The Toxicity of Ammonia to the Marine Organisms, Sheepshead Minnow (*Cyprinodon variegatus*), Mysid (*Mysidopsis bahia*), and Grass Shrimp (*Palaemonetes pugio*)

by

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

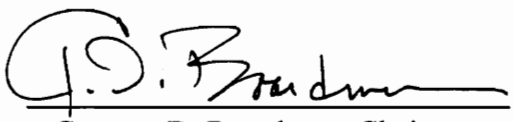
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In

Environmental Engineering

APPROVED:



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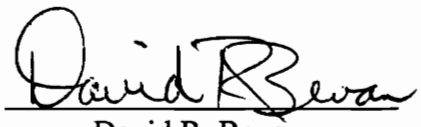

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July, 1996
Blacksburg, Virginia

Keywords: Ammonia, Marine, Toxicity, Chesapeake Bay, Bioassay

THE TOXICITY OF AMMONIA TO THE MARINE ORGANISMS, SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS*), MYSID (*MYSIDOPSIS BAHIA*), AND GRASS SHRIMP (*PALAEMONETES PUGIO*)

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Abstract

The discharge of wastewaters containing ammonia has become a major environmental concern, especially in the Chesapeake Bay watershed. A tentative instream limit of 1-2 mg/L for total ammonia has been proposed in Virginia to protect marine aquatic life. This limit was based upon the national chronic criterion for un-ionized ammonia (0.035 mg/L) which was calculated using both freshwater and saltwater toxicity data. Therefore, additional bioassays needed to be performed with marine organisms in order to refine this limit based solely on saltwater organisms.

Acute bioassays were conducted with one marine fish, the sheepshead minnow (*Cyprinodon variegatus*), and two marine invertebrates, the mysid (*Mysidopsis bahia*), and grass shrimp (*Palaemonetes pugio*). The acute LC_{50} and the no-observed-effect-concentration (NOEC) were determined for each organism and compared to data in the literature. The species mean acute values (SMAV) for the sheepshead minnow (13.5% difference) and the mysid (14.7% difference) compared well with the literature, while the grass shrimp was more tolerant (81.2% difference) to ammonia than expected. The SMAV were calculated based upon the LC_{50} data and used to assess both the national acute and chronic criterion for ammonia.

Seven day chronic bioassays were performed with the sheepshead minnow (*Cyprinodon variegatus*). The LC_{50} and the NOEC for both mortality and growth were calculated. The acute-chronic ratio was determined to be 6.95 for the sheepshead minnow and 4.35 for the mysid. Based upon these data, the refined national criteria for un-ionized ammonia was determined to be 0.054 mg/L. The instream limit can be set based upon the water pH, temperature, and salinity.

Acknowledgments

To begin with, I would like to thank my advisor, Dr. Gregory Boardman for his help and guidance during this research. I would also like to thank him for his good humor and his optimistic attitude, which made the research more enjoyable.

Thanks to the Commercial Fish and Shellfish Technologies (CFAST) Program for funding this research.

I must thank Dr. David Bevan and Dr. Robert Hoehn for serving on my committee and for their suggestions which enhanced this document.

I thank Olver Laboratories, Incorporated for allowing me to complete my research in their laboratory, and particularly, Lawrence Hoffman for his cooperation and suggestions in performing these experiments.

Thanks to Susan Mirlohi, Dirk Amtower, and Paul Jenkins for the training in toxicity tests and the friendship which we shared in the bioassay laboratory.

I would like to thank my roommates, Brian Lattimer and Charles Lattimer, for their support and friendship over the past two years.

Special thanks to Cathy Mills for all of her love and support and thanks to John and Allison Dyer for making me feel like part of the family.

Finally, I would like to thank my parents, Dr. and Mrs. H. Bland Hudgins, for their love over the first 26 years of my life. They have always inspired me to do my best.

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Introduction

1.1 Motivation

The marine environment is a sensitive ecosystem. Each year, human activities release both natural and synthetic chemicals into this environment. The population growth along the nation's coastline has constantly increased the volume of these chemicals. In order to protect these marine systems, effluent standards for specific chemicals must be adopted. These effluent standards are based predominantly upon toxicity data for the organisms that inhabit the marine ecosytems. One such effluent standard is the national criteria set for the discharge of ammonia into saltwater. However, a literature review revealed that although the criteria were developed based upon some saltwater toxicity data, freshwater toxicity data were also used to set the criteria. Therefore, in order to establish more appropriate standards, more ammonia toxicity research with saltwater organisms is needed.

