Protecting Our Water Environment

Metropolitan Water Reclamation District of Greater Chicago

RESEARCH AND DEVELOPMENT DEPARTMENT

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THE EFFECTS OF AGE ON THE TOXICITY RESPONSE OF PIMEPHALES PROMELAS IN ACUTE WHOLE EFFLUENT TOXICITY TESTS

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THE EFFECTS OF AGE ON THE TOXICITY RESPONSE OF <u>PIMEPHALES PROMELAS</u> IN ACUTE WHOLE EFFLUENT TOXICITY TESTS

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DISCLAIMER

Mention of proprietary equipment and chemicals in this report does not constitute endorsement by the Metropolitan Water Reclamation District of Greater Chicago.

SUMMARY AND CONCLUSIONS

The Metropolitan Water Reclamation District of Greater Chicago (District) conducts whole effluent toxicity (WET) tests on effluent samples from its seven water reclamation plants (WRPs). The Illinois Environmental Protection Agency (IEPA) has made conducting WET tests with the fathead minnow _Pimephales promelas_ a special condition of certain District National Pollutant Discharge Elimination System (NPDES) permits.

The United States Environmental Protection Agency (USEPA) has published test methods (USEPA, 1993) for conducting WET tests which the District follows for its NPDES permit related biomonitoring work.

The USEPA specifies that acute fish toxicity tests with _Pimephales promelas_ be conducted with test organisms 1- to 14-days old. The USEPA does not identify a specific age of test organism as optimal for toxicity testing. No studies have been published by the USEPA showing age dependent differences in the sensitivities of fish, in the 1- to 14-day age range, to various toxicants. Nor have any studies been published by the USEPA showing that laboratory precision is not related to the age of the test organisms in the 1- to 14-day range. This study was undertaken to determine:

1. Whether age of _Pimephales promelas_ affects its survival response when exposed to toxicants.

2. Whether age of _Pimephales promelas_ affects variability associated with its survival response when exposed to toxicants.

In this study the results of 48-hour acute toxicity tests separately conducted with 1- to 2-, 3- to 4-, 7- to 8-, 11- to 12-, and 13- to 14-day old fish and the reference toxicants potassium chloride (KCl), sodium lauryl sulfate (SDS), and KCl + SDS were compared.

The specific conclusions drawn from this study are enumerated below.

1. Standard analysis of variance (ANOVA) indicated that the mortality rates of 1- to 2-day old fish exposed to either of the toxicants KCl or SDS, individually, are significantly lower than the mortality rates of 3- to 14-day old fish exposed to these toxicants ($p \leq 0.05$). These results suggest that the 1- to 2-day old fish are less sensitive to the individual toxicants KCl and SDS than 3- to 14-day old fish.

2. Standard ANOVA indicated that there are no statistically significant differences in the mortality rates of 1- to 2-, 3- to 4-, 7- to 8-, 11- to 12-, or 13- to 14-day old age groups of fish exposed to the KCl + SDS toxicant combination.

3. Mean LC_{50} values for 1- to 2-day old age groups of fish exposed to the toxicants KCl and SDS individually, and the KCl + SDS combination are all numerically higher than the mean LC_{50} values for 3- to 4-, 7- to 8-, 11- to 12-, or 13- to 14-day old age groups of fish exposed to these toxicants. These results suggest that the 1- to 2-day old fish are less sensitive to the individual toxicants KCl, SDS, and the KCl + SDS combination than 3- to 14-day old fish. However, standard ANOVA indicated that these differences are not statistically significant.

4. Statistical analysis using a cross-validation method to select a regression model of LC_{50} as a function of age indicated that it was appropriate to analyze the LC_{50} data for both the KCl and the SDS data sets using a model with fewer age levels, specifically, the 1- to 2-day age group versus the rest of the age groups combined. In the case of KCl the results of a parametric two sample analysis showed that the expected LC_{50} value for 1- to 2-day old fish was significantly higher than the expected LC_{50} value for 3- to 14-day old fish ($p = 0.0225$). In the case of SDS the re-

sults of a nonparametric two sample analysis showed that the expected LC_{50} value for 1- to 2-day old fish is significantly higher than the expected LC_{50} value for 3- to 14-day old fish ($p = 0.021$). In other words, results of the two sample statistical analyses, which are in agreement with the analyses of the mortality data cited above, indicate that the 1- to 2-day old fish are less sensitive to the individual toxicants KCl and SDS than 3- to 14-day old fish.

These results are consistent with those of Markle et al. (2000) who reported that the LC_{50} values of 4-, 7-, 10-, and 14-day old fish (Pimephales promelas) were significantly lower than those of 1-day old fish in Cr^{+6} and SDS toxicity tests. These findings, from the present study, and the Markle et al. study, suggest that the USEPA should revise the promulgated method for acute WET tests with Pimephales promelas (USEPA, 1993) and eliminate 1- to 2-day old fish from the currently allowable age range of 1- to 14-day old fish because it has been shown that the 1- to 2-day old fish are less sensitive to certain toxicants, i.e., KCl, SDS, and Cr^{+6}, than the older fish in the allowable age range, i.e., 3- to 14-day old fish. Elimination of the 1- to 2-day old fish from the allowable age range would thus reduce the variability associated with acute Pimephales promelas WET tests. This would make the results of these tests more reliable. This is important to both the regulator (USEPA) and the regulated community (NPDES permit holders) in that it will help to ensure that both parties can have confidence in the WET test results from a sample, independent of the laboratory performing the test, provided that proper testing procedures were employed.

5. Coefficients of variation (CV) for all tests conducted with KCl, SDS, and the KCl + SDS toxicant combination were 11.0, 11.0, and 13.8 percent, respectively. These values are relatively low compared to the intra-laboratory precision for acute WET tests with fish reported by other laboratories. The USEPA reported CV values for acute WET tests with fish as high as 120 percent (USEPA, 1993). More recently the USEPA reported an interim CV of 16 percent for acute WET tests with fish (USEPA, 2000). This interim CV value represents the median CV observed within 21 labora-

tories, which reported the results of WET tests conducted with reference toxicants to the USEPA. Therefore, the precision of tests conducted for this study, as judged by the relatively low CV values of 11.0, 11.0, and 13.8, is good.

6. CVs for tests conducted with 1- to 14-day old fish were numerically greater than CVs for tests conducted with 3- to 14-day old fish for the toxicants KCl, SDS, and the KCl + SDS combination. However, these differences were not statistically significant at the 0.05 level for KCl + SDS. No statistical conclusions could be drawn about differences in the average CVs across the age groups for the tests conducted with SDS alone, because the LC_{50} data were not normally distributed, and there is no nonparametric test for homogeneous variance. These results suggest that LC_{50} data generated using the 1- to 2-day old fish are more variable than the LC_{50} data generated using 3- to 14-day old fish. These results were consistent with those of Markle et al. (2000) who found the response of 1-day old fish to the toxicants Cr^{+6}, NaPCP, SDS, and NH_3 to be more variable than 4- to 14-day old fish to these same

toxicants. These findings (in the present study and by Markle et al.) suggest that the variability associated with acute fish WET tests, as measured by CV values alone, could be reduced by eliminating 1- to 2-day old fish from the current allowable age range of 1- to 14-day old fish (USEPA, 1993) and that the USEPA should revise the promulgated method for acute WET tests with fish (USEPA, 1993) and exclude the use of 1- to 2-day old fish from the allowable age range of 1- to 14-day old fish.

In summation, the results of this study show that the mortality rates of fish exposed to either KCl alone or SDS alone are affected by age, i.e., the 1- to 2-day old fish are less sensitive to the individual toxicants KCl and SDS. The mortality rates of fish exposed to a KCl + SDS toxicant combinations were not affected by age in this study. When the mortality rate data were converted to LC_{50} data, the results of standard ANOVA indicated that the LC_{50} values of fish exposed to the toxicants KCl alone, SDS alone, or KCl + SDS were not affected by age. However, more sophisticated statistical analyses based upon a cross validation method indicated that the LC_{50} values of fish exposed to either KCl alone or SDS alone are affected by age, i.e., the 1- to 2-day old fish are less sensitive to the individual toxicants KCl and

SDS, while the LC_{50} values of fish exposed to the KCl + SDS toxicant combination were not affected by age in this study. The results of the more sophisticated statistical analyses conducted on the LC_{50} data are in agreement with the statistical analyses conducted on the mortality rates, and suggest that the data set may be too small to identify the effect of age on LC_{50} values using standard ANOVA. These results indicate that 1- to 2-day old fish are less sensitive to the individual toxicants KCl and SDS.

The results of this study, as well as those reported by Markle et al. (2000), suggest that the variability associated with acute fish WET tests, as measured by CV values alone, could be reduced by eliminating 1- to 2-day old fish from the allowable age range of 1- to 14-day old fish (USEPA, 1993) and that the USEPA should revise the promulgated method for acute WET tests with fish (USEPA, 1993) and exclude the use of 1- to 2-day old fish from the allowable age range of 1- to 14-day old fish.

The results of this study are consistent with some of those reported by Markle et al. (2000), who concluded that the age of organisms used for testing needs to be selected and/or specified to the laboratory conducting the bioassay in order to ensure uniform sensitivity and maximize precision.

The results of this study indicate that fish age as a cause of inter- and intra-laboratory variability has not been sufficiently addressed by the USEPA in the publication of standard methods for conducting WET tests with Pimephales promelas (USEPA, 1993). The results of this study also suggest that the USEPA should not rely upon the simple comparison of CV values to express precision. Better ways to measure inter- and intra-laboratory precision should be investigated.

INTRODUCTION

Biomonitoring in the District

The District conducts WET tests on effluent and upstream receiving water samples from its seven WRPs. The IEPA has made conducting WET tests with the fathead minnow Pimephales promelas a special condition of certain District NPDES permits. The District submits biomonitoring reports to the IEPA to meet the requirements of the NPDES permits. The results of WET tests are also used by the District in its own programs to assess the effectiveness of WRP operations. Acute WET tests for use in the NPDES Permit Program to identify wastewater treatment plant effluents containing toxic materials in toxic concentrations have been described by the USEPA (1993). The District uses the USEPA methods as specified in its NPDES permits.

Participation of the District in NPDES Discharge Monitoring Report - Quality Assurance Program

The USEPA and related state agencies conduct the NPDES Discharge Monitoring Report - Quality Assurance (DMR-QA) Program for NPDES permittees who must conduct WET tests on their effluents as specified in their permits. Participation of NPDES permittees in this program, including proper analyses, reporting and record retention, is mandatory based on the authority of Section 308(a) of the Clean Water Act. The IEPA has included acute WET testing of District effluents with the fathead minnow, _Pimephales promelas_, in District NPDES permits under special "biomonitoring conditions." The District must, therefore, conduct acute WET tests with _Pimephales promelas_ in DMR-QA toxicity studies.

Description of DMR-QA Toxicity Program

The DMR-QA Toxicity Program is administered by the Toxicity Coordinator in the USEPA Office of Wastewater and Compliance. Permittees order unknown toxicants and receive study instructions from the USEPA contractor for the DMR-QA Toxicity studies. The permittees then prepare "simulated effluent samples with the unknown toxicants and have acute WET tests conducted on these samples. The permittees report the results of toxicity tests to the USEPA contractor.

Determination of Acceptable Results for DMR-QA Studies

The USEPA contractor identifies a "true" value and an acceptable range of values for a particular test based upon a statistical bi-weight analysis of results submitted by all laboratories participating in the study. Results falling outside of this range are considered unacceptable. Laboratories which submit results judged to be unacceptable must prepare a response to their state EPA coordinator and demonstrate that corrective action has been taken.

Variability Associated with Pimephales promelas Acute Toxicity Tests

Although WET testing is a valuable tool, interpretation of results are often complicated by variability associated with the tests. In particular, the problems associated with false positive results (Type I errors) and "unacceptable results" due to both inter- and intra-laboratory variability are possible and have not been sufficiently addressed (Dhaliwal et al., 1995; Warren-Hicks et al., 1999; Moore et al., 2000). Positive WET tests on a WRP effluent could trigger expensive efforts to identify the source of the toxicity and ways to eliminate it. If the test results responsible for initiating an investigation are not due to toxicity but to variability, valuable resources will be wasted addressing a "toxicity problem" that does not exist. The reporting of "unacceptable values" for a DMR-QA Study due to variability would also lead to a waste of resources. Therefore, sources of variability must be considered when toxicity data are evaluated.

A number of sources contributing to acute toxicity test variability have been identified. These include undefined variability associated with: 1) a particular method, 2) the test species, and 3) the analyst (Burton et al., 1996). Another source of variability may be the age of test organisms used for a test.

The USEPA method for conducting acute toxicity tests with *Pimephales promelas* specifies that fish ranging in age from 1 to 14 days old be used (USEPA, 1993). In the District's Bio-monitoring Laboratory acute toxicity tests with *Pimephales promelas* ranging in age from 1 to 14 days old are conducted on District effluent samples, and on "simulated effluent" samples for the DMR-QA studies.

OBJECTIVES

The overall objective of this study was to determine whether there is a relationship between the response of Pimephales promelas to acute toxicants, and the age of the Pimephales promelas used in the test. The specific objectives in the initial phase of this study were: 1) to determine whether age of Pimephales promelas exposed to reference toxicants in the 48-hour acute toxicity test affects the survival response (LC_{50}), and 2) to determine whether age affects the variability associated with the survival response of Pimephales promelas organisms exposed to reference toxicants in the 48-hour acute toxicity test

At a later date, this study may be expanded to determine whether age affects the survival response of Pimephales promelas exposed to WRP effluent or receiving water in the 48-hour acute toxicity test, and to determine whether age affects the variability associated with the survival response of Pimephales promelas organisms exposed to WRP effluent or receiving water in the 48-hour acute toxicity test.

EXPERIMENTAL APPROACH

The experimental approach consisted of the following:

1. The 48-hour, acute, static, non-renewal *Pimephales promelas* bioassay was conducted with organisms in the age ranges of 1 to 2 days, 3 to 4 days, 7 to 8 days, 11 to 12 days, and 13 to 14 days using the reference toxicants KCl, SDS, and a mixture of KCl + SDS. These toxicants are often used by the USEPA in intra- and inter-laboratory precision studies (USEPA, 1993).
2. The data were analyzed statistically to determine whether the age of the test organism affected the survivability, LC_{50}, and/or variability of the test results.

MATERIALS AND METHODS

Pimephales promelas Cultures

A stock culture of the fathead minnow, *Pimephales promelas*, was purchased from Aquatic Research Organisms (ARO), Hampton, New Hampshire. ARO maintains a quality assurance program which includes health monitoring (inspections twice a year by a certified independent outside laboratory) and performance evaluation of organisms through the use of standard reference toxicants. The culture obtained from ARO has been maintained and used to stock breeding tanks in the District's Biomonitoring Laboratory.

Methods for culturing fathead minnows outlined by the USEPA (1993) were followed. All fathead minnows were cultured in aerated tap water in the District's Biomonitoring Laboratory. Four 29-gallon stock tanks and 16 10-gallon breeding tanks were maintained to provide a sufficient number of test organisms for this study. Larvae were maintained in 10-gallon tanks in 24 L of culture water. Solid organic wastes were removed, and water in all stock and rearing tanks was changed daily, 50 to 70 percent for each water change. Water in breeding tanks was changed and solid organics removed only after eggs were harvested.

Automatic aquarium heaters (Visitherm™, Aquarium Systems, Menton, Ohio) were used to maintain water temperatures at 25 $\pm$ 1°C in all tanks. Undertow™ under gravel filters (Penn-Plax, Inc., Garden City, New York) were used in all stock and breeding tanks. Sponge filters (Dirt Magnet™, Jungle Laboratories Corporation, Cibolo, Texas) were used in all larval holding tanks. The dissolved oxygen concentration in the water in all tanks was maintained near saturation by continuous aeration. Air was supplied with Quincy QRD 15 oil-less compressors (Quincy Compressor Division model number QRDS150-240, Collec Industries, Quincy, IL) fitted with Quincy filter elements (Quincy model number 110377E100). Bubble Walls™ (Penn-Plax) were used for aerating stock and larval holding tanks. Air stones (Top Fin™, Pacific Coast Distributing, Inc., Phoenix, Arizona) were used for aerating all breeding tanks.

Minnows in stock and breeding tanks were fed alternately TetraMin and TetraFin flake food (Tetra Sales, Blacksburg, Virginia) five to seven times per day or as much as would be eaten each workday. They were fed twice on Sundays. No feeding occurred on Saturdays. Minnows in stock and breeding tanks were also fed frozen brine shrimp (Fish King, Chicago, Illinois) one to two times per day (except Saturdays). Larvae were fed brine shrimp hatched from brine shrimp eggs (Argentemia, Argent Chemical Laboratories, Redmond, Washington) one to two times per day (except Saturdays).

Preparation of Laboratory Control and Dilution Water

Hard synthetic water with trace nutrients added was used as the laboratory control and dilution water for bioassays.

Hard synthetic water was chosen because it approximates the receiving waters in the District. It was prepared as outlined by the USEPA (1993) as follows. Laboratory tap water was purified with a Millipore Elix 10 water purification system, (Millipore Corp., Bedford, Massachusetts) and subsequently filtered through a Millipore Milli-Q® water purification system. The following reagent grade chemicals were then added to purified water to make hard synthetic water: 192.0 mg $NaHCO_3$/L, 120.0 mg $CaSO_4 \cdot 2H_2O$/L, 120.0 mg $MgSO_4$/L, and 8.0 mg KCl/L. Two hundred fifty (250.0) µg $Na_2EDTA \cdot 2H_2O$/L, and the following 14 trace nutrients (Elendt and Bias, 1990) were added to the hard synthetic water to prepare the control water: 99.6 µg $FeSO_4 \cdot 7H_2O$/L, 286.0 µg H_3BO_3/L, 36.1 µg $MnCl_2 \cdot 4H_2O$/L, 30.6 µg LiCl/L, 7.1 µg RbCl/L, 15.2 µg $SrCl_2 \cdot 6H_2O$/L, 1.6 µg NaBr/L, 6.3 µg $Na_2MoO_4 \cdot 2H_2O$/L, 1.7 µg $CuCl_2 \cdot 2H_2O$/L, 1.3 µg $ZnCl_2$/L, 1.0 µg $CoCl_2 \cdot 6H_2O$/L, 0.3 µg KI/L, 0.2 µg Na_2SeO_3/L, and 0.1 µg NH_4VO_3/L.

