium poisoning (selenosis) because the organic selenides are more toxic to mammals than the inorganic forms. The effects of the various types of selenium poisoning on animals and the nutritive importance of selenium are reviewed by Rosenfeld and Beath[58].

One of the first recorded studies of the effects of selenium on aquatic animals was completed by Ellis, et al.[59] using 80 mm goldfish and 160 mm channel catfish. In one experiment, goldfish were exposed to 2 mg/ℓ Se as sodium selenite for 46 days. Every 48 hr the fish were transferred to a fresh toxicant solution and fed. Under these conditions, fish ceased feeding within eight days, and the first animals perished in 18 days. Most mortalities were recorded between 25 and 37 days. The authors noted that goldfish died in four to ten days when exposed to 5 mg/ℓ Se as sodium selenite. Intraperitoneal injections of 3.0 mg Se/kg into 54 g catfish were found to be fatal to the fish in about 7 hr at 10° C. Injections of lesser amounts (0.2 mg/kg/day/5 days) produced exophthalmia, anemia, leukopenia and degeneration of liver, kidney and spleen. More recently, Weir and Hine[13] compared median lethal concentrations of selenium dioxide with those significantly affecting a conditioned behavior in 40 to 80 mm goldfish using static test conditions. The 9-day LC50 of 16.9 mg/ℓ SeO_2, which was derived from 48 hr exposure followed by 7 days holding in uncontaminated freshwater at 23° C, was 48 times greater than the concentration (0.035 mg/ℓ SeO_2) disrupting the ability of goldfish to respond to a light stimulus and seek a dark area.

The 9-day LC50 estimate obtained by Weir and Hine[13] is comparable to the LC50 of 17.2 mg/ℓ SeO_2 which we obtained for 168 hr continuous exposure, but about one-third that of our 48-hr LC50 of 46.5 mg/ℓ SeO_2 for the same species. Although the water used by Weir and Hine[13] was much softer (deionized water was reconstituted with 50 mg/ℓ $CaCO_3$) and of lower pH (range 6.0 to 6.9), it is probable, in light of the external pathology they noted, that substantial latent mortality occurred.

Our results indicate that for the given water quality and experimental conditions, selenium dioxide was most toxic to fathead minnows and least toxic to bluegills. Use of test speciments of equivalent age and size would affect the order of species sensitivity to some extent, as evidenced by the fact that the 96-hr LC50 for the much smaller fathead minnow fry was 40% that for juvenile minnows exposed for a comparable period. Nevertheless, direct comparison was possible for channel catfish, goldfish, and bluegill, which were of similar size and age.

The nature of the toxicity curves provides further insight into the biological action of selenium dioxide in addition to describing its differential effect on the various species. Because no lethal thresholds were observed for exposure periods up to 336 hr, it is evident that both toxicant concentration and exposure time influence toxicity. This conclusion is strengthened further by the observations that the fathead minnows, goldfish and bluegill, which were exposed for more than 168 hr, died at faster rates than would have been anticipated from extrapolation of the slopes of toxicity curves derived from exposures less than 168 hr. Selenium poisoning in fish evidently produces physiological dysfunction which manifests a lethal character upon prolonged exposure of the fish to the toxicant. Because of the external hemorrhaging observed by us for fathead minnow, channel catfish, brook trout and goldfish, and by Weir and Hine[13] for goldfish, it is likely that the peripheral blood, including either the blood or capillaries or both, was involved. This hypothesis is strengthened by the observation of erythrocyte destruction in catfish dosed with sodium selenite (Ellis, et al.[59]), and the common findings of damage to the hematopoietic system in mammals given inorganic selenium.

TOXICITY OF SODIUM ARSENITE

There are three important factors to consider in assessing the results of the toxicity tests of sodium arsenite. First, the presence of the toxicant altered the quality of the water, and in so doing, probably altered to some extent the magnitude of the responses of the test

animals. Secondly, there was no precipitation of the toxicant upon combination with the diluent; hence, the question of what form of the chemical was causing death was not as complicated. Finally, at least two modes of toxicity were evident from the curves relating median lethal concentration to exposure time.

In contrast to the acid conditions resulting from hydrolysis of the selenium, beryllium and lead compounds, hydrolysis of sodium arsenite rendered the water alkaline. At the highest toxicant concentrations employed, pH levels reached 8.9. pH values between 5.0 and 9.0 are generally not acutely lethal for most fish species (Doudoroff and Katz[60]; European Inland Fisheries Advisory Commission[61]), although chronic exposure to intermediate levels (pH 6.6) has been found to depress egg production and spawning of at least one freshwater fish species (Mount[62]). Jordan and Lloyd[63] determined that 15 days were required to kill 50% of a population of rainbow trout at a pH of 9.5, while four day exposures to pH 10.5 were necessary to produce 50% mortality in 39 mm bluegill (Cairns and Scheier[64]). In light of these findings, it appears that the highest levels of pH reached in the toxicity tests were near the lethal limits, and that sublethal pH stress may have interacted synergistically or additively with the toxicant to augment the responses of the test animals. Moreover, the sudden increase in ambient pH encountered by the fish, which had been acclimated to a level of approximately 7.5 to 8.0, might also act as a stresser. Jordan and Lloyd[63] found that the 24-hr median lethal pH levels were 9.86, 9.91 and 10.13 for rainbow trout previously acclimated to pH values of 6.55, 7.50 and 8.4. It is apparent that the possibility of a pH-toxicant interaction cannot be ignored for the high sodium arsenite concentrations. But since the log-probit, concentration-percent-mortality regression lines were calculated for the range of concentrations tested, including the lower concentrations where the pH interaction would likely be negligible, the bias would be effectively mitigated.

According to McKee and Wolf[4], the toxicity of arsenic is similar to that of selenium, although arsenic antagonizes the toxicity of selenium. In these studies, bimodal toxicity curves characterized the relationship between LC50 and exposure time for three of the species tested, fathead minnow, channel catfish and bluegill, whereas unimodal toxicity curves (resulting in median lethal thresholds) were observed for brook trout and goldfish. Differences in sensitivity, mediated perhaps by innate tolerance or a capability for metabolizing or excreting the chemical, may have distinguished the trout and goldfish from the other species. For the other species, the changes in slopes of the toxicity curves, each signifying increased mortality upon prolonged exposure to the lower concentrations, may represent the manifestation of a different toxicity mode, perhaps one denoting severe functional deterioration. Arsenic has been stated to be a cumulative poison (Jones[65]). Previous workers have either not conducted toxicant exposures sufficiently long to demonstrate whether a change in slope occurs or have conducted the tests in static systems wherein toxicant concentrations probably declined rather than remained stable. Grindley[66], in a study elaborated by Wuhrmann[50], exposed minnows (Phoxinus laevis) to sodium arsenite for 36 hr and observed a straight line relationship between the median time required for manifestiation of ataxia and toxicant concentration. Holland, et al.[67] exposed 0.30 g pink salmon fry [Oncorhynchus gorbuscha (Walbaum)] to sodium arsenite in a static system for ten days. When their percent mortality data were plotted against initial toxicant concentrations, mortality appeared to drop off and approach a median lethal threshold between 150 and 200 hr. Unfortunately, measured concentrations declined temporally, e.g., from 16.25 to 9.25 mg/ℓ at the highest level tested and from 1.50 to 0 mg/ℓ at the lowest.