This section will introduce the reader to the sources of ammonia, the potential criteria for Virginia's Chesapeake Bay watershed, and the toxicity tests which are necessary to determine if these potential criteria are valid. These topics will be covered as follows:

- Sources of ammonia
- Potential ammonia limits for the Chesapeake Bay

1.1.1 Sources of Ammonia

Ammonia is a ubiquitous constituent of the soil, the atmosphere, and the waters of the earth. Ammonia is present in most waters as a normal biological degradation product of

proteins, although the concentrations may be very small and subsequent conversion to nitrate (nitrification) may take place. For the most part, the sources and sinks of ammonia balance one another, causing few toxic effects. However, anthropogenic inputs of ammonia can have considerable impacts on the aquatic environment. This section details the major sources of ammonia from human activities. These sources are separated into two categories:

1. Point Sources
2. Nonpoint Sources

Due to the increased population along the nation's waterways and coastline and the resulting increase in industrial activity, there has been an increased potential in the amount of pollutants which can be discharged to surface waters. This trend has also been observed in the discharge of ammonia from various effluents of industrial sites and municipalities. Aside from accidental discharges and spills of ammonia, the predominant sources are from industrial and municipal wastewaters. Typical values for untreated domestic wastewater are between 12 and 50 mg NH_3-N/L [1] and treated wastewater without nitrification will contain between 10 and 40 mg NH_3-N/L. Plants with nitrification/denitrification will typically have 60-90 percent removal of total ammonia resulting in anywhere between 2 and 30 mg NH_3-N/L being discharged to the receiving water. Some industries may discharge wastewaters containing total ammonia concentrations much greater than these, depending on their National Pollution Discharge Elimination System (NPDES) permit [2].

In areas dominated by agriculture, the ammonia input to surface waters is dominated by nonpoint sources. This is predominantly due to nitrogen-rich fertilizers which are applied to crops and then are transported to surface waters during high periods of rain as runoff. This problem is intensified by improper agricultural practices such as overfertilization, cultivating up to the shoreline, and cultivating perpendicular to topographic lines. In

recent years much research has been done to understand the use of best management practices (BPMs) in reducing nonpoint source pollution from agriculture. Although still considered a problem, the levels of nutrients added to surface waters via agricultural inputs have decreased slightly in recent years through a cooperative effort between farmers and regulators.

1.1.2 Potential Ammonia Limits for the Chesapeake Bay

There are several point sources of ammonia in Virginia that until recently have gone unregulated. These point sources include seafood-processing companies whose wastewaters are characterized by low volume but high organic concentration. These companies process a variety of seafoods such as blue crab, shrimp, scallops, clams, and various types of fish. Due to the high concentration of proteins in many of these wastewaters, the total ammonia concentration can reach levels as high as 1000 mg/L but usually is around 200 mg/L [3]. Research has been conducted in the treatment of these wastewaters, especially crab-processing wastewaters, at Virginia Polytechnic Institute and State University and is detailed by McVeigh [3], Diz [4], and Wolfe [5]. Table 1.1 gives some characteristics of a wastewater produced by a crab-processing company. The data in Table 1.1 were taken from McVeigh.

Table 1.1 - Crab-processing wastewater characteristics [3].

Characteristic	Concentration (mg/L)
COD	20,000
TSS	1,200
TKN	2,200
Total NH_3-N	200

The Virginia Department of Environmental Quality (DEQ) has set a tentative limit for the Chesapeake Bay watershed of approximately 1-2 mg/L for total ammonia based upon the National Criteria for Ammonia [6]. This limit is referred to as the Final Chronic Value (FCV). The criteria document states that the saltwater aquatic organisms should not be affected unacceptably if the four-day average concentration of ammonia does not exceed the FCV more than once every three years. The FCV is based upon both acute and chronic toxicity data. This limit is still being debated and, at the time of this report, was not officially adopted by DEQ. The seafood-processing companies maintain that this chronic criterion is unreasonable since some of the toxicity data used to determine this limit were based upon tests involving freshwater organisms.

