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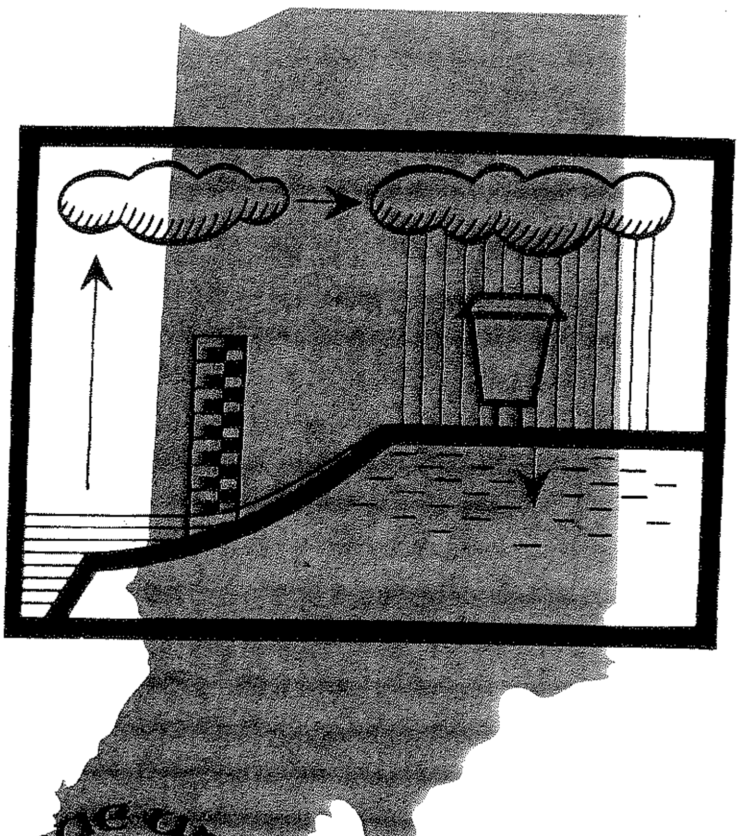

by

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and

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June 1983

PURDUE UNIVERSITY

WATER RESOURCES RESEARCH CENTER

WEST LAFAYETTE, INDIANA

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ABSTRACT

The acute toxicity of ammonium chloride was measured for eight fish species representing four families commonly found in midwestern streams. The 96-hour median lethal concentrations (LC50) at pH 7.5-7.9 and 22-27 C were: Catostomus commersoni, 0.65; Ictalurus punctatus, 1.19; Lepomis macrochirus, 1.32; Notemigonus chrysoleucas, 0.59; Notropis whipplei, 1.03; Notropis spilopterus, 1.11; Campostoma anomalum, 1.41; and Pimephales promelas, 1.49 mg NH_3-N/liter. The family mean acute value for Cyprinidae, expressed as a limit at high pH, was 1.45 mg NH_3-N/1. Fathead minnow (P. promelas) embryo-larvae, exposed for 28 days post-hatch, had significantly reduced growth at 0.28 mg NH_3-N/1 compared to controls. A chronic effect level of 0.18 mg NH_3-N/1, and an acute/chronic ratio of 8.28 were calculated fo the 28 day minnow test. Larval channel catfish (I. punctatus) exposed in a similar manner, had significantly reduced growth at 0.20 mg NH_3-N/1. The 30-day chronic effect level for catfish was 0.15 mg NH_3-N/1, with an acute/chronic ratio of 7.9. Larval growth rate was found to be more sensitive to ammonia than hatching success, occurrence of deformities, or 48-hour survival of larvae for both minnows and catfish.

A positive linear relationship was found between the LC50 for low dissolved oxygen, and the ambient concentration of ammonia in 96-hour exposures with fathead minnows. Proportional mortality could be described as a linear function of dissolved oxygen concentration and an interaction term between oxygen and ammonia. The results were also shown to follow a concentration-addition model for joint toxic action when ammonia and oxygen concentrations were expressed as "toxic units".

An ammonia criterion based on these results and other available toxicity information was calculated for sixteen families occurring in midwestern non-salmonid waters. A value of 0.2 mg NH_3-N/1 was obtained for the criterion maximum, or concentration that should not be exceeded. The maximum allowable 30-day average concentration was found to be 0.04 mg NH_3-N/1 using this approach. The amount of ammonia that may be discharged to a river depends on these two criterion limits, and the amount of dilution in the receiving water. Discharges are most limited during low flow events, which typically occur in late summer. An examination of the low flow characteristics for eleven Indiana rivers indicates that allowable discharge rates based on 30-day average

conditions are more restrictive than discharges based on a one-day maximum allowable concentration. The relative importance of the one-day and 30-day average low flow events depends on the ratio of the two flows, in comparison to the acute/chronic ratio used to derive the criterion.

INTRODUCTION

Ammonia is one of the most common water pollutants discharged to lakes and streams from either point or non-point sources (National Research Council, 1979). Municipal sewage effluents, industrial process effluents, and runoff from fertilizer or confined feedlot operations frequently contain significant amounts of ammonia or other nitrogen-containing organic materials such as urea that can form ammonia during decomposition. Dissolved ammonia in wastewater contributes to nitrogenous biochemical oxygen demand, which can impair aquatic communities by lowering dissolved oxygen levels. Ammonia may also cause direct toxicity to aquatic organisms.

Water quality criteria for ammonia are derived from available information on its acute and chronic toxicity to aquatic organisms. Criteria calculated from this toxicity information form the basis for designing stream standards and effluent limitation programs. For this reason, it is particularly important to derive realistic ammonia criteria that adequately protect aquatic life and related water resources, without being overly stringent. Since ammonia-nitrogen is incompletely removed from wastewater by conventional waste treatment, water quality standards requiring very low ammonia concentrations might prove difficult to meet, and might require the installation of expensive ammonia removal (nitrification) processes by municipalities and industries. Consequently, there has been active interest in the development of acceptable ammonia criteria in recent years.

The evaluation of ammonia toxicity in water is relatively complex because ammonia occurs in two forms - molecular (NH_3 or NH_4OH) and ionic (NH_4^+) - with differing toxicities. The equilibrium between the two forms is a function of temperature and pH, with high temperatures and alkaline conditions favoring the more toxic molecular form (Emerson et al, 1975). Toxicity results and criteria are usually expressed as concentrations of un-ionized ammonia. Nevertheless, the toxicity of the un-ionized form itself varies with temperature and pH (Thurston et al., 1981a). Low temperatures and pH appear to enhance its toxicity to fish and other aquatic organisms, making comparisons difficult between tests run under different conditions. Other water quality factors such as hardness, salinity, and dissolved oxygen may also affect the response of fish to ammonia.

The current recommended national water quality criterion for ammonia adopted by the U.S. Environmental Protection Agency (U.S. EPA 1977) is 0.02 NH_3

mg/l as un-ionized ammonia (although a newer criteria document is in review). Other agencies such as the International Joint Commission, ORSANCO, and European Inland Fisheries Commission have chosen values in the range of 0.02 to 0.05 mg/l. Until very recently, the toxicity data base supporting such criteria was rather small. Available test results were primarily acute rather than long-term. Many of the reported bioassays were unusable because of a lack of information on test conditions such as pH and size of organisms. Fish species were more frequently tested than aquatic invertebrates or plants, and among fish the family Salmonidae was heavily represented. The limited information available on non-salmonid families, based mainly on short-term tests was minnows (Cyprinidae), suggested that common warmwater families might be more resistant to ammonia This gave rise to criticism that the national criterion was overly protective for warmwater, non-salmonid, communities.

Since publication of the 1977 national criteria document, two newer approaches to water quality criteria have been proposed by the U.S. EPA and others. One is the use of a "two number" criterion specifying an instantaneous maximum allowable concentration of a pollutant, and a 30-day average concentration. The two numbers are derived from acute and chronic toxicities respectively (Stephan et al., 1983). In practice, the application of a "two number" criterion depends greatly on the flow regime of the receiving body, which governs the allowable discharge rates and determines whether the maximum or average conditions are more restrictive. An alternative approach, adopted by several states, has been to use separate criteria for summer and winter conditions.

There has also been growing interest in developing site-specific criteria for water bodies. In this approach, national criteria are modified to account for local water quality characteristics that could act to modify toxicity. Locations with particularly sensitive or insensitive communities of organisms would also be candidates for site-specific criteria. Guidelines for developing such site-specific water quality criteria have been described (U.S. EPA 1982). A number of agencies in the Midwest, including municipal and industrial dischargers in Indiana, have considered the possibility of developing site-specific ammonia criteria for rivers, having non-salmonid (warmwater) aquatic communities. Those same rivers also typically go through large fluctuations in flow during the year. In most Indiana streams, for example, lowest mean monthly flow occurs in late summer. The high temperatures and pH that generally accompany low flow also act to increase the percentage of un-ionized ammonia in effluents. Thus, the allowable quantity of total ammonia permitted at a discharge would be most restrictive during late summer low flow events. Other stressful conditions such as low dissolved oxygen could also interact to make aquatic organisms more susceptible to ammonia at that time of year.

Considering the biological and economic importance of adopting defensible criteria for ammonia, the authors felt that better toxicity information was needed on warmwater fish species. Regardless of whether or not such results would influence the choice of a national criterion, tests with additional species under conditions typical of midwestern streams would be helpful for developing future site-specific criteria in that region of the country. Consequently, the present study was designed to:

1) measure the acute toxicity of ammonia to additional species representing fish families typically found in midwestern warmwater streams.

2) determine the concentration of ammonia toxic to embryo and larval stages of two species in long-term exposures.

3) conduct the exposures under conditions of temperature, hardness, and pH similar to those occurring in the region during low flow conditions.

4) investigate the interaction of ammonia and low dissolved oxygen in short-term lethal exposures to minnows.

5) calculate an example of a "two number" ammonia criterion using available toxicity information on warmwater species, and apply it to representative streams in Indiana.

There has been a great deal of scientific interest in this topic during recent years. Since initiation of this project, a number of other authors have contributed to our understanding of ammonia toxicity in aquatic organisms. An up-to-date review of the ammonia literature was recently drafted as part of the revised national criteria document (U.S. EPA 1983). Every attempt has been made to incorporate these recent developments in the presentation and analysis of results in the following report.

I. TOXICITY TESTS WITH WARMWATER FISH

Methods and Materials

Exposure Systems. Dilution water for all tests conducted before July 1982 consisted of dechlorinated well water that had been passed through a series of four, 4 m^3 activated carbon filters. No chlorine residual was detectable by specific chlorine electrode during testing. Beginning in July, 1982, all tests were conducted with dilution water pumped directly from a well without chlorination. Plumbing used in the laboratory system was PVC, CPVC, and glass. Chemical characteristics of the dilution water are given in Table 1. Water temperature control in the range of 22-27C was achieved with an automatic water mixing valve on the influent line. The temperature, pH, and other water quality conditions were similar to those found during low flow periods in midwestern warmwater streams.

Flow-through acute toxicity tests were conducted in 60-liter glass aquaria supplied with solutions produced in a proportional diluter similar to that described by Lemke et al. (1978). A series of five ammonium chloride solutions having a dilution ratio of 50% or 70%, and a control, were distributed in duplicate to a total of 12 aquaria. The diluter was calibrated before each test and checked each day during the test. Flow rates of 0.5 - 0.6 liters/min to each tank provided more than the minimum water exchange rate suggested in bioassay protocols (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975).

The same system was used for embryo-larval tests, except that the larvae were held in smaller (5.5 liter) tanks that received test water siphoned at a rate of 0.06 liter/min from the unoccupied large aquaria. To begin the embryo-larval tests, fish embryos were held in oscillating incubation cups made of 6 cm lengths of 3.8 cm dia. CPVC pipe with 480 mesh Nitex nylon screening glued to one end. The cups were hung from a rocker arm assembly driven by a six rpm motor. This assembly produced a vertical oscillation of approximately 2 cm which provided an exchange of water inside the cups. When the fish hatched, the larvae were distributed to the 5.5 liter aquaria for the remainder of the test.

Acute lethal exposures to low dissolved oxygen were produced by supplying partially degassed influent water to 48-liter test aquaria. Dissolved oxygen

Table 1. Typical chemical characteristics of the dilution water used in all toxicity tests. Values are in mg/liter unless noted otherwise.

Alkalinity, as $CaCO_3$	260
Hardness, as $CaCO_3$	300
Conductivity, µmhos/cm	660
Total ammonia N	0.05
Nitrite - N	<0.005
Nitrate - N	0.27
Copper	<0.01
Aluminum	<0.05
Lead	<0.01
Zinc	0.01
Cadmium	<0.01
Iron	0.34

concentrations in the range of 0.5 to 7.0 mg/liter were generated by N_2 stripping and O_2 reaeration in a series of two columns, as described by Hicks and DeWitt (1970). Changes in the oxygen content once the water reached the open aquaria were prevented by floating plexiglass covers on top of the test water in each tank. This also prevented test fish from "gulping" air at the water surface during the 96-hour exposure.

Interactions between dissolved oxygen level and ammonia were tested in the same system by supplying all tanks with a constant concentration of ammonia during the oxygen tests. A multi-channel peristaltic pump (Gilson Medical Electronics Co.) was used to inject a stock ammonia solution. The combined flow rate was 0.2 liters/min into each aquarium.

Test Organisms. Test fish were obtained either from private dealers in Indiana, from laboratory cultures, or by seining local ponds. Fathead minnows (*Pimephales promelas*) and channel catfish (*Ictalurus punctatus*) were raised from cultures normally maintained in the toxicology laboratory. Fish brought to the laboratory from outside were quarantined for a minimum of 14 days before testing. Fish treated with therapeutic chemicals were likewise held for at least 14 days, and judged to be disease-free before being considered for use in toxicity tests.

Acute Lethality Tests. Ten or more fish were randomly distributed to each test aquarium at the beginning of a 96-hour toxicity test. Mortality observations were recorded on a time sequence of approximately: 0.25, 0.5, 1, 2, 4, 8, 12, 16, 24, 36, 48, 72, and 96 hours. Dead organisms were counted, removed, and preserved in 10% formalin at each observation time. The criterion for death was the lack of opercular movement and lack of response from gentle prodding. Weights of ten randomly selected fish were recorded after each test. Water chemistry analyses including total ammonia, dissolved oxygen, temperature, and pH were conducted at the beginning, at 48 hours, and at the conclusion of the test.

Thirty-day Embryo-larval Exposures. Fathead minnow embryos from multiple spawns at Lilly Research Laboratories, Greenfield, Indiana, were removed from spawning substrates and transported to the testing facility. After sorting the eggs according to developmental stage, 30 viable embryos, 10 to 18 hours old, were placed in the incubation cup suspended in each of the 12 test aquaria. Embryo mortality was recorded daily and dead or diseased eggs were removed at each observation time. Data on embryo mortality, hatching success, presence of deformities, and survival through hatching were recorded. After all live embryos had hatched, the surviving larvae were counted and released into the test chambers. Newly hatched brine shrimp (less than 12 hours old *Artemia salina* nauplii) were fed to the fish at least three times daily on weekdays, and at least once each Saturday and Sunday.

Water chemistry analyses were done prior to the beginning of the test and approximately every four days thereafter until the end of the test at 28 days post-hatch. Fry mortalities were recorded daily throughout the test. At 28 days, the fry were starved for 24 hours and sacrificed. Any unusual deformities were noted and individuals were measured for total length, standard length, and wet weight. The fish were preserved in 10% buffered formalin.

The embryo-larval test procedure was modified somewhat for the exposure of channel catfish because of the adhesive nature of the catfish egg masses. Separating and counting individual eggs for distribution to the incubation cups resulted in relatively high rates of mortality and fungal infection. Therefore, the exposure of separated embryos was terminated after hatching was complete. Data on embryonic mortality, hatching success, and deformities were recorded at that point. The test was then restarted with newly hatched (under 3 hours old) larvae that were collected from a normal laboratory spawn. Fifteen individuals were randomly apportioned to each of the 12 exposure tanks receiving 0.06 liters/min of test solution. Mortalities were recorded and removed daily. Number 00 fish meal (Ralston Purina Co.) was offered as food when swim-up activity began, and at two hour intervals for the next 12 hours. Fish were fed six times daily for the first week, at which time the number of feedings was reduced to three times each weekday and at least once each day of the weekend. Enough food was offered so that an excess generally occurred. This excess was siphoned from the tanks each morning. After 30 days, the fish were starved for 24 hours, sacrificed, and measured for weight and length.

Analytical Methods. Total ammonia concentrations in the test aquaria were measured with specific ammonia electrode (Orion Research Corp.). The results by this method were periodically verified by comparison to the indophenol colorimetric method of Scheiner (1976). When measuring low level ammonia concentrations with the probe, the internal filling solution was diluted 1:10 according to the manufacturer's instructions. Un-ionized ammonia concentrations were calculated according to pH and temperature using the relationship of Emerson et al. (1975). All ammonia results are expressed as un-ionized ammonia nitrogen (mg NH_3-N/liter) unless otherwise stated.

Water pH was measured with a glass combination electrode and millivolt meter (Orion Research Corp.) equipped with a thermistor to measure temperature of the sample. The meter was calibrated with buffer solutions at pH 7.0 and 10.0 (Fisher Scientific Co.).