Preparation of Toxicant Solutions

As stated previously, KCl + SDS were chosen as the toxicants for this study, as they are often used for toxicant testing. KCl (SigmaUltra) and SDS (SigmaUltra) were purchased from Sigma Chemical Company, St. Louis, Missouri. Test solutions were prepared by making dilutions of stock solutions of toxicants in laboratory control water. A stock solution containing 20,000 mg KCl/L was prepared weekly for tests by dissolving 20,000 mg of KCl in a quantity of laboratory control water sufficient to make 1.0 L. The following concentrations of KCl, in mg/L, were tested: 1,500, 1,250, 1,000, 500, and 250 (Table 1). A stock solution containing 400 mg SDS/L was prepared weekly for tests by dissolving 400 mg SDS in a quantity of laboratory control water sufficient to make 1.0 L. The following concentrations of SDS, in mg/L, were tested: 40, 35, 30, 20, and 10 (Table 2). A stock solution containing 15,000 mg/L KCl and 35 mg/L SDS was prepared weekly for tests by dissolving 15,000 mg KCl and 35 mg SDS in a quantity of laboratory control water sufficient to make 1.0 L. The following concentrations of KCl + SDS mixture were tested: 1500 mg KCl/L + 35 mg SDS/L, 1250 mg KCl/L + 29 mg SDS/L, 1000 mg KCl/L + 23 mg SDS/L, 500 mg KCl/L + 11.66 mg SDS/L, and 250 mg KCl/L + 5.83 mg SDS/L (Table 3).

Toxicity Tests

The Fathead Minnow, *Pimephales promelas*, Acute Toxicity Test (48-hour, static, non-renewal) was conducted with the toxicants KCl, SDS, and a mixture of KCl + SDS using the procedure specified by the USEPA (1993). Test conditions are presented in Tables 4, 5, and 6. Monthly quality assurance Fathead Minnow, *Pimephales promelas*, Acute Toxicity Tests (96-hour, static, non-renewal) with the reference toxicant NaCl

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE 1

PREPARATION OF KCl TEST SOLUTIONS

Concentrated Solution of KCl[1] (mL)	Laboratory Control Water (mL)	Total Volume Prepared (L)	Test Concentrations of KCl (mg KCl/L)
300	2700	3	1500
250	2750	3	1250
200	2800	3	1000
100	2900	3	500
50	2950	3	250

[1]This concentrated solution contained 15,000 mg KCl/L. It was prepared fresh weekly and used to make the test solutions.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE 2

PREPARATION OF SDS TEST SOLUTIONS

Concentrated Solution of SDS[1] (mL)	Laboratory Control Water (mL)	Total Volume Prepared (L)	Test Concentrations of SDS (mg SDS/L)
300	2700	3	40
262.5	2737.5	3	35
225	2775	3	30
150	2850	3	20
75	2925	3	10

[1]This concentrated solution contained 400 mg SDS/L. It was prepared fresh weekly and used to make the test solutions.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE 3

PREPARATION OF KCl + SDS (MIXTURE) SOLUTIONS

Concentrated Solution of KCl + SDS[1] (mL)	Laboratory Control Water (mL)	Total Volume Prepared (L)	Test Concentrations of KCl + SDS (mg KCl/L + mg SDS/L)
300	2700	3	1500 + 35
250	2750	3	1250 + 29
200	2800	3	1000 + 23
100	2900	3	500 + 11.66
50	2950	3	250 + 5.83

[1]This concentrated solution contained 15,000 mg KCl/L + 350 mg SDS/L. It was prepared fresh weekly and used to make the test solutions.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE 4

TEST CONDITIONS FOR ACUTE TOXICITY TESTS USING *PIMEPHALES PROMELAS* CONDUCTED WITH KCl

1.	Temperature (°C):	25 ± 1°C
2.	Light quality:	Ambient laboratory illumination
3.	Light intensity:	50 to 100 footcandles (ambient laboratory levels)
4.	Photoperiod:	16 hours light/8 hours darkness
5.	Size of test vessels:	0.50 L
6.	Volume of test solution:	0.25 L
7.	Age of fish (in days):	1 to 2; 3 to 4; 7 to 8; 11 to 12; 13 to 14 (24 hour range in age group)
8.	No. of fish/0.25 L:	5
9.	No. of replicate test vessels per concentration:	4
10.	Total no. organisms per concentration:	20
11.	Feeding regime:	Fish were fed *Artemia nauplii* while holding prior to the test
12.	Test chamber cleaning:	Cleaning was not required
13.	Concentrations used (mg KCl/L)	1,500; 1,250; 1,000; 500; 250
14.	Aeration:	None
15.	Dilution water:	Laboratory control water
16.	Test duration and type:	48-hour, acute, static, nonrenewal

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE 4 (Continued)

TEST CONDITIONS FOR ACUTE TOXICITY TESTS USING PIMEPHALES PROMELAS CONDUCTED WITH KCl

17.	Effect measured:	Mortality - no movement (LC_{50})
18.	Test acceptability:	90% or more survival in controls

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE 5

TEST CONDITIONS FOR ACUTE TOXICITY TESTS USING *PIMEPHALES PROMELAS* CONDUCTED WITH SDS

1.	Temperature (°C):	25 ± 1°C
2.	Light quality:	Ambient laboratory illumination
3.	Light intensity:	50 to 100 footcandles (ambient laboratory levels)
4.	Photoperiod:	16 hours light/8 hours darkness
5.	Size of test vessels:	0.50 L
6.	Volume of test solution:	0.25 L
7.	Age of fish (in days):	1 to 2; 3 to 4; 7 to 8; 11 to 12; 13 to 14 (24 hour range in age group)
8.	No. of fish/0.25 L:	5
9.	No. of replicate test vessels per concentration:	4
10.	Total no. organisms per concentration:	20
11.	Feeding regime:	Fish were fed *Artemia nauplii* while holding prior to the test
12.	Test chamber cleaning:	Cleaning was not required
13.	Concentrations used (mg SDS/L)	40, 35, 30, 20, 10
14.	Aeration:	None
15.	Dilution water:	Laboratory control water
16.	Test duration and type:	48-hour, acute, static, nonrenewal

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE 5 (Continued)

TEST CONDITIONS FOR ACUTE TOXICITY TESTS USING PIMEPHALES PROMELAS CONDUCTED WITH SDS

17.	Effect measured:	Mortality - no movement (LC_{50})
18.	Test acceptability:	90% or more survival in controls

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TABLE 6

TEST CONDITIONS FOR ACUTE TOXICITY TESTS USING PIMEPHALES PROMELAS CONDUCTED WITH A MIXTURE OF KCl + SDS

1.	Temperature (°C):	25 ± 1°C
2.	Light quality:	Ambient laboratory illumination
3.	Light intensity:	50 to 100 footcandles (ambient laboratory levels)
4.	Photoperiod:	16 hours light/8 hours darkness
5.	Size of test vessels:	0.50 L
6.	Volume of test solution:	0.25 L
7.	Age of fish (in days):	1 to 2; 3 to 4; 7 to 8; 11 to 12; 13 to 14 (24 hour range in age group)
8.	No. of fish/0.25 L:	5
9.	No. of replicate test vessels per concentration:	4
10.	Total no. organisms per concentration:	20
11.	Feeding regime:	Fish were fed Artemia nauplii while holding prior to the test
12.	Test chamber cleaning:	Cleaning was not required
13.	Concentrations used (mg KCl + mg SDS/L)	1500 + 35, 1250 + 29, 1000 + 23, 500 + 11.66, 250 + 5.83
14.	Aeration:	None
15.	Dilution water:	Laboratory control water
16.	Test duration and type:	48-hour, acute, static, nonrenewal

TABLE 6 (Continued)

TEST CONDITIONS FOR ACUTE TOXICITY TESTS USING *PIMEPHALES PROMELAS* CONDUCTED WITH A MIXTURE OF KCl + SDS

17.	Effect measured:	Mortality - no movement (LC_{50})
18.	Test acceptability:	90% or more survival in controls

were conducted using the procedure prescribed by the USEPA (1993).

Chemical and Physical Determinations

Dissolved oxygen, pH, and temperature were measured at the beginning and end of each 24-hour exposure period in all toxicant concentrations and laboratory control water. Alkalinity, hardness, and conductivity were measured at the beginning and end of each 24-hour exposure period in the highest toxicant concentration, the middle toxicant concentration, and in laboratory control water. Temperatures in the environmental chambers used for raising cultures and for bioassays were monitored continuously.

Data Quality Criteria

Data quality criteria used for this study are shown below.

LABORATORY CONTROL AND DILUTION WATER

Laboratory control and dilution water met the following criteria: Hardness was in the range of 160 to 180 mg $CaCO_3$/L. Alkalinity was in the range of 110 to 120 mg $CaCO_3$/L. pH was in the range of 7.6 to 8.3. Dissolved oxygen levels were greater than or equal to 4.0 mg/L. The laboratory control and dilution water used was not less than 48 hours old or more than two weeks old, except for one batch of dilution water which exceeded the two-week criteria. This deviation is discussed later in this report.

LABORATORY CULTURE WATER

Laboratory culture water was tap water from Lake Michigan, and dechlorinated by aeration for 24 hours.

PERFORMANCE CONTROLS

Laboratory culture water was used as performance control water in all tests.

TEMPERATURE OF TEST SOLUTIONS

Test solutions of toxicants, laboratory control water, and laboratory culture water were warmed to 25 ± 1°C in a waterbath before tests were set up, and then maintained in that range.

TEMPERATURE OF TEST CHAMBERS

The temperature in the test chamber was monitored continuously. The acceptable temperature of 25 ± 1°C for the test period was maintained.

TEST ORGANISMS

Test organisms were hatched within a 24-hour period and originated from at least three tiles of eggs. The density of fish fry in cultures was 150 fish fry per liter or less. Test organisms were fed two hours prior to the setup of tests.

CHEMICAL DATA

The pH of test solutions in bioassay cups was measured at the beginning and end of each 24-hour exposure period. The acceptable pH range is 6.0 to 9.0 and was maintained. Dissolved oxygen levels were measured in bioassay cups at the beginning and end of each 24-hour exposure period. The acceptable dissolved oxygen level is greater than or equal to 4.0 mg/L and was maintained.

AERATION OF TEST SOLUTIONS

It was not necessary to aerate test solutions during the course of this study. Dissolved oxygen levels did not fall below 4.0 mg/L.

LIGHT READINGS

Light readings were recorded daily. The acceptable range for light intensity is 50 to 100 foot-candles, and was maintained.

RANDOMIZATION

Test organisms were taken from a common pool and distributed randomly to the test chambers until the required number of organisms were placed in each. Test chambers were positioned randomly in holding trays. A computer program in Microsoft Quickbasic 4.0 was used to simplify and document the above procedure. The program uses data entry information and the "Randomize Timer" command to randomly assign the order the fish fry test organisms are added to the test vessels and randomly positioned test chambers.

NUMBER OF FISH PER TEST CHAMBER

Five fish were put in each test chamber, as previous experience in the District's Bioassay Laboratory has indicated that this works well. This procedure is also approved by the IEPA.

TEST ACCEPTABILITY

The criterion for test acceptability was 90 percent or greater survival of the control test organisms. This was achieved in all tests.

MONTHLY REFERENCE TOXICANT TESTS

Quality assurance tests with the reference toxicant NaCl were conducted monthly. These were 96-hour tests. Control charts were prepared to document ongoing laboratory performance.

Calculation of Mortality Rates

Mortality rates were calculated by dividing the number of mortalities observed for a treatment by the total number of fish exposed to the treatment.

Calculation of LC_{50} Values

LC_{50} values were calculated using the USEPA Toxicity Data Analysis Software (USEPA, 1994a and 1994b). Data were first entered into the Probit Analysis program. If the data were rejected or confidence limits were not generated by the Probit Analysis program, the data were re-entered into the Trimmed Spearman-Karber Program.

Precision

Precision was described as the percent coefficient of variation or CV of the calculated LC_{50} values (USEPA, 1991 and 1993). CVs were computed as the ratio of the standard deviation divided by the mean expressed as percentage. A non-parametric analogue to the CV was considered for the case of SDS. See the Results Section.

Statistical Analysis

Statistical analyses were performed using the following procedures.

CORRECTION FOR MORTALITY IN THE CONTROLS

Treatment responses for mortality were corrected for control mortality prior to statistical analysis by using Abbot's formula (USEPA, 1993):

$$r_c = \frac{r - r_0}{1 - r_0}$$

where

r_c = corrected mortality rate

r = calculated mortality rate

r_0 = mortality rate of controls

TESTS FOR NORMALITY AND HOMOGENEITY OF VARIANCE

Corrected mortality rates were tested for normality using the Shapiro-Wilk test (SAS Institute, 1995). Since the assumption of normality was questioned for these data (See Results Section), the assumption of homogeneity of variances was not tested. LC_{50} data were tested for normality using the Shapiro-Wilk test (SAS Institute, 1995). Bartlett's test for homogeneity of variance (Walpole and Meyers, 1989) was performed on data for which there was no reason to question the assumption of normality.

COMPARISON OF THE SENSITIVITIES OF DIFFERENT AGE GROUPS TO TOXICANTS

Standard nonparametric ANOVA was performed on the basis of corrected ranked mortality data across the five age groups studied (SAS Institute, 1995). The results of the nonparametric ANOVA were used to perform the Student-Newman-Keuls test (SAS Institute, 1995) on multiple comparisons.

Standard parametric ANOVA was performed on normally distributed data with equal variances (SAS Institute, 1995). Standard nonparametric ANOVA was performed on data not meeting these criteria (SAS Institute, 1995).

TEST FOR EQUALITY OF COEFFICIENTS OF VARIATION (CVs)

No specific test statistics are available to test CVs for equality. However, if the LC_{50} population means are equal and the variances are equal, it can be concluded that the CVs are equal. Therefore, CVs were tested for equality as follows.

The LC_{50} population means across all age groups studied were tested for normality using the Shapiro-Wilk test (SAS Institute, 1995) and for equal variances using Bartlett's test (Walpole and Myers, 1989). Equality of the LC_{50} population means across all age groups studied was tested using standard parametric ANOVA (SAS Institute, 1995) when the assumptions of normality and homogeneity of variance were met, and standard nonparametric ANOVA (SAS Institute, 1995) when they were not. When the results of ANOVA (the F-test) showed that population means are equal (but not zero), and Bartlett's test showed that variances are equal, then it was concluded that the CVs are equal, otherwise the CVs are not equal. The experiment-wise error rate was controlled as follows. If the significance level for the equality of CVs is α and the significance level for the F-test and Bartlett's test is α^*, then α^* is chosen such that $(1-\alpha^*)^2=1-\alpha$. The value of α was set to be 0.05. Therefore α^* is approximately 0.025.

It should be noted that if the population means are unequal and population variances are also unequal then the coefficient of variations can still be equal. But no statistical test or tests can confirm it. In this case no decision can be made regarding the equality of CVs.

RESULTS AND DISCUSSION

Quality Assurance

Control charts showing the results of monthly quality assurance tests with the reference toxicant sodium chloride are shown in Figures 1-4. The results of the tests performed in March through June 1999, when this study was conducted, all fell within the limits prescribed as acceptable by the USEPA (1993), that is, within two standard deviations from the cumulative mean LC_{50} values.

Data Quality Criteria

All data quality criteria specified by the USEPA were met with the following exception. The acceptable age for dilution water specified by the USEPA is 2 to 14 days. However, dilution water prepared on March 26, 1999 was used for tests set up on April 12, 14, 19, and 21, 1999. Eight tests were conducted with dilution water older than the 14-day acceptable limit. These tests were conducted with a dilution water aged within the range of 17 to 26 days.

This deviation did not have a significant effect on the test results. The controls indicated that all 60 tests conducted were valid.

Tests Conducted with KCl

The LC_{50} values for the tests conducted with KCl are shown in Table 7. The complete survival data are shown in Tables

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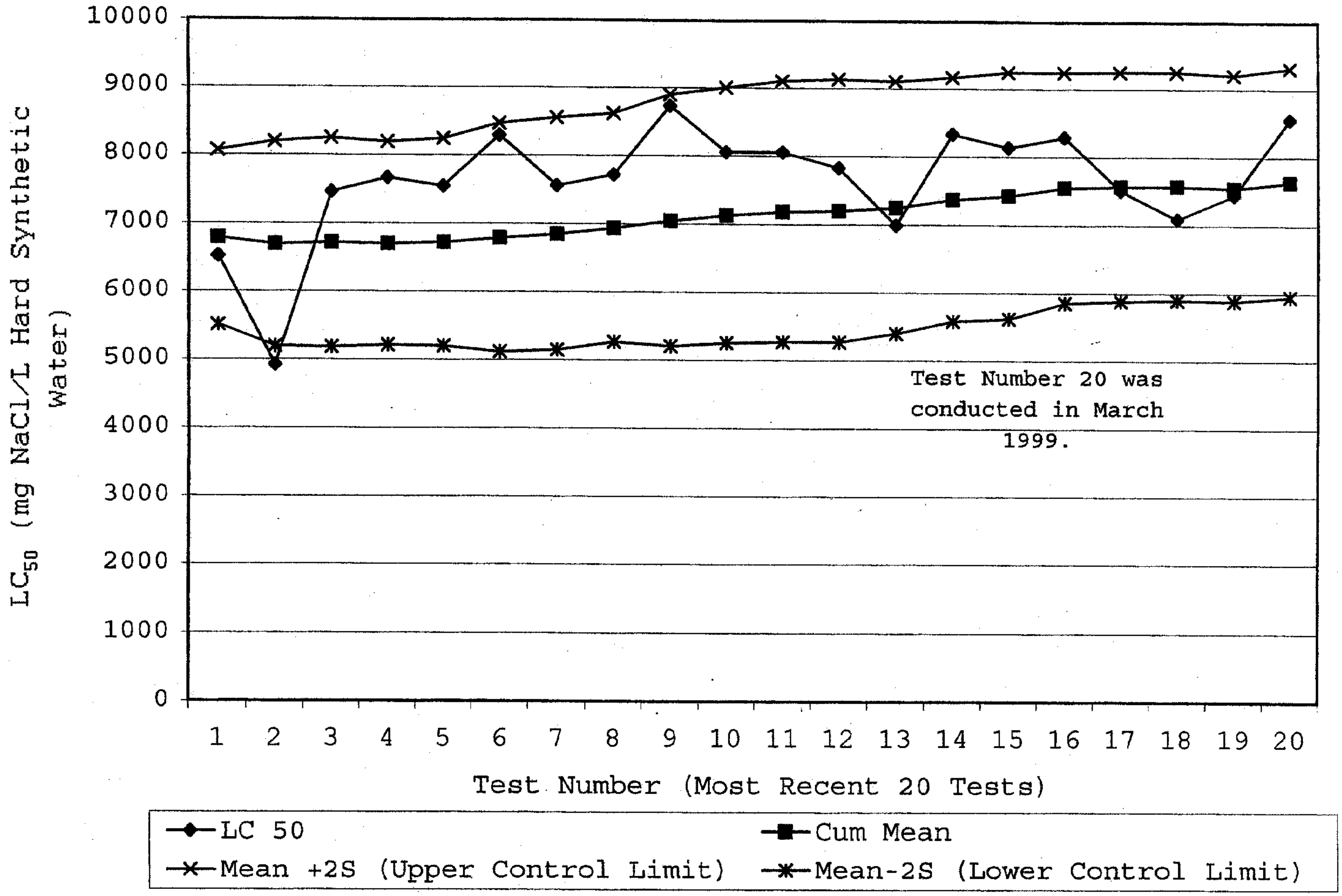
FIGURE 1