The toxicity data presented in this report were collected in order to reevaluate this limit based solely on saltwater ammonia toxicity data. The toxicity tests that are necessary to assess this limit are 48-hour and 96-hour acute tests and 7-day chronic tests with both vertebrates and invertebrates. This report is a first attempt at reevaluating the limit to determine if the limit set by DEQ is too stringent or possibly too lenient. More research is being conducted at Virginia Polytechnic Institute and State University with more saltwater organisms to further refine the limit.

1.2 Research Overview

Based upon the existing ammonia toxicity data for saltwater species, both acute and chronic toxicity tests need to be performed with both previously tested organisms and new species. The objectives of this research were threefold:

- Conduct acute bioassays with previously tested organisms to verify the data in the literature and to gain confidence in ammonia toxicity evaluation techniques.
- Expand the chronic toxicity data base and determine if the 7-day chronic test is useful for assessing the toxicity of ammonia to saltwater fishes.

- Use acute and chronic toxicity data from this research to refine the ammonia national criteria for both final acute values and final chronic values.

These three objectives are discussed in the following two sections:

1.2.1 Acute Toxicity

In order to establish limits for ammonia to protect aquatic life, acute toxicity bioassays need to be conducted. This research concentrated on two invertebrates and one fish. A set of conditions was chosen on the basis of average summer conditions in the Chesapeake Bay and the recommended temperature for warmwater species as described by "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms [7]. These conditions were 20°C, pH 8.2, and a salinity of 25 ppt.

The two invertebrates tested were the mysid (*Mysidopsis bahia*) and the grass shrimp (*Palaemonetes pugio*). The marine fish was the sheepshead minnow (*Cyprinodon variegatus*). The mysid and the sheepshead minnow were two of the most commonly tested saltwater organisms. There are data on the toxicity of ammonia for these two species, but this research concentrated on verifying the ammonia toxicity data under the conditions stated above. Both 48-hour static nonrenewal tests and 96-hour static renewal tests were conducted with larval sheepshead minnows and juvenile mysids. The results from these tests included the lethal concentration for 50 percent mortality (LC_{50}) and the no-observed-effect concentration (NOEC). The LC_{50} values from both the 48-hour and 96-hour tests were used later to help update the final acute value and the final chronic value for ammonia.

The grass shrimp was tested only under 48-hour toxicity conditions because the literature indicated that there was substantial toxicity data for the grass shrimp under 96-hour renewal conditions. The 48-hour LC_{50} and NOEC were determined based upon these tests and compared with data in the literature. The 48-hour LC_{50} for the grass shrimp was

used to help recalculate the final acute value based upon the national criteria as described in "Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989 [6].

1.2.2 Chronic Toxicity

Until this report, the chronic toxicity of ammonia had been assessed only with two saltwater organisms, the mysid (*Mysidopsis bahia*) and the inland silverside (*Menidia beryllina*). Research was conducted to expand the chronic toxicity database for ammonia. Also, since the inland silverside test was a 28-day test, the 7-day chronic test was chosen to determine if a more time efficient test could be used to assess the chronic toxicity of ammonia.

This research assessed the 7-day chronic toxicity of ammonia to the sheepshead minnow (*Cyprinodon variegatus*). The same average conditions as in the acute testing (pH 8.2 and 25 ppt salinity) were used, except for the temperature which was increased to 25°C as recommended in "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms [8]. Four tests for a total of 160 sheepshead minnows were exposed to five concentrations of total ammonia ranging from 10 to 50 mg/L. Results from these tests included the 7-day LC_{50}, NOEC based upon mortality, and NOEC based upon growth. The NOEC based upon growth was used along with the sheepshead minnow acute LC_{50} to determine an acute-chronic ratio. This acute-chronic ratio and the final acute value determined from the acute toxicity research were used later in assessing an updated final chronic value according to "Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989 [6].

2

Overview of Ammonia in the Aquatic Environment

As previously mentioned, ammonia is a by-product of most living creatures. In a healthy ecosystem, the rate of production of ammonia released into the system will equal the rate of uptake or conversion of ammonia into other forms. This balance can be disturbed, especially in an aquatic system, through the addition of ammonia from anthropogenic sources. Therefore, in order to understand the impacts of these sources of ammonia, the interaction of ammonia in the aquatic environment must be studied.