Dissolved oxygen concentrations were measured by probe (Yellow Springs Instrument Co. or Orion Research Corp.) and confirmed by Winkler titration (APHA 1981).

Statisticsl Analyses. The median lethal concentration (LC50) and 95% confidence limits for each acute ammonia or oxygen exposure were calculated by the trimmed Spearman-Karber method (Hamilton et al., 1977). The calculations, which included Abbott's adjustment for control mortalities (Finney 1971), were performed by a computer program as described by Hazel et al. (1979). Means for LC50's and other test results were calculated as geometric means rather than arithmetic means (Stephan et al. 1983). To determine whether two LC50's were significantly different,the confidence interval of the difference between log LC50 units was computed (U.S. EPA 1982). If the confidence interval about the difference included zero, the two results were not different.

Results from the dissolved oxygen-ammonia tests were analyzed in several different ways. First, LC50's for dissolved oxygen at each ambient ammonia level were calculated and compared. Then, a linear least-squares regression analysis (BMD-1R, Dixon and Brown 1979) was used to determine whether any relationship existed between the two responses. To test the hypothesis that environmental ammonia and oxygen interact to affect fish mortality, a multiple regression analysis (BMDP-1R) was performed, and the following model was fitted to the experimental data:

$$PM = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

where PM = 2*arcsin(sqrt(proportional mortality)

X_1 = mg NH_3-N/liter

X_2 = mg O_2/liter

$X_3 = X_1 * X_2$

To test whether there is a relationship between the dependent variable (PM) and the independent variables, the F statistic was used, where F = regression mean square/error mean square (Neter and Wasserman 1974).

The aptness of the model was examined by residual plots prior to making further inferences. If these plots suggested no deviations from the assumptions inherent in the regression analysis (Neter and Wasserman 1974), the error sum of squares was partitioned into pure error (SSPE) and lack of fit (SSLF). A significant F statistic computed from the respective mean squares (F=MSLF/MSPE) indicates that the proposed regression model is not adequate to describe the data, and the data should be considered non-linear.

The same oxygen-ammonia data were evaluated in terms of a concentration-addition model for joint toxic action, as reviewed by EIFAC (1980). Both ammonia and dissolved oxygen were expressed as effective concentrations by normalizing to their respective 96-hour LC50 values. In the case of oxygen, the inverse of concentration was used. These normalized proportions, called "toxic units" or TU, were used to calculate a total effective concentration for each experimental combination of oxygen and ammonia. The results were compared to a simple additive model in which $TU_X + TU_Y = 1.0$ for 50% lethality at 96 hours.

Fish weights obtained in the embryo-larval exposures were analyzed by analysis of variance for a randomized nested design, as discussed by McClave et al.(1981). In this analysis, the sum of squares for the tank duplicates (nested within treatment levels) could be pooled with the error sum of squares to test against the main treatment effect (fish weight). Where significant differences were found between treatments, comparisons between means were made using Dunnett's procedure (Dunnett 1964).

Dichotomous data (hatching success, occurrence of abnormalities, and early survival) obtained in the embryo-larval tests were analyzed using contingency tables (Sokal and Rohlf 1969).

Results

Acute Toxicity. The 96-hour median lethal concentration (LC50) of ammonium chloride was measured for eight fish species representing four families typically found in midwestern streams or lakes (Table 2). The corresponding mortality records and water quality conditions for each test are reported in Appendixes A1-A22. Of the species tested, the golden shiner (*Notemigonus chrysoleucas*) was found to be most sensitive (LC50 = 0.59 mg NH_3-N/liter), although the sensitivity of the white sucker (*Catostomus commersoni*) was not significantly different (LC50 = 0.65 mg NH_3-N/liter). The

Table 2. Acute toxicity of un-ionized ammonia to selected warmwater fish species.

Family: Species	96-h LC50 mg NH_3-N/ℓ (95% C.L.)	Significant differences[1]
Catostomidae:		
Catostomus commersoni	0.65 (0.60 - 0.69)	A
Ictaluridae:		
Ictalurus punctatus	1.19 (1.13 - 1.25)	D
Centrarchidae:		
Lepomis macrochirus	1.11 (1.02-1.22)	C,D
Lepomis macrochirus	1.44 (1.35 - 1.52)	E
Lepomis macrochirus	1.45 (1.29 - 1.62)	E
Cyprinidae:		
Notemigonus chrysoleucas	0.59 (0.50 - 0.69)	A
Notropis whipplei	1.03 (0.99 - 1.08)	B
Notropis spilopterus	1.11 (1.09 - 1.14)	C
Campostoma anomalum	1.41 (1.25 - 1.58	E
Pimephales promelas	1.44 (1.36 - 1.56)	E
Pimephales promelas	1.54 (1.44 - 1.63)	E

[1]LC50 values with common letters are not significantly different (P = 0.05).

five species within the minnow family (Cyprinidae) gave a geometric mean LC50 of 1.07 mg NH_3-N/liter, which was lower than all other families tested except suckers (Catostomidae). Nevertheless, the most resistant species tested was the fathead minnow (Pimephales promelas), which is commonly used in aquatic toxicity testing. Replicate tests with bluegill (Lepomis macrochirus) and fathead minnow showed good reproducibility, although one of the three bluegill results was relatively low. Eight of the eleven test results fell within the range of 1.11 to 1.54 mg NH_3-N/liter.

Dissolved Oxygen-Ammonia Relationship. Fathead minnows were more sensitive to low dissolved oxygen in the presence of ammonia. The 96 hour LC50 values for oxygen increased from 1.37 mg O_2/liter with no ammonia, to 4.05 mg O_2/liter in combination with 1.23 mg NH_3-N/l of un-ionized ammonia (Table 3). Thus there was a strong correlation between ambient ammonia and the dissolved oxygen concentration required for survival. Mortality records and water analyses for the oxygen tests are given in Appendixes A23-A36.

A least-squares regression model for the LC50 of oxygen as a function of ammonia (Figure 1) produced the estimated regression line:

Oxygen-LC50 = 1.06 + 2.13 (mg NH_3-N/l)

with correlation coefficient of r=0.96. Two test results were excluded from this regression analysis. The exposure to 1.64 mg/l un-ionized ammonia (Table 3) produced more than 50% mortality, and could not be used to calculate an LC50. The test at 1.14 mg/l un-ionized ammonia had a relatively large fluctuation in ammonia concentrations (relative standard deviation = 12% compared to 4-7% for other tests in the same range). That anomalous result is represented as an open circle in Figure 1.

A multiple regression analysis was also performed to test the hypothesis that proportional mortality is a function of oxygen, ammonia, and an interaction between the two variables. Two data sets were used for this analysis: the entire data set for proportional mortality (Appendixes A23-A36), and a truncated set consisting only of those treatments giving proportional mortalities greater than 0% but less than 100%. In the first analysis, the dependent variable:

PM = 2*arcsin(sqrt(proportional mortality))

was regressed against the two independent variables:

X_1 = mg NH_3-N/l

X_2 = mg O_2/l

Table 3. Median lethal concentration of low dissolved oxygen (LC50) for *Pimephales promelas* exposed to various concentrations of un-ionized ammonia at 26C.

Mean wt., g	Mean mg NH_3-N/1 + SD	Mean pH	Mean Temp., C	96-h LC50 mg O_2/1 (95% C.L.)
0.3	0	7.8 (7.8 - 7.8)[1]	25.9 (25.2 - 26.3)[1]	1.37 (1.27 - 1.47)
0.7	0.38 + 0.04 (0.30 - 0.49)[1]	7.7 (7.6 - 7.7)	26.8 (25.7 - 27.7)	1.48 (1.37 - 1.61)
0.3	1.14 + 0.14 (0.87 - 1.43)	7.7 (7.6 - 7.8)	25.5 (23.5 - 27.2)	1.70[2] (1.53 - 1.89)
0.4	0.71 + 0.05 (0.61 - 0.79)	7.8 (7.8 - 7.8)	25.8 (125.4 - 26.1)	2.52 (2.26 - 2.82)
0.3	1.25 + 0.06 (1.13 - 1.34)	7.7 (7.7 - 7.8)	26.1 (25.2 - 26.8)	3.54 (3.00 - 4.17)
0.5	1.23 + 0.05 (1.18 - 1.33)	7.8 (7.7 - 7.8)	27.5 (26.9 - 28.1)	4.05 (3.43 - 4.80)
0.5	1.64 + 0.07 (1.41 - 1.75)	7.7 (7.7 - 7.8)	25.6 (25.0 - 26.3)	*[3]

[1] Range

[2] Omitted from regression in Figure 1 because of relatively large variance in ammonia concentrations during the test.

[3] LC50 not calculable.

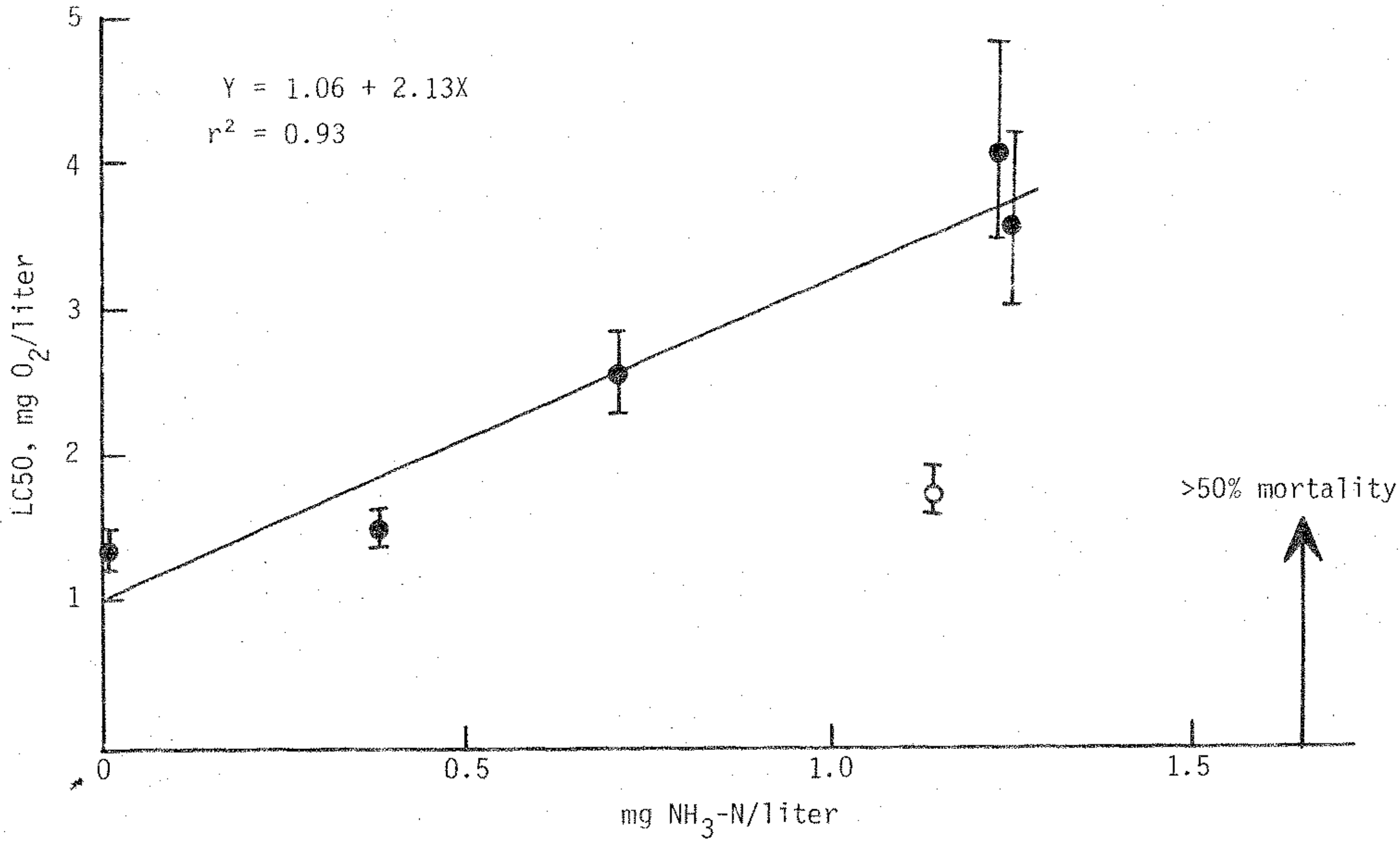

Figure 1. Relationship between ambient un-ionized ammonia concentration and low dissolved oxygen 96-hour LC50 (with 95% confidence limits) for <u>Pimephales promelas</u> at 26°C. The regression line is for solid circles only.

to give the relationship:

$$PM = 2.82 + 0.28X_1 + 0.94X_2 + 0.45(X_1 * X_2).$$

The F test for this equation confirmed that a significant relationship existed ($P < 0.01$) but the test for lack of fit was also significant ($P < 0.01$), meaning that the regression model did not adequately describe the data. This result suggests a non-linear relationship.

A second analysis was performed on the truncated data set, following the reasoning that 0% and 100% mortalities provide no information on the combinations of ammonia and oxygen that are likely to interact. Total mortality can result from either high ammonia or low oxygen. Conversely, complete survival can occur with low ammonia coupled with a wide range of oxygen concentrations (above about 2 mg/l). Analysis of the truncated data set gave the estimated regression line:

$$PM = 2.93 - 0.31X_1 - 1.33X_2 + 0.77(X_1 * X_2).$$

The test for linearity was greatly improved with the truncated data; the F value for lack of fit was only marginally significant at $P=.05$.

The next step was to determine whether all regression coefficients were necessary to the model. The regression coefficient for ammonia (β_1) was suspected of not contributing to the data. Therefore, the hypothesis that $\beta_1=0$ was tested. The calculated F indicated that the ammonia variable could be dropped from the equation, producing the simplified model:

$$PM = 2.56 - 1.19X_2 + 0.67(X_1 * X_2)$$

This equation is shown in Figure 2. The coefficient ($\beta_3 = 0.67$) for the interaction term ($X_1 * X_2$) was significantly different from zero, indicating that an interaction does exist between oxygen and ammonia toxicity. The interaction appears to be greater at high ammonia concentrations, as indicated by a change in the slopes of the ammonia lines in Figure 2 at greater oxygen concentrations.

An alternative approach to evaluating joint toxic action has traditionally been to assume additivity between chemicals (EIFAC 1980). In its simplest form, the additive model is expressed by calculating a toxic unit (TU) for each toxic component of a mixture, where:

TU = (concn. of component)/(LC50 of component)

By definition, a toxic unit of 1 for a single chemical produces 50% mortality in the test population. Assuming the joint effect of several toxicants is additive, the sum of all toxic units will also be equal to 1 if 50% mortality

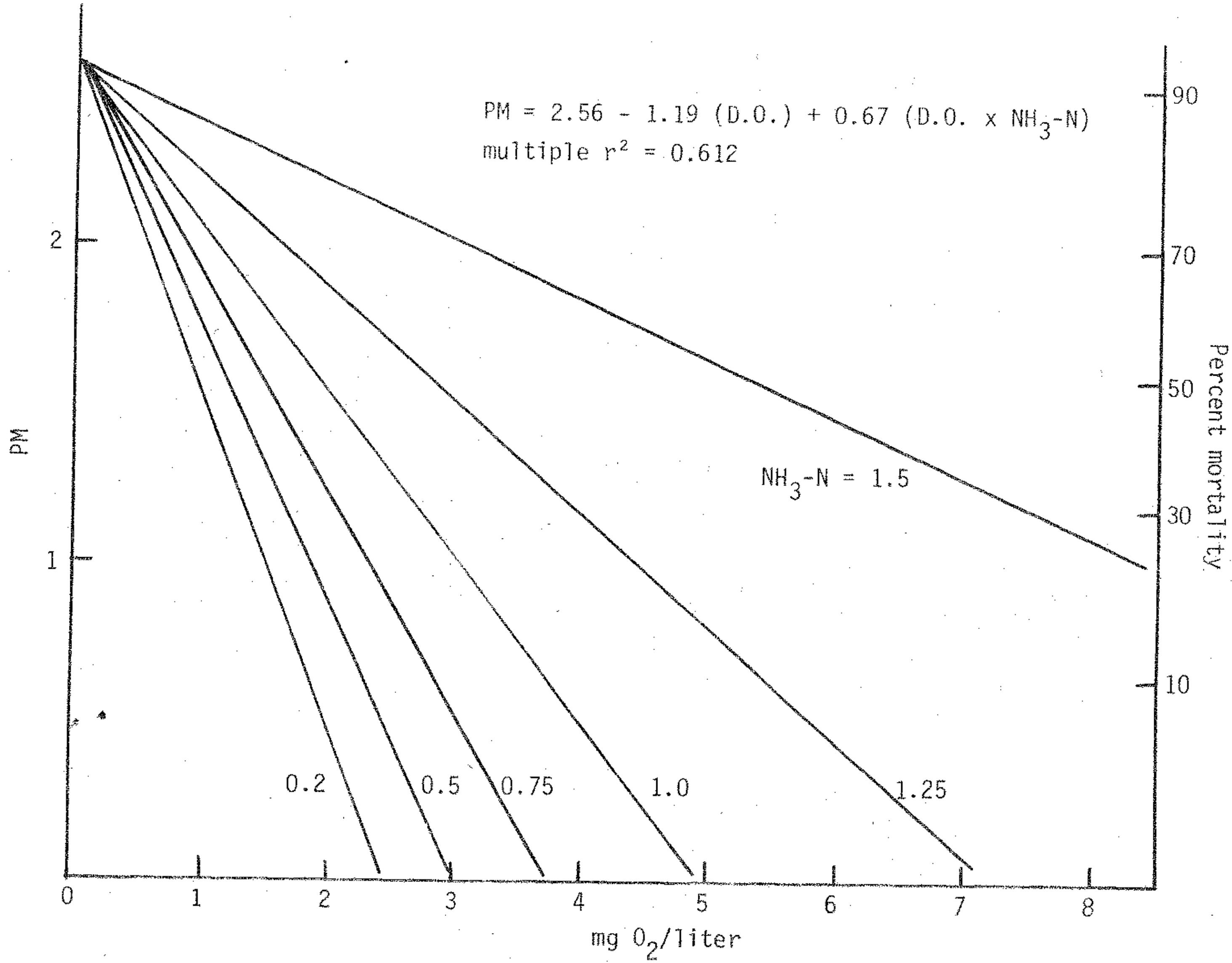

Figure 2. Multiple regression model for 96-hour proportional mortality of Pimephales promelas as a function of dissolved oxygen (D.O.) and ammonia (mg NH_3-N/ℓ), where PM = 2(arcsin (proportional mortality)$^{1/2}$).

occurs. For a binary mixture, the additive model predicting 50% mortality can be represented by a straight line with equation:

$$X + Y = 1 \qquad (\text{for } 0 \leq X \leq 1)$$

where X and Y are the TU values for each toxicant.