Median lethal concentrations for sodium arsenite obtained by others in laboratory and field experiments were generally lower than those determined in this program. Clemens and Sneed[68] determined that the 24-and 48-hr LC50 values for channel catfish were 47.9 and

25.9 mg/ℓ $NaAsO_2$, respectively. Mortalities ceased after 48 hr. The 48-and 96-hr LC50 estimates for catfish exposed in this study were 56.9 and 31.2 mg/ℓ $NaAsO_2$, respectively. Gilderhus[69] estimated 96-hr LC50 values of 34 mg/ℓ $NaAsO_2$ for goldfish and 35 mg/ℓ $NaAsO_2$ for bluegill in laboratory tests, then determined that as little as 4.0 mg/ℓ sodium arsenite inhibited growth of both 0.5 g immature and 15.8g adult bluegill and survival of the immature fish when sodium arsenite was applied at various intervals to outdoor pools. The 96-hr median lethal concentration determined for bluegill in our laboratory was twice as high (72 mg/ℓ) as the LC50 determined by Gilderhus[69], while the 336-hr LC50 (31.6 mg/ℓ) was more than 1,000 times higher than the concentration of 0.03 mg/ℓ $NaAsO_2$ that Gilderhus[69] determined would not affect survival or growth of bluegill when the toxicant was applied at a weekly rate for the control of aquatic vegetation.

TOXICITY OF BERYLLIUM SULFATE

Beryllium as beryllium sulfate was one of the two metals tested at concentrations exceeding its solubility in the moderately hard, alkaline diluent water. The most probable reaction is between beryllium and hydroxyl ions, with consequent rise in hydrogen ion concentration (i.e. $nBe^{++} + nH_2O \rightleftharpoons (BeOH)_n^{n+} + nH^+$)(Everest[37]). Since the solubility of beryllium hydroxide in distilled water (2 mg/ℓ, Lange[36]) is of the same order as the lowest concentration found to be lethal in the toxicity tests (336-hr LC50 of 2.2 mg/ℓ Be^{++} in bioassay using fathead minnows), it is evident that the toxicity tests had to be conducted above the solubility limit, i.e., in the presence of beryllium hydroxide.

In these toxicity tests, beryllium concentrations were measured as total metal from water samples collected in the middle of the test chambers. Because of precipitation, however, probably only a fraction of the nominal concentration was causing the toxicity. Since beryllium uptake in fasted freshwater fish would be primarily if not entirely via the gills, it would be expected that only dissolved beryllium could be causing the toxicity. Since all toxicity tests were conducted above the diluent water solubility of this chemical and since graded, concentration dependent kills were observed, it is possible that some of the precipitated material may have contributed significantly to mortality. Lloyd and Herbert[70] have advanced an hypothesis that may well account for the toxicity of mixtures of precipitated metals in general, which may include beryllium. Dissolution of the precipitated metal adhering to the branchial epithelium may be effected by the transiently high hydrogen ion concentrations (stemming from carbonic acid) existing at the gill-water interface because of excretion of carbon dioxide. Mount[71] recently invoked this hypothesis as a partial explanation of the toxicity of mixtures of precipitated zinc sulfate to 1 to 2 g fathead minnows. Tabata[72], however, believed that precipitated heavy metals would not harm most aquatic animals.

The change in pH of the diluent water was found to vary with the total concentration of beryllium sulfate. Hydrogen ion concentrations increased with increased beryllium sulfate concentrations, although the magnitude of the increase measured in the intermittent-flow system was not as great as that observed in static systems. The lowest pH levels recorded by Slonim[10] were approximately 3.7 and 4.1 at 200 and 100 mg/ℓ $BeSO_4$, respectively, in toxicity tests of guppies in hard water (hardness = 400 to 500 mg/ℓ $CaCO_3$ and initial pH of 7.8 to 8.2). In contrast, the lowest pH level we recorded in the intermittent-flow toxicity tests was 6.60 at 64 mg/ℓ $BeSO_4$. The smaller deviation in pH in the intermittent-flow test may have resulted from water-flow through

the chambers being sufficient to keep the precipitation reaction in an intermediate stage by displacing old toxicant and adding fresh. As demonstrated earlier, the precipitation reaction was much slower than the 90% molecular displacement time of nine hours.

There is little doubt that the increased acidity of the diluent water caused by the presence of beryllium sulfate also contributed to the toxicity of the metal, even though the maximum displacement in pH was only about one unit. Although the pH range of 5 to 9 is not directly lethal to fish (European Inland Fisheries Advisory Commission[61]), abrupt variation from the optimum, which occurred in the present toxicity tests as a function of toxicant concentration, is not anticipated to be well tolerated by most fish species and would constitute a stresser. Coupled with the increased solubility of beryllium under more acid conditions, the net effect probably would be to increase somewhat the toxicity of the beryllium mixtures at the lower levels of pH. Many other investigators have documented the positive effect of decreased pH on metal toxicity to fish (Wuhrmann[50]; Mount[71]; Freeman and Everhart[73]). In Mount's[62] recent investigation of the chronic effects of low pH on fathead minnows, he observed decreased egg production by adult fathead minnows chronically exposed to a pH of 6.6 relative to controls which had been reared at pH 7.5. Decreased fecundity is probably one of the functional manifestiations of sublethal stress.

The few investigations of the toxicity of beryllium to fish have been conducted under static conditions. Pomelee[9] added daily a complex of beryllium sulfate and tartaric acid to aquaria containing goldfish, minnows and snails for 12 days and did not observe any toxic effects on the animals at a total beryllium concentration of 28.5 mg/ℓ. Tarzwell and Henderson[74] studied the acute toxicity of beryllium sulfate to fathead minnows and bluegill. Ninety-six hour median lethal concentrations were estimated to be 0.2 mg/ℓ Be in soft water (20 mg/ℓ $CaCO_3$ hardness) and 11 mg/ℓ Be in hard water (400 mg/ℓ $CaCO_3$ hardness) for fathead minnows, and 1.3 mg/ ℓ Be in soft water and 12 mg/ℓ Be in

hard water for bluegill. Slonim[10] examined the relationship between hardness and beryllium sulfate toxicity using the adult and fry stages of guppies. Adult guppies were 100 times more tolerant in water having 400 to 500 mg/ℓ total hardness (96-hr LC50 = 27 mg/ℓ Be) than in water having only 20 to 25 mg/ℓ hardness (96-hr LC50 = 0.23 mg/ℓ Be). Neither age nor size appeared to affect sensitivity since survival times of fry and adults in beryllium solutions were similar (Slonim[10]). In a later study using adult guppies, Slonim and Slonim[11] examined the relationship between beryllium toxicity and four levels of hardness. The 96-hr LC50 estimates were 0.16, 6.1, 13.7 and 20.0 mg/ℓ Be for 22, 150, 275 and 400 mg/ℓ $CaCO_3$ hardness, respectively. For 96 hr toxicant exposure, the relationship between LC50 and water hardness could be expressed by the linear regression equation: log LC50 = - 3.044 + 1.706 (log hardness).

The median lethal concentrations detailed above are from 1.5 to 7 times higher on a beryllium ion basis than those found in our tests. For example, the 96-hr LC50 of 11 mg/ℓ Be obtained for fathead minnows exposed in hard water by Tarzwell and Henderson[74] was 3.4 times greater than the comparable LC50 of 3.25 mg/ℓ Be determined in our laboratory. Hardness appears to exert a substantial influence on beryllium toxicity as the data of Slonim and Slonim[11] suggests. Calcium is known to reduce the toxicity of many metals. (Tabata[72, 75]).

Under the intermittent-flow test conditions employed in the present study at a hardness averaging approximately 141 mg/ℓ $CaCO_3$ and pH averaging 7.84, two types of curves may have characterized the toxicity of beryllium sulfate. In the toxicity test using juvenile fathead minnows, a rectangular hyperbola, designating a median lethal threshold in the region of 240 hr, was in evidence. In the test using goldfish, the curve appeared to be sigmoid-shaped. Because of insufficient observations in the tests of flagfish, a straight line was used to tentatively establish the relationship between LC50 and beryllium toxicity in the 48 to 96-hr exposure interval. The data indicated a steadily declining LC50 between

72 and 240 hr and are in contrast with the observations of Slonim[10] and Slonim and Slonim[11], who found little change in percent mortality of guppies after 24 hr exposure. The difference may be due to the use of an intermittent-flow system in our studies and a static system without toxicant renewal in theirs. Although Slonim[10] indicated that there was little toxicant loss from the system in 96 hr, it is possible that some beryllium precipitated and settled out, as was demonstrated in the experiment described in the Materials and Methods Section and in Figure 5, leaving only a small amount of dissolved ion in the supernatant water at its solubility limit. As noted earlier, precipitated metal enmeshed in the gill filaments probably contributes to toxicity upon its dissolution by carbonic acid and uptake by the fish.