Once ammonia is released into a water environment, various chemical and biological interactions occur. These interactions have a direct impact on the conversion of ammonia to nitrate as well as the toxicity incurred on the organisms exposed to the ammonia. The study of these chemical and biological processes along with the toxicity data attained from toxicity experimentation allow for conservative estimates for ammonia discharge into these aquatic systems. These estimates are then used to set ammonia discharge limits for industries and Public Owned Treatment Works (POTW) based upon various parameters such as discharge volume, receiving stream or body, sensitivity of receiving stream or body, etc.

This chapter describes the primary biological and chemical interactions which affect the toxicity of ammonia to marine organisms as well as the process which is used to determine each dischargers Virginia Pollution Discharge Elimination System (VPDES) permit. These topics are covered in four sections:

2.1. The Nitrogen Cycle

2.2. Water Chemistry of Ammonia

2.3. Ammonia Toxicity

2.4. Ammonia Discharge Regulations

2.1 The Nitrogen Cycle

The nitrogen cycle is necessary for living organisms to assimilate proteins and nucleic acids. This cycle involves the following six processes which occur in aquatic environments as well as terrestrial environments:

- nitrogen fixation
- assimilation and biosynthesis
- decomposition
- ammonification
- nitrification
- denitrification

Each of these processes either directly or indirectly affects the concentration of free ammonia which is present in the aquatic environment.

In nitrogen fixation, a few kinds of bacteria convert N_2 to ammonia (NH_3) through a series of reduction reactions. In aquatic ecosystems, *Anabaena*, *Nostoc*, and other cyanobacteria are dominant nitrogen fixers. The ammonia is used by these nitrogen fixers to assimilate amino acids, proteins, and nucleic acids. This process is known as assimilation and biosynthesis in which organic nitrogen is formed in plants and animals either from ammonia, nitrates, or atmospheric N_2. Then, once these plants and animals die, ammonia and other nitrogen-containing substances are released through decomposition via various decay-inducing bacteria and fungi [1].

In ammonification the nitrogenous wastes such as urea and the remains of plants and animals are decomposed by some species of bacteria and fungi. The decomposers use the proteins and amino acids being released for their own growth. They also release the excess as ammonia or ammonium. The ammonia or ammonium that becomes available is metabolized by nitrifying bacteria. It is this process which contributes the majority of

ammonia in most systems. The discharge from industry and POTW contains ammonia predominantly from the breakdown of wastes via ammonification [1].

In a process called nitrification, bacteria, known as *Nitrosomonas*, strip the NH_3 or NH_4^+ of electrons, and nitrite (NO_2^{-1}) is released as a product of the reaction. Still other nitrifying bacteria, known as *Nitrobacter*, then use nitrite for energy metabolism, which yields nitrate (NO_3^{-2}) as a product. It is the process of nitrification which ultimately oxidizes the excess ammonia that is discharged into the receiving stream or body. If high concentrations of ammonia are released to the aquatic environment, it is possible that ammonia concentrations will increase. It is these elevated concentrations which can inflict a toxic effect on aquatic organisms living in this out-of-balance system. Therefore, when considering ammonia toxicity problems in an aquatic system, nitrification is arguably the most important process of the nitrogen cycle [1].

The final process of the nitrogen cycle is denitrification. In this process fixed nitrogen such as nitrite and/or nitrate is reduced to N_2 and a small amount of nitrous oxide (N_2O). The nitrogen cycle in shown in Fig. 2.1 [1].