While this approach has been used for certain pollutants such as cyanide and metals, it has not been applied successfully to low oxygen. Dissolved oxygen is an unusual 'toxicant' because lower concentrations are more lethal. Moreover, oxygen levels above a certain minimum level, near saturation, should have no direct effect on survival of organisms. Thus the scale for toxic units of oxygen must be inverted and normalized before it can be used in combination with toxic units of other chemicals.

This was done by using the inverse of oxygen LC50 data from Table 3 to compute toxic units according to:

TU-oxygen = (1/concn.)/(1/LC50) = 1.37/concn. in mg/l

Toxic units for ammonia with fathead minnows (Table 2) were calculated in the conventional way:

TU-ammonia = concn./LC50 = concn./1.49 mg/l

Total toxic units for the series of oxygen-ammonia tests were calculated (Table 4) and plotted for comparison to the additive model (Figure 3). The agreement between the model and bioassay results was surprisingly good, indicating that the joint toxic action is nearly additive within the range of oxygen-ammonia combinations tested.

The estimated regression line differs from the theoretical line (X + Y = 1) because, unlike other toxicants, oxygen toxic units calculated in this fashion can never be zero. Instead, a solution at 100% saturation (8.26 mg O_2/l at 25C) produces a minimum TU of approximately 0.2 units. One option would be to force the experimental regression line (TU oxygen = 1.03 - 0.892 TU ammonia) to fit the model (Y = 1-X) by transforming the oxygen toxic units as follows:

Y = (TU oxygen - 0.2)/0.83

so that the new value (Y) would range from 0 to 1. That approach would need to be validated with a second data set independent of the one used to develop the regression. Despite this peculiarity in the calculation, the simple additive model for oxygen and ammonia appears to be a reasonable approach, and its graphical form (Figure 3) is more easily interpreted than multiple regression models such as Figure 2.

Table 4. Calculation of toxic units (TU) for additivity model with oxygen and ammonia. Data are for *Pimephales promelas*, from Table 3.

Oxygen LC50, mg/l	TU-oxygen	mg NH_3-N/l	TU-ammonia	Total TU
1.37	1.0	0	0	1.0
1.48	0.926	0.38	0.255	1.18
2.52	0.544	0.71	0.477	1.02
3.54	0.387	1.25	0.840	1.23
4.05	0.338	1.23	0.826	1.21
7.20[1]	0.190	1.49[2]	1.00	1.19

[1]Ambient dissolved oxygen concentration during ammonia LC50 test.

[2]Geometric mean of replicate LC50 results for ammonia (Table 2).

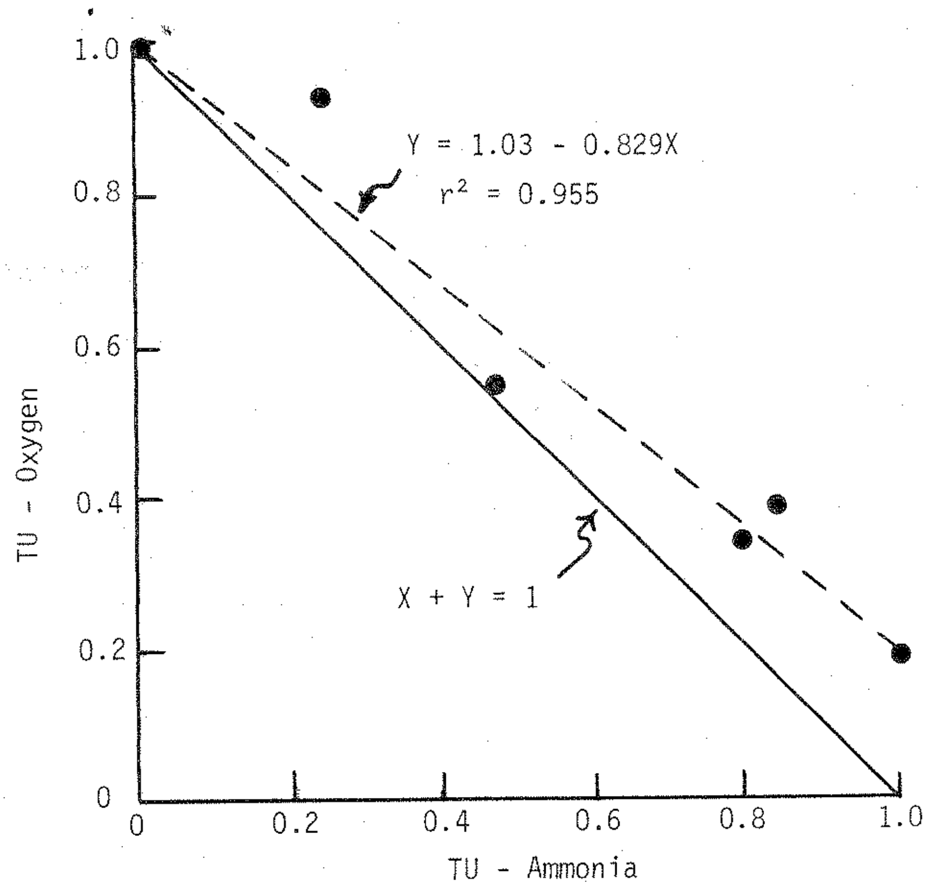

Figure 3. Joint toxicity of ammonia and low dissolved oxygen, expressed as toxic units. Dashed line is estimated regression for data given in Table 4; solid line is theoretical relationship in a concentration - addition model.

<u>Survival and Growth of Larvae</u>. Fathead minnow embryos exposed to 1.45 mg NH_3-N/1 had poor hatching success (38%) and complete mortality during early larval development. Individuals that did hatch exhibited abnormal development characterized by an unusually large thoracic region and a reduced caudal region. In contrast, fish exposed to 0.08 - 0.46 mg NH_3-N/1 had good hatching success and survival during the 28 day exposure (Table 5). Records for the fathead minnow embryo-larval test are given in Appendixes A37-39. Despite their good survival, larvae exposed to 0.28 or 0.46 mg NH_3-/N1 showed significantly reduced growth compared to controls (Table 5). The mean weight of the 28 day-old larvae at 0.46 mg NH_3-N/1 was only 60% of the weight for the control group. The highest level found not to affect growth significantly was 0.12 mg NH_3-N/1. Analysis of variance for the effect of ammonia on weights was highly significant (Table 6).

The effect of ammonia on hatching success and early survival was studied in an additional test with concentrations chosen within the range for mortality observed in the longer test. Although 1.45 mg NH_3-N/1 had caused poor hatching and complete mortality of larvae, a slightly lower concentration of 1.06 mg NH_3-N/1 gave better hatching success (Table 7). However fish at 1.06 and 0.99 mg NH_3-N/1 were deformed and died within 48 hours post-hatch. The highest ammonia concentration found not to affect hatching and early survival was 0.42 mg NH_3N/1 in this test. This level is considerably higher than the maximum no-effect level for larval growth, which was 0.18 mg NH_3-N/1 (geometric mean of 0.12 and 0.28).

A 30-day growth test with channel catfish larvae (Table 8) showed reduced survival at 0.68 mg NH_3-N/1 and significantly reduced growth at 0.20 and 0.38 mg NH_3-N/1. The highest concentration which produced no significant effect on growth was 0.11 mg NH_3-N/1, very similar to the result for minnows. The effect level for larval growth was calculated to be 0.15 mg NH_3-N/1. Records for the complete 30-day test with catfish are given in Appendixes A40-A43.

Analysis of variance for catfish weights at 30 days showed no significant effect of duplicate tanks or ammonia concentrations, when these factors were evaluated separately (Table 9). Since duplicate aquaria were non-significant,

Table 5. Effect of ammonia on survival and growth of Pimephales promelas larvae exposed for 28 days post-hatch.

Mean mg NH_3-N/1	% Survival	Number of fish	Mean wt., mg	SD	Dunnett's t
1.45	0	0	-	-	-
0.46	72	43	34.1	16.4	4.81**
0.28	70	42	42.6	17.7	3.33**
0.12	82	49	48.5	21.5	2.00
0.08	85	51	50.1	20.1	1.61
control	83	50	56.5	21.9	-

** Significantly less than controls ($p < 0.01$).

Table 6. Analysis of variance for Pimephales promelas weights after a 28 day exposure to ammonia.

Source of variation	Degrees of freedom	Mean squares	F[1]
Ammonia concn.	4	3258	25.1**
Duplicate tanks	5	129.7	0.33 NS
Error term	225	397.3	-
With pooled error terms:			
Ammonia concn.	4	3258	8.32**
Error term	230	391	

1 **, significant ($p < 0.01$).
NS, not significant.

Table 7. Chi-square tests for goodness of fit between observed and expected frequencies of hatching success, deformities, and 48 hour post-hatch survival for *Pimephales promelas* exposed to ammonia.

Treatment mg NH_3-N/l	n	% Hatching success	% Deformed larvae	% Survival @ 48 hours
1.06	20	95	100**	0**
0.99	20	100	100**	0**
0.42	20	95	5.3	94.7
0.28	20	100	5.0	95.0
0.10	20	100	0.0	100
Control	20	100	0.0	100

**Significantly different than controls ($p < 0.01$).

Table 8. Effect of ammonia on survival and growth of *Ictalurus punctatus* larvae exposed for 30 days post-hatch.

Mean mg NH_3-N·l	% Survival	Number of fish	Mean wt., mg	SD	Dunnett's t
0.68	17	5	97	24.0	79.84**
0.38	90	27	213	46.0	5.69**
0.20	80	24	267	110.1	3.23**
0.11	87	26	293	89.5	2.17
0.06	80	12[1]	301	66.0	1.46
control	77	23	343	84.5	-

**Significantly less than controls ($p < .01$).

[1]Represents one tank only. All others are total number of fish for two duplicate tanks.

their sums of squares were pooled with the error sums of squares, giving a more powerful statistical test. The effect of ammonia concentration on fish weights in this pooled analysis was highly significant ($P < 0.01$).

Hatching success and early survival of catfish larvae were evaluated in an additional test beginning with embryos separated from a normal catfish egg mass. Fungal development caused the loss of several treatment groups. Of the remaining groups (Table 10), the treatment at 1.26 mg NH_3-N/l showed significant reductions in hatching success and survival compared to controls. An ammonia level of 0.29 mg NH_3-N/l was not significantly different than controls. Thus, hatching success and early survival were found to be less sensitive to ammonia than larval growth rate.

Discussion

Acute Toxicity. A large number of acute toxicity tests with ammonia have been reported in the literature, which could be used to compare with those reported here if they were all conducted under the same water quality conditions. Direct comparisons are complicated, however, because un-ionized ammonia has been shown in decrease in toxicity with increasing pH (Thurston et al., 1981a). To a lesser extent, differences in temperature, size of fish, and dissolved oxygen levels can also cause differences between experimental results. A useful method of normalizing LC50 results to an upper limiting condition of pH has been proposed recently by the U.S. EPA (1983) in its final draft ammonia criteria document. According to this method, LC50's determined at a particular pH can be converted to an equivalent "limiting acute value" at high pH by the equation:

$$\text{Lim LC50} = \text{LC50}\,(1 + 10^{1.03\,(7.32 - \text{pH})})$$

where the pH during the test is used on the right side of the equation.

While low temperatures can enhance the toxicity of un-ionized ammonia, the available data suggest that tests conducted above 10C should give similar results (U.S. EPA 1983). Fish size may also be a factor to consider, but since most acute tests, including those reported here, are conducted with juveniles, it should not be a major concern. Dissolved oxygen is normally maintained at or near saturation during testing, and therefore should not cause significant differences between test results. For these reasons, pH was the only factor used to correct the reported acute values for purposes of comparison.

Table 9. Analysis of variance for *Ictalurus punctatus* weights after a 30 day exposure to ammonia.

Source of variation	Degrees of freedom	Mean squares	F[1]
Ammonia concn.	4	53.73	4.22 NS
Duplicate tanks	4	12.74	1.97 NS
Error term	103	6.46	-

With pooled error terms:

Ammonia concn.	4	53.73	8.02**
Pooled error	107	6.70	

[1] **, significant ($p < 0.01$).
NS, not significant.

Table 10. Chi-square tests for goodness of fit between observed and expected frequencies of hatching success, deformities, and 48 hour post-hatch survival for *Ictalurus punctatus* exposed to ammonia.

Treatment mg NH_3-N/1	n	% Hatching success	% Deformed larvae	% Survival @ 48 hours
1.26	20	15**	100**	0.0**
0.29	20	75	0.0	100
Control	20	75	0.0	100

**Significantly different than control ($p < 0.01$).

Table 11 summarizes the acute results of this study and the acute data cited by U.S. EPA (1983), normalized to a limiting pH. There is overall agreement between the two sets, with no particular trend toward high or low results in either set. The poorest agreement is for the catostomid and ictalurid tests, which differed by about 50% between authors. The catostomid result of 1.29 mg/l cited by U.S. EPA from Reinbold and Pescitelli (unpublished report, 1982) was for a lower temperature (15C) and higher pH (8.0-8.3) than our test. Otherwise, the tests appear to have been run under similar conditions. Reinbold and Pescitelli also reported LC50 results for channel catfish of 1.45-1.44 mg NH_3-N/l compared to 1.19 mg/l (uncorrected for pH) found in this study. Earlier authors, however, reported higher LC50's for catfish in the range of 1.7 - 3.5 mg NH_3-N/l (Colt and Tchobanoglous, 1976; Roseboom and Richey, 1977).

Literature values for bluegill, Lepomis macrochirus, were in the range of 0.7 to 3.8 mg NH_3-N/l (uncorrected). The value of 1.63 from Reinbold and Pescitelli for pH of 8 and temperature of 25C agreed reasonably well with our mean for three bluegill tests: 1.32 mg/l (uncorrected). The minnow family, Cyprinidae, gave the closest agreement between our results and the values compiled by the U.S. EPA. The family means were particularly close considering that different species were used for the two calculations (Table 11). The relatively narrow range among cyprinid acute results lends support to the theory that toxicities are more similar within families than between families.

Oxygen-Ammonia Relationship. Earlier reports have noted that low dissolved oxygen increases the toxicity of ammonia to freshwater fish. Alabaster (1979) found that the 24-hour LC50 for Atlantic salmon (Salmo salar) was approximately twice as great at 3.5-3.1 mg/l oxygen as it was at 9.6-9.5 mg/l dissolved oxygen. Likewise, Thurston et al. (1981b) reported a 1.4 fold increase in toxicity of ammonia to rainbow trout (Salmo gairdneri) at 5.0 mg/l compared to 8.5 mg/l, and Lloyd (1961) measured a 2.5 fold increase for the same species when dissolved oxygen was reduced from saturation to 40% of saturation. The lethality of ammonia to carp (Cyprinus carpio) in ponds was approximately 6X greater at 5 mg/l oxygen than at 10 mg/l (Selěsi and Vamoš, 1976). Others finding enhanced toxicity or reduced survival times for ammonia at low dissolved oxygen concentrations include Downing and Merkens (1955), Danecker (1964), and Allan (1955).

Table 11. Comparison between 96-hour ammonia LC50 values determined in this study and data cited in U.S. EPA (1983). Un-ionized ammonia concentrations are expressed as mg NH_3-N/liter[a].