TOXICITY OF LEAD CHLORIDE

Lead chloride was not found to be acutely lethal in a four day period to the two most sensitive freshwater fish tested, brook trout and fathead minnows. Precipitation of lead occurred rapidly, probably in the form of lead carbonate. According to Davies and Everhart[76], the solubility of lead in hard water (353 mg/ℓ $CaCO_3$), is of the order of 30 μg/ℓ. The solubility of lead as $PbCl_2$ in our laboratory water was found to be approximately 1.5 mg/ℓ. Because the toxicity of lead is limited by its low solubility in natural waters, it is not considered to be a very important pollutant in terms of acute toxicity (Aronson[77]). However, the 96-hr LC50 for lead chloride to fathead minnows has been estimated to be 2.4 mg/ℓ Pb in soft water (20 mg/ℓ $CaCO_3$) and greater than 75 mg/ℓ Pb in hard water (400 mg/ℓ $CaCO_3$) (Tarzwell and Henderson[74]), suggesting a substantial acute toxicity in soft waters. In studies of the short-and long-term effects of lead nitrate on juvenile brook trout, Dorfman and Whitworth[78] calculated a 96-hr LC50 of 3.12 mg/ℓ Pb for much smaller specimens (8 to 10 g) than employed for our studies. They used a reconstituted, deionized water having a pH of 6.8 to 7.2 and alkalinity of 27 to 34 mg/ℓ $CaCO_3$.

When single doses of lead nitrate were administered daily (5 times/week) and allowed to flush out, the level not affecting growth and survival of the fish was between 10 and 15 mg/ℓ Pb. Even though fish may not experience mortality upon acute exposure to lead in hard waters, functional disturbances may result. In sea water spiked with 10 mg/ℓ lead as lead nitrate, Jackim[79] observed a 22% decline in the activity of the enzyme, γ-aminolevulinate, after 96 hr in mummichogs [Fundulus heteroclitus (Linnaeus)] and a 66% decline in the enzyme's activity in winter flounder [Pseudopleuronectes americanus (Walbaum)] exposed for one week. Dawson[80] employed massive concentrations of lead acetate (100, 000 ppm) to invoke severe disturbances in the peripheral blood and hematopoietic system of brown bullheads [Ictalurus nebulosus (Lesueur)] after 16 to 183 days.

SECTION VII

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SECTION VIII

APPENDIX

Appendix Table 1. CHARACTERISTICS OF TEST SPECIES EXPOSED TO SODIUM PENTACHLOROPHENATE

Species	Approximate age at testing, months	Developmental stage	Test specimen density, g fish/ℓ	Total length, mm	Wet body weight, g
Fathead minnow	3	J[a]	0.06	22.7 ±3.2[d]	0.098 ±0.047[d]
Bluegill	12	J	3.15	70.4 ±7.2	5.2 ±1.6
Bluegill	12	J	0.50	43.6 ±6.1	0.84 ±0.49
Brook trout	18	A[b]	25.25	218.0 ±16.0	101.0 ±21.0
Goldfish	12	A[c]	1.70	65.9 ±12.4	2.8 ±1.3
Goldfish	12	A[c]	4.24	80.5 ±14.6	7.0 ±3.0

[a] Juvenile

[b] Adult, but not sexually mature

[c] Adult

[d] Means ± standard deviation are given

Appendix Table 2. CHARACTERISTICS OF FISH EXPOSED TO SODIUM CYANIDE

Species	Approximate age at testing, months	Developmental stage	Test specimen density, g fish/ℓ	Total length, mm	Wet body weight, g
Fathead minnow	3	J[a]	0.12	28.1[c] ± 4.4	0.205[c] ±0.105
Goldfish	6	J	1.76	54.3 ± 7.9	2.9 ±1.3
Brook trout	18	A[b]	20.33	211.0 ±11.0	81.3 ±16.2
Channel catfish	6	J	0.69	51.8 ± 7.1	1.14 ±0.57
Bluegill	6	J	0.12	28.1 ± 4.4	0.20 ±0.10

[a] Juvenile.

[b] Adult.

[c] Means ± standard deviation are given.

Appendix Table 3. CHARACTERISTICS OF FISH EXPOSED TO SELENIUM DIOXIDE

Species	Approximate age at testing, months	Developmental stage	Test specimen density, g fish/ℓ	Total length, mm	Wet body weight, g
Fathead minnow	(1 day)	yolk-sac fry	---	5.0 [c] ± 1.0	---
Fathead minnow	3	J [a]	0.05	20.7 ± 3.0	0.085 [c] ±0.045
Flagfish	2	J	0.04	14.7 ± 2.9	0.059 ±0.039
Brook trout	18	A [b]	24.90	211.0 ±13.0	99.6 ±18.5
Channel catfish	6	J	1.45	64.5 ± 6.9	2.4 ±0.8
Goldfish	6	J	1.45	62.0 ± 4.9	2.4 ±0.5
Bluegill	6	J	2.42	65.3 ± 6.4	4.0 ±1.4

[a] Juvenile.

[b] Adult, but not sexually mature. Gonads of male trout comprised 0.0096 mg/g body weight, while those of female trout comprised 0.0288 mg/g.

[c] Means ± 1 standard deviation are given.

Appendix Table 4 . CHARACTERISTICS OF FISH EXPOSED TO SODIUM ARSENITE

Species	Approximate age at testing, months	Developmental stage	Test specimen density, g fish/ℓ	Total length, mm	Wet body weight, g
Goldfish	6	J[a]	0.15	62.0[c] ± 4.9	2.4[c] ±0.5
Fathead minnow	3	J	0.02	21.0 ± 2.7	0.085 ±0.035
Brook trout	18	A[b]	21.18	200.0 ±14.0	84.7 ±19.0
Bluegill	6	J	1.27	51.8 ± 7.7	2.1 ±1.1
Channel catfish	6	J	1.46	66.4 ± 8.7	2.4 ±0.8

[a] Juvenile.

[b] Adult.

[c] Means ± 1 standard deviation are given.

Appendix Table 5. CHARACTERISTICS OF FISH EXPOSED TO BERYLLIUM SULFATE

Species	Approximate age at testing, months	Developmental stage	Test specimen density, g fish/ℓ	Total length, mm	Wet body weight, g
Flagfish	(1-4 days)	yolk-sac fry	---	3.0[c] ± 0.5	---
Fathead minnow	3	J[a]	0.08	25.4 ± 2.9	0.139[c] ±0.050
Goldfish	6	J	1.52	64.3 ± 7.0	2.5 ±1.1
Channel catfish	6	J	1.27	63.9 ± 6.4	2.1 ±0.6
Brook trout	18	A[b]	31.00	229.0 ±10.0	124.0 ±22.0

[a] Juvenile.

[b] Adult.

[c] Means ± 1 standard deviation are given.

Appendix Table 6. REPRODUCIBILITY AND ACCURACY OF CHEMICAL ANALYSES OF SELENIUM DIOXIDE

Replicate	Absorbance of spiked concentrations of selenium dioxide as Se (mg/ℓ)[a]					
	Reagent[b] blank	5[c]	Standards			
			5	10	15	20
1	0.0000	0.0168	0.0168	0.0313	0.0434	0.0550
2	--	0.0168	0.0170	0.0301	0.0432	0.0558
3	--	0.0168	-	-	-	-
4	--	0.0173	-	-	-	-
5	--	0.0170	-	-	-	-
Mean	0.0000	0.0169	0.0169	0.0307	0.0433	0.0554
Standard deviation	--	0.0002	-	-	-	-
Standard error	--	0.0001	-	-	-	-
Coefficient of variation, %	--	1.1	-	-	-	-

[a]Read at a wavelength of 196 nm.