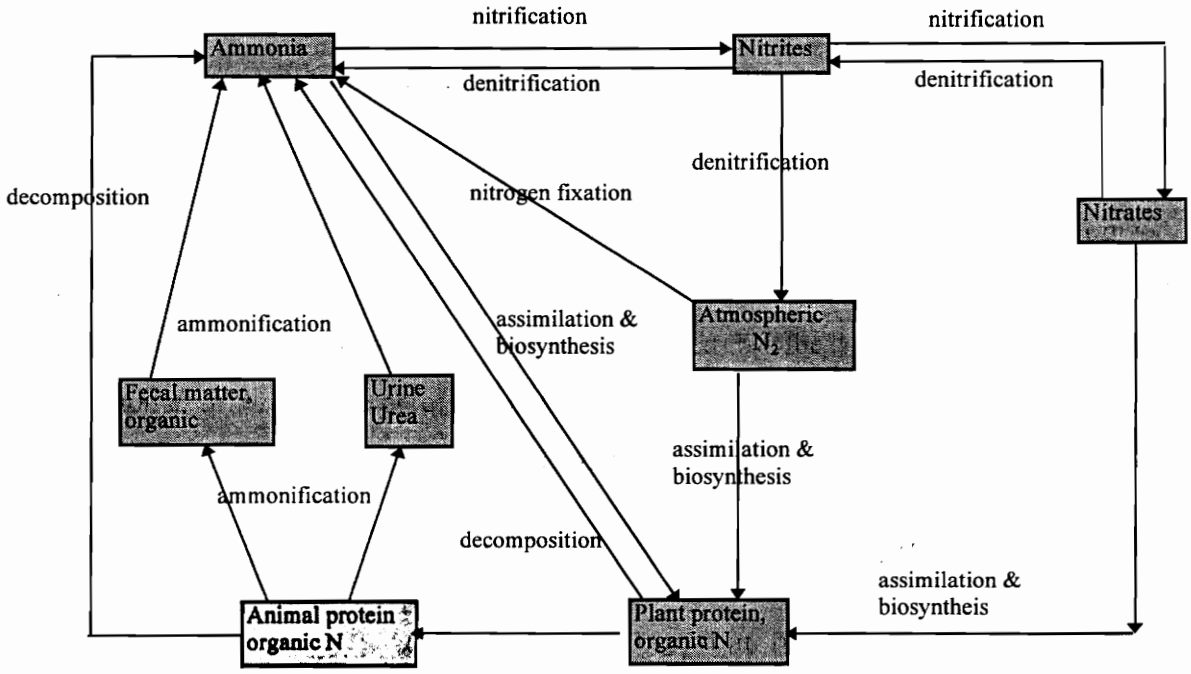

Figure 2.1 - Nitrogen cycle [1].

2.2 Water Chemistry of Ammonia

In discussing ammonia toxicity in the aquatic environment, one must consider the characteristics of the receiving water. Changes in water parameters such as pH, temperature, and salinity can alter the apparent toxicity to the aquatic life. These environmental factors affect the toxic concentration of ammonia to aquatic vertebrates and invertebrates and these factors may account for some of the inconsistencies shown in early data. These differences arise predominantly from the shifting of ambient concentrations of the un-ionized (NH_3) to the ionized (NH_4^+) or vice versa. In aqueous solution ammonia is present in two forms, un-ionized and ionized, represented by the equilibrium equation:

$$NH_4^+ + H_2O \Leftrightarrow NH_3 + H_3O^+ \qquad (2.1)$$

The shift between un-ionized and ionized ammonia given in equation 2.1 is governed by the ionization constant (25°C) given as followed:

$$K_a = \frac{[NH_3][H^+]}{[NH_4^+]} = 10^{-9.2} \qquad \textbf{(2.2)}$$

Initially research done by Wuhrmann and Woker [9] determined that the most toxic form is un-ionized ammonia because it is uncharged and lipid soluble, whereas the permeability of plasma membranes to charged, hydrated ammonium ions (NH_4^+) is relatively low [10; 11]. This indicates that the ammonium ion is toxic but that it is not absorbed in high enough concentrations to exhibit a toxic effect on either aquatic vertebrates or invertebrates. Thus, the concentration of un-ionized ammonia (NH_3) is the principal chemical species involved in ammonia toxicity, and several properties of water affect the concentration of un-ionized ammonia. Therefore, the percentage of ammonia in the un-ionized form is influenced greatly by characteristics or water chemistry of the receiving bay or stream [12]. This section will discuss the key water parameters and their influence on ammonia toxicity, including pH, temperature, salinity, carbon dioxide, dissolved oxygen, and alkalinity.

The relative proportions of NH_4^+ and NH_3 in solution depend chiefly on temperature and pH and, to a lesser extent, on salinity or ionic strength. Concentrations of un-ionized ammonia increase with elevated temperatures and pH values, and decrease with higher salinities [12].

2.2.1 Water pH

According to Equation 2.1, ammonia is present in two forms, NH_4^+ and NH_3, and the relative concentration of each species is determined by the concentration of hydrogen ion in solution. As pH increases, the hydrogen ion concentration decreases, and the equilibrium shift is toward the NH_3 species. Within the pH range acceptable to most

vertebrate and invertebrate species, increase of one pH unit will increase the NH_3 concentration by an order of magnitude.