Family: Species	This study LC50	This study pH	This study Limiting acute value	EPA Limiting acute value
Catostomidae:				
Catostomus commersoni	0.65	7.8	0.86	1.29
Ictaluridae:				
Ictalurus punctatus	1.19	7.8	1.57	2.36
Centrarchidae:				1.23[c]
Lepomis macrochirus	1.32	7.8	1.74	1.19
Cyprinidae:			1.45[d]	1.58[d]
Notemigonus chrysoleucas	0.59	7.5	0.98	1.15
Notropis whipplei	1.03	7.9	1.29	-
Notropis spilepterus	1.11	7.9	1.39	1.36
Campostoma anomalum	1.41	7.8	1.86	-
Pimephales promelas	1.49	7.8	1.97	1.50

[a]To convert to mg NH_3/liter, multiply by 1.217.

[b]Limiting acute values were calculated as:
Lim. AV = LC50 (1 + 10 EXP(1.03(7.32 - pH))).

[c]Family value = geometric mean of four species values.

[d]Family value = geometric mean of five species values.

A positive linear relationship between ammonia LC50 (as mg NH_3/l) and dissolved oxygen (mg/l) was reported by Thurston et al. (1981b) for rainbow trout:

$$\text{Ammonia-LC50} = 0.1903 + 0.06712 \text{ D.O.}$$
$$r = 0.9346$$

Although the trend is similar, the results for fathead minnows shown in Figure 1 are not directly comparable because the independent and dependent variables were reversed in the present study. That is, ammonia exposure was held constant while dissolved oxygen was varied in the minnow experiments. This produced a relationship,

$$\text{Oxygen-LC50} = 1.06 + 2.13 \text{ (mg } NH_3\text{-N/l)}$$
$$r = 0.96$$

with about the same degree of correlation. However, when the rainbow trout and minnow results were analyzed in terms of proportional mortality (p), the relative importance of the oxygen and ammonia terms were not identical in the two studies. Thurston et al. (1981b) found that both NH_3 and D.O. terms were significant, but that the interaction term was not, when the entire data set was included for analysis. If a truncated set was used (deleting 0 and 100% mortalities), all three coefficients were significantly different from zero. Only the oxygen and ammonia-oxygen interaction terms (Figure 2) were found to be significant in the trunctated data set for fathead minnows. The most suitable transformations for p were also different in the two studies.

Despite the fact that clear positive relationships were established between ammonia and dissolved oxygen for both fathead minnows and rainbow trout, the linear regression models for these data are rather inconsistent and seem to depend on the particular circumstances of the experiment. In the long run, it would be more satisfying to develop a model based on joint-toxic action, rather than on purely descriptive correlations. Then, deviations from the "ideal" could be described as needed. This is the approach taken with the ideal gas law and many other relationships in chemistry. Joint-toxic action has been described by concentration-addition and response-addition models for a variety of toxicants with fish. It would seem worthwhile to do the same with oxygen as a "toxicant" contributing to overall mortality rates. The proposed model shown in Figure 3 relating oxygen and ammonia toxic units is such an attempt. The "fit" seems rather good for the fathead minnow data, but it would have to be validated with independent data sets such as the one from Thurston et al.(1981b). Other tests on fathead minnow survival with oxygen and ammonia are apparently available (Thurston et al., unpublished, cited by U.S. EPA 1983) which could be used to evaluate the concentration-addition model given in Figure 3.

The current recommended dissolved oxygen criterion, which has become a stream standard in many states, is a minimum concentration of 5.0 mg/l (U.S. EPA 1977). That level represents a "toxic unit" of approximately 0.1 according to the adjusted concentration-addition model (where Y = ((1.37/D.O.) - 0.2)/0.83), so it would not be expected to increase the lethality of ammonia greatly. Fathead minnows can survive very low oxygen concentrations, at least during short exposures (Table 3). But these values should not be applied automatically to other species. Salmonids and a number of other taxa such as *Etheostoma* have relatively high dissolved oxygen requirements, so that substantial interaction between oxygen and ammonia may occur at 5.0 mg/l or above. Thurston et al. (1981b) concluded that 5.0 mg/l dissolved oxygen would reduce trout tolerance to ammonia by 30%. Low flow conditions, when low oxygen and high temperature are likely to occur, probably reduce the ability of most species to tolerate ammonia and combinations of ammonia with other toxicants.

Embryo-Larval Tests. Growth of fathead minnow larvae was found to be a more sensitive response to ammonia than survival or hatching success. Exposure to 0.42 mg NH_3-N/l produced no significant reduction in hatching success or 48-hour fry survival but 0.28 mg NH_3-N/l significantly reduced fish weights after 28 days post-hatch. A mean chronic effect level based on larval growth was 0.18 mg NH_3-N/l (range 0.12 - 0.28), which gives an acute/chronic ratio of 8.28 for fathead minnows.

The final draft ammonia criteria document (U.S. EPA 1983) cited results of two full life-cycle exposures to fathead minnows, ending after 60-days exposure to the progeny (Thurston et al., unpublished). No effect on hatching success at 0.072 mg NH_3-N/l or lower was found. However, there was significant mortality of the progeny at 0.16 to 0.33 mg NH_3-N/l after 30 and 60 days post-hatch. The chronic effect level cited was 0.107 mg NH_3-N/l (0.13 mg NH_3/l as given) and the acute/chronic ratio was 18. Thus, the full chronic exposure produced a chronic value about 60% of the 30-day embryo-larval result, which is to be expected for the longer test. That difference also accounts for most of the discrepancy between the acute/chronic ratios calculated from the two studies.

Larval growth was also the most sensitive response in the 30-day exposure of channel catfish. A chronic effect level based on larval growth reduction was found to be 0.15 mg NH_3-N/l (range 0.11 - 0.20). This is slightly lower than the chronic value of 0.233 mg NH_3-N/l selected for the national criteria document (U.S. EPA 1983) from the data of Reinbold and Pescitelli, who found growth reduction of fry at 0.32 mg NH_3-N/l and above. Robinette (1976) reported growth retardation at lower levels of 0.123-0.132 mg NH_3-N/l. Colt and Tchobanglous (1978) measured a 50% reduction in growth of juvenile catfish at 0.52 mg NH_3-N/l, as compared to this study, where 0.38 mg NH_3-N/l caused a 62% reduction in larval growth (Table 8). The acute/chronic ratio for the present study, calculated to be 7.9, is in overall agreement with the value of 6.2 used by U.S. EPA (1983).

Embryo-larval or juvenile exposures are particularly important for developing toxicity data for water quality criteria in midwestern streams. Such streams typically undergo large fluctuations in flow, and if they receive relatively constant point source inputs of pollutants, the resulting toxicant concentrations will also fluctuate during the course of a year. In regions such as Indiana, where low flow conditions usually occur in summer or fall, the highest toxicant concentrations of the year are apt to occur during spawning or early development of the fish. The "critical period" determines year-class strength and growth characteristics for the fish population later on. Thus, an accurate estimate of toxic effect levels to early life stages is particularly important.

II. CALCULATION OF AMMONIA CRITERIA FOR WARMWATER STREAMS DURING LOW FLOW

Methods

A procedure has been developed for deriving national water quality criteria based on acute and chronic toxicity information (Stephan et al. 1983). If toxicity data are available for a sufficient number of taxa, a "two number" water quality criterion consisting of a maximum allowable concentration and a 30-day average concentration can be calculated. The two values needed for this computation are the "final acute value" (FAV) and acute/chronic ratio. The rationale and complete description for this procedure are given by Stephan et al. (1983). In brief, the FAV is computed from a list of family mean acute values (FMAV), where each family mean is the geometric mean of all available species mean acute values (expressed as LC50 concentrations) in that family. This approach was taken with the idea that toxicity varies more between families than between species within families. A numerical procedure is then used to estimate the LC50 represented by a cumulative frequency of P = 0.05 for the list of family means. This value is intended to provide reasonable protection for all but the few most sensitive families (5 in 100). Especially sensitive commercial or sport species may cause this value to be lowered in special cases. The cumulative proportion (P) for each FMAV is found according to:

$$P = R/(N + 1)$$

where R is the rank order of the FMAV and N is the total number of such values. The four lowest FMAV values are then used to calculate the FAV as follows:

$$M^2 = \frac{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}{\Sigma((\ln \text{FMAV})^2) - ((\Sigma(\ln \text{FMAV}))^2/4)}$$

$$\overline{X} = (\Sigma(\ln \text{FMAV}))/4$$

$$\overline{Y} = (\Sigma(\sqrt{P}))/4$$

$$A = \overline{X} + \frac{\sqrt{0.05} - \overline{Y}}{M}$$

$$\text{FAV} = e^A$$

The "criterion maximum concentration", which is not to be exceeded at any time, is taken as one-half the resulting FAV.

The chronic value for any suitable chronic or partial-life-cycle toxicity test is calculated as the geometric mean between the highest no-effect concentration and the lowest concentration giving a significant effect (such as growth, hatching success, survival, etc.). If a comparable acute value is available for the same species and test conditions, an acute/chronic ratio is calculated as the geometric mean of all available species values. The "final chronic value" (FCV) used to develop the water quality criterion is found by dividing the FAV by the final acute/chronic ratio. The FCV is used as the "criterion average", or the highest average concentration allowed during any 30 day period.

The final draft of the national water quality criteria for ammonia (U.S. EPA 1983) evaluated the current body of literature on ammonia and tabulated acute and chronic values for 18 families of freshwater fish and invertebrates. All of these families ordinarily occur in Indiana warmwater streams except for Dendrocoelidae (flatworm) and Salmonidae (salmon, whitefish, and trout). A final acute value (FAV) for warmwater streams was therefore calculated using 16 of the 18 families listed in the EPA summary. The calculation was made using the cited literature values, and also with the family means found in the present study. Criterion maximum and average ammonia concentrations (mg NH_3-N/l) were then calculated as described above. Toxicities were adjusted to a pH of 7.5, a typical value for Indiana rivers, and a temperature of 28C, the highest temperature expected during summertime low flow.

The ammonia criteria calculated in this manner were translated into allowable discharge rates (Kg/day) for representative Indiana rivers during low flow conditions. Data on flow regimes for 11 rivers having 30 years or more of flow records were selected from the compilation of Horner (1976). The streams were chosen only as examples - not because of any existing or proposed ammonia discharges. The yearly records for each river were examined to determine the lowest flows (in ft^3/sec) occurring for one and 30 consecutive days at recurrence intervals of 2 and 10 years. Recurrence interval for a particular flow is defined as: $(N + 1)/M$ where N is the period of record and M is the rank order of the flow. Allowable discharge rates were calculated by multiplying flow, in liters/day, by the ammonia criteria, in kg/l. The criterion maximum (0.2 mg/l) was used with the one-day low flow duration, and the criterion average (0.04 mg/l) with the 30-day duration. It should be noted that a flow with a recurrence interval of 2 years is equivalent to the median flow for the period of record.

Results and Discussion

The calculation of the final acute value (FAV) was similar, whether national values were used, or results from this study were substituted for four of the fish families (Table 12). In either case, the four lowest values are actually the only ones used to determine the FAV. The result is influenced to a great extent by the low family acute value for Percidae, which is derived from two tests with orangethroat darter (Etheostoma spectabile) and walleye (Stizostedion vitreum vitreum) (U.S. EPA 1983). Salmonidae, one of the most sensitive families, was not included in the calculation. Since the family acute value for Centrarchidae in this study was relatively high, and the result for Catostomidae relatively low compared to the data cited by U.S. EPA, the net result was a similar FAV for the two family lists.

The criterion average calculation is dependent on the acute/chronic ratio chosen. In the final draft U.S. EPA (1983) document, an acute/chronic ratio of 16 was chosen to develop the national ammonia criteria. That value appeared to be too high for use in a warmwater criterion. The acute/chronic ratios for the six available fish species other than salmonids reported in the final draft document were: Pimephales promelas, 18; Catostomus commersoni, 25; Ictalurus punctatus, 6.2; Lepomis cyanellus, 5.6; Lepomis macrochirus, 11.2; and Micropterus dolomieui, 5.45. The geometric mean for these (9.9) is similar to the ratio of 8.05 found for the mean of the catfish and minnow acute/chronic ratios in the present study. The acute/chronic ratios for invertebrates appear to be smaller based on results for Daphnia magna (1.3-4.9). Thus the use of a value of 8.05 or 9.9 to calculate the final chronic value should offer a reasonable level of protection for other taxa as well.

Site-specific ammonia criteria have been calculated by a similar method of family deletion for several rivers in the north-central and western states (U.S. EPA 1983). Salmonids were deleted from some but not all of these example. The range of criterion maximum values for the rivers without salmonids was 0.07 to 0.48 mg NH_3-N/1, which encompasses the results of Table 12. The criterion averages for the same site-specific examples were 0.025 to 0.06 mg NH_3-N/1. These are also in general agreement with the chronic values of Table 12 even though the acute/chronic ratio used to derive the final chronic values for the U.S. EPA sites was 16.

Table 12. Calculation of a final acute value (FAV) and criteria for ammonia (mg NH_3-N/1) using fish and invertebrate families found in midwestern warmwater streams. Family acute values are from the national summary (U.S. EPA 1983) and from this study (*) adjusted to pH = 7.5.

Family	National acute value	Rank	Substitutions from this study	Rank
Elmidae	4.13	16	4.13	16
Ephemerellidae	3.09	15	3.09	15
Ascellidae	1.92	14	1.92	14
Actacidae	1.72	13	1.72	13
Tubificidae	1.51	12	1.51	12
Ictaluridae	1.43	11	0.95*	6
Perlodidae	1.34	10	1.34	11
Poeciliidae	1.28	9	1.28	10
Baetidae	1.17	8	1.17	9
Percichthyidae	1.14	7	1.14	8
Cyprinidae	0.96	6	0.88*	4
Daphnidae	0.91	5	0.91	5
Cottidae	0.82	4	0.82	3
Catostomidae	0.78	3	0.52*	2
Centrarchidae	0.74	2	1.05*	7
Percidae	0.405	1	0.405	1
FAV	0.413		0.368	
Criterion maximum	0.207		0.184	
Acute/chronic ratio	9.9		8.05	
FCV or criterion average	0.042		0.046	
Total ammonia, mg N/1, at 28C:				
Criterion maximum	9.54		8.48	
Criterion average	1.94		2.12	

Both the results of Table 12 and the site-specific examples of U.S. EPA (1983) are significantly higher than the national criteria developed in the same draft EPA report. The recommended national criteria for pH 7.5 and 28C would be 0.075 and 0.018 mg NH_3-N/1 for the maximum and average concentrations respectively. Those are approximately twice the values given in Table 12.

Ultimately the limitation that is important to dischargers is not the in-stream ammonia concentration, but rather the allowable rate of discharge of ammonia, measured in Kg/day. The rate of discharge is determined by a combination of water quality criteria and flow regime of the particular receiving stream. The allowable discharge rate, and the relative importance of the criterion maximum and 30-day average are related to mean flow and variability in flow. A 'flashy' stream would have more extreme deviations from the mean flow, and would perhaps be more restricted by the criterion maximum. A stream with relatively constant flow would be governed primarily by the 30-day average, although the exact relationship would depend on the difference between the two numbers making up the criterion.

The results of Table 12 were used to calculate allowable discharge rates for representative Indiana streams as an illustration of the use of "two number" criteria for ammonia (Table 13). The flow regimes for these midwestern rivers are such that the 30-day average ammonia concentration is more restrictive than the one-day maximum. For example, the Flatrock River could receive 0.49 Kg/d of un-ionized ammonia if the criterion maximum was the only consideration. However, the 30-day criterion average permits only 0.38 Kg/d under the same conditions. This occurs because the ratio between 30 day and one-day low flows is usually less than a factor of 5. If an acute/chronic ratio of 10 is used to derive the criterion, then there is a ratio of 5:1 between the maximum and average criteria (the maximum being one-half the final acute value). Any flow regime with a proportion of less than 5 for the one-day and 30-day low flows should be "controlled" by the 30-day average discharge limitations. The Mississinewa River happens to have a 5:1 ratio between 30-day and one-day low flows at a 10 year recurrence, and is thus equally limited by maximum and average criteria in this example.

The rivers listed in Table 13 are ranked from lowest to highest mean annual discharge. However, there are variations in the deviations about the mean, and the low flows are not in the same order. The Whitewater River at Alpine, with a mean annual flow of 15 m^3/s, has less extreme low flow events

Table 13. Maximum allowable discharges of un-ionized ammonia, in Kg/day, to representative Indiana rivers during low flow, based on a one-day maximum of 0.2 mg/1 and 30-day average of 0.04 mg NH_3-N/1. The ammonia discharges shown here would produce concentrations at or above the criteria at recurrence intervals of 2 or 10 years. Stream flow data from Horner (1976).

River	Recurrence:	2 yr		10 yr	
	Duration:	1 d	30 d	1 d	30 d
Hart Ditch, Munster Low flow: Aug.		1.68	0.50	1.02	0.35
Flatrock, St. Paul Low flow: Sept.		3.56	1.27	0.49	0.28
Big Blue, Shelbyville Low flow: Sept.		28.3	6.82	18.0	4.31
Elkhart, - Goshen Low flow: Sept.		51.2	14.4	14.6	8.69
Whitewater, Alpine Low flow: Oct.		34.4	8.39	21.4	5.85
St. Mary's, Ft. Wayne Low flow: Sept.		7.31	2.22	3.61	1.13
Blue, White Cloud Low flow: Oct.		10.7	2.99	5.36	1.69
Mississinewa, Marion Low flow: Aug.		8.77	4.55	2.73	2.73
White, Indianapolis Low flow: Sept.		55.8	16.2	22.2	6.94
Wabash, Lafayette Low flow: Sept.		23.9	6.82	13.6	3.85
Kankakee, Shelby Low flow: Sept.		263	58.1	197	43.5

than the St. Mary's River at Ft. Wayne, where the mean annual flow is 16 m^3/s. Consequently the Whitewater River could receive higher rates of ammonia discharge than the St. Mary's, while complying with water quality limitations.