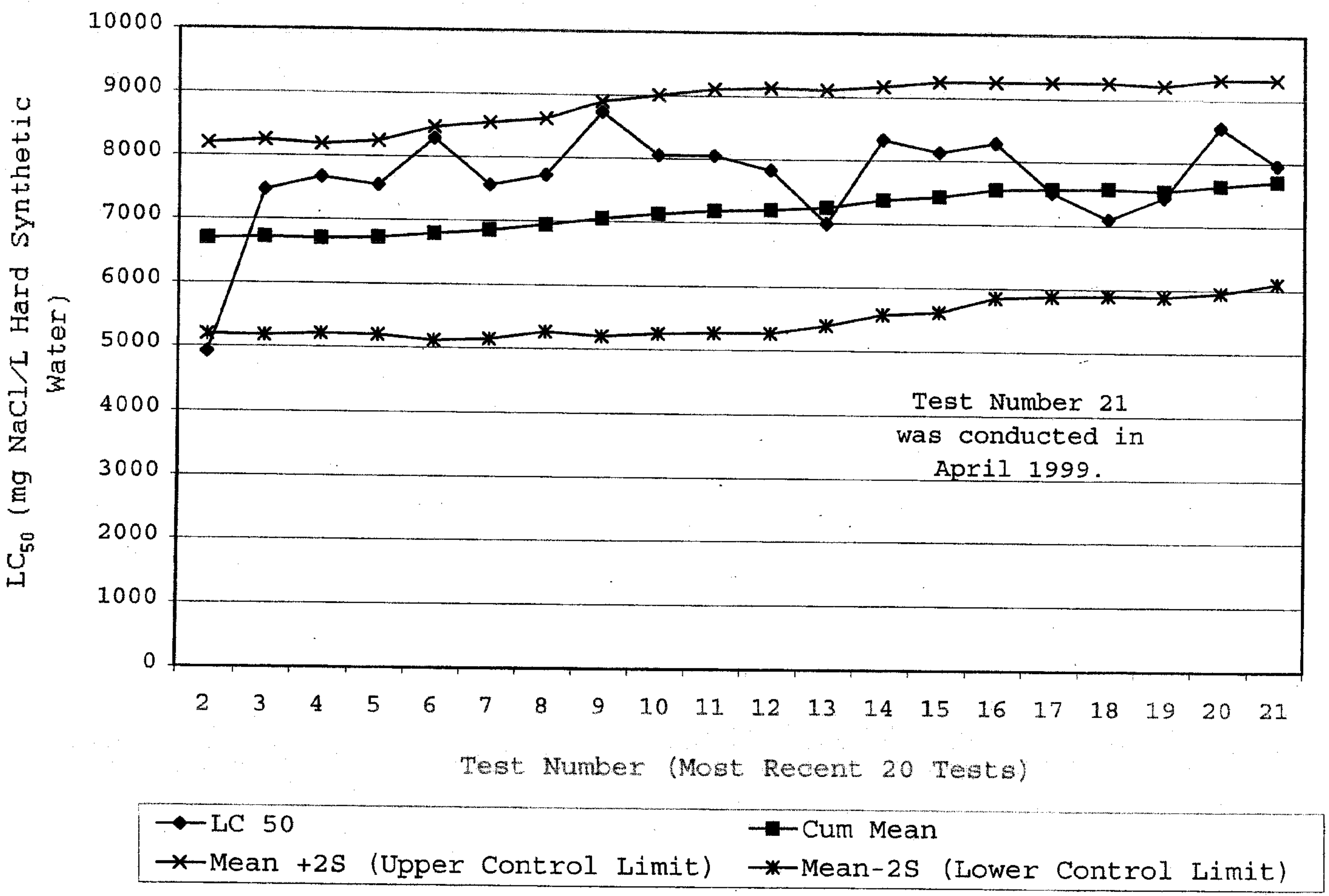
CONTROL CHART FOR ACUTE FATHEAD MINNOW TESTS WITH NaCl, MARCH 1999



METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

FIGURE 2

CONTROL CHART FOR ACUTE FATHEAD MINNOW TESTS WITH NaCl, APRIL 1999



METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

FIGURE 3

CONTROL CHART FOR ACUTE FATHEAD MINNOW TESTS WITH NaCl, MAY 1999

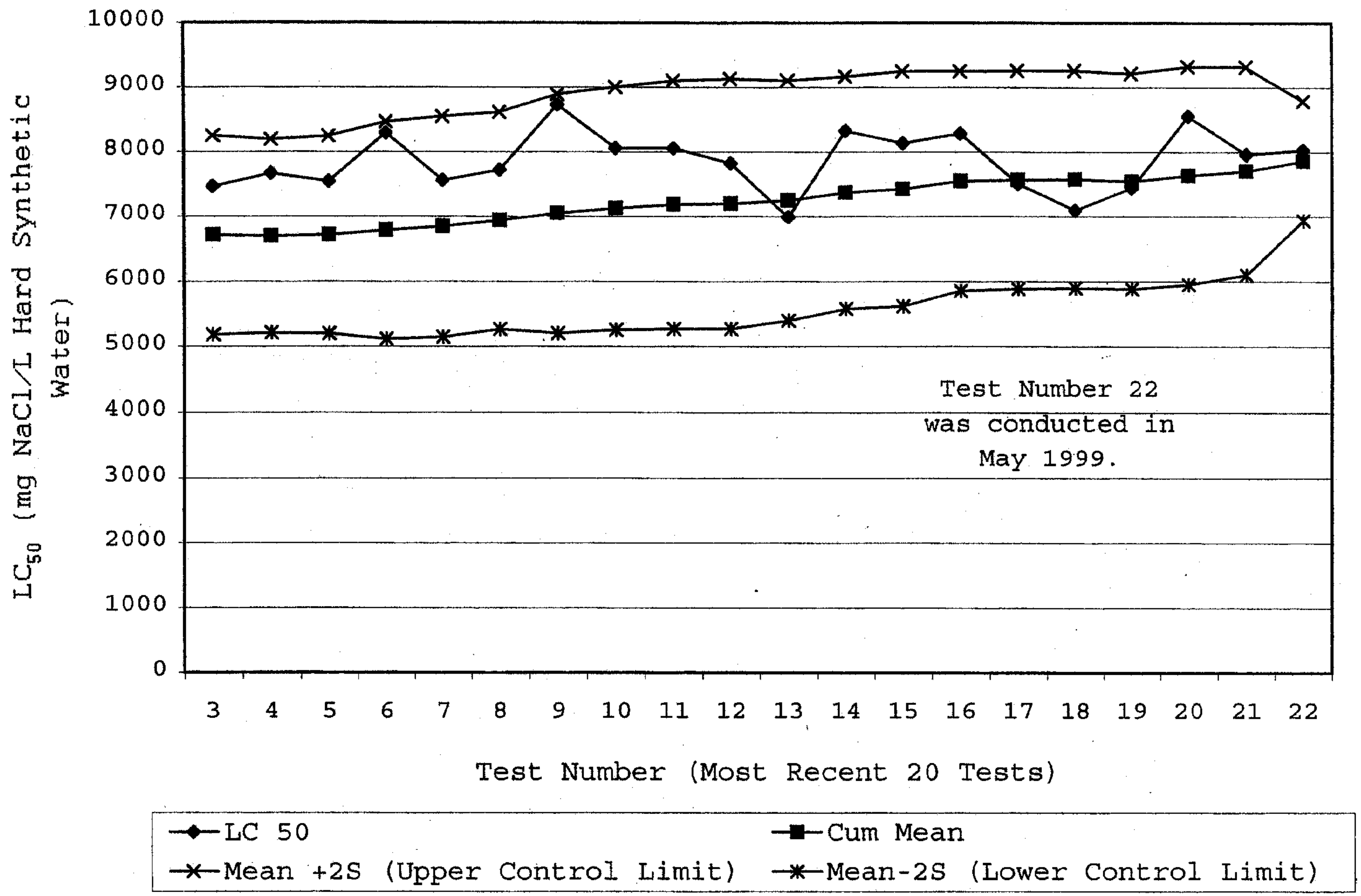


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FIGURE 4

CONTROL CHART FOR ACUTE FATHEAD MINNOW TESTS WITH NaCl, JUNE 1999

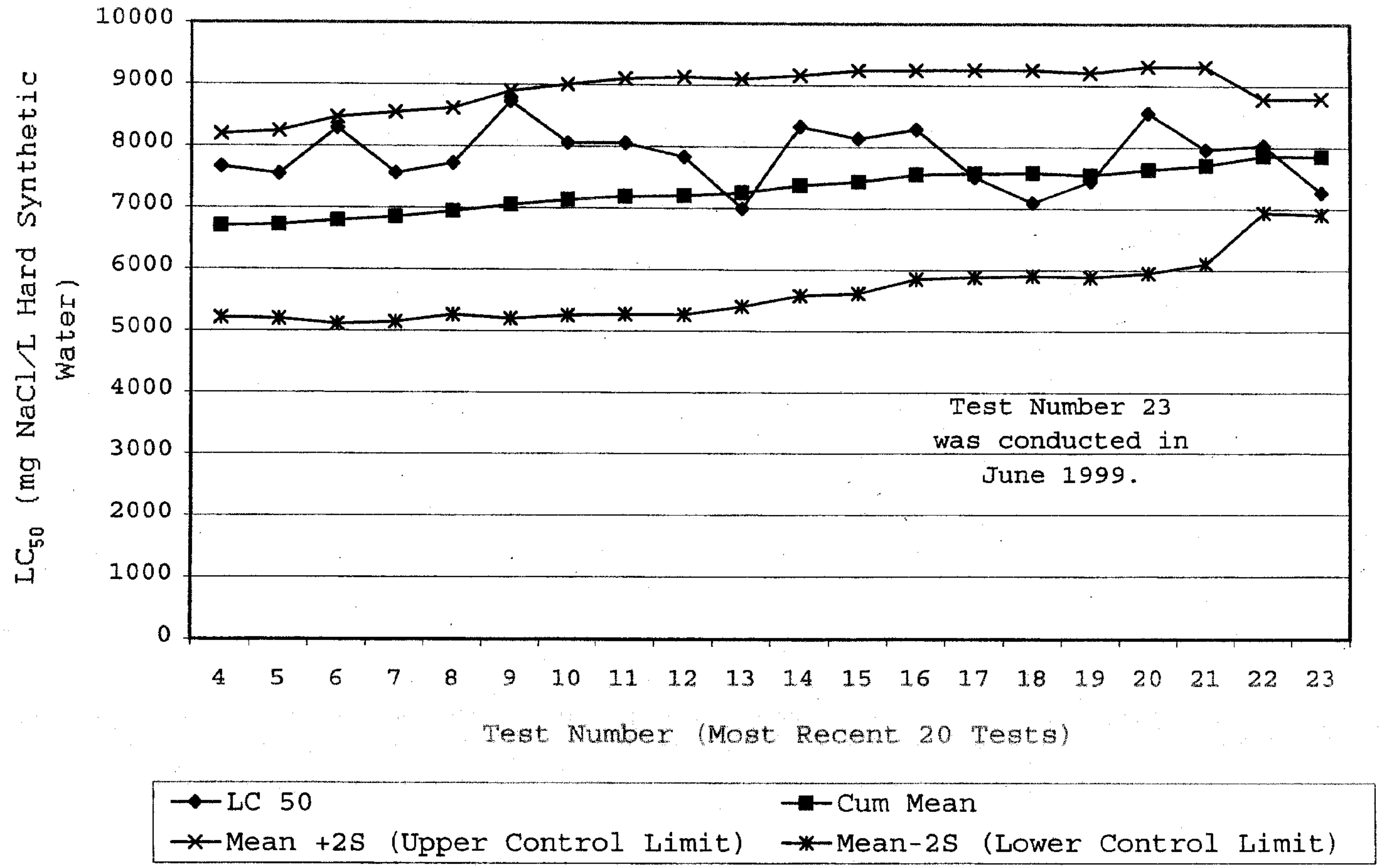

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TABLE 7

RESULTS OF 48-HOUR ACUTE TOXICITY TESTS WITH PIMEPHALES PROMELAS USING THE TOXICANT KCl[1]

Age of Fish (days)	LC_{50}[2]	Mean LC_{50}	Method[3]	Date Set Up
1 to 2	1,595.2		P	3/8/99
	1,189.6		P	3/17/99
	1,239.4		P	3/17/99
	1,347.5		P	4/12/99
		(1342.9)		
3 to 4	1,131.0		P	3/24/99
	1,008.4		P	4/19/99
	1,136.8		S	4/21/99
	1,350.9		S	4/28/99
		(1156.8)		
7 to 8	1,148.5		P	3/24/99
	1,176.2		S	4/14/99
	1,088.1		P	4/19/99
	1,130.6		P	4/21/99
		(1135.9)		

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TABLE 7 (Continued)

RESULTS OF 48-HOUR ACUTE TOXICITY TESTS WITH PIMEPHALES PROMELAS USING THE TOXICANT KCl[1]

Age of Fish (days)	LC_{50}[2]	Mean LC_{50}	Method[3]	Date Set Up
11 to 12	1,195.5		P	3/15/99
	1,041.5		P	3/22/99
	1,285.1		S	4/12/99
	1,255.5		P	4/14/99
		1,194.4		
13 to 14	1,332.4		P	3/10/99
	1,209.3		S	3/15/99
	1,076.0		S	5/3/99
	1,275.2		S	5/3/99
		1,223.2		
1 to 14		1,210.6		

[1]KCl = Potassium chloride.
[2]LC_{50} = Concentration of toxicant in mg/L lethal to 50 percent of test organisms.
[3]LC_{50} values were calculated by the Probit Method (P)(USEPA, 1994a). When acute toxicity test data did not meet the requirements for use of the Probit Method, LC_{50} values were calculated by the Trimmed Spearman to Karber Method(s)(USEPA, 1994b).

AI-1 - AI-20. The mean LC_{50} values for tests conducted with 1- to 2-, 3- to 4-, 7- to 8-, 11- to 12-, and 13- to 14-day old fish were calculated to be 1,342.9, 1,156.8, 1,135.9, 1,194.4, and 1,223.2 mg KCl/L, respectively. These data are shown in Figure 5. The mean LC_{50} value with 1- to 2-day old fish is numerically higher than the mean LC_{50} values for fish in all other age groups. Thus, 1- to 2-day old fish appear to be less sensitive to the toxicant KCl than fish in the 3- to 14-day old age range.

The mean LC_{50} value for all of the tests conducted (1- to 14-day old fish) was calculated to be 1,210.6 mg KCl/L. This LC_{50} value is higher than the mean LC_{50} value of 896 mg KCl/L reported by the USEPA for 203 laboratories which submitted data for a 1991 inter-laboratory precision study (USEPA, 1993). However, the results are not strictly comparable for the following reason. The laboratory control water used by the laboratories reporting data to the USEPA for the 1991 study was moderately hard synthetic water. Hard synthetic water was used in this study because it approximates the hardness of District effluents and receiving waters (USEPA, 2000b). This could account for some of the difference between the mean LC_{50} value for this study and that reported by the USEPA for the 1991 study. The composition of moderately hard and hard synthetic freshwater is shown in Table 8. Subsequent to the completion of this study, two tests were conducted with KCl in the District's Bioassay Laboratory using moderately

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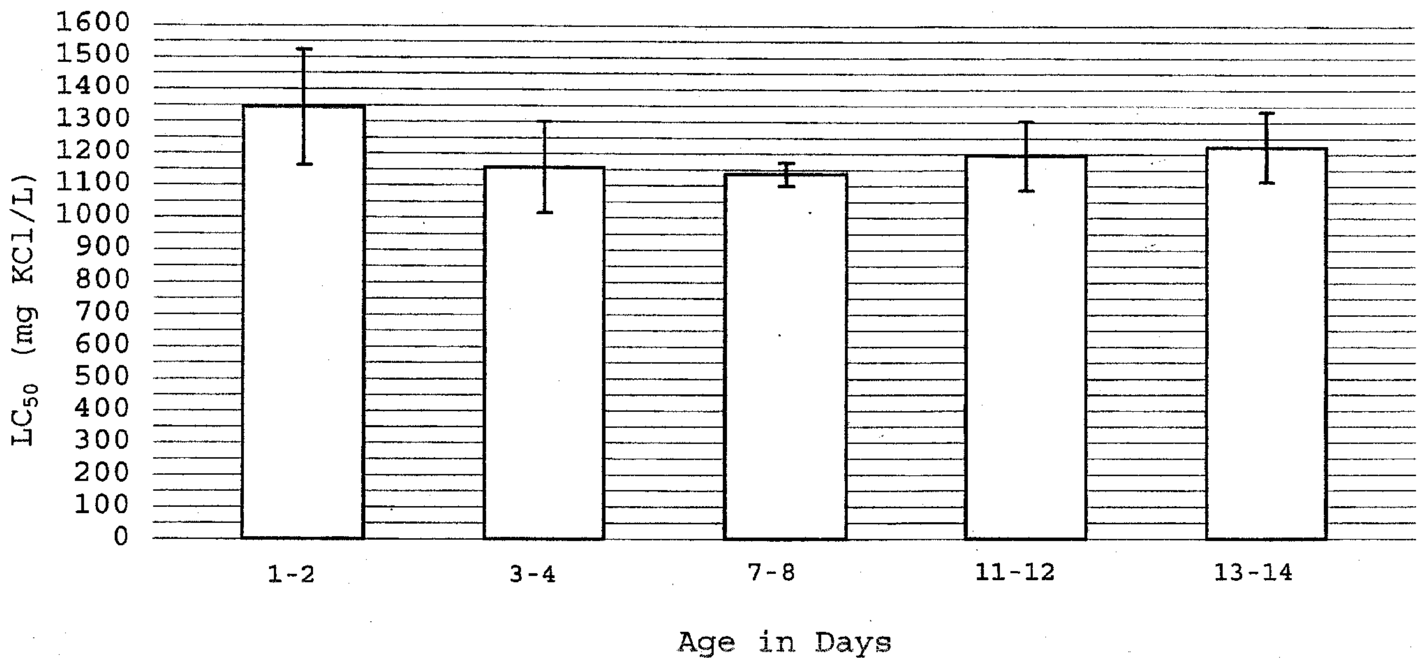
FIGURE 5

RESULTS OF TOXICITY TESTS CONDUCTED WITH THE KCl AND FIVE AGE GROUPS OF FISH: MEAN LC_{50} VALUES[1]



[1] Error bars indicate plus or minus one standard deviation.