As previously stated, NH_3 is the toxic form; however, to date no method for determining the un-ionized ammonia concentration has been developed. Therefore, the un-ionized ammonia is reported as a percentage of the total ammonia concentration which can be measured by several standard test methods outlined by the American Public Health Association *et al.* [13]. The percent un-ionized ammonia in freshwater is calculated from the measured value of total ammonia by using the equation:

$$\textbf{\% un-ionized ammonia} = 100 / 1 + \text{antilog}\,(pK_a - pH) \qquad \textbf{(2.3)}$$

where pK_a and pH are defined as the negative logarithm of the ionization constant and hydrogen ion concentration, respectively.

Thus, for example, an increase in pH from 7.0 to 7.3 would double the concentration of un-ionized ammonia [14]. The pK_a for ammonia at 20 °C is 9.4. Table 2.1 illustrates the relationship between pH and percent un-ionized ammonia as determined by Emerson *et al.* [15]:

Table 2.1 - Percent NH_3 in aqueous ammonia solution at 20 °C [15].

pH	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
% NH_3	0.04	0.13	0.40	1.24	3.82	11.2	28.4	55.4	79.9

Therefore, for a given total ammonia concentration, as the pH increases, the concentration of NH_3 increases, resulting in an increase in toxicity to aquatic life. The effects of a pH change in seawater will be discussed in Section 2.2.3 of this report.

2.2.2 Water Temperature

Water temperature affects the concentration of un-ionized ammonia in aqueous solution. This arises because pK_a is a function of temperature. As the water temperature increases, the ionization constant increases or the pK_a decreases which increases the fraction of NH_3. Emerson *et al.* [15] determined that in the temperature range from 0 to 30 °C, pK_a can be determined by the following equation

$$pK_a = 0.09018 + 2729.92 / T \qquad (2.4)$$

Values for the ionization constant and percent NH_3 as a function of temperature are listed in Table 2.2.

Table 2.2 - Temperature dependence of percent NH_3 at pH 7 and 8, respectively.

Temp (°C)	pK_a	pH 7 % NH_3	pH 8 % NH_3
0	10.08	0.083	0.82
5	9.90	0.125	1.23
10	9.73	0.183	1.83
15	9.56	0.273	2.67
20	9.40	0.396	3.82
25	9.24	0.566	5.38
30	9.09	0.799	7.46

Therefore, according to Table 2.2, the toxicity of ammonia to aquatic life would be expected to increase with increasing water temperature for a constant total ammonia concentration. This trend would be due to the increasing concentration of un-ionized ammonia in solution. However, dated studies by Burrows [16] showed that at lower temperatures, un-ionized ammonia became markedly more toxic to Chinook salmon and

by Brown [17] suggested that at 3°C the threshold LC_{50} of un-ionized ammonia for rainbow trout is about half that at 10 °C. Both studies showed that below 10°C, the salmonids appeared to be more susceptible to ammonia poisoning. The authors suggested that the lower metabolism of the salmonids may be the cause for the increased vulnerability.

2.2.3 Water Salinity or Ionic Strength

The relative proportions of NH_3 and NH_4^+ in solution depend on salinity to a lesser degree than temperature and pH. As either the salinity or ionic strength increases, the un-ionized ammonia concentration decreases due to the decrease in the ionization constant. Table 2.3 lists the ionization constants for various dilutions of seawater at 20 °C and 1 atm pressure [12].

Table 2.3 - Mean values for pK_a in seawater at 20 °C and 1 atm pressure [12].

Molal ionic strength	Salinity (%)	pK_a	$\%NH_3$ (pH 8)
~0	0.0	9.40	3.82
0.4	20	9.46	3.41
0.5	25	9.48	3.19
0.6	30	9.49	3.12
0.75	36	9.51	2.98

From Table 2.3 it is expected that as the salinity of the aqueous solution increases, the toxicity should decrease due to the decreasing fraction of NH_3. Studies have shown that the toxicity of ammonia to rainbow trout decreases as salinity increases [14]. Alabaster *et al.* [18] determined the 24-hour LC_{50} for Atlantic salmon smolts to be 0.15 mg NH_3/L (un-ionized ammonia) in freshwater and 0.3 mg NH_3/L (un-ionized ammonia) in 30%

seawater. This increase in ammonia tolerance with increased salinity was also observed in mysids [19]. Both authors attributed this increased tolerance to reduced osmotic stress on the fish and the invertebrates. Both authors hypothesized that salinity increases the ammonia tolerance of some fishes and invertebrates by two mechanisms: salinity shifts the ammonia fraction towards the ionized form and also biochemically reduces the toxicity of the un-ionized form.