The choice of a recurrence interval for applying water quality standards is a question that needs to be addressed. The two intervals used in Table 13 for illustrative purposes are not meant to be taken as "acceptable" recurrence intervals. The two-year interval is clearly unacceptable because it would generate stream standard violations, on the average, once every two years. The 10-year interval is a traditional choice, since most low flow modelling is based on a 7Q10. Other intervals such a 5 or 20 years should be considered, both from biological and economic view points.

CONCLUSIONS

The acute toxicity of un-ionized ammonia to the eight fish species tested ranged from 0.59 mg NH_3-N/1 for golden shiner, to 1.54 mg NH_3-N/1 for fathead minnow. Eight of the eleven 96-hour LC50 results fell between 1.11 and 1.54 mg NH_3-N/1, and were in general agreement with other acute values reported in the literature. Reduced growth was the most sensitive response of fathead minnow and channel catfish larvae to long-term ammonia exposures. Hatching success, number of deformities, and 48-hour survival of larvae after hatching were less sensitive measures of ammonia toxicity. The chronic effect levels based on reduced larval growth were 0.18 (0.12 - 0.28) mg NH_3-N/1 for fathead minnow, and 0.15 (0.11 - 00.20) mg NH_3-N/1 for catfish. The calculated acute/chronic ratios, 8.28 and 7.9 respectively, were also quite similar for the two species.

A linear relationship was established between the acute toxicity of low dissolved oxygen and the ambient concentration of ammonia in tests with fathead minnows. The proportional mortality at 96 hours could be described as a function of dissolved oxygen concentration and an interaction term between oxygen and ammonia. The results also appeared to follow a concentration-addition model when oxygen and ammonia were expressed as toxic units. If this pattern is validated with additional species, the concentration-addition model will greatly improve predictions about the influence of dissolved oxygen on toxicity of ammonia and perhaps other toxicants. The current recommended criterion of 5.0 mg/l oxygen (U.S. EPA, 1977) would contributed approximately 0.1 toxic unit, which would enhance the acute toxicity of ammonia to fathead minnows by about 10%. However other warmwater species with higher oxygen requirements might be more sensitive at that concentration, as has been shown for rainbow trout (Thurston et al. 1981b).

Calculation of a "two number" ammonia criterion using available toxicity information on 16 families of fish and invertebrates found in midwestern warmwater rivers produced values that were consistent with other site-specific criteria. A maximum allowable concentration of approximately 0.2 mg NH_3-N/1, and a 30-day average of 0.04 mg NH_3-N/1 were obtained using either the bioassay results from this study, or values taken from the national criteria document (U.S. EPA, 1983). The criterion average is nearly identical to the current maximum allowable concentration of 0.05 mg NH_3/1 (or 0.04 mg NH_3-N/1) adopted by many states. However, it is about twice as high as the proposed national ammonia criterion (U.S. EPA 1983) which recommends a maximum of 0.075 and 30-day average of 0.018 mg NH_3-N/1 at 25C and pH 7.5.

The allowable discharge of ammonia to rivers (in Kg/day total ammonia) is ultimately limited by low flow conditions. In midwestern rivers, low flow events typically occur in late summer, when high temperatures (perhaps combined with high pH or low dissolved oxygen) make restrictions based on un-ionized ammonia most stringent. The relative importance of one-day and 30-day low flow events in limiting allowable discharges is related to the ratio of the two low flow conditions. For most Indiana rivers, the difference between one-day and 30-day low flow is less than a factor of 5. The "two number" ammonia criterion of 0.2 and 0.04 mg NH_3-N/1 is in the ratio of 5:1, based on an acute/chronic ratio of approximately 10:1. If such a criterion is adopted, the 30-average condition will be more restrictive to dischargers than the one-day maximum allowable limit. For that reason, the selection of chronic bioassay results for developing an ammonia criterion is especially important.

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PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 hour Acute

BEGINNING DATE: Oct. 17, 1980

SPECIES: Catostomus commersoni (white sucker)

TOXICANT: Ammonia

ENDING DATE: Oct. 21, 1980

NUMBER OF ORGANISMS PER AQUARIUM: 12*

AVERAGE WEIGHT OF ORGANISMS, g: 11.4

FLOW RATE, ℓ/MIN: 0.6

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 22.5

AVERAGE pH: 7.8

AVERAGE D.O., mg O_2/ℓ: 7.4

LC50: 0.65

95% CONFIDENCE LEVEL

UPPER: 0.69

LOWER: 0.60

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	1.55	1.28	0.79	0.80*	0.46	0.44	0.25	0.25	0.11	0.07	c	c
0.25												
0.5												
1	30	33										
2	100	83	17									
4		100	42									
8			50	9								
12			58	27								
16												
24				36								
36												
48			75	55								
60												
72				73								
96	100	100	83	91	0	0	0	0	0	0	0	0

Comments: *11 fish at 0.80 mg/ℓ concentration.

Test: 96 hour acute

Test species: Catostomus commersoni

Date initiated: October 17, 1980

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
9 hours	55.7	55.5	32.4	32.4	17.5	15.8	8.5	8.9	0.3	3.5	ND	ND
48 hours	62.1	61.3	35.9	37.0	18.4	18.5	9.3	9.6	4.4	4.4	ND	ND
96 hours	58.4	57.7	32.6	33.1	12.2	12.2	6.8	6.9	2.4	2.3	ND	ND
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.54	1.16	0.74	0.74	0.44	0.54	0.30	0.29	0.12	0.13	--	--
48 hours	NM	NM	0.76	0.78	0.53	0.51	0.27	0.26	0.13	0.14	--	--
96 hours	NM	NM	0.97	0.95	0.44	0.42	0.24	0.25	0.08	0.08	--	--
pH												
0 hours	7.77	7.64	7.69	7.68	7.73	7.87	7.87	7.84	7.89	7.89	8.03	7.97
48 hours	7.74	7.64	7.68	7.68	7.82	7.80	7.82	7.78	7.84	7.86	7.98	7.94
96 hours	7.89	7.82	7.80	7.81	7.92	7.89	7.91	7.92	7.91	7.90	7.96	7.91
Temperature °C												
0 hours	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
48 hours	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
96 hours	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Dissolved Oxygen, mg O_2/ℓ												
0 hours	7.4	7.4	7.4	7.3	7.3	7.2	7.3	7.5	7.5	7.5	7.6	7.5
96 hours	7.4	7.3	7.5	7.4	7.5	6.9	7.1	7.5	7.4	7.1	7.3	7.1

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

BEGINNING DATE: Oct. 4, 1982

SPECIES: *Ictalurus punctatus* (channel catfish)

TOXICANT: Ammonia

ENDING DATE: Oct. 8, 1982

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.5

FLOW RATE, ℓ/MIN: 0.5

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 25.7

AVERAGE pH: 7.8

AVERAGE D.O., mg O_2/ℓ: 7.1

LC50: 1.19

95% CONFIDENCE LEVEL

UPPER: 1.25

LOWER: 1.13

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	2.31	2.13	1.58	1.53	1.00	1.00	0.71	0.66	c	c		
0.25												
0.5		10										
1												
2	30											
4	40	30	20	10								
8	60	50										
12	70	70	40			10						
16	90		50									
24												
36	100	100	80	60								
48												
60			100	90	10							
72												
96	100	100	100	100	10	10	0	0	0	0		

Comments:

Test: 96 hour acute

Test species: Ictalurus punctatus

Date initiated: October 4, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	72.5	74.9	48.4	46.5	30.3	30.6	19.2	19.6	ND	ND		
48 hours	70.6	73.6	46.8	48.2	28.6	29.2	18.7	17.2	ND	ND		
96 hours	70.2	72.1	47.8	49.1	29.6	30.5	20.1	19.3	ND	ND		
Un-ionized Ammonia, mg N/ℓ												
0 hours	2.13	2.31	1.56	1.52	1.05	1.03	0.67	0.67	ND	ND		
48 hours	2.14	2.33	1.57	1.65	1.00	0.98	0.70	0.65	ND	ND		
96 hours	2.10	2.17	1.47	1.58	0.95	1.00	0.77	0.67	ND	ND		
pH												
0 hours	7.71	7.74	7.75	7.76	7.79	7.78	7.79	7.79	8.00	8.01		
48 hours	7.70	7.73	7.75	7.76	7.78	7.77	7.80	7.80	7.99	8.00		
96 hours	7.73	7.73	7.74	7.76	7.76	7.77	7.84	7.79	7.96	8.01		
Temperature °C												
0 hours	25.7	25.6	25.7	25.7	25.6	25.4	25.6	25.5	25.6	25.5		
48 hours	26.4	26.2	26.4	26.3	26.0	25.6	26.3	26.3	26.4	26.3		
96 hours	25.5	25.4	25.4	25.5	25.3	25.4	25.4	25.5	25.3	25.4		
Dissolved Oxygen, mg O_2/ℓ												
0 hours	6.76	6.84	6.83	6.99	7.13	6.84	6.92	6.82	7.01	6.95		
48 hours	7.03	7.14	6.97	7.01	7.09	7.19	7.21	7.11	7.05	6.94		
96 hours	7.11	7.25	7.38	7.25	7.20	7.26	7.13	7.07	7.15	7.28		

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

BEGINNING DATE: Jan. 22, 1981

SPECIES: *Lepomis macrochirus* (bluegill sunfish)

TOXICANT: Ammonia

ENDING DATE: Jan. 26, 1981

NUMBER OF ORGANISMS PER AQUARIUM: 14*

AVERAGE WEIGHT OF ORGANISMS, g: 0.9

FLOW RATE, ℓ/MIN: 0.6

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 24.2

AVERAGE pH: 7.8

AVERAGE D.O., mg O_2/ℓ: 6.4

LC50: 1.11

95% CONFIDENCE LEVEL

UPPER: 1.22

LOWER: 1.02

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	1.73*	1.53	1.34	1.32	0.68	0.67	0.34	0.33	0.29	0.22	c	c
0.25												
0.5												
1	31	14										
2	54	21										
4	92	93	29	7								
8	100	100	43	50								
12												
16												
24			50	57								
36												
48			57									
60												
72				64								
96	100	100	57	64	0	0	0	0	0	0	0	0

Comments: *13 per tank at 1.73 ppm

Test: 96 hour acute

Test species: Lepomis macrochirus

Date initiated: January 22, 1981

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	66.4	66.4	57.4	56.7	32.5	30.3	12.9	13.9	11.6	9.0	ND	ND
48 hours	58.8	59.3	50.2	51.4	28.3	27.2	10.7	11.6	10.1	7.9	ND	ND
96 hours	64.9	65.8	55.5	55.2	29.5	28.1	11.5	10.9	9.1	7.7	ND	ND
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.78	1.60	1.43	1.43	0.68	0.70	0.41	0.30	0.34	0.24	--	--
48 hours	1.77	1.38	1.22	1.25	0.69	0.68	0.30	0.30	0.30	0.20	--	--
96 hours	1.65	1.62	1.30	1.33	0.64	0.65	0.32	0.38	0.25	0.22	--	--
pH												
0 hours	7.75	7.71	7.72	7.73	7.64	7.69	7.83	7.65	7.79	7.75	7.80	7.81
48 hours	7.76	7.64	7.66	7.66	7.67	7.68	7.73	7.69	7.75	7.69	7.80	7.79
96 hours	7.67	7.65	7.63	7.64	7.59	7.63	7.70	7.81	7.70	7.73	7.81	7.79
Temperature °C												
0 hours	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
48 hours	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5
96 hours	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Dissolved Oxygen, mg O_2/ℓ												
0 hours	6.8	6.8	6.7	6.7	6.7	6.6	6.6	6.7	6.7	6.8	6.6	6.4
96 hours	6.0	6.1	6.1	6.1	6.2	6.1	6.1	6.2	6.4	5.9	6.3	6.3

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

BEGINNING DATE: July 20, 1982

SPECIES: Lepomis macrochirus (bluegill sunfish)

TOXICANT: Ammonia

ENDING DATE: July 24, 1982

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.9

FLOW RATE, ℓ/MIN: 0.5

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 26.5

AVERAGE pH: 7.6

AVERAGE D.O., mg O_2/ℓ: 7.0

LC50: 1.44

95% CONFIDENCE LEVEL

UPPER: 1.52

LOWER: 1.35

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	1.84	1.79	1.32	1.39	1.09	1.17	0.67	0.80	0.49	0.52	c	c
0.25												
0.5												
1		10										
2		20										
4									10			
8	20	60	10									
12	50											
16												
24	100	100	20	10				10				
36												
48			30	20								
60												
72			50									
96	100	100	60	30	0	0	0	10	10	0	0	0

Comments:

Test: 96 hour acute

Test species: Lepomis macrochirus

Date initiated: July 20, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	89.2	85.5	71.6	75.0	45.5	46.3	24.7	34.7	18.0	18.8	ND	ND
48 hours	NM	NM	58.4	56.2	44.4	41.8	25.0	30.7	16.0	17.2	ND	ND
96 hours	NM	NM	51.2	59.6	45.2	46.0	23.9	31.4	15.7	17.4	ND	ND
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.84	1.79	1.63	1.63	1.10	1.22	0.67	0.83	0.52	0.53	--	--
48 hours	NM	NM	1.27	1.26	1.10	1.12	0.68	0.79	0.49	0.50	--	--
96 hours	NM	NM	1.06	1.27	1.07	1.17	0.65	0.77	0.46	0.54	--	--
pH												
0 hours	7.55	7.56	7.59	7.58	7.63	7.66	7.67	7.62	7.71	7.70	7.78	7.77
48 hours	NM	NM	7.54	7.56	7.59	7.64	7.63	7.61	7.67	7.66	7.75	7.77
96 hours	NM	NM	7.52	7.54	7.58	7.62	7.64	7.60	7.67	7.70	7.79	7.78
Temperature °C												
0 hours	25.8	25.7	25.9	25.6	25.7	25.6	25.8	25.7	25.7	25.7	25.7	25.7
48 hours	NM	NM	26.8	26.8	27.2	26.8	27.2	27.2	27.4	27.3	27.3	27.3
96 hours	NM	NM	26.8	26.6	26.8	26.5	26.9	26.7	27.1	27.0	26.9	26.9
Dissolved Oxygen, mg O_2/ℓ												
0 hours	7.12	7.11	7.07	7.05	7.23	7.22	7.07	6.98	7.20	7.24	7.12	7.06
48 hours	NM	NM	6.89	6.84	7.00	7.01	6.96	6.90	7.07	7.19	7.14	7.08
96 hours	NM	NM	6.90	6.70	6.90	7.10	6.75	6.75	6.95	7.00	7.00	7.01

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

BEGINNING DATE: Sept. 28, 1982

SPECIES: Lepomis macrochirus (bluegill sunfish)

TOXICANT: Ammonia

ENDING DATE: Oct. 2, 1982

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 1.2

FLOW RATE, ℓ/MIN: 0.5

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 26.6

AVERAGE pH: 7.8

AVERAGE D.O., mg O_2/ℓ: 7.2

LC50: 1.45

95% CONFIDENCE LEVEL

UPPER: 1.62

LOWER: 1.29

Cumulative Percent Mortality

Observation Time, Hr. \ Toxicant Concn mg NH_3-N/ℓ	2.29	1.88	1.-2	0.88	0.84	c
0.25						
0.5						
1						
2	30	20				
4	50	30				
8	90					
12	100	50	10			
16		70				
24			20			
36						
48						
60						
72		90				
96	100	90	20	0	0	0

Comments:

Test: 96 hour acute

Test species: Lepomis macrochirus

Date initiated: September 28, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	76.3		61.7		34.3		16.4		18.3		ND	
48 hours	77.2		61.0		37.2		26.0		20.9		ND	
96 hours	74.7		61.7		34.8		25.9		20.1		ND	
Un-ionized Ammonia, mg N/ℓ												
0 hours	2.33		1.99		1.29		0.72		0.81		ND	
48 hours	2.39		1.82		1.27		0.98		0.85		ND	
96 hours	2.16		1.83		1.09		0.94		0.87		ND	
pH												
0 hours	7.68		7.71		7.79		7.85		7.86		8.00	
48 hours	7.70		7.68		7.74		7.78		7.81		7.86	
96 hours	7.70		7.71		7.74		7.81		7.88		7.95	
Temperature °C												
0 hours	27.2		27.2		26.7		27.0		27.0		26.7	
48 hours	26.8		27.0		26.8		27.0		27.0		26.9	
96 hours	25.9		25.8		25.7		25.7		25.8		25.7	
Dissolved Oxygen, mg O_2/ℓ												
0 hours	7.30		7.15		7.37		6.99		7.26		7.15	
48 hours	7.14		7.09		7.25		7.11		7.16		7.07	
96 hours	7.21		7.28		7.04		7.16		7.05		7.22	

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

BEGINNING DATE: Nov. 28, 1980

SPECIES: Notemigonus chrysoleucas (golden shiner)