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TABLE 8

COMPOSITION OF SYNTHETIC FRESHWATER
USING REAGENT GRADE CHEMCALS[1]

Water Type	Reagent Added (mg/L)[2]				Final Water Quality		
	$NaHCO_3$	$CaSO_4.2H_2O$	$MgSO_4$	KCl	pH[3]	Hardness[4]	Alkalinity[4]
Moderately Hard	96.0	60.0	60.0	4.0	7.4-7.8	80-100	60-70
Hard	192.0	120.0	120.0	8.0	7.6-8.0	160-180	110-120

[1]Taken from USEPA (1993).
[2]Add reagent grade chemicals to deionzed water.
[3]Approximate equilibrium after 24 h of aeration.
[4]Expressed as mg $CaCO_3$/L.

hard synthetic water. The LC_{50} values calculated for these tests were 960.3 and 983.2 mg KCl/L for 1- and 7-day old fish, respectively. These lower LC_{50} values are much closer to the mean LC_{50} value of results reported to the USEPA in 1991. These results support the explanation that the lower mean LC_{50} value of results reported to the USEPA in 1991 for tests conducted with KCl is explained, at least in part, by the use of moderately hard synthetic water for that study. These data are compared in Table 9.

Tests Conducted with SDS

The LC_{50} values for the tests conducted with SDS are shown in Table 10. The complete survival data are shown in Tables AI-21 - AI-40. The mean LC_{50} values for tests conducted with 1- to 2-, 3- to 4-, 7- to 8-, 11- to 12-, and 13- to 14-day old fish were calculated to be 28.1, 22.9, 24.6, 25.5, and 24.6 mg SDS/L, respectively. These data are shown in Figure 6. The mean LC_{50} value with 1- to 2-day old fish is numerically higher than the mean LC_{50} values for fish in all other age groups. Thus, 1- to 2-day old fish appear to be less sensitive to the toxicant SDS than fish in the 3- to 14-day old age range. The mean LC_{50} value for all of the tests conducted (1- to 14-day old fish) was calculated to be 25.2 mg SDS/L.

Tests Conducted with KCl + SDS

The LC_{50} values for the tests conducted with KCl + SDS are shown in Table 11. The complete survival data are shown in

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TABLE 9

COMPARISON OF LC_{50} VALUES (mg/L OF TEST SOLUTION) FOR THE TOXICANT KCl OBTAINED USING MODERATELY HARD AND HARD SYNTHETIC WATER FOR 48-HOUR ACUTE TOXICITY TESTS WITH <u>PIMEPHALES PROMELAS</u>

Water Type	District	Other Laboratories
Moderately Hard	971.8	896[1]
Hard	1,210.6	No data Reported[2]

[1]From a national study of interlaboratory precision of toxicity test data performed in 1991 by the Environmental Monitoring Systems Laboratory, USEPA, Cincinnati, Ohio (USEPA, 1993).
[2]No data reported for the study cited above.

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TABLE 10

RESULTS OF 48-HOUR ACUTE TOXICITY TESTS WITH PIMEPHALES PROMELAS USING THE TOXICANT SDS

Age of Fish (days)	LC_{50}[2]	Mean LC_{50}	Method[3]	Date Set Up
1 to 2	29.0		S	3/3/99
	25.0		P	3/17/99
	29.0		S	3/17/99
	29.2		S	6/2/99
		28.1		
3 to 4	17.1		S	3/24/99
	25.9		P	5/13/99
	24.9		P	5/24/99
	23.8		S	5/26/99
		22.9		
7 to 8	25.5		P	3/24/99
	26.6		P	5/19/99
	24.8		S	5/19/99
	21.5		S	6/2/99
		24.6		

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TABLE 10 (Continued)

RESULTS OF 48-HOUR ACUTE TOXICITY TESTS WITH <u>PIMEPHALES PROMELAS</u> USING THE TOXICANT SDS

Age of Fish (days)	LC_{50}[2]	Mean LC_{50}	Method[3]	Date Set Up
11 to 12	24.2		S	3/15/99
	28.6		S	3/22/99
	24.8		S	3/29/99
	24.5		S	3/29/99
		25.5		
13 to 14	24.8		S	3/15/99
	24.8		S	5/24/99
	25.5		P	6/1/99
	23.3		P	6/1/99
		24.6		
1 to 14		25.2		

[1]SDS = Sodium dodecyl sulfate.
[2]LC_{50} = Concentration of toxicant in mg/L lethal to 50 percent of test organisms.
[3]LC_{50} values were calculated by the Probit Method (P)(USEPA, 1994a). When acute toxicity test data did not meet the requirements for use of the Probit Method, LC_{50} values were calculated by the Trimmed Spearman to Karber Method(s) (USEPA, 1994b).

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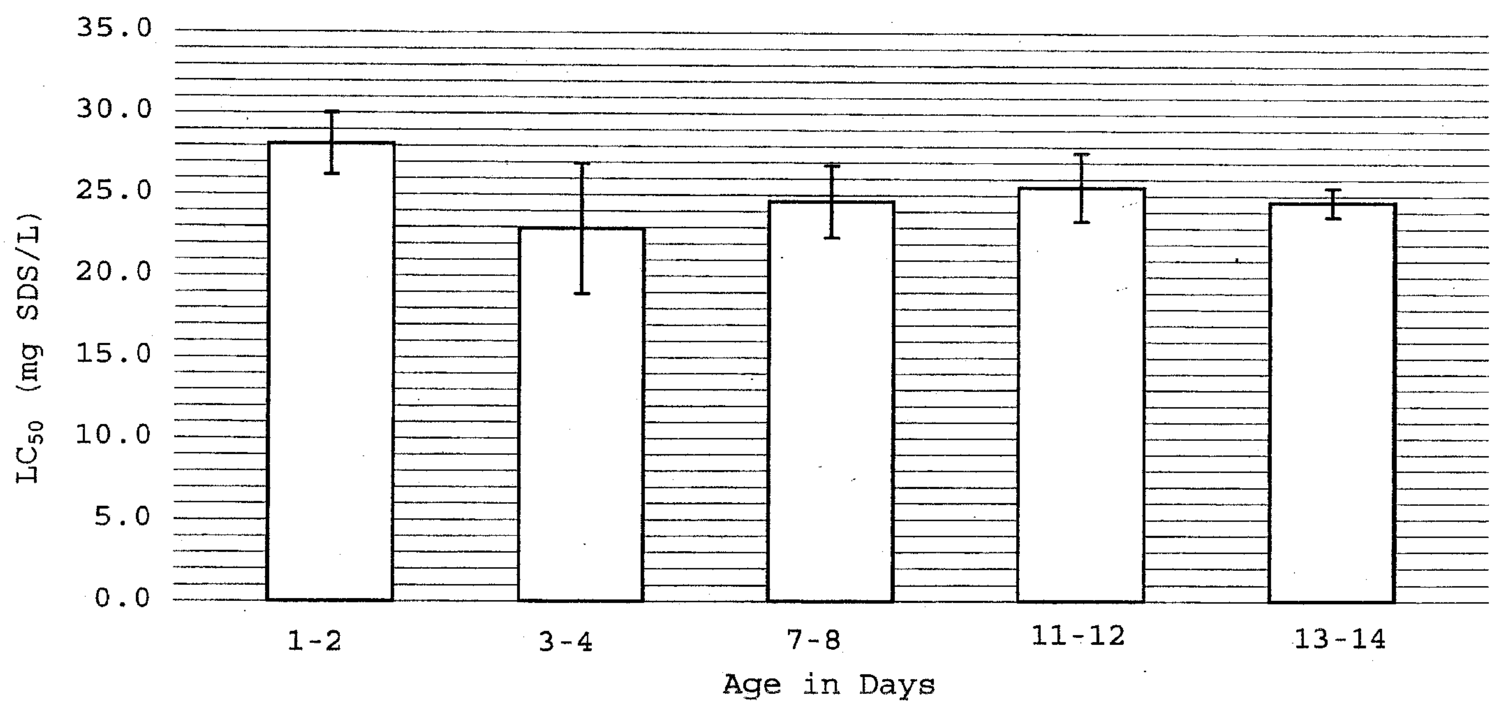
FIGURE 6

RESULTS OF TOXICITY TESTS CONDUCTED WITH THE SDS AND FIVE AGE GROUPS OF FISH: MEAN LC_{50} VALUES[1]



[1] Error bars indicate plus or minus one standard deviation.

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TABLE 11

RESULTS OF 48-HOUR ACUTE TOXICITY TESTS WITH PIMEPHALES PROMELAS USING A MIXTURE OF THE TOXICANTS KCl[1] + SDS[2]

Age of Fish (days)	LC_{50}[3]	Mean LC_{50}	Method[4]	Date Set Up
1 to 2	72.1		P	3/1/99
	65.0		S	3/3/99
	61.3		S	3/8/99
	42.5		S	5/6/99
		60.2		
3 to 4	56.2		P	3/1/99
	43.7		S	3/8/99
	51.7		S	5/10/99
	62.8		S	5/12/99
		53.6		
7 to 8	52.9		S	3/1/99
	49.4		S	3/3/99
	45.5		S	5/5/99
	53.4		S	5/12/99
		50.3		

TABLE 11 (Continued)

RESULTS OF 48-HOUR ACUTE TOXICITY TESTS WITH *PIMEPHALES PROMELAS* USING A MIXTURE OF THE TOXICANTS KCl[1] + SDS[2]

Age of Fish (days)	LC_{50}[3]	Mean LC_{50}	Method[4]	Date Set Up
11 to 12	54.1		S	3/1/99
	56.6		S	3/3/99
	52.9		S	5/10/99
	55.3		S	5/13/99
		54.7		
13 to 14	47.1		S	3/10/99
	51.5		P	3/8/99
	53.3		S	5/5/99
	63.6		S	5/6/99
		53.9		
1 to 14		54.5		

[1]KCl = Potassium chloride.
[2]Sodium dodecyl sulfate.
[3]LC_{50} = Percentage of a solution containing both KCl (1,500 mg/L) and SDS (35 mg/L) lethal to 50 percent of test organisms.
[4]LC_{50} values were calculated by the Probit Method (P)(USEPA, 1994a). When acute toxicity test data did not meet the requirements for use of the Probit Method, LC_{50} values were calculated by the Trimmed Spearman to Karber Method (S)(USEPA, 1994b).

Tables AI-41 - AI-60. The mean LC_{50} values for tests conducted with 1- to 2-, 3- to 4-, 7- to 8-, 11- to 12-, and 13- to 14-day old fish were calculated to be 60.2, 53.6, 50.3, 54.7, and 53.9 percent of the test solution containing 1,500 mg KCl + 35 mg SDS/L, respectively. These data are shown in Figure 7. The mean LC_{50} value with 1- to 2-day old fish is numerically higher than the mean LC_{50} values for fish in all other age groups. Thus, 1- to 2-day old fish appear to be less sensitive to the KCl + SDS toxicant combination than fish in the 3- to 14-day old age range. The mean LC_{50} value for all of the tests conducted (1- to 14-day old fish) was calculated to be 54.5 percent of the test solution containing 1,500 mg KCl + 35 mg SDS/L.

Precision

Precision of the tests conducted for each of the five age groups with the toxicants KCl, SDS, and KCl + SDS combination is shown in Table 12. The coefficients of variation (CV) for all of the tests conducted (1- to 14-day old fish) for each of these toxicants are 11.0, 11.0, and 13.8 percent, respectively. Until recently the USEPA gave no numerical criteria for demonstrating acceptable laboratory performance by judging intra-laboratory precision expressed as CV values (USEPA, 1993). The USEPA only stated elsewhere that "the closer the CV is to zero the better" (USEPA, 1991). Recent guidance (USEPA, 2000a) provided an "interim method CV" of 16 percent

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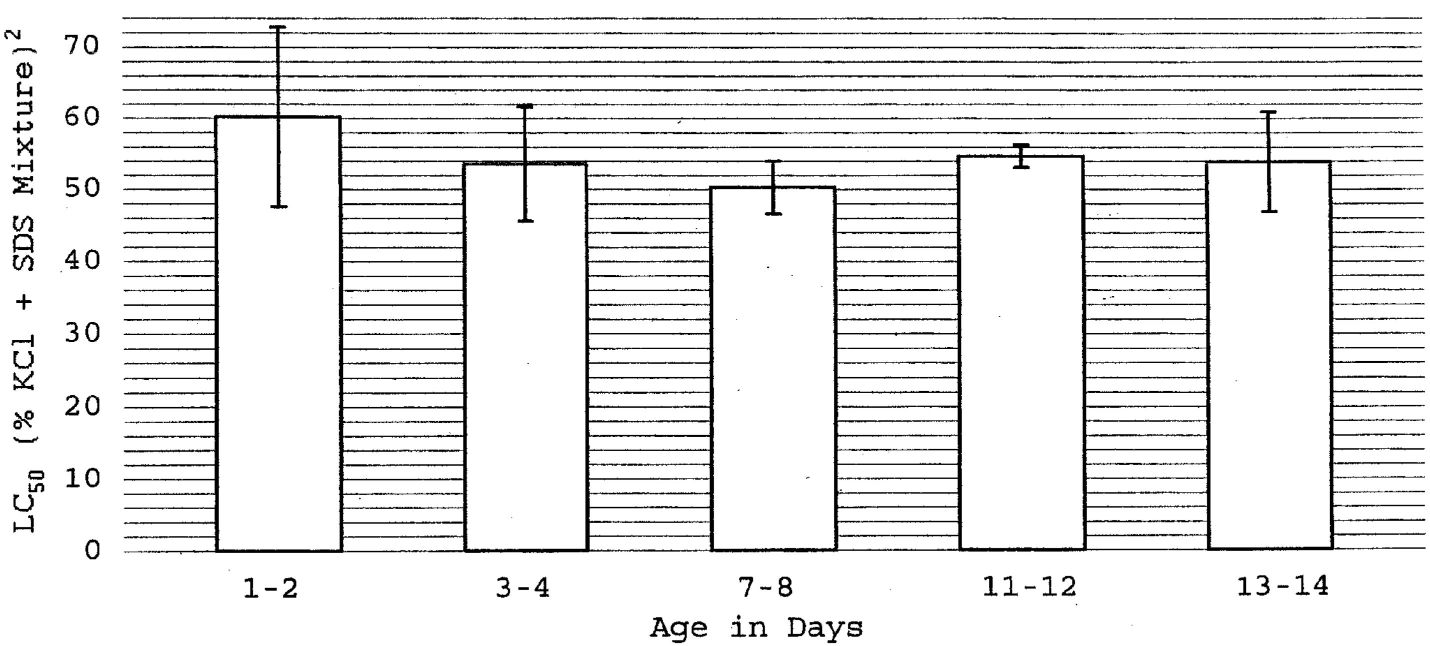
FIGURE 7

RESULTS OF TOXICITY TESTS CONDUCTED WITH THE KCl AND FIVE AGE GROUPS OF FISH: MEAN LC50 VALUES[1]



[1] Error bars indicate plus or minus one standard deviation.

[2]Note: Percentage of a solution containing both KCl (1,500 mg/L) and SDS (35 mg/L) lethal to 50 percent of test organisms.

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TABLE 12

PRECISION OF 48-HOUR ACUTE TOXICITY TESTS CONDUCTED WITH FIVE AGE GROUPS OF <u>PIMEPHALES</u> <u>PROMELAS</u> TESTED: CV VALUES[1]

Age of Fish (Days)	Toxicant		
	KCl	SDS	KCl + SDS
1 to 2	13.5	6.9	21.0
3 to 4	12.3	17.3	15.0
7 to 8	3.3	8.9	7.3
11 to 12	9.1	8.1	2.9
13 to 14	9.0	3.8	13.0
1 to 14[2]	11.0	11.0	13.8
3 to 14[2]	8.6	10.1	10.1

[1]Coefficient of Variation of LC_{50} values of four tests from each group.
[2]Combination of data from the above age groups.

for acute WET tests conducted with *Pimephales promelas*. This interim CV represented the median CV, or 50th percentile CV, observed within 21 laboratories (for WET tests conducted with reference toxicants). The USEPA recommended calculating warning and control limits based on the 75th and 90th percentiles, respectively, of the method CV, which were reported to be 19 and 33 percent, respectively. The CVs reported for this study, 11.0, 11.0, and 13.8 percent, are well below the 75th percentile CV of 19 percent, and even the 50th percentile CV of 16 percent, and demonstrate acceptable precision as defined in the recent USEPA guidance cited above.

The CVs for the tests conducted with KCl and 1- to 2-, 3- to 4-, 7- to 8-, 11- to 12-, and 13- to 14-day old fish are 13.5, 12.3, 3.3, 9.1, and 9.0 percent, respectively. The CVs for the tests conducted with SDS and 1- to 2-, 3- to 4-, 7- to 8-, 11- to 12-, and 13- to 14-day old fish are 6.9, 17.3, 8.9, 8.1, and 3.8 percent, respectively. The CVs for the tests conducted with the KCl + SDS combination and 1- to 2-, 3- to 4-, 7- to 8-, 11- to 12-, and 13- to 14-day old fish are 21.0, 15.0, 7.3, 2.9, and 13.0 percent, respectively.

Parametric CV values were reported in the previous paragraph for the SDS data, even though the LC_{50} data for the toxicant SDS, as demonstrated in the next section, provide significant evidence of non-normality. The use of means and standard deviations is closely associated with the assumption of normality, and so is the use of a CV. This is not the case for the other two types of toxicants. For this reason, a nonparametric analogue to the CV for the SDS toxicant data was considered.

The median is an alternative nonparametric measure of central tendency that may be used in place of the mean when normality is not ascertained with the data collected, while the interquartile range is an alternative measure of spread that may be used in place of the standard deviation. Thus, a natural analogue to the parametric CV is the nonparametric CV computed as the ratio of the interquartile range divided by the median expressed as percentage. Nonparametric CVs for the SDS data are shown in Table 13. Note that the parametric CVs for SDS (Table 12) and the nonparametric CVs for SDS reported in Table 13 vary with age in a similar fashion, and so it seems reasonable in this case to use the parametric CVs even though normality is questionable.