[b]Distilled water.

[c]Spiked into filtered (Whatman No. 1 filter paper) water collected from tank containing control fish.

Appendix Table 7. REPRODUCIBILITY AND ACCURACY OF CHEMICAL ANALYSES OF SODIUM ARSENITE

Replicate	Absorbance of spiked concentration of sodium arsenite as As (mg/ℓ)[a]					
	Reagent[b] blank	2[c]	Standards			
			2	5	10	20
1	0.0000	0.0057	0.0054	0.0139	0.0278	0.0562
2	-	0.0061	-	-	-	-
3	-	0.0057	-	-	-	-
4	-	0.0054	-	-	-	-
5	-	0.0054	-	-	-	-
Mean	0.0000	0.0057	0.0054	0.0139	0.0278	0.0562
Standard deviation	-	0.0003	-	-	-	-
Standard error	-	0.0001	-	-	-	-
Coefficient of variation, %	-	5.3	-	-	-	-

[a]Read at a wavelength of 193.7 nm.

[b]Distilled water.

[c]Spiked into filtered (Whatman No. 1 filter paper) water collected from tank containing control fish.

Appendix Table 8 . REPRODUCIBILITY AND ACCURACY OF CHEMICAL ANALYSES OF BERYLLIUM SULFATE

Replicate	Absorbance of spiked concentrations of beryllium sulfate as Be (mg/ℓ)[a]			
	Reagent[b] blank	1.0[c]	Standards 0.5	Standards 1.0
1	0.0000	0.1249	0.0610	0.1249
2	-	0.1249	-	-
3	-	0.1249	-	-
4	-	0.1238	-	-
5	-	0.1232	-	-
Mean	0.0000	0.1242	0.0610	0.1249
Standard deviation	-	0.00074	-	-
Standard error	-	0.00033	-	-
Coefficient of variation, %	-	0.59	-	-

[a]Read at a wavelength of 234.9 nm.

[b]Distilled water.

[c]Spiked into filtered (Whatman No. 1 filter paper) water collected from tank containing control fish.

Appendix Table 9. REPRODUCIBILITY AND ACCURACY OF CHEMICAL ANALYSES OF LEAD CHLORIDE

Replicate	Absorbance of spiked concentrations of lead chloride as Pb (mg/ℓ)[a]					
	Reagent[b] blank	5[c]	Standards 5	10	15	20
1	0.0004	0.0379	0.0362	0.0716	0.1073	0.1427
2	-	0.0379	-	-	-	-
3	-	0.0379	-	-	-	-
4	-	0.0372	-	-	-	-
5	-	0.0372	-	-	-	-
Mean	0.0004	0.0376	0.0362	0.0716	0.1073	0.1427
Standard deviation	-	0.0004	-	-	-	-
Standard error	-	0.0001	-	-	-	-
Coefficient of variation, %	-	1.1	-	-	-	-

[a]Read at a wavelength of 283.3 nm.

[b]Distilled water.

[c]Spiked into filtered (Whatman No. 1 filter paper) water collected from tank containing control fish.

Appendix Table 10. REPRODUCIBILITY AND ACCURACY OF CHEMICAL ANALYSES OF SODIUM CYANIDE

Replicate	Absorbance of spiked concentrations of sodium cyanide as CN^- ($\mu g/\ell$)[a]						
				Standard			
	Blank[b]	Control[c]	5[d]	5	10	15	20
1	0.000	0.003	0.123	0.118	0.264	0.399	0.575
2	-	-	0.130	-	-	-	-
3	-	-	0.133	-	-	-	-
4	-	-	0.131	-	-	-	-
5	-	-	0.131	-	-	-	-
Mean	0.000	0.003	0.130	0.118	0.264	0.399	0.575
Standard deviation	-	-	0.004	-	-	-	-
Standard error	-	-	0.002	-	-	-	-
Coefficient of variation, %	-	-	3.1	-	-	-	-

[a]Read at a wavelength of 620 nm.

[b]Distilled water.

[c]Water taken from tank containing control fish.

[d]Spiked into filtered (Whatman No. 1 filter paper) water collected from tank containing control fish.

Appendix Table 11. REPRODUCIBILITY AND ACCURACY OF CHEMICAL ANALYSES OF SODIUM PENTACHLOROPHENATE

Replicate	Peak height, mm			
	Reagent[a] blank	Control	0.2 mg/ℓ Spike[b]	0.2 mg/ℓ Standard
1	0	0	110.6	101.1
2	-	-	101.2	-
3	-	-	101.3	-
4	-	-	100.1	-
5	-	-	94.4	-
Mean	0	0	101.52	101.1
Standard deviation	-	-	5.82	-
Standard error	-	-	2.60	-
Coefficient of variation, %	-	-	5.73	-

[a]Distilled water.

[b]Spiked into filtered (Whatman No. 1 filter paper) water collected from tank containing control fish.

Appendix Table 12. MEASURED CONCENTRATIONS OF SODIUM PENTACHLOROPHENATE (NaPCP) IN TOXICITY TESTS

Fish Species	No. measurements/tank	Measured NaPCP concentration, mg/ℓ					
		Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Brook trout	3	0[a]	0.091[b]	0.110	0.162	0.230	0.347
		-	±0.014	±0.023	±0.057	±0.042	±0.046
Fathead minnow	3	0	0.125	0.212	0.260	0.408	0.581
		-	±0.050	±0.040	±0.102	±0.094	±0.141
Goldfish							
Test 1	3	0	0.082	0.107	0.156	0.188	0.232
		-	±0.011	±0.006	±0,002	±0.004	±0.019
Test 2	1	0	---	---	0.432	0.650	0.921
		-			---	---	---
Bluegill							
Test 1	2	0	0.313	0.375	0.502	0.719	0.810
		-	±0.007	±0.023	±0.099	±0.030	±0.018
Test 2	0	0	0.075	0.100	0.133	0.178	0.237
		-	---	---	---	---	---

[a]No pentachlorophenate detected.

[b]Means ± 1 standard deviation are given.

Appendix Table 13. MEASURED CONCENTRATIONS OF SODIUM CYANIDE (NaCN) IN TOXICITY TESTS

Fish species	No. measurements/tank	Measured concentration of NaCN, mg/ℓ					
		Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Fathead minnow							
Test 1	3	0[a]	0.077[b]	0.100	0.146	0.173	0.245
		-	±0.015	±0.023	±0.044	±0.061	±0.056
Test 2	1	0	0.268	0.342	0.424	0.572	0.783
		-	----	----	----	----	----
Bluegill	1	0	0.160	0.184	0.252	0.364	0.440
		-	----	----	----	----	----
Brook trout							
Test 1	3	0	0.16	0.22	0.28	0.39	0.49
		-	±0.02	±0.04	±0.03	±0.05	±0.03
Test 2	4	0	0.093	0.178	0.224	0.306	0.397
		-	±0.054	±0.015	±0.009	±0.021	±0.042
Channel catfish	2	0	0.146	0.240	0.254	0.458	0.577
		-	±0.083	±0.047	±0.066	±0.018	±0.064
Goldfish							
Test 1	4	0	0.293	0.383	0.480	0.634	0.840
		-	±0.114	±0.165	±0.196	±0.217	±0.352
Test 2	2	0	0.616	0.817	1.12	1.34	2.13
		-	±0.020	±0.090	±0.04	±0.02	±0.01

[a]No cyanide detected.

[b]Means ± 1standard deviation are given.