TOXICANT: Ammonia

ENDING DATE: Dec. 2, 1980

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 8.7

FLOW RATE, ℓ/MIN: 0.6

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 24.5

AVERAGE pH: 7.5

AVERAGE D.O., mg O_2/ℓ: 7.7

LC50: 0.59

95% CONFIDENCE LEVEL

UPPER: 0.69

LOWER: 0.50

Cumulative Percent Mortality

Observation Time, Hr. \ Toxicant Concn mg NH_3-N/ℓ	1.08	1.02	0.60	0.58	0.31	0.30	0.15	0.14	0.07	0.07	c	c
0.25												
0.5												
1	48	20										
2	50											
4	70	50		10	10							
8												
12		100										
16												
24	90											
36												
48	100			20								
60												
72			30	40		10						
96	100	100	30	50	10	20	0	0	0	0	0	0

Comments:

Test: 96 hour acute

Test species: Notemigonus chrysoleucas

Date initiated: November 28, 1980

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	74.8	75.9	41.9	42.1	20.2	20.0	8.7	9.2	4.2	4.1	ND	ND
48 hours	61.1	65.2	36.5	36.0	18.9	16.4	6.3	6.8	3.3	3.2	ND	ND
96 hours	60.0	61.1	34.4	34.0	18.4	14.8	6.1	6.7	3.0	3.0	ND	ND
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.23	1.16	0.83	0.78	0.33	0.39	0.20	0.19	0.10	0.08	--	--
48 hours	1.11	1.00	0.59	0.59	0.31	0.29	0.14	0.14	0.07	0.07	--	--
96 hours	0.90	0.91	0.39	0.38	0.28	0.22	0.10	0.09	0.05	0.05	--	--
pH												
0 hours	7.51	7.46	7.58	7.55	7.48	7.55	7.64	7.59	7.66	7.57	7.66	7.66
48 hours	7.51	7.44	7.46	7.46	7.46	7.48	7.60	7.56	7.58	7.59	7.59	7.60
96 hours	7.47	7.47	7.35	7.34	7.48	7.48	7.51	7.43	7.52	7.52	7.56	7.58
Temperature °C												
0 hours	24.0	24.3	24.2	24.2	24.6	24.9	24.3	24.6	24.4	24.5	24.3	24.5
48 hours	25.2	25.3	25.4	25.3	25.5	25.9	25.3	25.5	25.5	25.5	25.4	25.5
96 hours	23.8	23.8	23.6	23.7	23.9	23.3	23.6	23.6	23.6	23.7	23.4	23.5
Dissolved Oxygen, mg O_2/ℓ												
9 hours	7.4	7.0	6.9	7.3	7.3	7.1	7.4	7.0	7.1	7.3	7.5	7.2
48 hours	8.1	8.1	8.0	7.8	7.9	7.7	7.9	8.1	8.1	8.0	8.0	8.1
96 hours	7.9	8.0	7.8	7.9	7.8	7.7	8.0	8.1	7.8	7.8	7.7	7.9

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

SPECIES: Notropis whipplei

TOXICANT: Ammonia

NUMBER OF ORGANISMS PER AQUARIUM: *

AVERAGE WEIGHT OF ORGANISMS, g: 0.5

FLOW RATE, ℓ/MIN: 0.5

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 25.7

AVERAGE pH: 7.9

AVERAGE D.O., mg O_2/ℓ: 7.3

BEGINNING DATE: Oct. 19, 1982

ENDING DATE: Oct. 23, 1982

LC50: 1.03

95% CONFIDENCE LEVEL

UPPER: 1.08

LOWER: 0.99

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	1.29	0.89	0.88	c
0.25	0	0	0	0
0.5				
1				
2				
4				
8				
12				
16				
24				
36				
48				
60				
72				
96	100	18.2	0	0

* Comments:

Total No.	5	11	7	9

Test included several species of Notropis. Identifications were made at 96 hours.

Test: 96 hour acute

Test species: Notropis whipplei

Date initiated: October 19, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	33.6		25.1		19.4		16.3		ND			
48 hours	35.2		27.4		19.4		17.2		ND			
96 hours	32.6		24.8		17.3		15.0		ND			
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.30		1.03		0.94		0.88		ND			
48 hours	1.32		1.16		0.91		0.92		ND			
96 hours	1.26		1.05		0.82		0.83		ND			
pH												
0 hours	7.85		7.87		7.93		7.98		8.04			
48 hours	7.82		7.88		7.92		7.98		8.07			
96 hours	7.83		7.88		7.91		7.98		8.01			
Temperature °C												
0 hours	25.4		25.5		25.4		25.8		25.6			
48 hours	25.8		25.4		25.7		25.8		25.6			
96 hours	26.0		25.6		26.1		26.1		25.9			
Dissolved Oxygen, mg O_2/ℓ												
0 hours	7.62		7.18		7.49		7.73		7.18			
48 hours	7.05		7.19		7.14		7.28		7.41			
96 hours	7.00		7.23		7.08		7.31		7.19			

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

SPECIES: Notropis spilopterus

TOXICANT: Ammonia

NUMBER OF ORGANISMS PER AQUARIUM: *

AVERAGE WEIGHT OF ORGANISMS, g: 0.5

FLOW RATE, ℓ/MIN: 0.5

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 25.7

AVERAGE pH: 7.9

AVERAGE D.O., mg O_2/ℓ: 7.3

BEGINNING DATE: Oct. 19, 1982

ENDING DATE: Oct. 23, 1982

LC50: 1.11

95% CONFIDENCE LEVEL

UPPER: 1.14

LOWER: 1.09

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	1.29	1.08	0.89	0.88	c
0.25	0	0	0	0	0
0.5					
1					
2					
4					
8					
12					
16					
24					
36					
48					
60					
72					
96	100	30.8	0	0	0

* Comments:

Total No.:	19	26	15	18	19

Test included several species of Notropis. Identifications were made at 96 hours.

Test: 96 hour acute

Test species: Notropis spilopterus

Date initiated: October 19, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	33.6		25.1		19.4		16.3		ND			
48 hours	35.2		27.4		19.4		17.2		ND			
96 hours	32.6		24.8		17.3		15.0		ND			
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.30		1.03		0.94		0.88		ND			
48 hours	1.32		1.16		0.91		0.92		ND			
96 hours	1.26		1.05		0.82		0.83		ND			
pH												
0 hours	7.85		7.87		7.93		7.98		8.04			
48 hours	7.82		7.88		7.92		7.98		8.07			
96 hours	7.83		7.88		7.91		7.98		8.01			
Temperature °C												
0 hours	25.4		25.5		25.4		25.8		25.6			
48 hours	25.8		25.4		25.7		25.8		25.6			
96 hours	26.0		25.6		26.1		26.1		25.9			
Dissolved Oxygen, mg O_2/ℓ												
0 hours	7.62		7.18		7.49		7.73		7.18			
48 hours	7.05		7.19		7.14		7.28		7.41			
96 hours	7.00		7.23		7.08		7.31		7.19			

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute
SPECIES: *Campostoma anomalum*
TOXICANT: Ammonia
NUMBER OF ORGANISMS PER AQUARIUM: 13*
AVERAGE WEIGHT OF ORGANISMS, g: 2.1
FLOW RATE, ℓ/MIN: 0.5
SIZE OF AQUARIA, ℓ: 60
AVERAGE TEMPERATURE, °C: 25.7
AVERAGE pH: 7.8
AVERAGE D.O., mg O_2/ℓ: 6.4

BEGINNING DATE: Aug. 23, 1982
ENDING DATE: Aug. 27, 1982

LC50: 1.41
95% CONFIDENCE LEVEL
UPPER: 1.58
LOWER: 1.25

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	1.88	1.36	0.83	0.56	0.45	c
0.25						
0.5						
1	31					
2						
4	46	15	8			
8	54					
12						
16	69	23				
24						
36						
48						
60						
72						
96	100	23	8	0	0	0

* Comments:
Treatment levels 0.56, 0.45 and c began with 12 individuals

Test: 96 hour acute

Test species: Campostoma anomalum

Date initiated: August 23, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	57.1		38.8		25.4		15.7		12.4		ND	
48 hours	NM		39.5		22.8		16.0		12.8		ND	
96 hours	NM		40.2		23.7		17.7		13.3		ND	
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.88		1.41		0.89		0.56		0.44		ND	
48 hours	NM		1.30		0.77		0.53		0.43		ND	
96 hours	NM		1.36		0.84		0.60		0.47		ND	
pH												
0 hours	7.74		7.78		7.77		7.77		7.77		7.80	
48 hours	7.78		7.77		7.78		7.78		7.79		7.81	
96 hours	7.80		7.79		7.81		7.79		7.81		7.82	
Temperature °C												
0 hours	26.3		26.5		26.5		26.5		26.4		26.4	
48 hours	25.6		25.3		25.5		25.3		25.3		25.3	
96 hours	25.5		25.2		25.3		25.1		25.2		25.4	
Dissolved Oxygen, mg O_2/ℓ												
0 hours	6.02		6.14		6.12		6.05		6.09		6.98	
48 hours	6.44		6.38		6.33		6.19		6.29		6.71	
96 hours	6.31		6.73		6.58		6.77		6.36		6.09	

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

BEGINNING DATE: Aug. 9, 1982

SPECIES: *Pimephales promelas* (fathead minnow)

TOXICANT: Ammonia

ENDING DATE: Aug. 13, 1982

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.2

FLOW RATE, ℓ/MIN: 0.5

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 25.9

AVERAGE pH: 7.78

AVERAGE D.O., mg O_2/ℓ: 7.1

LC50: 1.44

95% CONFIDENCE LEVEL

UPPER: 1.51

LOWER: 1.36

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	1.91	1.83	1.56	1.54	1.10	1.14	0.85	0.85	0.61	0.58	0	0
0.25												
0.5		10										
1	30	60										
2	60	90										
4	90	100	30	10								
8			40	20								
12												
16	90		60	30								
24	100		70	40								
36												
48			80	50								
60												
72												
96	100	100	80	60	0	0	0	0	0	0	0	0

Comments:

Test: 96 hour acute

Test species: Pimephales promelas

Date initiated: August 9, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	69.0	70.1	47.4	47.6	31.0	32.3	23.0	25.0	15.2	12.2	ND	ND
48 hours	NM	NM	52.4	51.4	35.4	36.1	25.0	24.7	17.3	15.4	ND	ND
96 hours	NM	NM	47.3	46.8	31.6	32.2	23.4	22.5	16.1	16.4	ND	ND
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.91	1.83	1.42	1.41	1.00	1.03	0.81	0.83	0.54	0.47	ND	ND
48 hours	NM	NM	1.57	1.42	1.09	1.09	0.85	0.78	0.61	0.56	ND	ND
96 hours	NM	NM	1.69	1.79	1.21	1.31	0.90	0.94	0.69	0.70	ND	ND
pH												
0 hours	7.64	7.63	7.68	7.68	7.71	7.72	7.76	7.73	7.76	7.80	7.79	7.81
48 hours	NM	NM	7.72	7.68	7.74	7.74	7.78	7.75	7.80	7.82	7.89	7.87
96 hours	NM	NM	7.82	7.84	7.85	7.87	7.87	7.88	7.89	7.89	7.91	7.85
Temperature °C												
0 hours	27.0	26.7	26.9	26.8	28.1	26.6	26.8	26.9	26.8	26.8	26.6	26.7
48 hours	NM	NM	25.5	25.7	25.4	25.3	25.5	25.6	25.6	25.4	25.7	25.5
96 hours	NM	NM	25.2	25.4	25.1	25.2	24.5	25.4	25.5	25.5	25.4	25.3
Dissolved Oxygen, mg O_2/ℓ												
0 hours	6.82	6.78	6.82	6.72	6.92	6.87	6.86	6.72	7.09	7.01	6.81	6.63
48 hours	NM	NM	7.31	7.30	7.27	7.34	7.33	7.28	7.45	7.43	7.33	7.24
96 hours	NM	NM	7.23	7.27	7.33	7.15	7.19	7.13	7.24	7.09	7.21	7.08

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 Hour Acute

SPECIES: Pimephales promelas (fathead minnow)

TOXICANT: Ammonia

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.5

FLOW RATE, ℓ/MIN: 0.5

SIZE OF AQUARIA, ℓ: 60

AVERAGE TEMPERATURE, °C: 25.6

AVERAGE pH: 7.8

AVERAGE D.O., mg O_2/ℓ: 7.2

BEGINNING DATE: Oct. 11, 1982

ENDING DATE: Oct. 15, 1982

LC50: 1.54

95% CONFIDENCE LEVEL

UPPER: 1.63

LOWER: 1.44

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg NH_3-N/ℓ	2.41	2.22	1.59	1.56	1.03	0.99	0.75	0.71	0.34	0.30	0	0
0.25												
0.5	30	10		10								
1		30	10									
2	60	50		30								
4	80	60										
8		70	20	40				10				
12	90	100										
16	100			60								
24			30								10	
36												
48			40	70								
60												
72												
96	100	100	40	70	0	0	0	10	0	0	10	0

Comments:

Test: 96 hour acute

Test species: Pimephales promelas

Date initiated: October 11, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
9 hours	75.7	78.2	52.2	50.5	30.3	32.6	20.1	19.0	8.6	9.9	ND	ND
48 hours	NM	NM	49.8	53.8	31.7	29.6	22.3	21.3	7.7	8.4	ND	ND
96 hours	NM	NM	49.9	51.0	33.1	29.6	22.1	20.8	8.0	8.9	ND	ND
Un-ionized Ammonia, mg N/ℓ												
0 hours	2.22	2.41	1.64	1.56	1.05	1.11	0.72	0.68	0.31	0.36	ND	ND
48 hours	NM	NM	1.56	1.65	0.98	0.93	0.77	0.73	0.29	0.31	ND	ND
96 hours	NM	NM	1.48	1.56	1.06	0.93	0.76	0.73	0.29	0.36	ND	ND
pH												
0 hours	7.70	7.71	7.73	7.72	7.76	7.76	7.78	7.78	7.79	7.79	7.81	7.80
48 hours	NM	NM	7.72	7.75	7.75	7.77	7.80	7.80	7.83	7.83	7.88	7.89
96 hours	NM	NM	7.73	7.74	7.76	7.76	7.78	7.79	7.83	7.86	7.88	8.00
Temperature °C												
0 hours	26.2	26.3	26.1	26.1	26.4	26.3	26.4	26.2	26.1	26.3	26.3	26.3
48 hours	NM	NM	25.2	25.1	25.0	24.8	25.1	25.1	25.2	25.1	25.2	25.0
96 hours	NM	NM	25.1	25.3	25.4	25.2	25.8	25.7	24.9	25.6	25.6	25.8
Dissolved Oxygen, mg O_2/ℓ												
0 hours	7.34	7.41	7.06	7.41	7.16	7.25	7.48	7.44	7.37	7.72	7.32	7.40
48 hours	NM	NM	7.15	7.02	7.18	7.09	7.16	6.99	7.07	7.22	7.37	7.28
96 hours	NM	NM	7.00	6.98	7.11	7.05	7.22	6.79	6.98	7.12	7.06	7.15

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 hour acute
SPECIES: *Pimephales promelas*
TOXICANT: Oxygen (low)
NUMBER OF ORGANISMS PER AQUARIUM: 10
AVERAGE WEIGHT OF ORGANISMS, g: 0.34
FLOW RATE, ℓ/MIN: 0.2
SIZE OF AQUARIA, ℓ: 40
AVERAGE TEMPERATURE, °C: 25.9
AVERAGE pH: 7.8
AVERAGE mg NH_3-N/ℓ: No ammonia

BEGINNING DATE: August 17, 1982

ENDING DATE: August 21, 1982

LC50: 1.37 mg O_2/ℓ
95% CONFIDENCE LEVEL
UPPER: 1.47
LOWER: 1.27

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg O_2/ℓ	0.71	0.78	1.11	1.17	1.29	1.33	2.13	2.27	2.27	2.67	2.78	3.89
0.25												
0.5	30	30	10		10							
1	50	50	40	20								
2												
4	100	100	70	60	20	10						
8												
12												
16												
24			90	100	50	30						
36			100									
48												
60												
72												
96	100	100	100	100	50	30	0	0	0	0	0	0

Comments:

Test: 96 hour acute

Test species: *Pimephales promelas*

Date initiated: August 17, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
Un-ionized Ammonia, mg N/ℓ												
pH												
0 hours	7.78	7.77	7.78	7.78	7.78	7.78	7.78	7.79	7.77	7.78	7.80	7.78
96 hours	NM	NM	NM	NM	7.79	7.80	7.80	7.80	7.81	7.79	7.80	7.80
Temperature °C												
0 hours	25.6	25.5	25.6	25.7	25.2	25.3	25.5	25.7	25.6	25.5	25.6	25.8
48 hours	NM	NM	NM	NM	26.3	26.2	26.2	26.3	26.2	26.4	26.0	26.0
96 hours	NM	NM	NM	NM	26.0	26.1	26.3	26.1	25.9	26.1	26.2	26.1
Dissolved Oxygen, mg O_2/ℓ												
0 hours	0.71	0.78	1.12	1.29	1.31	1.45	2.25	2.28	2.27	2.66	2.71	3.98
12 hours	NM	NM	1.10	1.04	0.91	1.41	1.71	2.39	2.03	2.52	2.58	3.76
24 hours	NM	NM	NM	NM	1.32	1.40	2.03	2.25	2.50	2.55	2.80	3.70
48 hours	NM	NM	NM	NM	1.25	1.37	2.34	2.06	1.96	2.70	2.92	3.85
72 hours	NM	NM	NM	NM	1.41	1.43	2.29	2.14	2.58	2.75	2.88	4.15
96 hours	NM	NM	NM	NM	1.55	1.49	2.13	2.50	2.28	2.82	2.81	3.91