Markle et al. (2000), who conducted 48-hour acute toxicity tests with 1-, 4-, 7-, 10-, and 14-day old fish (*Pimephales promelas*) and four different toxicants, Cr^{+6}, sodium pentachlorophenate (NaPCP), SDS, and NH_3, reported that LC_{50} data generated using 1-day old fish were the most variable. In this study the CV for the tests conducted with KCl and 3- to 14-day old fish is 8.6 percent compared to a CV of 11.0 percent for tests conducted with 1- to 14-day old fish. The CV for tests conducted with SDS and 3- to 14-day old is 10.1 compared to a CV of 11.0 for tests conducted with 1- to 14-day old fish. The CV

TABLE 13

PRECISION OF 48-HOUR ACUTE TOXICITY TESTS CONDUCTED WITH FIVE AGE GROUPS OF *PIMEPHALES PROMELAS* TESTED (NONPARAMETRIC COEFFICIENTS OF VARIATION)

Toxicant	Age of Fish (Days)	Nonparametric CV[1] (Percent)
SDS[2]	1 to 2	6.9
	3 to 4	20.3
	7 to 8	11.5
	11 to 12	9.5
	13 to 14	4.4
	1 to 14[3]	7.6
	3 to 14[3]	6.0

[1]Nonparametric coefficient of variation of LC_{50} values of four tests from each age group. The nonparametric CV is computed as the ratio of the interquartile range divided by the median expressed as percentage. Nonparametric CVs were calculated because the LC_{50} data for SDS were not shown to be normally distributed.
[2]Sodium dodecyl sulfate.
[3]Combination of data from the above age groups.

for the tests conducted with the KCl + SDS toxicant combination and 3- to 14-day old fish is 10.1 percent compared to a CV of 13.8 percent for tests conducted with 1- to 14-day old fish. Thus, LC_{50} data generated with 1- to 2-day old fish appear to be more variable than LC_{50} data generated with 3- to 14-day old fish for KCl, SDS, and KCl + SDS, as shown in this study, and in the cases of Cr^{+6}, NaPCP, SDS, and NH_3, as shown by Markle et al. (2000). The CVs for this study were, in general, much lower than those reported by Markle et al., but the patterns observed in both laboratories were essentially the same. These findings suggest that the use of 1- to 2-day old fish contributes significantly to the variability associated with the acute fish WET test.

Statistical Analysis

CORRECTION FOR MORTALITY IN THE CONTROLS

Observed and corrected mortality rates of fish exposed to the toxicants KCl, SDS, and KCl + SDS are shown in Tables AII-1, AII-2, and AII-3, respectively.

TESTS FOR NORMALITY AND HOMOGENEITY OF VARIANCE

Mortality Rates. Results of the Shapiro-Wilk test showed that the corrected mortality rates were not normally distributed for any of the toxicants used (Table 14). For this reason, only nonparametric results are reported below for corrected mortality rates. No further tests were performed to

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TABLE 14

RESULTS OF THE SHAPIRO-WILK TEST FOR NORMALITY CONDUCTED ON CORRECTED MORTALITY RATES

Toxicant	P-Value	Normally Distributed
KCl	0.0001	No
SDS	0.0001	No
KCl + SDS	0.0001	No

verify the assumption of homogeneity of variance across the different age groups.

LC_{50} Data. For LC_{50} data across different age groups, the Shapiro-Wilk test for normality was significant only in the case of the SDS toxicant (Table 15). For this reason, non-parametric ANOVA results are reported below for LC_{50} data in the SDS toxicant case. For each of the other two toxicants cases, KCl and KCl + SDS, Bartlett's test for equal variances (Walpole and Meyers, 1989) is non-significant (Table 15). Thus, in these two cases, there is no reason to question the assumptions of normality and equal variances necessary for the standard parametric ANOVA, and so parametric ANOVA results are reported below in these cases.

COMPARISON OF THE SENSITIVITIES OF DIFFERENT AGE GROUPS TO TOXICANTS

Mortality Rates. The results of nonparametric ANOVA performed on the basis of ranked corrected mortality rates are summarized in Table 16. These results show that the linear model is highly adequate to explain the variation observed (SAS Institute, 1995). These results also show that age did affect the mortality rates of fish exposed to either of the toxicants KCl or SDS, but not the combination of KCl + SDS. Results of the associated Student-Newman-Keuls test showed that 1 to 2-day old fish exposed to either of the toxicants KCl or SDS had significantly lower mortality rates than the

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TABLE 15

RESULTS OF THE SHAPIRO-WILK TEST AND BARTLETT'S TEST CONDUCTED ON LC_{50} DATA

Toxicant	Number of LC_{50} Values	Significance Probability for Normality (Shapiro-Wilk Test)	Significance Probability for Homogeneous Variance (Bartlett's Test)
KCl	20	p = 0.18[1]	>0.025[2]
SDS	20	p = 0.01	NA[3]
KCl + SDS	20	p = 0.58[1]	>0.025[2]

[1]Data are normally distributed.
[2]Variances across the five age groups are equal.
[3]Not Applicable. There is no nonparametric test for homogeneous variance.

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TABLE 16

RESULTS OF NONPARAMETRIC ANALYSIS OF VARIANCE PERFORMED ON THE BASIS OF RANKED CORRECTED MORTALITY RATES

Toxicant	Percent of Variation Explained[1]	Significance Probability on Age Effects
KCl	82	0.0001[2]
SDS	89	0.0001[2]
KCl + SDS	90	0.1100

[1] R^2 x 100.
[2] Significant.

older fish exposed to these toxicants ($p \leq 0.05$). The results are summarized in Table 17.

LC$_{50}$ Data. The results of parametric and nonparametric (for SDS) standard ANOVA (Table 18) show that age had no statistically significant effect upon the LC$_{50}$ values for the toxicants KCl, SDS, or KCl + SDS.

The one-way ANOVA model used above is a five parameter model (not counting the constant variance parameter) while the data set has only 20 observations. It is possible that this model may be too complex relative to the size of the data set for the associated F-test to identify effectively any significant differences of a simple nature that may exist between a single age level and all remaining age levels combined. This was not the case for the analysis of the mortality data, but may be the case for the analysis of the LC$_{50}$ data. For this reason, alternative regression models for the LC$_{50}$ data were considered.

In particular, the age classification levels were transformed into numerical age scores, "x" by average ages within each age level (i.e., 1 to 2 is coded as 1.5, 3 to 4 as 3.5, etc.). Regression models were then considered for the expected LC$_{50}$ value as a possibly power-transformed function of "x" that is:

$$E(LC_{50} | x = \alpha + \beta \cdot x^{p}),\ p \neq 0$$

with the limiting case as $p \rightarrow 0$

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TABLE 17

RESULTS OF THE STUDENT-NEWMAN-KEULS TEST ON MULTIPLE COMPARISONS[1]

Toxicant	Age of Fish in Days	Rank
KCl	1 to 2	1[2]
	3 to 4, 7 to 8, 11 to 12, 13 to 14	2[3]
SDS	1 to 2	1
	3 to 4, 7 to 8, 11 to 12, 13 to 14	2

[1]Ranking of mortality rates of different fish age groups exposed to KCl and SDS.
[2]Lower mortality rate.
[3]Mortality rates are higher than 1- to 2-day old, but insignificant within this group.

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TABLE 18

RESULTS OF ANALYSIS OF VARIANCE ON LC_{50} VALUES
(F-TEST)

Toxicant	ANOVA	p-value
KCl	parametric	0.20[1]
SDS	Non-parametric	0.09[1]
KCl + SDS	parametric	0.49[1]

[1]p-values <0.05 are significant at the 0.05 level. The results of this test indicates that there is no age effect on the concentration of toxicant causing 50 percent mortality.

$$E(LC_{50} | (x) = \alpha + \beta \cdot \ln(x)$$

For each transform of "x", the parameters were estimated using ordinary least squares. The power parameter "p" was chosen using cross-validation, that is, by minimizing the associated predicted residual sum of squares (PRESS) over a grid of powers (SAS Institute, 1990).

For each fixed power "p", the above model has only two parameters (other than the constant variance parameter), substantially less than the five parameters of the one-way analysis model for these data. For this reason, regression models should be better able to identify a dependency, should one exist, of LC_{50} on age, than is possible using the one-way analysis of variance model.

In the case of the LC_{50} data for the KCl toxicant, the PRESS score decreased as the power decreased over negative values, but the decreases were eventually negligible. For example, the PRESS score for p = -5 was 3.506×10^5 while the score for p = -5.5 was 3.505×10^5. The estimated expected LC_{50} value is displayed in terms of "x" through the transform "x^{-5}" in <u>Figure 8</u>.

This expected value function is essentially the same as the one corresponding to the one-way ANOVA model identified for the mortality data, that is, the one which groups age into

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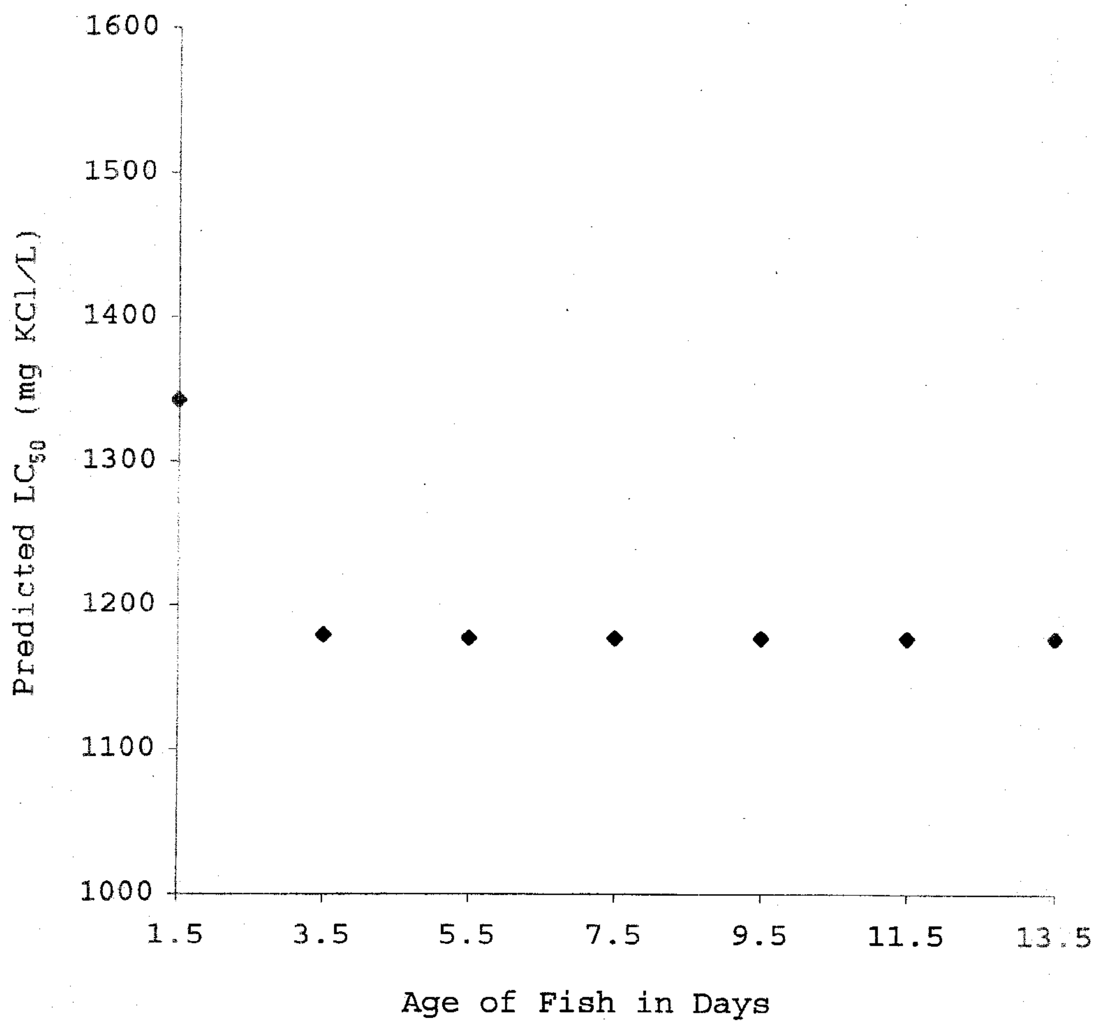
FIGURE 8

PREDICTED LC_{50} VALUES FOR THE TOXICANT KCl



two groups consisted of age level 1 to 2 separate from levels 3 to 4, 7 to 8, 11 to 12, and 13 to 14 combined. This two-group model has an associated PRESS score with the negligibly better score of 3.502×10^5. Furthermore, the PRESS score for the constant model is 3.770×10^5, a tangible 7.7 percent larger value than for the two-group model. This constant model is the one that could not be rejected using the full five level ANOVA model. These cross-validation results indicate that the one-way ANOVA results for the KCl toxicant are adversely affected by sample size, and that LC_{50} values actually do depend on age through the same two-group model as identified for mortality data.

In the case of the LC_{50} data for the SDS toxicant, the PRESS score decreased as the power decreased over negative values, but the decreases were eventually negligible. For example, the PRESS score for $p = -5$ was 124.5 while the score for $p = -5.5$ was 124.3. The estimated expected LC_{50} value is displayed in terms of x through the transform x^{-5} in Figure 9.

Note that this expected value function is essentially the same as the one corresponding to the one-way ANOVA model identified for the mortality data, that is, the one which groups age into two groups consisting of level 1- to 2-day age group separate from levels 3- to 4-, 7- to 8-, 11- to 12-, and 13-

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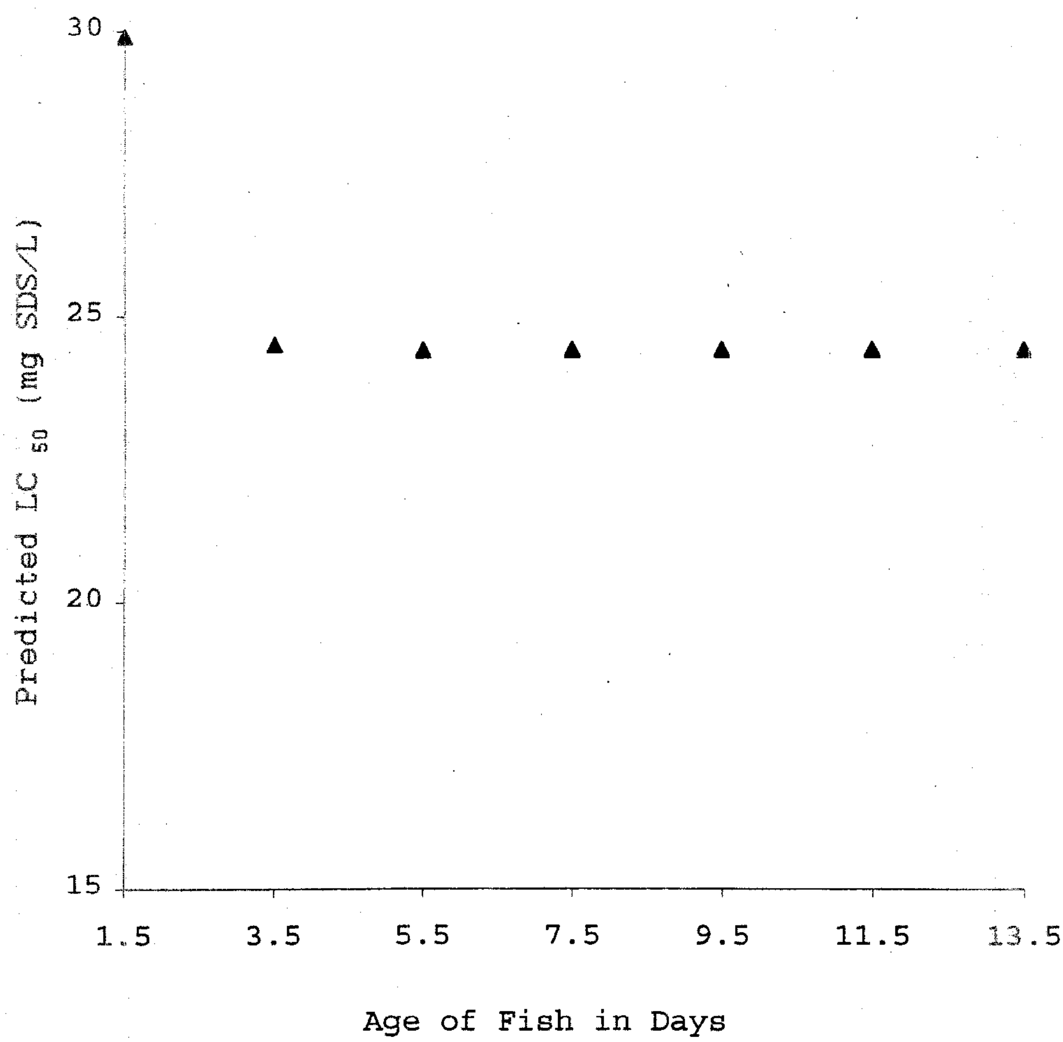
FIGURE 9

PREDICTED LC_{50} VALUES FOR THE TOXICANT SDS



to 14-day age group combined. This two-group model has an associated PRESS score with the negligibly better score of 123.9. Furthermore, the PRESS score for the constant model is 161.9, a substantial 30.7 percent larger value than for the two-group model. This constant model is the one that could not be rejected using the full five level analysis of variance model. These cross-validation results indicate that the one-way analysis of variance results for the SDS toxicant are adversely affected by sample size, and that LC_{50} values actually do depend on age through the same two-group model as identified for mortality data.

As explained above, it is appropriate to analyze the LC_{50} data for both the KCl and the SDS data sets using a model with fewer age levels, specifically, the 1- to 2-day age group versus the rest of the age groups combined. In the case of KCl the results of parametric ANOVA showed that the expected LC_{50} value for 1- to 2-day old fish is significantly higher than the expected LC_{50} value for 3- to 14-day old fish ($p = 0.0225$). In the case of SDS the results of nonparametric ANOVA showed that the expected LC_{50} value for 1- to 2-day old fish is significantly higher than the expected LC_{50} value for 3- to 14-day old fish ($p = 0.021$).

In the case of the LC_{50} data for the KCl + SDS toxicant, the PRESS score was smallest within ± 0.5 for the case p = 0 corresponding to the natural log transform ln(x) with value 1.323×10^3. On the other hand, the PRESS score for the constant model was the substantially smaller value of 1.190×10^3, indicating that LC_{50} values for the toxicant KCl + SDS may be reasonably treated as constant in age as they were for the mortality data.

Therefore, sophisticated statistical analyses using cross-validation to select a regression model of LC_{50} as a function of age indicated that it was appropriate to analyze the LC_{50} data for both the KCl and SDS data sets using a model with fewer age groups, specifically, the 1- to 2-day age group versus the rest of the age groups combined. Two sample analyses of the data on LC_{50} values of fish exposed to either KCl or SDS indicated that the LC_{50} values for these toxicants are affected by fish age, i.e., that fish 1 to 2 days old are more tolerant of these toxicants than the older fish in the age group of 3 to 14 days. In this study the LC_{50} values for the KCl + SDS data set were not shown to be affected by age.

TEST FOR EQUALITY OF COEFFICIENTS OF VARIATION (CVs)

The KCl and KCl + SDS LC_{50} population means across all age groups studied were not shown to be unequal or to have unequal variances (Table 15). Therefore, there is no significant difference in the CVs across the age groups studied for the toxicant KCl or the toxicant combination KCl + SDS even though numerical differences were observed. No conclusion regarding the CVs for SDS could be made because there is no non-parametric test for testing the homogeneity of variance. These results are summarized in Table 19. These results indicate that it would be appropriate, in general, to report the results of statistical analyses when CV values are used to evaluate precision as done in this study. Relying exclusively upon CV values to make sweeping judgments about the reproducibility of a test based on the magnitude of the CV values across the board may not be appropriate or desirable in certain instances.