Appendix Table 14. MEASURED CONCENTRATIONS OF SELENIUM DIOXIDE (SeO_2) IN TOXICITY TESTS

Fish Species	No. measurements/tank	Measured SeO_2 concentration, mg/ℓ					
		Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Fathead minnow							
Test 1	3	0[a]	2.9[b]	4.2	5.6	8.2	11.2
		-	± 1.5	± 1.5	± 1.8	± 1.7	± 2.1
Test 2	2	0	17.6	22.4	29.7	40.1	54.1
		-	± 5.0	± 5.4	± 7.4	± 8.2	±16.8
Brook trout	2	0	25.6	33.7	51.1	67.6	102.2
		-	± 0.4	± 0.8	± 4.0	± 8.3	± 7.1
Channel catfish	2	0	17.9	25.4	36.5	45.4	63.2
		-	----	± 4.8	± 0	± 0.6	± 0
Flagfish	2	0	11.2	16.9	21.8	27.9	37.6
		-	± 1.2	± 1.6	± 2.2	± 2.3	± 0.2
Goldfish							
Test 1	5	0	7.0	10.4	14.0	20.0	33.0
		-	± 1.4	± 1.3	± 0.4	± 3.2	± 4.4
Test 2	2	0	38.8	51.7	72.0	86.3	114.1
		-	± 1.6	± 1.6	± 0.8	± 1.2	± 0
Bluegill							
Test 1	2	0	----	----	70.9	95.4	133.0
		-	----	----	± 9.4	± 2.6	± 0.3
Test 2	4	0	8.8	11.9	18.2	23.9	33.1
		-	± 1.3	± 1.2	± 3.2	± 2.2	± 2.9

[a]No selenium detected.

[b]Means ± 1 standard deviation are given.

Appendix Table 15. MEASURED CONCENTRATIONS OF SODIUM ARSENITE ($NaAsO_2$) IN TOXICITY TESTS

Fish Species	No. measurements/tank	Measured concentration of $NaAsO_2$, mg/ℓ					
		Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Brook trout							
Test 1	4	0[a]	8.6[b]	10.6	15.5	19.9	25.9
		-	±3.5	±3.5	±3.3	±2.8	±3.1
Test 2	2	0	27.7	38.4	42.4	71.5	84.7
		-	±1.1	±1.8	±2.3	±1.7	±2.9
Fathead minnow							
Test 1	4	0	6.7	8.8	13.3	17.3	25.6
		-	±2.4	±1.2	±2.0	±3.1	±4.3
Test 2	1	0	25.5	33.7	44.1	59.9	82.1
		-	----	----	----	----	----
Channel catfish	2	0	19.8	25.1	41.5	51.7	72.4
		-	±1.4	±0.4	±0.4	±1.6	±8.9
Goldfish							
Test 1	3	0	21.4	24.6	32.1	40.1	57.4
		-	±2.3	±3.3	±2.6	±1.2	±2.9
Test 2	1	0	66.6	90.8	129.7	182.6	272.0
		-	----	----	----	----	----
Flagfish	2	0	19.8	25.1	41.5	51.7	72.4
		-	±1.4	±0.4	±0.4	±1.6	±8.9
Bluegill	3	0	16.3	23.9	31.6	41.5	67.9
		-	± 2.6	± 2.2	± 0.7	± 2.4	± 2.5

[a]No arsenic detected.

[b]Means ± 1 standard deviation are given.

Appendix Table 16. MEASURED CONCENTRATIONS OF BERYLLIUM SULFATE ($BeSO_4$) IN TOXICITY TESTS

Fish species	No. measurements/tank	Measured $BeSO_4$ concentration, mg/ℓ					
		Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Fathead minnow	3	0[a] -	18.8[b] ± 2.5	25.1 ± 2.7	28.6 ± 2.0	37.6 ± 2.3	47.8 ± 2.2
Flagfish	3	0 -	18.8 ± 2.5	25.1 ± 2.7	28.6 ± 2.0	37.6 ± 2.3	47.8 ± 2.2
Goldfish	3	0 -	18.8 ± 3.0	24.6 ± 0.7	36.8 ± 4.6	46.9 ± 3.9	59.0 ±13.0

[a]No beryllium detected.

[b]Means ± 1 standard deviation are given.

Appendix Table 17. STANDARD CONCENTRATION - PERCENT MORTALITY DATA SUPPLIED BY COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS

Data Set	Percent Mortality						
	Toxicant Concentrations, µg/ℓ						
	Control	7.8	13	22	36	60	100
A	0	0	0	10	100	100	100
B	0	0	0	70	100	100	100
C	0	0	0	10	40	100	100
D	0	0	0	20	70	100	100
E	0	0	0	20	30	100	100

Appendix Table 18. COMPARISON OF MEDIAN LETHAL CONCENTRATIONS AND 95% CONFIDENCE LIMITS WITH OTHER AQUATIC TOXICOLOGY LABORATORIES

Data set	Our laboratory				Average of eight other laboratories[a]	
	Litchfield and Wilcoxon (1949)		Computer program			
	LC50, µg/ℓ	95% Confidence limits for LC50	LC50, µg/ℓ	95% Confidence limits for LC50	LC50,[b] µg/ℓ	95% Confidence limits for LC50
A	25.4	22.1 - 29.2	--[c]	--	25.3 ±1.9	20.4 - 32.0 ±5.1 ±4.8
B	21.2	18.8 - 23.9	--	--	20.4 ±0.9	14.8 - 49.3 ±4.0 ±57.0
C	35.5	28.2 - 44.7	35.6	30.3 - 41.7	35.3 ±2.4	27.5 - 45.1 ±3.3 ±3.6
D	29.8	23.9 - 37.2	29.5	25.0 - 34.7	29.4 ±0.8	23.5 - 36.9 ±1.9 ±1.2
E	35.5	27.1 - 46.9	35.5	29.1 - 43.3	36.5 ±3.4	28.2 - 46.8 ±3.5 ±4.3

[a] Results obtained through use of both manual and computer methods.

[b] Means ± 1 standard deviation are given for concentrations in µg/ℓ.

[c] Calculations not made since computer was not programmed to handle data having only one partial kill.

Appendix Table 19. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR ADULT BROOK TROUT EXPOSED TO SODIUM PENTACHLOROPHENATE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	Slope function[a]
24	0.315	0.294 - 0.337	1.07
32	0.230	0.215 - 0.246	1.07
48	0.180	0.168 - 0.193	1.07
72	0.153	0.143 - 0.164	1.07
96	0.138	0.129 - 0.148	1.07
152	0.128	0.119 - 0.138	1.08
219	0.118	0.110 - 0.126	1.07
336	0.118	0.110 - 0.126	1.07

[a] All median lethal concentrations calculated according to Litchfield and Wilcoxon[29]. The slope function, S, is the antilogarithm of the standard deviation of the population tolerance frequency distribution.

Appendix Table 20. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE FATHEAD MINNOWS EXPOSED TO SODIUM PENTACHLOROPHENATE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
8	0.469	0.437 - 0.504	0.0721	$9.56 + 13.87 (\log x_i)$
12	0.358	0.334 - 0.383	0.0797	$10.61 + 12.55 (\log x_i)$
21	0.343	0.315 - 0.374	0.0872	$10.33 + 11.47 (\log x_i)$
72	0.309	0.285 - 0.336	0.0953	$10.34 + 10.49 (\log x_i)$
96	0.285	0.267 - 0.305	0.0769	$12.09 + 13.01 (\log x_i)$
120	0.276	0.258 - 0.295	0.0758	$12.38 + 13.20 (\log x_i)$
173	0.255	0.237 - 0.274	0.0844	$12.04 + 11.85 (\log x_i)$
216	0.235	0.210 - 0.263	0.1315	$9.78 + 7.60 (\log x_i)$
240	0.217	0.194 - 0.243	0.1307	$10.08 + 7.65 (\log x_i)$
288	0.185	0.155 - 0.221	0.1772	$9.13 + 5.64 (\log x_i)$
336	0.153	0.123 - 0.188	0.1680	$9.86 + 5.95 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 21. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR GOLDFISH EXPOSED TO SODIUM PENTACHLOROPHENATE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$[a]	Log-probit regression equation[b]
21	0.369	0.336 - 0.406	0.1100	$8.94 + 9.09 (\log x_i)$
46	0.270	0.246 - 0.297	0.1099	$10.18 + 9.10 (\log x_i)$
120	0.253	0.231 - 0.277	0.0910	$11.56 + 11.00 (\log x_i)$
168	0.241	0.219 - 0.265	0.0961	$11.43 + 10.41 (\log x_i)$
268	0.202	0.171 - 0.239	0.2180	$8.19 + 4.59 (\log x_i)$
336	0.189	0.162 - 0.219	0.1955	$8.71 + 5.12 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 22. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR BLUEGILL EXPOSED TO SODIUM PENTACHLOROPHENATE