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 hour acute

SPECIES: Pimephales promelas

TOXICANT: Oxygen with constant NH_3

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.8

FLOW RATE, ℓ/MIN: 0.2

SIZE OF AQUARIA, ℓ: 40

AVERAGE TEMPERATURE, °C: 26.8

AVERAGE pH: 7.7

AVERAGE mg NH_3-N/ℓ: 0.38

BEGINNING DATE: July 11, 1982

ENDING DATE: July 15, 1982

LC50: 1.48 mg O_2/ℓ

95% CONFIDENCE LEVEL

UPPER: 1.61

LOWER: 1.37

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg O_2/ℓ	0.45	0.76	0.95	1.19	1.79	1.97	2.63	2.76	3.44	3.99	4.22	4.37
0.25												
0.5	100	20										
1		80										
2		100										
4												
8												
12			20	20								
16			50	40	10							
24			60	60		10						
36												
48			70	70								
60			90									
72				90								
96	100	100	90	100	10	10	0	0	0	0	0	0

Comments:

Test: 96 hour acute

Test species: <u>Pimephales promelas</u>

Date initiated: July 11, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	12.0	13.0	12.4	13.6	12.9	13.4	12.1	11.7	12.1	12.5	12.3	12.4
48 hours	NM	NM	13.2	13.5	14.7	13.8	16.1	12.3	13.7	15.7	12.4	12.3
96 hours	NM	NM	13.1	13.5	14.3	14.9	14.1	13.1	12.3	14.1	12.8	12.9
Un-ionized Ammonia, mg N/ℓ												
0 hours	0.35	0.43	0.39	0.43	0.40	0.43	0.35	0.35	0.36	0.38	0.40	0.39
48 hours	NM	NM	0.38	0.36	0.40	0.37	0.38	0.31	0.30	0.33	0.32	0.30
96 hours	NM	NM	0.43	0.44	0.43	0.49	0.41	0.38	0.35	0.35	0.37	0.35
pH												
0 hours	7.68	7.72	7.71	7.72	7.70	7.72	7.68	7.68	7.70	7.69	7.72	7.71
48 hours	NM	NM	7.69	7.67	7.67	7.67	7.61	7.64	7.56	7.56	7.65	7.61
96 hours	NM	NM	7.71	7.70	7.67	7.70	7.64	7.65	7.64	7.58	7.65	7.62
Temperature °C												
0 hours	26.6	27.0	26.9	26.8	26.9	26.9	26.7	26.7	26.3	26.8	26.8	27.0
48 hours	NM	NM	25.9	25.8	26.0	25.7	25.7	25.7	26.3	25.8	25.7	26.1
96 hours	NM	NM	27.5	27.6	27.4	27.5	27.6	27.5	27.7	27.5	27.5	27.5
Dissolved Oxygen, mg O_2/ℓ												
0 hours	0.45	0.76	0.93	1.06	1.45	1.66	2.29	2.35	3.25	2.85	4.07	4.05
24 hours	NM	NM	0.85	1.03	1.60	1.66	2.25	2.30	3.10	3.49	3.77	3.76
48 hours	NM	NM	0.92	1.05	1.83	2.12	2.55	2.63	3.52	4.02	4.12	4.43
96 hours	NM	NM	1.09	1.60	2.29	2.45	3.43	3.77	3.90	4.59	4.90	5.23

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 hour acute

SPECIES: Pimephales promelas

TOXICANT: Oxygen with constant NH_3

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.4

FLOW RATE, ℓ/MIN: 0.2

SIZE OF AQUARIA, ℓ: 40

AVERAGE TEMPERATURE, °C: 25.8

AVERAGE pH: 7.8

AVERAGE mg NH_3-N/ℓ: 0.71

BEGINNING DATE: September 28, 1982

ENDING DATE: October 2, 1982

LC50: 2.52 mg O_2/ℓ

95% CONFIDENCE LEVEL

UPPER: 2.82

LOWER: 2.26

Cumulative Percent Mortality

Observation Time, Hr. \ Toxicant Concn mg O_2/ℓ	0.74	0.81	1.11	1.19	1.79	2.24	3.36	3.43	4.79	5.06	6.55	6.75
0.25		10										
0.5	30	40										
1	90	80			10							
2	100	100	40	30								
4				60		10						
8			70		20							
12				80								
16				100								
24			100		40	30	10					
36					60							
48						40	20					
60					100							
72						50						
96	100	100	100	100	100	60	20	0	0	0	0	0

Comments:

Test: 96 hour acute

Test species: Pimephales promelas

Date initiated: September 28, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	21.7	21.6	20.4	22.0	21.2	22.3	20.8	21.6	21.8	22.2	20.2	20.5
48 hours	19.7	18.8	21.2	18.6	19.1	20.0	20.3	18.8	20.4	20.8	19.9	19.6
96 hours	21.0	22.6	22.0	22.1	21.5	20.3	19.9	22.3	23.0	19.7	22.4	21.7
Un-ionized Ammonia, mg N/ℓ												
0 hours	0.74	0.73	0.69	0.76	0.74	0.77	0.72	0.72	0.73	0.75	0.70	0.71
48 hours	0.64	0.61	0.68	0.62	0.63	0.65	0.66	0.61	0.65	0.69	0.66	0.63
96 hours	0.72	0.79	0.76	0.76	0.74	0.71	0.69	0.76	0.78	0.69	0.77	0.73
pH												
0 hours	7.78	7.77	7.77	7.78	7.78	7.77	7.78	7.77	7.77	7.77	7.78	7.77
48 hours	7.76	7.76	7.75	7.77	7.77	7.76	7.76	7.76	7.75	7.76	7.77	7.76
96 hours	7.77	7.78	7.77	7.77	7.78	7.78	7.78	7.78	7.77	7.77	7.77	7.77
Temperature °C												
0 hours	25.8	25.9	25.9	25.8	25.9	26.0	25.9	25.7	25.8	25.7	25.9	26.0
48 hours	25.6	25.4	25.6	25.6	25.5	25.7	25.5	25.4	25.6	25.8	25.6	25.6
96 hours	25.9	26.0	26.1	26.1	25.9	26.0	25.9	25.7	25.9	26.1	26.1	25.7
Dissolved Oxygen, mg O_2/ℓ												
0 hours	0.74	0.81	1.02	1.19	1.68	2.14	3.42	4.57	4.92	4.86	6.01	6.28
12 hours	NM	NM	1.26	1.14	1.72	2.43	3.09	3.25	5.01	5.29	6.26	6.57
24 hours	NM	NM	1.19	1.26	1.93	2.29	3.17	3.35	4.74	5.11	6.47	6.66
48 hours	NM	NM	NM	NM	1.82	2.08	3.52	3.68	4.62	5.30	6.80	7.22
72 hours	NM	NM	NM	NM	1.78	2.17	3.37	3.19	4.90	5.01	6.74	7.01
96 hours	NM	NM	NM	NM	NM	2.31	3.61	3.54	4.55	4.79	6.99	6.74

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 hour acute

SPECIES: <u>Pimephales promelas</u>

TOXICANT: Oxygen with constant NH_3

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.3

FLOW RATE, ℓ/MIN: 0.2

SIZE OF AQUARIA, ℓ: 40

AVERAGE TEMPERATURE, °C: 25.5

AVERAGE pH: 7.7

AVERAGE mg NH_3-N/ℓ: 1.14

BEGINNING DATE: July 28, 1982

ENDING DATE: August 1, 1982

LC50: 1.70 mg O_2/ℓ

95% CONFIDENCE LEVEL

UPPER: 1.89

LOWER: 1.53

Cumulative Percent Mortality

Observation Time, Hr. / Toxicant Concn mg O_2/ℓ	1.09	1.10	1.54	1.56	2.11	2.15	3.70	3.73	3.93	4.31	4.58	4.86
0.25												
0.5												
1												
2	10											
4	70	100										
8	100		30	10							10	
12												
16												
24			40	20								
36					10							
48			50	30			10	10				
60			60	50								
72			70									
96	100	100	70	50	10	0	10	10	0	0	10	0

Comments:

Test: 96 hour acute

Test species: Pimephales promelas

Date initiated: July 28, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	40.9	42.2	44.3	42.0	44.7	41.5	40.3	39.9	38.6	40.3	43.2	41.7
48 hours	NM	NM	39.9	43.3	37.3	43.1	42.7	43.1	42.3	42.5	41.3	41.9
96 hours	NM	NM	36.8	35.6	37.6	36.0	38.3	37.1	36.3	35.4	35.7	36.8
Un-ionized Ammonia, mg N/ℓ												
0 hours	0.98	1.04	1.20	1.14	1.17	1.14	1.02	1.05	0.90	0.87	1.01	0.91
48 hours	NM	NM	1.15	1.43	1.14	1.37	1.12	1.13	1.07	1.18	1.15	1.11
96 hours	NM	NM	1.16	1.34	1.34	1.03	1.38	1.35	1.03	1.11	1.18	1.28
pH												
0 hours	7.68	7.70	7.73	7.74	7.73	7.74	7.71	7.72	7.66	7.64	7.67	7.64
48 hours	NM	NM	7.70	7.75	7.72	7.73	7.65	7.65	7.64	7.67	7.68	7.65
96 hours	NM	NM	7.71	7.77	7.75	7.66	7.76	7.77	7.66	7.70	7.72	7.75
Temperature °C												
0 hours	23.7	23.7	24.0	23.7	23.5	23.7	23.6	23.7	24.0	23.6	23.8	23.5
48 hours	NM	NM	25.7	26.3	26.1	26.2	26.2	26.2	25.8	26.2	26.1	26.1
96 hours	NM	NM	26.9	27.3	27.1	27.1	27.0	27.0	26.8	27.0	27.2	27.1
Dissolved Oxygen, mg O_2/ℓ												
0 hours	1.09	1.10	1.86	1.81	2.29	2.47	3.90	3.86	4.90	4.20	5.13	4.72
12 hours	NM	NM	1.55	1.41	1.93	1.91	3.59	3.64	4.67	4.06	4.88	4.55
24 hours	NM	NM	1.40	1.42	2.01	2.06	3.75	3.69	4.56	3.89	4.93	4.45
48 hours	NM	NM	1.45	1.66	2.25	2.30	3.48	3.60	4.03	3.77	4.33	3.97
72 hours	NM	NM	1.45	1.33	1.88	1.88	3.81	3.86	3.56	3.70	4.92	4.73
96 hours	NM	NM	1.62	1.59	2.31	2.26	3.82	3.55	4.11	3.95	4.98	5.03

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 hour acute
SPECIES: Pimephales promelas
TOXICANT: oxygen with constant NH_3
NUMBER OF ORGANISMS PER AQUARIUM: 10
AVERAGE WEIGHT OF ORGANISMS, g: 0.5
FLOW RATE, ℓ/MIN: 0.2
SIZE OF AQUARIA, ℓ: 40
AVERAGE TEMPERATURE, °C: 27.5
AVERAGE pH: 7.8
AVERAGE mg NH_3-N/ℓ: 1.23

BEGINNING DATE: April 7, 1982
ENDING DATE: April 11, 1982
LC50: 4.05 mg O_2/ℓ
95% CONFIDENCE LEVEL
UPPER: 4.80
LOWER: 3.43

Cumulative Percent Mortality

Observation Time, Hr. \ Toxicant Concn mg O_2/ℓ	1.10	1.13	1.17	1.45	1.48	1.48	2.20	2.32	2.68	2.72	3.15	3.33
0.25												
0.5												
1												
2												
4												
8												
12												
16												
24	30	10	40	10		10	10		10			10
36												
48	100	50	60	40	20	80	40	30	30	30	20	
60												
72		100	100	100	100	100	50	70	30	50		30
96	100	100	100	100	100	100	50	70	30	50	50	30

Comments:

Test: 96 hour acute

Test species: Pimephales promelas

Date initiated: April 7, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	32.8	32.5	32.9	32.9	33.2	33.2	33.5	32.1	33.1	33.4	32.8	32.7
48 hours	31.6	31.6	31.6	31.2	31.6	31.2	31.3	31.3	30.9	31.2	32.0	31.4
96 hours	30.8	31.5	30.8	30.3	31.2	31.5	31.2	30.6	31.4	30.5	31.5	31.2
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.24	1.19	1.22	1.26	1.27	1.22	1.27	1.21	1.26	1.27	1.24	1.24
48 hours	1.29	1.20	1.30	1.33	1.25	1.18	1.20	1.19	1.18	1.19	1.07	1.08
96 hours	1.21	1.21	1.20	1.18	1.26	1.28	1.22	1.25	1.27	1.22	1.26	1.25
pH												
0 hours	7.77	7.77	7.77	7.77	7.77	7.76	7.78	7.76	7.77	7.77	7.77	7.77
48 hours	7.81	7.79	7.82	7.83	7.79	7.77	7.79	7.77	7.77	7.77	7.71	7.73
96 hours	7.79	7.78	7.78	7.78	7.79	7.79	7.79	7.79	7.79	7.78	7.78	7.78
Temperature °C												
0 hours	27.6	27.1	27.3	27.5	27.6	27.3	27.3	27.7	27.5	27.6	27.6	27.5
48 hours	27.3	26.9	27.0	27.3	27.5	27.4	27.2	27.5	27.5	27.6	27.6	27.5
96 hours	27.5	27.4	27.5	27.5	27.9	27.8	27.7	28.0	27.8	28.1	28.0	28.0
Dissolved Oxygen, mg O_2/ℓ												
0 hours	1.00	1.10	1.10	1.50	1.55	1.50	2.50	2.20	2.90	2.80	3.30	3.60
48 hours	1.00	1.10	1.15	1.40	1.45	1.40	2.40	2.30	2.60	2.70	3.05	3.20
96 hours	1.30	1.20	1.25	1.45	1.45	1.55	2.05	2.10	2.55	2.65	3.10	3.20

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 hour acute

SPECIES: Pimephales promelas

TOXICANT: Oxygen with constant NH_3

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.3

FLOW RATE, ℓ/MIN: 0.2

SIZE OF AQUARIA, ℓ: 40

AVERAGE TEMPERATURE, °C: 26.1

AVERAGE pH: 7.7

AVERAGE mg NH_3-N/ℓ: 1.25

BEGINNING DATE: August 24, 1982

ENDING DATE: August 28, 1982

LC50: 3.54 mg O_2/ℓ

95% CONFIDENCE LEVEL

UPPER: 4.17

LOWER: 3.00

Cumulative Percent Mortality

Observation Time, Hr. \ Toxicant Concn mg O_2/ℓ	0.85	0.92	1.25	1.29	2.76	2.93	3.50	3.62	4.07	4.02	5.16	5.27
0.25	10		10									
0.5	50	60	30	20								
1	100	100	60	50	20			10				
2			90	90		10						
4			100	100						10		10
8							10	20				
12					40							
16						20	20				10	
24												20
36						40		30				
48						50	30		20			
60					60			40				
72						70	50					
96	100	100	100	100	60	70	60	40	20	10	10	20

Comments:

Test: 96 hour acute

Test species: Pimephales promelas

Date initiated: August 24, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	39.9	38.4	39.5	37.8	37.3	37.9	40.9	40.0	35.3	40.2	38.6	39.1
48 hours	36.2	36.0	38.1	36.0	37.0	36.0	35.9	36.9	36.0	35.5	36.0	37.2
96 hours	40.8	42.1	42.7	41.0	40.8	42.7	43.3	43.7	41.9	44.3	42.9	43.9
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.28	1.27	1.28	1.25	1.24	1.28	1.32	1.30	1.25	1.34	1.32	1.29
48 hours	1.14	1.14	1.19	1.17	1.16	1.18	1.21	1.28	1.18	1.17	1.13	1.15
96 hours	1.22	1.27	1.26	1.27	1.24	1.27	1.30	1.35	1.25	1.32	1.27	1.28
pH												
0 hours	7.72	7.74	7.73	7.74	7.73	7.74	7.72	7.73	7.76	7.75	7.75	7.74
48 hours	7.73	7.73	7.72	7.74	7.73	7.74	7.75	7.74	7.74	7.75	7.73	7.72
96 hours	7.72	7.73	7.72	7.74	7.73	7.72	7.72	7.74	7.71	7.73	7.72	7.72
Temperature °C												
0 hours	26.6	26.6	26.7	26.5	26.8	26.7	26.6	26.6	26.8	26.4	26.7	26.5
48 hours	26.2	26.3	26.2	26.3	26.1	26.4	26.4	26.3	26.3	26.1	26.2	26.2
96 hours	25.8	25.5	25.4	25.6	25.6	25.6	25.6	25.4	25.8	25.2	25.5	25.4
Dissolved Oxygen, mg O_2/ℓ												
0 hours	0.85	0.92	1.25	1.29	2.66	2.68	3.41	3.48	4.04	3.95	5.50	5.41
12 hours	NM	NM	NM	NM	3.01	2.97	3.39	3.40	4.16	4.09	5.19	5.38
24 hours	NM	NM	NM	NM	2.51	3.00	3.66	3.75	4.21	4.25	5.25	5.15
48 hours	NM	NM	NM	NM	2.78	2.79	3.80	3.67	4.05	4.00	4.98	5.29
72 hours	NM	NM	NM	NM	2.99	3.10	3.25	3.52	3.86	3.95	4.90	5.22
96 hours	NM	NM	NM	NM	2.65	3.04	3.48	3.89	4.11	3.88	5.11	5.19