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TABLE 19

SUMMARY OF STATISTICAL ANALYSES CONDUCTED ON LC_{50} DATA TO TEST CVs ACROSS ALL AGE GROUPS FOR EQUALITY

Toxicant	Normally Distributed[1]	Homogeneous Variance[1]	Equal Means[2]	Conclusion Regarding CVs Across All Age Groups
KCl	Yes	Yes	Yes	Equal
SDS	No	NA[3]	Yes	No Conclusion Can Be Drawn
KCl plus SDS	Yes	Yes	Yes	Equal

[1]Table 15.
[2]Table 18.
[3]Not applicable. There is no nonparametric test for testing homogeneity of variance.

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APPENDIX AI

SURVIVAL DATA

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-1

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 8-10, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	4	5	5
250	5	5	5	5
500	5	5	5	5
1000	5	4	5	5
1250	5	4	5	5
1500	4	3	2	2

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TABLE AI-2

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 17-19, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	5	4	5	4
1250	3	1	1	2
1500	1	0	0	0

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TABLE AI-3

SURVIVAL DATA FOR 2-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 17-19, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	4	5	5	4
1250	2	1	1	4
1500	0	1	2	1

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TABLE AI-4

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 12-14, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	5	5	5	5
1250	2	4	4	3
1500	2	0	0	3

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TABLE AI-5

SURVIVAL DATA FOR 3-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 24-26, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	3	4	3	5
1250	1	2	2	1
1500	0	0	0	1

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TABLE AI-6

SURVIVAL DATA FOR 4-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 19-21, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	3	1	3	3
1250	2	0	1	0
1500	0	0	0	0

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TABLE AI-7

SURVIVAL DATA FOR 3-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 21-23, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	4	5	5	5
1000	4	4	4	5
1250	1	0	2	4
1500	0	0	1	0

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TABLE AI-8

SURVIVAL DATA FOR 3-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 28-30, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	4	5	5	5
1000	5	5	5	5
1250	3	4	3	3
1500	1	1	2	2

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TABLE AI-9

SURVIVAL DATA FOR 7-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 24-26, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	5	2	4	3
1250	2	2	2	3
1500	0	0	0	1

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TABLE AI-10

SURVIVAL DATA FOR 8-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 14-16, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	5	5	5	5
1250	1	2	1	1
1500	0	0	0	0

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TABLE AI-11

SURVIVAL DATA FOR 7-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 19-21, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	4	2	2	4
1250	1	3	0	3
1500	0	0	0	0

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TABLE AI-12

SURVIVAL DATA FOR 8-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 21-23, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	4	4	5	5
1250	0	1	2	0
1500	0	0	0	0

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TABLE AI-13

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 15-17, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	4	5	5
250	5	5	5	5
500	5	5	5	5
1000	4	5	4	4
1250	1	3	1	3
1500	0	0	1	0

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TABLE AI-14

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 22-24, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	4	2	2	2
1250	2	3	0	2
1500	0	0	0	0

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TABLE AI-15

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 12-14, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	5	5	5	4
1250	5	4	5	2
1500	0	0	0	0

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TABLE AI-16

SURVIVAL DATA FOR 12-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), APRIL 14-16, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	5	5	4	5
1250	1	2	3	2
1500	0	2	0	2

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TABLE AI-17

SURVIVAL DATA FOR 13-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 10-12, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	3	5	5	5
1250	4	3	4	4
1500	1	1	2	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-18

SURVIVAL DATA FOR 13-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 15-17, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	4	5	5	5
1250	3	2	3	2
1500	0	0	0	0

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TABLE AI-19

SURVIVAL DATA FOR 14-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 3-5, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	4	5
1000	5	4	5	4
1250	4	3	0	4
1500	1	0	1	2

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-20

SURVIVAL DATA FOR 14-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 3-5, 1999

KCl Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250	5	5	5	5
500	5	5	5	5
1000	4	4	4	5
1250	2	1	0	0
1500	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-21

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 3-5, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	3	3	4	2
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-22

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 17-19, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	3	5	5	5
30	0	0	3	1
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-23

SURVIVAL DATA FOR 2-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 17-19, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	5	1	2	4
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-24

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), JUNE 2-4, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	4	2	4	2
35	1	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-25

SURVIVAL DATA FOR 3-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 24-26, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	4	3	0	0
30	0	0	0	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-26

SURVIVAL DATA FOR 3-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 13-15, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	4	5
30	2	0	1	1
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-27

SURVIVAL DATA FOR 4-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 24-26, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	4	5
10	5	5	5	5
20	5	5	5	4
30	0	1	0	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-28

SURVIVAL DATA FOR 3-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 26-28, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	4	5
30	0	0	0	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-29

SURVIVAL DATA FOR 7-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 24-26, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	4	5	5
30	0	0	2	1
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-30

SURVIVAL DATA FOR 7-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 19-21, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	4	5	5
30	4	0	2	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-31

SURVIVAL DATA FOR 8-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 19-21, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	1	0	0	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-32

SURVIVAL DATA FOR 7-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), JUNE 2-4, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	4	5	5
20	4	4	3	4
30	0	0	0	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-33

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 15-17, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	4	5	5
10	5	5	5	5
20	5	5	5	4
30	0	0	0	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-34

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 22-24, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	3	3	4	1
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-35

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 29-31, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	0	0	1	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-36

SURVIVAL DATA FOR 12-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 29-31, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	0	0	0	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-37

SURVIVAL DATA FOR 13-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 15-17, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	0	0	0	1
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-38

SURVIVAL DATA FOR 14-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 24-26, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	5	5	5	5
30	0	0	0	1
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-39

SURVIVAL DATA FOR 13-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), JUNE 1-3, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	4	5	5	5
30	0	2	0	1
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-40

SURVIVAL DATA FOR 14-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), JUNE 1-3, 1999

SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
10	5	5	5	5
20	4	4	5	4
30	1	0	0	0
35	0	0	0	0
40	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-41

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 1-3, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	4	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	5	4	4	3
1250 (KCl) + 29 (SDS)	1	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-42

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 3-5, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	2	3	5	4
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-43

SURVIVAL DATA FOR 1-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 8-10, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	4	5	5
1000 (KCl) + 23 (SDS)	3	3	4	3
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-44

SURVIVAL DATA FOR 2-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 6-8, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	4	4	4	5
1000 (KCl) + 23 (SDS)	0	0	0	0
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-45

SURVIVAL DATA FOR 4-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 1-3, 1999

KCl + SDS Concentration mg/L	Number of Survivors A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	4
1000 (KCl) + 23 (SDS)	0	3	3	2
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-46

SURVIVAL DATA FOR 3-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 8-10, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	4	5	5	5
500 (KCl) + 11.66 (SDS)	3	5	4	5
1000 (KCl) + 23 (SDS)	0	1	0	0
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-47

SURVIVAL DATA FOR 4-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 10-12, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	1	0	2	1
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-48

SURVIVAL DATA FOR 3-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 12-14, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	4	5	5	5
1000 (KCl) + 23 (SDS)	1	4	5	4
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-49

SURVIVAL DATA FOR 7-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 1-3, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	1	1	1	2
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-50

SURVIVAL DATA FOR 7-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 3-5, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	2	0	0	0
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-51

SURVIVAL DATA FOR 8-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 5-7, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	4	5	5
1000 (KCl) + 23 (SDS)	0	0	0	0
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-52

SURVIVAL DATA FOR 8-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 12-14, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	4	5	5
1000 (KCl) + 23 (SDS)	1	2	3	1
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-53

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 1-3, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	2	2	1	1
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-54

SURVIVAL DATA FOR 12-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 3-5, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	0	2	3	3
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-55

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 10-12, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	0	2	1	2
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-56

SURVIVAL DATA FOR 11-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 13-15, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	3	0	2	2
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-57

SURVIVAL DATA FOR 13-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 8-10, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	4
1000 (KCl) + 23 (SDS)	0	0	2	2
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-58

SURVIVAL DATA FOR 13-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MARCH 10-12, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	5
500 (KCl) + 11.66 (SDS)	5	5	5	5
1000 (KCl) + 23 (SDS)	0	0	0	0
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-59

SURVIVAL DATA FOR 13-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 5-7, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	4	5
500 (KCl) + 11.66 (SDS)	5	4	5	5
1000 (KCl) + 23 (SDS)	2	3	0	1
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-60

SURVIVAL DATA FOR 14-DAY OLD FATHEAD MINNOWS (PIMEPHALES PROMELAS) EXPOSED TO KCl + SDS (48-HOUR, STATIC, NON-RENEWAL, ACUTE TOXICITY TEST), MAY 6-8, 1999

KCl + SDS Concentration mg/L	Number of Survivors			
	A	B	C	D
0	5	5	5	5
250 (KCl) + 5.83 (SDS)	5	5	5	4
500 (KCl) + 11.66 (SDS)	5	4	5	5
1000 (KCl) + 23 (SDS)	4	3	2	4
1250 (KCl) + 29 (SDS)	0	0	0	0
1500 (KCl) + 35 (SDS)	0	0	0	0

APPENDIX AII

OBSERVED AND CORRECTED MORTALITY RATES

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-1

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
1	250	A1	0.00	0.00
2	250	A1	0.00	0.00
3	250	A1	0.00	0.00
4	250	A1	0.00	0.00
5	250	A1	0.00	0.00
6	250	A1	0.00	0.00
7	250	A1	0.00	0.00
8	250	A1	0.00	0.00
9	250	A1	0.00	0.00
10	250	A1	0.00	0.00
11	250	A1	0.00	0.00
12	250	A1	0.00	0.00
13	250	A1	0.00	0.00
14	250	A1	0.00	0.00
15	250	A1	0.00	0.00
16	250	A1	0.00	0.00
17	250	A2	0.00	0.00
18	250	A2	0.00	0.00
19	250	A2	0.00	0.00
20	250	A2	0.00	0.00
21	250	A2	0.00	0.00
22	250	A2	0.00	0.00
23	250	A2	0.00	0.00
24	250	A2	0.00	0.00
25	250	A2	0.00	0.00
26	250	A2	0.00	0.00
27	250	A2	0.00	0.00
28	250	A2	0.00	0.00
29	250	A2	0.00	0.00
30	250	A2	0.00	0.00
31	250	A2	0.00	0.00
32	250	A2	0.00	0.00
33	250	A3	0.00	0.00
34	250	A3	0.00	0.00

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
35	250	A3	0.00	0.00
36	250	A3	0.00	0.00
37	250	A3	0.00	0.00
38	250	A3	0.00	0.00
39	250	A3	0.00	0.00
40	250	A3	0.00	0.00
41	250	A3	0.00	0.00
42	250	A3	0.00	0.00
43	250	A3	0.00	0.00
44	250	A3	0.00	0.00
45	250	A3	0.00	0.00
46	250	A3	0.00	0.00
47	250	A3	0.00	0.00
48	250	A3	0.00	0.00
49	250	A4	0.00	0.00
50	250	A4	0.00	0.00
51	250	A4	0.00	0.00
52	250	A4	0.00	0.00
53	250	A4	0.00	0.00
54	250	A4	0.00	0.00
55	250	A4	0.00	0.00
56	250	A4	0.00	0.00
57	250	A4	0.00	0.00
58	250	A4	0.00	0.00
59	250	A4	0.00	0.00
60	250	A4	0.00	0.00
61	250	A4	0.00	0.00
62	250	A4	0.00	0.00
63	250	A4	0.00	0.00
64	250	A4	0.00	0.00
65	250	A5	0.00	0.00
66	250	A5	0.00	0.00
67	250	A5	0.00	0.00
68	250	A5	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
69	250	A5	0.00	0.00
70	250	A5	0.00	0.00
71	250	A5	0.00	0.00
72	250	A5	0.00	0.00
73	250	A5	0.00	0.00
74	250	A5	0.00	0.00
75	250	A5	0.00	0.00
76	250	A5	0.00	0.00
77	250	A5	0.00	0.00
78	250	A5	0.00	0.00
79	250	A5	0.00	0.00
80	250	A5	0.00	0.00
81	500	A1	0.00	0.00
82	500	A1	0.00	0.00
83	500	A1	0.00	0.00
84	500	A1	0.00	0.00
85	500	A1	0.00	0.00
86	500	A1	0.00	0.00
87	500	A1	0.00	0.00
88	500	A1	0.00	0.00
89	500	A1	0.00	0.00
90	500	A1	0.00	0.00
91	500	A1	0.00	0.00
92	500	A1	0.00	0.00
93	500	A1	0.00	0.00
94	500	A1	0.00	0.00
95	500	A1	0.00	0.00
96	500	A1	0.00	0.00
97	500	A2	0.00	0.00
98	500	A2	0.00	0.00
99	500	A2	0.00	0.00
100	500	A2	0.00	0.00
101	500	A2	0.00	0.00
102	500	A2	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
103	500	A2	0.00	0.00
104	500	A2	0.00	0.00
105	500	A2	0.20	0.20
106	500	A2	0.00	0.00
107	500	A2	0.00	0.00
108	500	A2	0.00	0.00
109	500	A2	0.20	0.20
110	500	A2	0.00	0.00
111	500	A2	0.00	0.00
112	500	A2	0.00	0.00
113	500	A3	0.00	0.00
114	500	A3	0.00	0.00
115	500	A3	0.00	0.00
116	500	A3	0.00	0.00
117	500	A3	0.00	0.00
118	500	A3	0.00	0.00
119	500	A3	0.00	0.00
120	500	A3	0.00	0.00
121	500	A3	0.00	0.00
122	500	A3	0.00	0.00
123	500	A3	0.00	0.00
124	500	A3	0.00	0.00
125	500	A3	0.00	0.00
126	500	A3	0.00	0.00
127	500	A3	0.00	0.00
128	500	A3	0.00	0.00
129	500	A4	0.00	0.00
130	500	A4	0.00	0.00
131	500	A4	0.00	0.00
132	500	A4	0.00	0.00
133	500	A4	0.00	0.00
134	500	A4	0.00	0.00
135	500	A4	0.00	0.00
136	500	A4	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
137	500	A4	0.00	0.00
138	500	A4	0.00	0.00
139	500	A4	0.00	0.00
140	500	A4	0.00	0.00
141	500	A4	0.00	0.00
142	500	A4	0.00	0.00
143	500	A4	0.00	0.00
144	500	A4	0.00	0.00
145	500	A5	0.00	0.00
146	500	A5	0.00	0.00
147	500	A5	0.00	0.00
148	500	A5	0.00	0.00
149	500	A5	0.00	0.00
150	500	A5	0.00	0.00
151	500	A5	0.00	0.00
152	500	A5	0.00	0.00
153	500	A5	0.00	0.00
154	500	A5	0.00	0.00
155	500	A5	0.20	0.20
156	500	A5	0.00	0.00
157	500	A5	0.00	0.00
158	500	A5	0.00	0.00
159	500	A5	0.00	0.00
160	500	A5	0.00	0.00
161	1000	A1	0.00	0.00
162	1000	A1	0.20	0.19
163	1000	A1	0.00	0.00
164	1000	A1	0.00	0.00
165	1000	A1	0.00	0.00
166	1000	A1	0.20	0.19
167	1000	A1	0.00	0.00
168	1000	A1	0.20	0.19
169	1000	A1	0.20	0.19
170	1000	A1	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
171	1000	A1	0.00	0.00
172	1000	A1	0.20	0.19
173	1000	A1	0.00	0.00
174	1000	A1	0.00	0.00
175	1000	A1	0.00	0.00
176	1000	A1	0.00	0.00
177	1000	A2	0.40	0.40
178	1000	A2	0.20	0.20
179	1000	A2	0.40	0.40
180	1000	A2	0.00	0.00
181	1000	A2	0.40	0.40
182	1000	A2	0.80	0.80
183	1000	A2	0.40	0.40
184	1000	A2	0.40	0.40
185	1000	A2	0.20	0.20
186	1000	A2	0.20	0.20
187	1000	A2	0.20	0.20
188	1000	A2	0.00	0.00
189	1000	A2	0.00	0.00
190	1000	A2	0.00	0.00
191	1000	A2	0.20	0.20
192	1000	A2	0.00	0.00
193	1000	A3	0.00	0.00
194	1000	A3	0.60	0.59
195	1000	A3	0.20	0.19
196	1000	A3	0.40	0.39
197	1000	A3	0.00	0.00
198	1000	A3	0.00	0.00
199	1000	A3	0.00	0.00
200	1000	A3	0.00	0.00
201	1000	A3	0.20	0.19
202	1000	A3	0.60	0.59
203	1000	A3	0.60	0.59
204	1000	A3	0.20	0.19

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
205	1000	A3	0.20	0.19
206	1000	A3	0.20	0.19
207	1000	A3	0.00	0.00
208	1000	A3	0.00	0.00
209	1000	A4	0.20	0.19
210	1000	A4	0.00	0.00
211	1000	A4	0.20	0.19
212	1000	A4	0.20	0.19
213	1000	A4	0.20	0.19
214	1000	A4	0.60	0.59
215	1000	A4	0.60	0.59
216	1000	A4	0.60	0.59
217	1000	A4	0.00	0.00
218	1000	A4	0.00	0.00
219	1000	A4	0.00	0.00
220	1000	A4	0.20	0.19
221	1000	A4	0.00	0.00
222	1000	A4	0.00	0.00
223	1000	A4	0.20	0.19
224	1000	A4	0.00	0.00
225	1000	A5	0.40	0.40
226	1000	A5	0.00	0.00
227	1000	A5	0.00	0.00
228	1000	A5	0.00	0.00
229	1000	A5	0.20	0.20
230	1000	A5	0.00	0.00
231	1000	A5	0.00	0.00
232	1000	A5	0.00	0.00
233	1000	A5	0.00	0.00
234	1000	A5	0.20	0.20
235	1000	A5	0.00	0.00
236	1000	A5	0.20	0.20
237	1000	A5	0.20	0.20
238	1000	A5	0.20	0.20

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
240	1000	A5	0.00	0.00
241	1250	A1	0.00	0.00
242	1250	A1	0.20	0.19
243	1250	A1	0.00	0.00
244	1250	A1	0.00	0.00
245	1250	A1	0.40	0.39
246	1250	A1	0.80	0.80
247	1250	A1	0.80	0.80
248	1250	A1	0.60	0.59
249	1250	A1	0.60	0.59
250	1250	A1	0.80	0.80
251	1250	A1	0.80	0.80
252	1250	A1	0.20	0.19
253	1250	A1	0.60	0.59
254	1250	A1	0.20	0.19
255	1250	A1	0.20	0.19
256	1250	A1	0.40	0.39
257	1250	A2	0.80	0.80
258	1250	A2	0.60	0.60
259	1250	A2	0.60	0.60
260	1250	A2	0.80	0.80
261	1250	A2	0.60	0.60
262	1250	A2	1.00	1.00
263	1250	A2	0.80	0.80
264	1250	A2	1.00	1.00
265	1250	A2	0.80	0.80
266	1250	A2	1.00	1.00
267	1250	A2	0.60	0.60
268	1250	A2	0.20	0.20
269	1250	A2	0.40	0.40
270	1250	A2	0.20	0.20
271	1250	A2	0.40	0.40
272	1250	A2	0.40	0.40