Exposure time, hr	LC 50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [a]	Log - probit regression equation[b]
5	0.828	0.798-0.860	0.0372	$7.21 + 26.90 (\log x_i)$
6.5	0.719	0.691-0.749	0.0403	$8.56 + 24.80 (\log x_i)$
9.5	0.534	0.497-0.574	0.0716	$8.80 + 14.00 (\log x_i)$
30	0.303	0.283-0.324	0.0564	$14.20 + 17.73 (\log x_i)$
243	0.251	0.222-0.284	0.1236	$9.86 + 8.09 (\log x_i)$
313	0.226	0.196-0.261	0.1443	$9.48 + 6.93 (\log x_i)$
390	0.207	0.170-0.252	0.1971	$8.47 + 5.07 (\log x_i)$
406	0.188	0.162-0.218	0.1470	$9.95 + 6.80 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}i = a+b (\log x_i)$.

Appendix Table 23. MEDIAN LETHAL CONCENTRATIONS (LC50) AS CN^- FOR FATHEAD MINNOWS EXPOSED TO SODIUM CYANIDE

Exposure time, hr	LC50, mg/ℓ	95% confidence limits for LC50, mg/ℓ	$\hat{\sigma}$[a]	Log-probit regression equation[b]
1.5	0.326	0.299 - 0.356	0.0870	$10.59 + 11.49 (\log x_i)$
2.5	0.216	0.208 - 0.225	0.0449	$19.81 + 22.25 (\log x_i)$
3.0	0.196	0.189 - 0.205	1.0710	---
6.0	0.181	0.174 - 0.187	1.0640	---
33.0[c]	0.142	---	---	---
120.0	0.120	0.106 - 0.136	0.1255	$12.33 + 7.97 (\log x_i)$
144.0	0.117	0.104 - 0.131	0.1156	$13.07 + 8.65 (\log x_i)$
192.0	0.114	0.102 - 0.127	0.1066	$13.84 + 9.38 (\log x_i)$
240.0	0.114	0.102 - 0.127	0.1066	$13.84 + 9.38 (\log x_i)$

[a]Standard deviation of the logarithm of the population tolerance frequency distribution.

[b]Probit $\hat{y}_i = a + b (\log x_i)$.

[c]Median lethal time.

Appendix Table 24. MEDIAN LETHAL CONCENTRATIONS (LC50) AS CN^- FOR BLUEGILL EXPOSED TO SODIUM CYANIDE

Exposure time, hr	LC50, mg/ℓ	95% confidence limits for LC50, mg/ℓ	$\hat{\sigma}$[a]	Log-probit regression equation[b]
1.5	0.326	0.299 - 0.356	0.0870	$10.59 + 11.49 (\log x_i)$
2.0	0.216	0.208 - 0.225	0.0449	$19.81 + 22.25 (\log x_i)$
6.0	0.183	0.176 - 0.191	1.0720	---
24.0	0.149	0.143 - 0.154	1.0587	---
48.0[c]	0.134	0.126 - 0.142	1.0887	---
120.0	0.124	0.108 - 0.142	0.1735	$10.23 + 5.77 (\log x_i)$
168.0	0.116	0.103 - 0.130	0.1515	$11.19 + 6.60 (\log x_i)$

[a]Standard deviation of the logarithm of the population tolerance frequency distribution.

[b]Probit $\hat{y}_i = a + b (\log x_i)$, where x_i = concentration of CN^-.

[c]LC50 based on truncated data.

Appendix Table 25. MEDIAN LETHAL CONCENTRATIONS (LC50) AS CN^- OF ADULT BROOK TROUT EXPOSED TO SODIUM CYANIDE

Exposure time, hr	LC50, mg/ℓ	95% confidence limits for LC50, mg/ℓ	Slope function, S[a]
Test No. 1			
6	0.260	0.245 - 0.277	1.063
12	0.210	0.196 - 0.225	1.083
24	0.196	0.185 - 0.209	1.074
48	0.163	0.152 - 0.175	1.083
96	0.156	0.145 - 0.167	1.083
168	0.152	0.142 - 0.164	1.084
240	0.141	0.103 - 0.193	1.512
240[b]	0.131	0.117 - 0.147	0.1320
264	0.127	0.100 - 0.162	1.377
264[b]	0.124	0.111 - 0.139	0.1284
Test No. 2			
7	0.223	0.184 - 0.269	1.248
10	0.170	0.159 - 0.182	1.192
21	0.158	0.148 - 0.168	1.075
264	0.133	0.110 - 0.159	1.277
288	0.126	0.105 - 0.151	1.277

[a]Antilogarithm of $\hat{\sigma}$.

[b]Calculations by computer program. Log-probit regression equations were Probit $\hat{y}_i = 11.68 + 7.58 (\log x_i)$ for 240-hr LC50 and Probit $\hat{y}_i = 12.06 + 7.79 (\log x_i)$ for 264-hr LC50.

Appendix Table 26. MEDIAN LETHAL CONCENTRATIONS (LC50) AS CN^- FOR CHANNEL CATFISH EXPOSED TO SODIUM CYANIDE

Exposure time, hr	LC50, mg/ℓ	95% confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
6	0.249	0.212 - 0.293	0.1865	$8.24 + 5.36 (\log x_i)$
10	0.187	0.169 - 0.207	0.1181	$11.17 + 8.47 (\log x_i)$
20	0.166	0.153 - 0.180	0.0930	$13.40 + 10.75 (\log x_i)$
26	0.161	0.149 - 0.174	0.0997	$12.96 + 10.03 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 27. MEDIAN LETHAL CONCENTRATIONS (LC50) AS CN^- FOR JUVENILE GOLDFISH EXPOSED TO SODIUM CYANIDE

Exposure time, hr	LC50, mg/ℓ	95% confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
4	1.134	0.962 - 1.337	0.1646	---
6	0.856	0.730 - 1.004	0.1600	---
8	0.595	0.531 - 0.665	0.1447	---
12	0.403	0.355 - 0.457	0.1636	---
24	0.330	0.287 - 0.378	0.1581	---
24[c]	0.446	0.415 - 0.480	0.0732	$9.79 + 13.66 (\log x_i)$
36	0.350	0.335 - 0.366	1.0865	---
48	0.345	0.329 - 0.361	1.0739	---
96	0.318	0.300 - 0.337	0.0670	$12.43 + 14.92 (\log x_i)$
120	0.309	0.290 - 0.329	0.0711	$12.16 + 14.06 (\log x_i)$
168	0.298	0.281 - 0.317	0.0696	$12.56 + 14.36 (\log x_i)$
240	0.278	0.260 - 0.298	0.0786	$12.07 + 12.73 (\log x_i)$
336	0.261	0.238 - 0.287	0.0947	$11.16 + 10.56 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

[c] Statistics calculated from a second toxicity test conducted for 336 hr.