PURDUE AQUATIC TOXICOLOGY LABORATORY

TEST: 96 hour acute

SPECIES: Pimephales promelas

TOXICANT: Oxygen with constant NH_3

NUMBER OF ORGANISMS PER AQUARIUM: 10

AVERAGE WEIGHT OF ORGANISMS, g: 0.5

FLOW RATE, ℓ/MIN: 0.2

SIZE OF AQUARIA, ℓ: 40

AVERAGE TEMPERATURE, °C: 25.6

AVERAGE pH: 7.7

AVERAGE mg NH_3-N/ℓ: 1.64

BEGINNING DATE: September 2, 1982

ENDING DATE: September 6, 1982

LC50: *

95% CONFIDENCE LEVEL

UPPER:

LOWER:

Cumulative Percent Mortality

Observation Time, Hr. \ Toxicant Concn mg O_2/ℓ	0.71	0.85	2.00	2.20	3.26	3.42	5.00	5.28	6.13	6.39	7.66	7.81
0.25	40	30	10									
0.5	100	100	30			10				10		
1			40	20	10			10				
2						30			10		10	10
4			60	40	20				20			
8				50		60		20		30		
12			90		70	70	40		30			30
16				90	90			30	40	40	20	
24			100	100		90	60		70	50		
36						100	70					
48								60	80	60	30	50
60					100							
72							90			70		
96	100	100	100	100	100	100	100	80	90	80	40	70

Comments: * LC50 not calculable

Test: 96 hour acute

Test species: Pimephales promelas

Date initiated: September 2, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
0 hours	49.6	53.0	51.6	52.1	52.4	52.3	53.2	51.4	53.7	52.1	51.8	53.0
48 hours	53.6	54.8	54.8	55.3	54.8	53.3	55.5	53.8	54.5	52.6	56.0	54.3
96 hours	54.3	52.6	54.3	53.6	53.1	55.8	54.8	53.1	55.1	56.1	52.8	53.3
Un-ionized Ammonia, mg N/ℓ												
0 hours	1.60	1.48	1.54	1.70	1.62	1.63	1.64	1.41	1.58	1.59	1.52	1.66
48 hours	1.67	1.72	1.67	1.72	1.70	1.65	1.72	1.70	1.75	1.63	1.70	1.63
96 hours	1.62	1.70	1.69	1.63	1.60	1.67	1.64	1.70	1.64	1.66	1.60	1.61
pH												
0 hours	7.76	7.69	7.72	7.75	7.75	7.75	7.74	7.69	7.73	7.74	7.73	7.76
48 hours	7.73	7.72	7.72	7.73	7.73	7.73	7.72	7.74	7.74	7.74	7.72	7.73
96 hours	7.73	7.75	7.74	7.74	7.73	7.73	7.73	7.75	7.73	7.72	7.73	7.74
Temperature °C												
0 hours	25.5	25.5	25.6	25.5	25.4	25.4	25.4	25.5	25.2	25.3	25.1	25.0
48 hours	26.0	26.3	25.8	25.8	25.9	25.8	26.1	26.0	26.0	25.7	25.8	25.4
96 hours	25.2	25.8	25.6	25.3	25.3	25.4	25.4	25.8	25.2	25.4	25.4	25.1
Dissolved Oxygen, mg O_2/ℓ												
0 hours	0.71	0.85	2.11	2.25	3.48	3.91	4.86	5.06	6.03	6.11	7.86	7.84
12 hours	NM	NM	2.31	1.98	3.13	3.25	4.99	5.41	5.85	6.72	7.73	7.92
24 hours	NM	NM	2.18	1.77	3.19	3.44	5.02	5.27	6.41	6.37	7.77	7.63
48 hours	NM	NM	NM	NM	3.22	3.08	4.77	5.22	6.28	6.24	7.49	7.82
72 hours	NM	NM	NM	NM	NM	NM	5.13	5.36	6.11	6.34	7.61	7.85
96 hours	NM	NM	NM	NM	NM	NM	5.23	5.38	6.09	6.55	7.50	7.77

Test: 30 Day Growth

Test species: <u>Pimephales promelas</u>

Date initiated: November 10, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
Nov. 10	32.2	32.9	14.7	14.1	8.5	8.1	4.1	4.2	3.0	3.0	ND	ND
Nov. 15	31.1	27.5	13.2	12.6	7.8	7.4	3.2	2.2	0.8	0.7	ND	ND
Nov. 19	NM	NM	13.8	13.6	7.7	7.6	3.0	2.9	1.2	1.3	ND	ND
Un-ionized Ammonia, mg N/ℓ												
Nov. 10	1.45	1.50	0.70	0.67	0.44	0.43	0.23	0.24	0.20	0.21	ND	ND
Nov. 15	1.33	1.52	0.71	0.64	0.41	0.46	0.19	0.12	0.06	0.05	ND	ND
Nov. 19	NM	NM	0.41	0.44	0.26	0.26	0.10	0.09	0.05	0.06	ND	ND
pH												
Nov. 10	7.92	7.93	7.93	7.93	7.97	7.99	8.01	8.03	8.09	8.11	8.13	8.08
Nov. 15	7.93	8.05	7.99	8.01	8.03	8.13	8.09	8.06	8.17	8.14	8.22	8.22
Nov. 19	NM	NM	7.72	7.76	7.77	7.78	7.78	7.75	7.88	7.91	8.01	8.03
Temperature °C												
Nov. 10	25.2	25.2	25.6	25.6	25.6	25.3	25.4	25.4	25.5	25.4	25.3	25.4
Nov. 15	23.9	23.8	23.9	23.9	23.8	23.2	23.8	23.9	24.2	23.8	23.6	23.6
Nov. 19	NM	NM	25.6	25.6	25.5	25.4	25.5	25.4	25.6	25.5	25.5	25.6
Dissolved Oxygen, mg O_2/ℓ												
Nov. 10	7.80	7.85	7.66	7.69	7.32	7.77	7.63	7.77	7.96	7.96	7.91	7.85
Nov. 15	7.48	7.68	7.46	7.47	7.22	7.77	7.51	7.57	7.84	7.90	8.08	7.99
Nov. 19	NM	NM	6.74	6.23	6.55	6.81	7.03	6.32	6.95	6.18	6.94	6.76

Test: 30 Day Growth

Test species: Pimephales promelas

Date initiated: November 10, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
Nov. 23	NM	NM	12.3	12.3	5.8	6.4	2.7	2.6	1.2	1.2	ND	ND
Nov. 26	NM	NM	11.5	11.4	6.8	8.5	2.9	3.2	1.3	1.5	ND	ND
Nov. 30	NM	NM	12.2	11.8	6.9	7.8	3.4	2.7	1.2	1.5	ND	ND
Un-ionized Ammonia, mg N/ℓ												
Nov. 23	NM	NM	0.35	0.32	0.21	0.20	0.08	0.07	0.05	0.06	ND	ND
Nov. 26	NM	NM	0.32	0.29	0.26	0.21	0.07	0.08	0.06	0.07	ND	ND
Nov. 30	NM	NM	0.45	0.47	0.28	0.24	0.11	0.10	0.07	0.08	ND	ND
pH												
Nov. 23	NM	NM	7.74	7.70	7.85	7.77	7.74	7.72	7.90	7.97	7.98	7.99
Nov. 26	NM	NM	7.68	7.66	7.80	7.62	7.64	7.63	7.91	7.93	8.01	8.00
Nov. 30	NM	NM	7.79	7.84	7.84	7.74	7.75	7.80	7.98	7.99	8.08	8.06
Temperature °C												
Nov. 23	NM	NM	24.4	24.5	24.4	24.4	24.6	24.5	24.8	24.5	24.6	24.4
Nov. 26	NM	NM	25.8	25.5	26.6	26.3	26.1	26.3	25.7	26.3	25.7	25.9
Nov. 30	NM	NM	26.4	26.1	26.3	25.5	26.2	26.3	26.3	26.0	25.8	25.9
Dissolved Oxygen, mg O_2/ℓ												
Nov. 23	NM	NM	6.98	6.48	6.59	6.71	6.94	7.03	6.96	6.89	6.72	6.83
Nov. 26	NM	NM	7.14	6.85	7.01	6.86	7.10	7.14	7.03	6.93	6.75	6.92
Nov. 30	NM	NM	6.93	6.96	6.96	7.07	7.03	7.06	6.78	6.81	7.00	6.91

Test: 30 Day Growth

Test species: Pimephales promelas

Date initiated: November 10, 1982

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
Dec. 3	NM	NM	13.0	12.5	7.8	7.2	3.0	3.1	1.5	1.3	ND	ND
Dec. 8	NM	NM	12.4	12.7	7.7	7.6	2.8	3.0	1.3	1.3	ND	ND
Dec. 15	NM	NM	13.2	12.4	6.6	6.9	3.5	3.3	1.2	1.3	ND	ND
Un-ionized Ammonia, mg N/ℓ												
Dec. 3	NM	NM	0.46	0.43	0.26	0.21	0.09	0.11	0.07	0.05	ND	ND
Dec. 8	NM	NM	0.37	0.37	0.21	0.21	0.08	0.09	0.05	0.05	ND	ND
Dec. 15	NM	NM	0.43	0.40	0.21	0.23	0.11	0.11	0.04	0.05	ND	ND
pH												
Dec. 3	NM	NM	7.76	7.77	7.74	7.71	7.71	7.80	7.92	7.83	8.17	8.14
Dec. 8	NM	NM	7.79	7.79	7.75	7.78	7.77	7.79	7.89	7.94	8.00	8.07
Dec. 15	NM	NM	7.79	7.78	7.78	7.79	7.79	7.81	7.82	7.87	8.01	8.03
Temperature °C												
Dec. 3	NM	NM	2.66	26.1	26.4	25.9	26.4	26.1	26.7	26.5	25.9	26.3
Dec. 8	NM	NM	23.2	23.1	23.2	22.7	23.2	23.7	23.4	23.3	23.3	22.8
Dec. 15	NM	NM	24.8	24.8	24.7	24.8	24.6	24.6	24.8	24.9	24.8	24.9
Dissolved Oxygen, mg O_2/ℓ												
Dec. 3	NM	NM	7.08	6.79	7.23	7.16	6.92	7.05	7.09	6.94	7.12	6.68
Dec. 8	NM	NM	6.76	6.49	7.01	6.99	6.76	6.65	7.03	6.98	6.71	7.05
Dec. 15	NM	NM	6.83	6.93	6.91	7.09	6.95	6.88	7.11	7.18	6.88	7.13

Test: Embryo-Larvae 30-day Growth

Test species: Channel catfish (Ictalurus punctatus)

Date initiated: July 25, 1980

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
July 25	21.9	21.0	12.4	12.4	6.3	5.9	2.7	3.1	1.3	1.5	ND	ND
July 28	19.9	20.0	11.7	11.5	6.2	5.7	2.7	3.1	1.3	1.1	ND	ND
July 30	19.6	19.5	11.5	11.3	5.8	5.2	2.5	2.5	1.1	1.0	ND	ND
Un-ionized Ammonia, mg N/ℓ												
July 25	0.65	0.53	0.25	0.23	0.13	0.12	0.06	0.08	0.04	0.04	ND	ND
July 28	0.74	0.72	0.39	0.40	0.23	0.20	0.11	0.14	0.07	0.05	ND	ND
July 30	0.53	0.48	0.28	0.29	0.14	0.14	0.07	0.08	0.04	0.03	ND	ND
pH												
July 25	7.69	7.62	7.52	7.48	7.54	7.54	7.55	7.63	7.67	7.66	7.72	7.60
July 28	7.81	7.79	7.76	7.77	7.81	7.78	7.85	7.89	7.95	7.87	7.88	7.85
July 30	7.74	7.70	7.68	7.70	7.69	7.73	7.78	7.80	7.88	7.81	7.84	7.86
Temperature °C												
July 25	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.9
July 28	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
July 30	23.6	23.6	24.0	23.8	23.6	23.5	23.6	23.6	23.7	23.5	23.6	23.6
Dissolved Oxygen, mg O_2/ℓ												
July 25	5.85	5.65	5.40	5.10	5.35	5.00	5.50	5.50	5.80	5.60	6.05	5.50
July 28	6.20	6.00	6.40	6.15	6.20	6.00	6.55	6.55	6.75	6.50	6.70	6.60
August 5	5.80	5.90	6.70	6.40	6.30	6.20	5.90	6.10	6.50	6.40	6.60	6.40

Test: Embryo-Larvae 30-day Growth

Test species: Channel catfish (Ictalurus punctatus)

Date initiated: July 25, 1980

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
August 1	18.0	17.9	11.5	10.8	5.6	5.2	2.4	2.4	1.1	1.1	ND	ND
August 5	19.8	20.0	12.7	12.2	6.5	5.8	2.5	2.6	1.1	1.1	ND	ND
August 13	23.5	20.2	11.6	11.7	5.8	6.3	2.6	2.9	0.9	1.2	ND	ND
Un-ionized Ammonia, mg N/ℓ												
August 1	0.50	0.45	0.37	0.38	0.16	0.14	0.08	0.08	0.04	0.04	ND	ND
August 5	0.54	0.54	0.38	0.34	0.19	0.17	0.08	0.08	0.05	0.04	ND	ND
August 13	0.86	0.77	0.45	0.43	0.24	0.23	0.11	0.12	0.04	0.05	ND	ND
pH												
August 1	7.63	7.59	7.69	7.65	7.63	7.73	7.71	7.79	7.76	7.76	7.81	7.77
August 5	7.61	7.60	7.65	7.62	7.64	7.64	7.67	7.67	7.80	7.73	7.81	7.77
August 13	7.74	7.76	7.77	7.74	7.79	7.74	7.82	7.81	7.82	7.83	7.87	7.87
Temperature °C												
August 1	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5
August 5	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0
August 13	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0
Dissolved Oxygen, mg O_2/ℓ												
August 10	4.80	5.10	4.80	4.70	5.10	4.90	5.00	5.40	4.90	5.30	5.30	5.20
August 11	3.10	3.20	3.30	4.10	3.80	3.60	3.90	3.50	3.60	4.20	4.00	3.90
August 14	5.90	6.10	6.50	6.40	6.70	6.40	6.20	6.50	6.60	6.50	6.70	6.50

Test: Embryo-Larvae 30-day Growth

Test species: Channel catfish (*Ictalurus punctatus*)

Date initiated: July 25, 1980

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
August 14	22.6	20.1	11.2	11.8	4.7	5.6	2.2	2.7	1.0	1.0	ND	ND
August 18	25.0	24.8	11.6	11.6	5.4	5.8	2.6	2.7	1.4	1.3	ND	ND
August 22	24.9	23.8	11.4	10.8	5.6	6.5	2.6	2.8	1.4	1.9	ND	ND
Un-ionized Ammonia, mg N/ℓ												
August 14	0.70	0.67	0.38	0.36	0.19	0.17	0.08	0.10	0.04	0.04	ND	ND
August 18	0.82	0.75	0.41	0.35	0.19	0.19	0.11	0.10	0.07	0.06	ND	ND
August 22	0.86	0.79	0.44	0.40	0.23	0.23	0.12	0.13	0.07	0.09	ND	ND
pH												
August 14	7.68	7.71	7.72	7.67	7.79	7.67	7.75	7.75	7.84	7.80	7.89	7.83
August 18	7.72	7.68	7.75	7.68	7.74	7.73	7.85	7.78	7.93	7.91	7.98	7.95
August 22	7.74	7.73	7.79	7.77	7.82	7.76	7.89	7.87	7.92	7.87	8.01	7.98
Temperature °C												
August 14	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5
August 18	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0
August 22	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0
Dissolved Oxygen, mg O_2/ℓ												
August 27	5.90	6.40	6.40	6.60	6.20	5.90	5.90	6.30	6.20	6.50	6.80	6.70

Test: Embryo-Larvae 30-day Growth

Test species: Channel catfish (*Ictalurus punctatus*)

Date initiated: July 25, 1980

Replicate Treatment Levels

	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Total Ammonia, mg N/ℓ												
August 27	24.4	22.4	11.5	10.7	5.9	6.8	2.6	3.3	1.8	2.2	ND	ND
Un-ionized Ammonia, mg N/ℓ												
August 27	0.91	0.83	0.54	0.45	0.32	0.31	0.14	0.18	0.12	0.15	ND	ND
pH												
August 27	7.76	7.76	7.87	7.82	7.93	7.86	7.94	7.95	8.04	8.04	8.14	8.11
Temperature °C												
August 27	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5
Dissolved Oxygen, mg O_2/ℓ												

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