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
273	1250	A3	0.60	0.59
274	1250	A3	0.60	0.59
275	1250	A3	0.60	0.59
276	1250	A3	0.40	0.39
277	1250	A3	0.80	0.80
278	1250	A3	0.60	0.59
279	1250	A3	0.80	0.80
280	1250	A3	0.80	0.80
281	1250	A3	0.80	0.80
282	1250	A3	0.40	0.39
283	1250	A3	1.00	1.00
284	1250	A3	0.40	0.39
285	1250	A3	1.00	1.00
286	1250	A3	0.80	0.80
287	1250	A3	0.60	0.59
288	1250	A3	1.00	1.00
289	1250	A4	0.80	0.80
290	1250	A4	0.40	0.39
291	1250	A4	0.80	0.80
292	1250	A4	0.40	0.39
293	1250	A4	0.60	0.59
294	1250	A4	0.40	0.39
295	1250	A4	1.00	1.00
296	1250	A4	0.60	0.59
297	1250	A4	0.00	0.00
298	1250	A4	0.20	0.19
299	1250	A4	0.00	0.00
300	1250	A4	0.60	0.59
301	1250	A4	0.80	0.80
302	1250	A4	0.60	0.59
303	1250	A4	0.40	0.39
304	1250	A4	0.60	0.59
305	1250	A5	0.20	0.20
306	1250	A5	0.40	0.40

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
307	1250	A5	0.20	0.20
308	1250	A5	0.20	0.20
309	1250	A5	0.40	0.40
310	1250	A5	0.60	0.60
311	1250	A5	0.40	0.40
312	1250	A5	0.60	0.60
313	1250	A5	0.20	0.20
314	1250	A5	0.40	0.40
315	1250	A5	1.00	1.00
316	1250	A5	0.20	0.20
317	1250	A5	0.60	0.60
318	1250	A5	0.80	0.80
319	1250	A5	1.00	1.00
320	1250	A5	1.00	1.00
321	1500	A1	0.20	0.19
322	1500	A1	0.40	0.39
323	1500	A1	0.60	0.59
324	1500	A1	0.60	0.59
325	1500	A1	0.80	0.80
326	1500	A1	1.00	1.00
327	1500	A1	1.00	1.00
328	1500	A1	1.00	1.00
329	1500	A1	1.00	1.00
330	1500	A1	0.80	0.80
331	1500	A1	0.60	0.59
332	1500	A1	0.80	0.80
333	1500	A1	0.60	0.59
334	1500	A1	1.00	1.00
335	1500	A1	1.00	1.00
336	1500	A1	0.40	0.39
337	1500	A2	1.00	1.00
338	1500	A2	1.00	1.00
339	1500	A2	1.00	1.00
340	1500	A2	0.80	0.80

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
341	1500	A2	1.00	1.00
342	1500	A2	1.00	1.00
343	1500	A2	1.00	1.00
344	1500	A2	1.00	1.00
345	1500	A2	1.00	1.00
346	1500	A2	1.00	1.00
347	1500	A2	0.80	0.80
348	1500	A2	1.00	1.00
349	1500	A2	0.80	0.80
350	1500	A2	0.80	0.80
351	1500	A2	0.60	0.60
352	1500	A2	0.60	0.60
353	1500	A3	1.00	1.00
354	1500	A3	1.00	1.00
355	1500	A3	1.00	1.00
356	1500	A3	0.80	0.80
357	1500	A3	1.00	1.00
358	1500	A3	1.00	1.00
359	1500	A3	1.00	1.00
360	1500	A3	1.00	1.00
361	1500	A3	1.00	1.00
362	1500	A3	1.00	1.00
363	1500	A3	1.00	1.00
364	1500	A3	1.00	1.00
365	1500	A3	1.00	1.00
366	1500	A3	1.00	1.00
367	1500	A3	1.00	1.00
368	1500	A3	1.00	1.00
369	1500	A4	1.00	1.00
370	1500	A4	1.00	1.00
371	1500	A4	0.80	0.80
372	1500	A4	1.00	1.00
373	1500	A4	1.00	1.00
374	1500	A4	1.00	1.00

TABLE AII-1 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT KCl FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
375	1500	A4	1.00	1.00
376	1500	A4	1.00	1.00
377	1500	A4	1.00	1.00
378	1500	A4	1.00	1.00
379	1500	A4	1.00	1.00
380	1500	A4	1.00	1.00
381	1500	A4	1.00	1.00
382	1500	A4	0.60	0.59
383	1500	A4	1.00	1.00
384	1500	A4	0.60	0.59
385	1500	A5	0.80	0.80
386	1500	A5	0.80	0.80
387	1500	A5	0.60	0.60
388	1500	A5	1.00	1.00
389	1500	A5	1.00	1.00
390	1500	A5	1.00	1.00
391	1500	A5	1.00	1.00
392	1500	A5	1.00	1.00
393	1500	A5	0.80	0.80
394	1500	A5	1.00	1.00
395	1500	A5	0.80	0.80
396	1500	A5	0.60	0.60
397	1500	A5	1.00	1.00
398	1500	A5	1.00	1.00
399	1500	A5	1.00	1.00
400	1500	A5	1.00	1.00

[1]A1: 1 to 2-day old fish; A2: 3 to 4-day old fish; A3: 7 to 8-day old fish; A4: 11 to 12-day old fish; A5: 13 to 14-day old fish.

[2]Mortality data corrected using Abbott's formula.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-2

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
1	10	A1	0.00	0.00
2	10	A1	0.00	0.00
3	10	A1	0.00	0.00
4	10	A1	0.00	0.00
5	10	A1	0.00	0.00
6	10	A1	0.00	0.00
7	10	A1	0.00	0.00
8	10	A1	0.00	0.00
9	10	A1	0.00	0.00
10	10	A1	0.00	0.00
11	10	A1	0.00	0.00
12	10	A1	0.00	0.00
13	10	A1	0.00	0.00
14	10	A1	0.00	0.00
15	10	A1	0.00	0.00
16	10	A1	0.00	0.00
17	10	A2	0.00	0.00
18	10	A2	0.00	0.00
19	10	A2	0.00	0.00
20	10	A2	0.00	0.00
21	10	A2	0.00	0.00
22	10	A2	0.00	0.00
23	10	A2	0.00	0.00
24	10	A2	0.00	0.00
25	10	A2	0.00	0.00
26	10	A2	0.00	0.00
27	10	A2	0.00	0.00
28	10	A2	0.00	0.00
29	10	A2	0.00	0.00
30	10	A2	0.00	0.00
31	10	A2	0.00	0.00
32	10	A2	0.00	0.00
33	10	A3	0.00	0.00
34	10	A3	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
35	10	A3	0.00	0.00
36	10	A3	0.00	0.00
37	10	A3	0.00	0.00
38	10	A3	0.00	0.00
39	10	A3	0.00	0.00
40	10	A3	0.00	0.00
41	10	A3	0.00	0.00
42	10	A3	0.00	0.00
43	10	A3	0.00	0.00
44	10	A3	0.00	0.00
45	10	A3	0.00	0.00
46	10	A3	0.20	0.20
47	10	A3	0.00	0.00
48	10	A3	0.00	0.00
49	10	A4	0.00	0.00
50	10	A4	0.00	0.00
51	10	A4	0.00	0.00
52	10	A4	0.00	0.00
53	10	A4	0.00	0.00
54	10	A4	0.00	0.00
55	10	A4	0.00	0.00
56	10	A4	0.00	0.00
57	10	A4	0.00	0.00
58	10	A4	0.00	0.00
59	10	A4	0.00	0.00
60	10	A4	0.00	0.00
61	10	A4	0.00	0.00
62	10	A4	0.00	0.00
63	10	A4	0.00	0.00
64	10	A4	0.00	0.00
65	10	A5	0.00	0.00
66	10	A5	0.00	0.00
67	10	A5	0.00	0.00
68	10	A5	0.00	0.00

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
69	10	A5	0.00	0.00
70	10	A5	0.00	0.00
71	10	A5	0.00	0.00
72	10	A5	0.00	0.00
73	10	A5	0.00	0.00
74	10	A5	0.00	0.00
75	10	A5	0.00	0.00
76	10	A5	0.00	0.00
77	10	A5	0.00	0.00
78	10	A5	0.00	0.00
79	10	A5	0.00	0.00
80	10	A5	0.00	0.00
81	20	A1	0.00	0.00
82	20	A1	0.00	0.00
83	20	A1	0.00	0.00
84	20	A1	0.00	0.00
85	20	A1	0.40	0.40
86	20	A1	0.00	0.00
87	20	A1	0.00	0.00
88	20	A1	0.00	0.00
89	20	A1	0.00	0.00
90	20	A1	0.00	0.00
91	20	A1	0.00	0.00
92	20	A1	0.00	0.00
93	20	A1	0.00	0.00
94	20	A1	0.00	0.00
95	20	A1	0.00	0.00
96	20	A1	0.00	0.00
97	20	A2	0.20	0.19
98	20	A2	0.40	0.39
99	20	A2	1.00	1.00
100	20	A2	1.00	1.00
101	20	A2	0.00	0.00
102	20	A2	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
103	20	A2	0.20	0.19
104	20	A2	0.00	0.00
105	20	A2	0.00	0.00
106	20	A2	0.00	0.00
107	20	A2	0.00	0.00
108	20	A2	0.20	0.19
109	20	A2	0.00	0.00
110	20	A2	0.00	0.00
111	20	A2	0.20	0.19
112	20	A2	0.00	0.00
113	20	A3	0.00	0.00
114	20	A3	0.20	0.20
115	20	A3	0.00	0.00
116	20	A3	0.00	0.00
117	20	A3	0.00	0.00
118	20	A3	0.20	0.20
119	20	A3	0.00	0.00
120	20	A3	0.00	0.00
121	20	A3	0.00	0.00
122	20	A3	0.00	0.00
123	20	A3	0.00	0.00
124	20	A3	0.00	0.00
125	20	A3	0.20	0.20
126	20	A3	0.20	0.20
127	20	A3	0.40	0.40
128	20	A3	0.20	0.20
129	20	A4	0.00	0.00
130	20	A4	0.00	0.00
131	20	A4	0.00	0.00
132	20	A4	0.20	0.19
133	20	A4	0.00	0.00
134	20	A4	0.00	0.00
135	20	A4	0.00	0.00
136	20	A4	0.00	0.00

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
137	20	A4	0.00	0.00
138	20	A4	0.00	0.00
139	20	A4	0.00	0.00
140	20	A4	0.00	0.00
141	20	A4	0.00	0.00
142	20	A4	0.00	0.00
143	20	A4	0.00	0.00
144	20	A4	0.00	0.00
145	20	A5	0.00	0.00
146	20	A5	0.00	0.00
147	20	A5	0.00	0.00
148	20	A5	0.00	0.00
149	20	A5	0.00	0.00
150	20	A5	0.00	0.00
151	20	A5	0.00	0.00
152	20	A5	0.00	0.00
153	20	A5	0.20	0.20
154	20	A5	0.00	0.00
155	20	A5	0.00	0.00
156	20	A5	0.00	0.00
157	20	A5	0.20	0.20
158	20	A5	0.20	0.20
159	20	A5	0.00	0.00
160	20	A5	0.20	0.20
161	30	A1	0.40	0.40
162	30	A1	0.40	0.40
163	30	A1	0.20	0.20
164	30	A1	0.60	0.60
165	30	A1	1.00	1.00
166	30	A1	1.00	1.00
167	30	A1	0.40	0.40
168	30	A1	0.80	0.80
169	30	A1	0.00	0.00
170	30	A1	0.80	0.80

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
171	30	A1	0.60	0.60
172	30	A1	0.20	0.20
173	30	A1	0.20	0.20
174	30	A1	0.60	0.60
175	30	A1	0.20	0.20
176	30	A1	0.60	0.60
177	30	A2	1.00	1.00
178	30	A2	1.00	1.00
179	30	A2	1.00	1.00
180	30	A2	1.00	1.00
181	30	A2	0.60	0.59
182	30	A2	1.00	1.00
183	30	A2	0.80	0.80
184	30	A2	0.80	0.80
185	30	A2	1.00	1.00
186	30	A2	0.80	0.80
187	30	A2	1.00	1.00
188	30	A2	1.00	1.00
189	30	A2	1.00	1.00
190	30	A2	1.00	1.00
191	30	A2	1.00	1.00
192	30	A2	1.00	1.00
193	30	A3	1.00	1.00
194	30	A3	1.00	1.00
195	30	A3	0.60	0.60
196	30	A3	0.80	0.80
197	30	A3	0.20	0.20
198	30	A3	1.00	1.00
199	30	A3	0.60	0.60
200	30	A3	1.00	1.00
201	30	A3	0.80	0.80
202	30	A3	1.00	1.00
203	30	A3	1.00	1.00
204	30	A3	1.00	1.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
205	30	A3	1.00	1.00
206	30	A3	1.00	1.00
207	30	A3	1.00	1.00
208	30	A3	1.00	1.00
209	30	A4	1.00	1.00
210	30	A4	1.00	1.00
211	30	A4	1.00	1.00
212	30	A4	1.00	1.00
213	30	A4	0.40	0.39
214	30	A4	0.40	0.39
215	30	A4	0.20	0.19
216	30	A4	0.80	0.80
217	30	A4	1.00	1.00
218	30	A4	1.00	1.00
219	30	A4	0.80	0.80
220	30	A4	1.00	1.00
221	30	A4	1.00	1.00
222	30	A4	1.00	1.00
223	30	A4	1.00	1.00
224	30	A4	1.00	1.00
225	30	A5	1.00	1.00
226	30	A5	1.00	1.00
227	30	A5	1.00	1.00
228	30	A5	0.80	0.80
229	30	A5	1.00	1.00
230	30	A5	1.00	1.00
231	30	A5	1.00	1.00
232	30	A5	0.80	0.80
233	30	A5	1.00	1.00
234	30	A5	0.60	0.60
235	30	A5	1.00	1.00
236	30	A5	0.80	0.80
237	30	A5	0.80	0.80
238	30	A5	1.00	1.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
239	30	A5	1.00	1.00
240	30	A5	1.00	1.00
241	35	A1	1.00	1.00
242	35	A1	1.00	1.00
243	35	A1	1.00	1.00
244	35	A1	1.00	1.00
245	35	A1	1.00	1.00
246	35	A1	1.00	1.00
247	35	A1	1.00	1.00
248	35	A1	1.00	1.00
249	35	A1	1.00	1.00
250	35	A1	1.00	1.00
251	35	A1	1.00	1.00
252	35	A1	1.00	1.00
253	35	A1	0.80	0.80
254	35	A1	1.00	1.00
255	35	A1	1.00	1.00
256	35	A1	1.00	1.00
257	35	A2	1.00	1.00
258	35	A2	1.00	1.00
259	35	A2	1.00	1.00
260	35	A2	1.00	1.00
261	35	A2	1.00	1.00
262	35	A2	1.00	1.00
263	35	A2	1.00	1.00
264	35	A2	1.00	1.00
265	35	A2	1.00	1.00
266	35	A2	1.00	1.00
267	35	A2	1.00	1.00
268	35	A2	1.00	1.00
269	35	A2	1.00	1.00
270	35	A2	1.00	1.00
271	35	A2	1.00	1.00
272	35	A2	1.00	1.00

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
273	35	A3	1.00	1.00
274	35	A3	1.00	1.00
275	35	A3	1.00	1.00
276	35	A3	1.00	1.00
277	35	A3	1.00	1.00
278	35	A3	1.00	1.00
279	35	A3	1.00	1.00
280	35	A3	1.00	1.00
281	35	A3	1.00	1.00
282	35	A3	1.00	1.00
283	35	A3	1.00	1.00
284	35	A3	1.00	1.00
285	35	A3	1.00	1.00
286	35	A3	1.00	1.00
287	35	A3	1.00	1.00
288	35	A3	1.00	1.00
289	35	A4	1.00	1.00
290	35	A4	1.00	1.00
291	35	A4	1.00	1.00
292	35	A4	1.00	1.00
293	35	A4	1.00	1.00
294	35	A4	1.00	1.00
295	35	A4	1.00	1.00
296	35	A4	1.00	1.00
297	35	A4	1.00	1.00
298	35	A4	1.00	1.00
299	35	A4	1.00	1.00
300	35	A4	1.00	1.00
301	35	A4	1.00	1.00
302	35	A4	1.00	1.00
303	35	A4	1.00	1.00
304	35	A4	1.00	1.00
305	35	A5	1.00	1.00
306	35	A5	1.00	1.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
307	35	A5	1.00	1.00
308	35	A5	1.00	1.00
309	35	A5	1.00	1.00
310	35	A5	1.00	1.00
311	35	A5	1.00	1.00
312	35	A5	1.00	1.00
313	35	A5	1.00	1.00
314	35	A5	1.00	1.00
315	35	A5	1.00	1.00
316	35	A5	1.00	1.00
317	35	A5	1.00	1.00
318	35	A5	1.00	1.00
319	35	A5	1.00	1.00
320	35	A5	1.00	1.00
321	40	A1	1.00	1.00
322	40	A1	1.00	1.00
323	40	A1	1.00	1.00
324	40	A1	1.00	1.00
325	40	A1	1.00	1.00
326	40	A1	1.00	1.00
327	40	A1	1.00	1.00
328	40	A1	1.00	1.00
329	40	A1	1.00	1.00
330	40	A1	1.00	1.00
331	40	A1	1.00	1.00
332	40	A1	1.00	1.00
333	40	A1	1.00	1.00
334	40	A1	1.00	1.00
335	40	A1	1.00	1.00
336	40	A1	1.00	1.00
337	40	A2	1.00	1.00
338	40	A2	1.00	1.00
339	40	A2	1.00	1.00
340	40	A2	1.00	1.00

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH
EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
341	40	A2	1.00	1.00
342	40	A2	1.00	1.00
343	40	A2	1.00	1.00
344	40	A2	1.00	1.00
345	40	A2	1.00	1.00
346	40	A2	1.00	1.00
347	40	A2	1.00	1.00
348	40	A2	1.00	1.00
349	40	A2	1.00	1.00
350	40	A2	1.00	1.00
351	40	A2	1.00	1.00
352	40	A2	1.00	1.00
353	40	A3	1.00	1.00
354	40	A3	1.00	1.00
355	40	A3	1.00	1.00
356	40	A3	1.00	1.00
357	40	A3	1.00	1.00
358	40	A3	1.00	1.00
359	40	A3	1.00	1.00
360	40	A3	1.00	1.00
361	40	A3	1.00	1.00
362	40	A3	1.00	1.00
363	40	A3	1.00	1.00
364	40	A3	1.00	1.00
365	40	A3	1.00	1.00
366	40	A3	1.00	1.00
367	40	A3	1.00	1.00
368	40	A3	1.00	1.00
369	40	A4	1.00	1.00
370	40	A4	1.00	1.00
371	40	A4	1.00	1.00
372	40	A4	1.00	1.00
373	40	A4	1.00	1.00
374	40	A4	1.00	1.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-2 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE TOXICANT SDS FOR 48 HOURS

Observation	Dose Level (mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
375	40	A4	1.00	1.00
376	40	A4	1.00	1.00
377	40	A4	1.00	1.00
378	40	A4	1.00	1.00
379	40	A4	1.00	1.00
380	40	A4	1.00	1.00
381	40	A4	1.00	1.00
382	40	A4	1.00	1.00
383	40	A4	1.00	1.00
384	40	A4	1.00	1.00
385	40	A5	1.00	1.00
386	40	A5	1.00	1.00
387	40	A5	1.00	1.00
388	40	A5	1.00	1.00
389	40	A5	1.00	1.00
390	40	A5	1.00	1.00
391	40	A5	1.00	1.00
392	40	A5	1.00	1.00
393	40	A5	1.00	1.00
394	40	A5	1.00	1.00
395	40	A5	1.00	1.00
396	40	A5	1.00	1.00
397	40	A5	1.00	1.00
398	40	A5	1.00	1.00
399	40	A5	1.00	1.00
400	40	A5	1.00	1.00

[1]A1: 1 to 2-day old fish; A2: 3 to 4-day old fish; A3: 7 to 8-day old fish. A4: 11 to 12-day old fish; A5: 13 to 14-day fish.