Appendix Table 28. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE FATHEAD MINNOWS EXPOSED TO SELENIUM DIOXIDE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$[a]	Log-probit regression equation[b]
18.5	31.2	26.9 - 36.3	0.1945	$-2.68 + 5.14 (\log x_i)$
21.5	27.9	24.1 - 32.4	0.1917	$-2.55 + 5.22 (\log x_i)$
24.5	24.3	16.4 - 36.0	0.1846	$-2.50 + 5.42 (\log x_i)$
30	19.5	16.8 - 22.7	0.1931	$-1.68 + 5.18 (\log x_i)$
42	15.6	13.4 - 18.3	0.1806	$-1.61 + 5.54 (\log x_i)$
72	10.9	9.4 - 12.7	0.2181	$0.24 + 4.59 (\log x_i)$
96	7.3	5.7 - 9.2	0.3062	$2.19 + 3.27 (\log x_i)$
120	4.5	3.4 - 6.0	0.3777	$3.27 + 2.65 (\log x_i)$
144	3.2	2.6 - 3.9	0.2477	$2.97 + 4.04 (\log x_i)$
168	2.9	2.5 - 3.3	0.1657	$2.22 + 6.03 (\log x_i)$
220[c]	2.9	--	--	--

[a] $\hat{\sigma}$ is the standard deviation of the logarithm of the population tolerance frequency distribution.

[b] probit $\hat{y}_i = a + b (\log x_i)$.

[c] Median lethal time for a concentration of 2.9 ± 1.5 mg SeO_2/ℓ calculated to have 95% confidence limits between 129 and 374 hr and a $\hat{\sigma}$ value of 0.308.

Appendix Table 29. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR ADULT BROOK TROUT EXPOSED TO SELENIUM DIOXIDE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC 50, mg/ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
6	87.3	74.2 - 102.6	0.1622	$-6.97 + 6.17 (\log x_i)$
7	70.3	63.7 - 77.6	0.0986	$-13.74 + 10.14 (\log x_i)$
24	36.3[c]	---	--	---
48	23.8[c]	---	--	---
64	23.0[d]	---	--	---
96	14.3	13.1 - 15.5	0.0688	$-11.78 + 14.54 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

[c] Derived from regression equation $\log \hat{y}_i$ (LC50) = $3.95 - 1.65 \log x_i$ (exposure time) based on LC50 values for 6, 7, 64, and 96 hr.

[d] Median lethal time of 64.0 hr for a concentration of 23.0 mg/ℓ.

Appendix Table 30. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE CHANNEL CATFISH EXPOSED TO SELENIUM DIOXIDE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
23	46.7	42.1 - 51.8	0.1028	$-11.24 + 9.73 (\log x_i)$
28	40.2	37.8 - 42.8	0.0617	$-21.00 + 16.20 (\log x_i)$
52	24.9	20.0 - 31.1	0.0592	$-8.27 + 9.50 (\log x_i)$
94 [c]	19.1	17.1 - 21.4	1.1504	---

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

[c] LC50 calculated by method of Litchfield and Wilcoxon[29].

Appendix Table 31. MEDIAN LETHAL TIMES (LT50) FOR JUVENILE FLAGFISH EXPOSED TO SELENIUM DIOXIDE (SeO_2)

Measured SeO_2 concentration, mg/ℓ	LT50, hr	95% Confidence limits for LT50, hr	$\hat{\sigma}$[a]	Log-probit regression equation[b]
11.2	83.1	55.1 - 125.2	0.2371	$-3.10 + 4.22 (\log x_i)$
16.9	67.2	48.7 - 92.8	0.1863	$-4.81 + 5.37 (\log x_i)$
21.8	68.3	43.9 - 106.3	0.2557	$-2.17 + 3.91 (\log x_i)$
27.9	55.4	42.3 - 72.[illegible]	0.1565	$-6.14 + 6.39 (\log x_i)$
37.6	44.3	35.7 - 55.0	0.1250	$-8.17 + 8.00 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 32. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE GOLDFISH EXPOSED TO SELENIUM DIOXIDE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$[a]	Log-probit regression equation[b]
12[c]	110.0	100.7 - 120.1	1.125	---
18[c]	76.5	70.6 - 82.9	1.184	---
24	71.3	65.8 - 77.2	0.1022	$-13.14 + 9.79\ (\log x_i)$
36	60.2	54.6 - 66.3	0.1122	$-10.86 + 8.92\ (\log x_i)$
48	46.5	42.6 - 50.7	0.1120	$-9.89 + 8.93\ (\log x_i)$
60	41.2	36.1 - 46.9	0.1515	$-5.66 + 6.60\ (\log x_i)$
96	36.6	26.7 - 50.2	0.3159	$+0.05 + 3.17\ (\log x_i)$
120	32.7	22.7 - 47.0	0.3626	$+0.82 + 2.76\ (\log x_i)$
144	22.3	16.4 - 30.3	0.3523	$+1.17 + 2.84\ (\log x_i)$
168	17.2	13.1 - 22.5	0.3123	$+1.05 + 3.20\ (\log x_i)$
216	13.0	10.1 - 16.6	0.2873	$+1.13 + 3.48\ (\log x_i)$
264	11.5	9.9 - 13.3	0.1707	$-1.21 + 5.86\ (\log x_i)$
336	8.8	7.8 - 9.9	0.1194	$-2.92 + 8.38\ (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b\ (\log x_i)$.

[c] Calculated according to method of Litchfield and Wilcoxon[29].

Appendix Table 33. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE BLUEGILL EXPOSED TO SELENIUM DIOXIDE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$[a]	Log-probit regression equation[b]
8	126.6	104.5 - 153.4	0.1921	$-5.95 + 5.21 (\log x_i)$
18	82.1	76.3 - 88.4	0.0743	$-20.75 + 13.45 (\log x_i)$
24	77.3	72.1 - 82.8	0.0709	$-21.64 + 14.11 (\log x_i)$
168	30.7	27.2 - 34.5	0.1360	$-5.93 + 7.35 (\log x_i)$
192	27.7	24.3 - 31.6	0.1519	$-4.50 + 6.58 (\log x_i)$
240	23.6	20.8 - 26.9	0.1620	$-3.48 + 6.17 (\log x_i)$
288	20.5	18.6 - 22.7	0.1249	$-5.50 + 8.01 (\log x_i)$
336	17.6	16.4 - 18.9	0.0826	$-10.10 + 12.11 (\log x_i)$

[a] $\hat{\sigma}$ is the standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 34. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR ADULT BROOK TROUT EXPOSED TO SODIUM ARSENITE[a]

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [b]	Log-probit regression equation[c]
22	54.1	28.5 - 102.9	0.1840	$-4.42 + 5.43 (\log x_i)$
24	53.9	49.2 - 59.1	0.0913	$-13.97 + 10.96 (\log x_i)$
30	40.8	39.6 - 42.1	0.0315	$-46.13 + 31.74 (\log x_i)$
31	42.2	36.4 - 49.0	0.1723	$-4.44 + 5.81 (\log x_i)$
48	27.8	23.9 - 32.4	0.1522	$-4.49 + 6.57 (\log x_i)$
93	25.8	23.7 - 28.1	0.0836	$-11.89 + 11.96 (\log x_i)$
144	20.0	18.3 - 21.8	0.0870	$-9.95 + 11.49 (\log x_i)$
164	19.4	17.8 - 21.2	0.0888	$-9.50 + 11.26 (\log x_i)$
262	18.0	17.1 - 19.0	0.0506	$-19.83 + 19.77 (\log x_i)$

[a] One test conducted for 48 hr and one for 262 hr.

[b] Standard deviation of the logarithm of the population tolerance frequency distribution.