[2]Mortality data corrected using Abbott's formula.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
1	0.17	A1	0.00	0.00
2	0.17	A1	0.00	0.00
3	0.17	A1	0.00	0.00
4	0.17	A1	0.00	0.00
5	0.17	A1	0.00	0.00
6	0.17	A1	0.00	0.00
7	0.17	A1	0.00	0.00
8	0.17	A1	0.00	0.00
9	0.17	A1	0.00	0.00
10	0.17	A1	0.00	0.00
11	0.17	A1	0.00	0.00
12	0.17	A1	0.00	0.00
13	0.17	A1	0.00	0.00
14	0.17	A1	0.00	0.00
15	0.17	A1	0.00	0.00
16	0.17	A1	0.00	0.00
17	0.17	A2	0.00	0.00
18	0.17	A2	0.00	0.00
19	0.17	A2	0.00	0.00
20	0.17	A2	0.00	0.00
21	0.17	A2	0.20	0.20
22	0.17	A2	0.00	0.00
23	0.17	A2	0.00	0.00
24	0.17	A2	0.00	0.00
25	0.17	A2	0.00	0.00
26	0.17	A2	0.00	0.00
27	0.17	A2	0.00	0.00
28	0.17	A2	0.00	0.00
29	0.17	A2	0.00	0.00
30	0.17	A2	0.00	0.00
31	0.17	A2	0.00	0.00
32	0.17	A2	0.00	0.00
33	0.17	A3	0.00	0.00
34	0.17	A3	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
35	0.17	A3	0.00	0.00
36	0.17	A3	0.00	0.00
37	0.17	A3	0.00	0.00
38	0.17	A3	0.00	0.00
39	0.17	A3	0.00	0.00
40	0.17	A3	0.00	0.00
41	0.17	A3	0.00	0.00
42	0.17	A3	0.00	0.00
43	0.17	A3	0.00	0.00
44	0.17	A3	0.00	0.00
45	0.17	A3	0.00	0.00
46	0.17	A3	0.00	0.00
47	0.17	A3	0.00	0.00
48	0.17	A3	0.00	0.00
49	0.17	A4	0.00	0.00
50	0.17	A4	0.00	0.00
51	0.17	A4	0.00	0.00
52	0.17	A4	0.00	0.00
53	0.17	A4	0.00	0.00
54	0.17	A4	0.00	0.00
55	0.17	A4	0.00	0.00
56	0.17	A4	0.00	0.00
57	0.17	A4	0.00	0.00
58	0.17	A4	0.00	0.00
59	0.17	A4	0.00	0.00
60	0.17	A4	0.00	0.00
61	0.17	A4	0.00	0.00
62	0.17	A4	0.00	0.00
63	0.17	A4	0.00	0.00
64	0.17	A4	0.00	0.00
65	0.17	A5	0.00	0.00
66	0.17	A5	0.00	0.00
67	0.17	A5	0.00	0.00
68	0.17	A5	0.00	0.00

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
69	0.17	A5	0.00	0.00
70	0.17	A5	0.00	0.00
71	0.17	A5	0.00	0.00
72	0.17	A5	0.00	0.00
73	0.17	A5	0.00	0.00
74	0.17	A5	0.00	0.00
75	0.17	A5	0.20	0.20
76	0.17	A5	0.00	0.00
77	0.17	A5	0.00	0.00
78	0.17	A5	0.00	0.00
79	0.17	A5	0.00	0.00
80	0.17	A5	0.20	0.20
81	0.34	A1	0.00	0.00
82	0.34	A1	0.00	0.00
83	0.34	A1	0.00	0.00
84	0.34	A1	0.00	0.00
85	0.34	A1	0.00	0.00
86	0.34	A1	0.00	0.00
87	0.34	A1	0.00	0.00
88	0.34	A1	0.00	0.00
89	0.34	A1	0.00	0.00
90	0.34	A1	0.20	0.20
91	0.34	A1	0.00	0.00
92	0.34	A1	0.00	0.00
93	0.34	A1	0.20	0.20
94	0.34	A1	0.20	0.20
95	0.34	A1	0.20	0.20
96	0.34	A1	0.00	0.00
97	0.34	A2	0.00	0.00
98	0.34	A2	0.00	0.00
99	0.34	A2	0.00	0.00
100	0.34	A2	0.20	0.20
101	0.34	A2	0.40	0.40
102	0.34	A2	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
103	0.34	A2	0.20	0.20
104	0.34	A2	0.00	0.00
105	0.34	A2	0.00	0.00
106	0.34	A2	0.00	0.00
107	0.34	A2	0.00	0.00
108	0.34	A2	0.00	0.00
109	0.34	A2	0.20	0.20
110	0.34	A2	0.00	0.00
111	0.34	A2	0.00	0.00
112	0.34	A2	0.00	0.00
113	0.34	A3	0.00	0.00
114	0.34	A3	0.00	0.00
115	0.34	A3	0.00	0.00
116	0.34	A3	0.00	0.00
117	0.34	A3	0.00	0.00
118	0.34	A3	0.00	0.00
119	0.34	A3	0.00	0.00
120	0.34	A3	0.00	0.00
121	0.34	A3	0.00	0.00
122	0.34	A3	0.20	0.20
123	0.34	A3	0.00	0.00
124	0.34	A3	0.00	0.00
125	0.34	A3	0.00	0.00
126	0.34	A3	0.20	0.20
127	0.34	A3	0.00	0.00
128	0.34	A3	0.00	0.00
129	0.34	A4	0.00	0.00
130	0.34	A4	0.00	0.00
131	0.34	A4	0.00	0.00
132	0.34	A4	0.00	0.00
133	0.34	A4	0.00	0.00
134	0.34	A4	0.00	0.00
135	0.34	A4	0.00	0.00
136	0.34	A4	0.00	0.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
137	0.34	A4	0.00	0.00
138	0.34	A4	0.00	0.00
139	0.34	A4	0.00	0.00
140	0.34	A4	0.00	0.00
141	0.34	A4	0.00	0.00
142	0.34	A4	0.00	0.00
143	0.34	A4	0.00	0.00
144	0.34	A4	0.00	0.00
145	0.34	A5	0.00	0.00
146	0.34	A5	0.00	0.00
147	0.34	A5	0.00	0.00
148	0.34	A5	0.00	0.00
149	0.34	A5	0.00	0.00
150	0.34	A5	0.00	0.00
151	0.34	A5	0.00	0.00
152	0.34	A5	0.20	0.20
153	0.34	A5	0.00	0.00
154	0.34	A5	0.20	0.20
155	0.34	A5	0.00	0.00
156	0.34	A5	0.00	0.00
157	0.34	A5	0.00	0.00
158	0.34	A5	0.20	0.20
159	0.34	A5	0.00	0.00
160	0.34	A5	0.00	0.00
161	0.67	A1	0.00	0.00
162	0.67	A1	0.20	0.20
163	0.67	A1	0.20	0.20
164	0.67	A1	0.40	0.40
165	0.67	A1	0.60	0.60
166	0.67	A1	0.40	0.40
167	0.67	A1	0.00	0.00
168	0.67	A1	0.20	0.20
169	0.67	A1	0.40	0.40
170	0.67	A1	0.40	0.40

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
171	0.67	A1	0.20	0.20
172	0.67	A1	0.40	0.40
173	0.67	A1	1.00	1.00
174	0.67	A1	1.00	1.00
175	0.67	A1	1.00	1.00
176	0.67	A1	1.00	1.00
177	0.67	A2	1.00	1.00
178	0.67	A2	0.40	0.40
179	0.67	A2	0.40	0.40
180	0.67	A2	0.60	0.60
181	0.67	A2	1.00	1.00
182	0.67	A2	0.80	0.80
183	0.67	A2	1.00	1.00
184	0.67	A2	1.00	1.00
185	0.67	A2	0.80	0.80
186	0.67	A2	1.00	1.00
187	0.67	A2	0.60	0.60
188	0.67	A2	0.80	0.80
189	0.67	A2	0.80	0.80
190	0.67	A2	0.20	0.20
191	0.67	A2	0.00	0.00
192	0.67	A2	0.20	0.20
193	0.67	A3	0.80	0.80
194	0.67	A3	0.80	0.80
195	0.67	A3	0.80	0.80
196	0.67	A3	0.60	0.60
197	0.67	A3	0.60	0.60
198	0.67	A3	1.00	1.00
199	0.67	A3	1.00	1.00
200	0.67	A3	1.00	1.00
201	0.67	A3	1.00	1.00
202	0.67	A3	1.00	1.00
203	0.67	A3	1.00	1.00
204	0.67	A3	1.00	1.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
205	0.67	A3	0.80	0.80
206	0.67	A3	0.60	0.60
207	0.67	A3	0.40	0.40
208	0.67	A3	0.80	0.80
209	0.67	A4	0.60	0.60
210	0.67	A4	0.60	0.60
211	0.67	A4	0.80	0.80
212	0.67	A4	0.80	0.80
213	0.67	A4	1.00	1.00
214	0.67	A4	0.60	0.60
215	0.67	A4	0.40	0.40
216	0.67	A4	0.40	0.40
217	0.67	A4	1.00	1.00
218	0.67	A4	0.60	0.60
219	0.67	A4	0.80	0.80
220	0.67	A4	0.60	0.60
221	0.67	A4	0.40	0.40
222	0.67	A4	1.00	1.00
223	0.67	A4	0.60	0.60
224	0.67	A4	0.60	0.60
225	0.67	A5	1.00	1.00
226	0.67	A5	1.00	1.00
227	0.67	A5	1.00	1.00
228	0.67	A5	1.00	1.00
229	0.67	A5	1.00	1.00
230	0.67	A5	1.00	1.00
231	0.67	A5	0.60	0.60
232	0.67	A5	0.60	0.60
233	0.67	A5	0.60	0.60
234	0.67	A5	0.40	0.40
235	0.67	A5	1.00	1.00
236	0.67	A5	0.80	0.80
237	0.67	A5	0.20	0.20
238	0.67	A5	0.40	0.40

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
239	0.67	A5	0.60	0.60
240	0.67	A5	0.20	0.20
241	0.83	A1	0.80	0.80
242	0.83	A1	1.00	1.00
243	0.83	A1	1.00	1.00
244	0.83	A1	1.00	1.00
245	0.83	A1	1.00	1.00
246	0.83	A1	1.00	1.00
247	0.83	A1	1.00	1.00
248	0.83	A1	1.00	1.00
249	0.83	A1	1.00	1.00
250	0.83	A1	1.00	1.00
251	0.83	A1	1.00	1.00
252	0.83	A1	1.00	1.00
253	0.83	A1	1.00	1.00
254	0.83	A1	1.00	1.00
255	0.83	A1	1.00	1.00
256	0.83	A1	1.00	1.00
257	0.83	A2	1.00	1.00
258	0.83	A2	1.00	1.00
259	0.83	A2	1.00	1.00
260	0.83	A2	1.00	1.00
261	0.83	A2	1.00	1.00
262	0.83	A2	1.00	1.00
263	0.83	A2	1.00	1.00
264	0.83	A2	1.00	1.00
265	0.83	A2	1.00	1.00
266	0.83	A2	1.00	1.00
267	0.83	A2	1.00	1.00
268	0.83	A2	1.00	1.00
269	0.83	A2	1.00	1.00
270	0.83	A2	1.00	1.00
271	0.83	A2	1.00	1.00
272	0.83	A2	1.00	1.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
273	0.83	A3	1.00	1.00
274	0.83	A3	1.00	1.00
275	0.83	A3	1.00	1.00
276	0.83	A3	1.00	1.00
277	0.83	A3	1.00	1.00
278	0.83	A3	1.00	1.00
279	0.83	A3	1.00	1.00
280	0.83	A3	1.00	1.00
281	0.83	A3	1.00	1.00
282	0.83	A3	1.00	1.00
283	0.83	A3	1.00	1.00
284	0.83	A3	1.00	1.00
285	0.83	A3	1.00	1.00
286	0.83	A3	1.00	1.00
287	0.83	A3	1.00	1.00
288	0.83	A3	1.00	1.00
289	0.83	A4	1.00	1.00
290	0.83	A4	1.00	1.00
291	0.83	A4	1.00	1.00
292	0.83	A4	1.00	1.00
293	0.83	A4	1.00	1.00
294	0.83	A4	1.00	1.00
295	0.83	A4	1.00	1.00
296	0.83	A4	1.00	1.00
297	0.83	A4	1.00	1.00
298	0.83	A4	1.00	1.00
299	0.83	A4	1.00	1.00
300	0.83	A4	1.00	1.00
301	0.83	A4	1.00	1.00
302	0.83	A4	1.00	1.00
303	0.83	A4	1.00	1.00
304	0.83	A4	1.00	1.00
305	0.83	A5	1.00	1.00
306	0.83	A5	1.00	1.00

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
307	0.83	A5	1.00	1.00
308	0.83	A5	1.00	1.00
309	0.83	A5	1.00	1.00
310	0.83	A5	1.00	1.00
311	0.83	A5	1.00	1.00
312	0.83	A5	1.00	1.00
313	0.83	A5	1.00	1.00
314	0.83	A5	1.00	1.00
315	0.83	A5	1.00	1.00
316	0.83	A5	1.00	1.00
317	0.83	A5	1.00	1.00
318	0.83	A5	1.00	1.00
319	0.83	A5	1.00	1.00
320	0.83	A5	1.00	1.00
321	1.00	A1	1.00	1.00
322	1.00	A1	1.00	1.00
323	1.00	A1	1.00	1.00
324	1.00	A1	1.00	1.00
325	1.00	A1	1.00	1.00
326	1.00	A1	1.00	1.00
327	1.00	A1	1.00	1.00
328	1.00	A1	1.00	1.00
329	1.00	A1	1.00	1.00
330	1.00	A1	1.00	1.00
331	1.00	A1	1.00	1.00
332	1.00	A1	1.00	1.00
333	1.00	A1	1.00	1.00
334	1.00	A1	1.00	1.00
335	1.00	A1	1.00	1.00
336	1.00	A1	1.00	1.00
337	1.00	A2	1.00	1.00
338	1.00	A2	1.00	1.00
339	1.00	A2	1.00	1.00
340	1.00	A2	1.00	1.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
341	1.00	A2	1.00	1.00
342	1.00	A2	1.00	1.00
343	1.00	A2	1.00	1.00
344	1.00	A2	1.00	1.00
345	1.00	A2	1.00	1.00
346	1.00	A2	1.00	1.00
347	1.00	A2	1.00	1.00
348	1.00	A2	1.00	1.00
349	1.00	A2	1.00	1.00
350	1.00	A2	1.00	1.00
351	1.00	A2	1.00	1.00
352	1.00	A2	1.00	1.00
353	1.00	A3	1.00	1.00
354	1.00	A3	1.00	1.00
355	1.00	A3	1.00	1.00
356	1.00	A3	1.00	1.00
357	1.00	A3	1.00	1.00
358	1.00	A3	1.00	1.00
359	1.00	A3	1.00	1.00
360	1.00	A3	1.00	1.00
361	1.00	A3	1.00	1.00
362	1.00	A3	1.00	1.00
363	1.00	A3	1.00	1.00
364	1.00	A3	1.00	1.00
365	1.00	A3	1.00	1.00
366	1.00	A3	1.00	1.00
367	1.00	A3	1.00	1.00
368	1.00	A3	1.00	1.00
369	1.00	A4	1.00	1.00
370	1.00	A4	1.00	1.00
371	1.00	A4	1.00	1.00
372	1.00	A4	1.00	1.00
373	1.00	A4	1.00	1.00
374	1.00	A4	1.00	1.00

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3 (Continued)

OBSERVED AND CORRECTED MORTALITY RATES OF FISH EXPOSED TO THE KCl + SDS TOXICANT COMBINATION FOR 48 HOURS

Observation	Dose Level (% max mg/L)	Age Group[1]	Observed Mortality	Corrected Mortality[2]
375	1.00	A4	1.00	1.00
376	1.00	A4	1.00	1.00
377	1.00	A4	1.00	1.00
378	1.00	A4	1.00	1.00
379	1.00	A4	1.00	1.00
380	1.00	A4	1.00	1.00
381	1.00	A4	1.00	1.00
382	1.00	A4	1.00	1.00
383	1.00	A4	1.00	1.00
384	1.00	A4	1.00	1.00
385	1.00	A5	1.00	1.00
386	1.00	A5	1.00	1.00
387	1.00	A5	1.00	1.00
388	1.00	A5	1.00	1.00
389	1.00	A5	1.00	1.00
390	1.00	A5	1.00	1.00
391	1.00	A5	1.00	1.00
392	1.00	A5	1.00	1.00
393	1.00	A5	1.00	1.00
394	1.00	A5	1.00	1.00
395	1.00	A5	1.00	1.00
396	1.00	A5	1.00	1.00
397	1.00	A5	1.00	1.00
398	1.00	A5	1.00	1.00
399	1.00	A5	1.00	1.00
400	1.00	A5	1.00	1.00

[1]A1: 1 to 2-day old fish; A2: 3 to 4-day old fish; A3: 7 to 8-day old fish; A4: 11 to 12-day old fish; A5: 13 to 14-day old fish.

[2]Mortality data corrected using Abbott's formula.