[c] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 35. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE FATHEAD MINNOWS EXPOSED TO SODIUM ARSENITE

Exposure time, hr	LC50, mg/ℓ	95% confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
14	45.3	39.6 - 51.9	0.1749	$-4.47 + 5.72 (\log x_i)$
16	41.7	37.9 - 45.9	0.1228	$-8.20 + 8.14 (\log x_i)$
24	36.2	33.2 - 39.5	0.1123	$-8.88 + 8.91 (\log x_i)$
48	31.3	29.1 - 33.8	0.0875	$-12.09 + 11.43 (\log x_i)$
72	29.2	27.0 - 31.6	0.0795	$-13.42 + 12.57 (\log x_i)$
96	27.0	24.7 - 29.4	0.0861	$-11.62 + 11.61 (\log x_i)$
187	24.9	22.1 - 28.0	0.0961	$-9.52 + 10.40 (\log x_i)$
283	21.7	16.5 - 28.6	0.3555	$1.24 + 2.81 (\log x_i)$
336	18.2	13.3 - 25.0	0.4082	$1.91 + 2.45 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 36. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE CHANNEL CATFISH EXPOSED TO SODIUM ARSENITE

Exposure time, hr	LC50, mg/ℓ	95% confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
43	60.8	55.4 - 66.8	0.0937	$-14.03 + 10.67 (\log x_i)$
48	56.9	52.5 - 61.8	0.0822	$-16.36 + 12.17 (\log x_i)$
67	47.5	42.0 - 53.6	0.1405	$-6.93 + 7.12 (\log x_i)$
77	44.5	39.8 - 49.8	0.1291	$-7.77 + 7.75 (\log x_i)$
92	33.3	29.0 - 38.3	0.1775	$-3.58 + 5.63 (\log x_i)$
96	31.2	27.6 - 35.4	0.1612	$-4.27 + 6.21 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 37. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE GOLDFISH EXPOSED TO SODIUM ARSENITE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50 mg/ℓ	$\hat{\sigma}$[a]	Log-probit regression equation[b]
6	211.2	179.3 - 248.8	0.1637	$-9.20 + 6.11 (\log x_i)$
18	103.1	92.6 - 114.8	0.1508	$-8.36 + 6.63 (\log x_i)$
24	89.6	78.8 - 101.9	0.1666	$-6.72 + 6.00 (\log x_i)$
36	60.8	54.8 - 67.4	0.1344	$-8.28 + 7.44 (\log x_i)$
48	54.6	48.9 - 60.9	0.1107	$-10.69 + 9.04 (\log x_i)$
72	50.0	44.5 - 56.0	0.1159	$-9.66 + 8.63 (\log x_i)$
96	44.9	40.3 - 50.1	0.1095	$-10.09 + 9.13 (\log x_i)$
168	36.2	33.4 - 39.4	0.0953	$-11.37 + 10.50 (\log x_i)$
240	33.1	18.4 - 59.6	0.0711	$-16.38 + 14.07 (\log x_i)$
336	32.1	29.9 - 34.4	0.0716	$-16.03 + 13.96 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

Appendix Table 38. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR FLAGFISH FRY EXPOSED TO SODIUM ARSENITE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
29	69.8	57.1 - 85.4	0.1427	$-7.92 + 7.01 (\log x_i)$
67	60.8	53.0 - 69.8	0.1597	$-6.17 + 6.26 (\log x_i)$
93[c]	55.5	42.1 - 73.2	0.2341	$-2.45 + 4.27 (\log x_i)$
93[c]	40.4	23.7 - 68.8	0.1561	$-5.29 + 6.40 (\log x_i)$
93[c]	49.6	38.5 - 63.9	0.2320	$-2.31 + 4.31 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

[c] The average LC50 of the 93-hr LC50 estimates, 48.5 mg/ℓ, was used for plotting the toxicity curve for this species. The 93-hr LC50 values are equivalent to 96-hr LC50's and were derived from three replicates.

Appendix Table 39. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE BLUEGILL EXPOSED TO SODIUM ARSENITE

Exposure time, hr	LC50, mg/ℓ	95% Confidence limits for LC50, mg/ ℓ	$\hat{\sigma}$ [a]	Log-probit regression equation[b]
96	72.0	55.5 - 93.5	1.5299[c]	-
120	67.3	58.1 - 77.8	0.1460	$-7.52 + 6.85 (\log x_i)$
168	61.7	51.9 - 73.2	0.1717	$-5.42 + 5.82 (\log x_i)$
240	47.8	41.2 - 55.6	0.1738	$-4.66 + 5.75 (\log x_i)$
264	42.2	35.6 - 50.0	0.2193	$-2.41 + 4.56 (\log x_i)$
288	37.0	32.0 - 42.9	0.1896	$-3.27 + 5.27 (\log x_i)$
336	31.6	27.9 - 35.8	0.1617	$-4.27 + 6.18 (\log x_i)$

[a] $\hat{\sigma}$ is the standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

[c] Computations performed by the method of Litchfield and Wilcoxon [29] $\hat{\sigma}$ is expressed as the slope function, S, the antilogarithm of $\hat{\sigma}$.

Appendix Table 40. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE FATHEAD MINNOWS EXPOSED TO BERYLLIUM SULFATE

Exposure time, hr	LC50,[a] mg/ℓ	95% confidence limits for LC50, mg/ℓ	$\hat{\sigma}$[b]	Log-probit regression equation[c]
75[d]	47.8	--	-	-
92[e]	40.2	27.6 - 58.5	0.0910	$-12.62 + 10.98 (\log x_i)$
96[e]	37.9	27.5 - 52.3	0.0764	$-15.66 + 13.09 (\log x_i)$
121	30.8	29.4 - 32.3	0.0623	$-18.91 + 16.06 (\log x_i)$
164	27.7	26.1 - 29.3	0.0733	$-14.66 + 13.64 (\log x_i)$
192	27.4	25.9 - 29.0	0.0723	$-14.88 + 13.83 (\log x_i)$
283	26.1	24.4 - 27.9	0.0777	$-13.24 + 12.87 (\log x_i)$
336	25.4	23.9 - 27.0	0.0691	$-15.32 + 14.46 (\log x_i)$

[a] As $BeSO_4$.

[b] Standard deviation of the logarithm of the population tolerance frequency distribution.

[c] Probit $\hat{y}_i = a + b (\log x_i)$.

[d] Median lethal time.

[e] Heterogenous concentration - percent mortality data.

Appendix Table 41. MEDIAN LETHAL CONCENTRATIONS (LC50) AND MEDIAN LETHAL TIMES (LT50) FOR FLAGFISH FRY EXPOSED TO BERYLLIUM SULFATE

Group	Median response estimate	95% confidence limits	$\hat{\sigma}$ [a]	Log-probit regression equation [b]
96-hr LC50 (mg/ℓ $BeSO_4$)				
I [c]	46.3 mg/ℓ	43.9 - 48.8 mg/ℓ	1.053	---
II	41.1 mg/ℓ	37.2 - 45.3 mg/ℓ	0.0801	---
III	41.1 mg/ℓ	38.4 - 44.0 mg/ℓ	0.0488	---
LT50 for 47.8 ± 2.2 mg/ℓ $BeSO_4$				
I	41.3 hr	10.5 -162.1 hr	0.4996	$1.77 + 2.00 (\log x_i)$
II	74.8 hr	61.1 - 91.4 hr	0.0821	$-17.81 + 12.18 (\log x_i)$
III	55.4 hr	48.3 - 63.5 hr	0.0560	$-26.13 + 17.86 (\log x_i)$

[a] Standard deviation of the logarithm of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

[c] Calculation by method of Litchfield and Wilcoxon[29].

Appendix Table 42. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR JUVENILE GOLDFISH EXPOSED TO BERYLLIUM SULFATE

Exposure time, hr	LC50,[a] mg/ℓ	95% confidence limits for LC50, mg/ℓ	$\hat{\sigma}$[b]	Log-probit regression equation[c]
96	55.9	49.0 - 63.7	0.1510	$-6.57 + 6.63 (\log x_i)$
120	49.3	44.0 - 55.3	0.1325	$-7.78 + 7.55 (\log x_i)$
168	48.3	42.7 - 54.6	0.1595	$-5.56 + 6.27 (\log x_i)$
186	46.5	40.8 - 53.1	0.1526	$-5.93 + 6.55 (\log x_i)$
216	41.6	37.2 - 46.6	0.1122	$-9.44 + 8.92 (\log x_i)$
240	38.4	34.4 - 43.0	0.1115	$-9.21 + 8.97 (\log x_i)$

[a] As $BeSO_4$.

[b] Standard deviation of the logarithm of the population tolerance frequency distribution.

[c] Probit $\hat{y}_i = a + b (\log x_i)$.