A detailed table of the percent un-ionized ammonia (NH_3) as a function of pH, temperature, and salinity is given in Appendix A.

2.2.4 Other Factors (Carbon Dioxide, Dissolved Oxygen, and Alkalinity)

Several other minor factors affect the un-ionized ammonia fraction and the toxicity of NH_3 to aquatic life. These include free carbon dioxide, dissolved oxygen, and alkalinity. However, to date, the influence that these parameters have on marine species has not been investigated.

Increasing the free carbon dioxide concentration, decreases the pH, thereby reducing the fraction of un-ionized ammonia. Therefore, free carbon dioxide reduces the toxicity of ammonia. A reduction in the level of dissolved oxygen in the water increases the toxicity of ammonia to freshwater fishes. Alabaster *et al.* [18] showed that a reduction in the oxygen content of the water to 50 percent of the air saturation value reduced the survival times of several species of fish in lethal NH_3 concentrations. So far as is known, alkalinity affects the toxicity of ammonia only through its role in determining the pH value of the water in conjunction with the level of free carbon dioxide.

2.3 Routes of Exposure and Mechanisms of Ammonia Toxicity

In the previous section, the toxicity of ammonia was mentioned in order to illustrate the influence of water characteristics on ammonia. However, little information was given on

the mechanisms of un-ionized ammonia toxicity such as absorption, metabolism, or excretion. This section will begin to investigate these mechanisms as well as routes of exposure and toxicity indicators. These subjects will be covered as follows:

1. Routes of Entry, Absorption, and Excretion
2. Modes of Action and Mechanisms of Toxicity
3. Indicators of Toxicity

2.3.1 Routes of Entry, Absorption, and Excretion

In order for ammonia to be toxic to an aquatic organism, it must first gain entry into the organism or on a molecular level, it must pass through the cell membrane into the cytoplasm and mitochondria. The predominant route of entry for ammonia is at the gills for both vertebrates and invertebrates. Once again, the un-ionized ammonia (NH_3) rather than the ionic (NH_4^+) is the form which can pass readily through cell membranes at the gill surface. This neutral form is readily soluble in the lipid segments of the membrane, and apparently needs no active transport (diffusion only), while the ionic form occurs as a larger hydrated and charged molecule which cannot readily pass through the charged micropores of the hydrophobic membrane. Table 2.4 lists the diffusion coefficient of ammonia and ammonium ion for different cell types of various organisms [20].

Table 2.4 - Simple diffusion of ammonia and ammonium ions.

Compound	Diffusion Coeff. (cm^2/s)	Cell Type
NH_3	0.01	Human erythrocyte
	0.007	Human kidney cells
	0.006	Squid giant axon
NH_4^+	4.9×10^{-6}	Turtle bladder
	3.5×10^{-7}	Mouse hybridoma
	6.8×10^{-8}	Squid giant axon

As seen in Table 2.4, the diffusion coefficient for un-ionized ammonia is three to five orders of magnitude greater than the values of ionized ammonia. The diffusion coefficient was defined by Fick's first law of diffusion. Fick's first law of diffusion is:

$$J_e = -D\left(\frac{dC_e}{dx}\right) \qquad \textbf{(2.5)}$$

where J_e is the flow of solute (ammonia) in units of mass, or mole, per unit area per unit time, D is the diffusion coefficient of the solute in a particular solvent (water), and C_e is the concentration of solute. From equation 2.5 it can be seen that the diffusion coefficient is a measure of the rate that a specific solute migrates through a specific solvent. Therefore, as shown in Table 2.4, the rate of transport of the ammonium ion across most cell membranes is low. With such a low rate of transport, the NH_4^+ can never accumulate toxic concentrations of ammonia in the blood stream.

In most fishes and macroinvertebrates, most nitrogen is excreted directly as ammonia without any detoxicative metabolism. This potentially toxic product is rapidly eliminated by the gills of these organisms [21]. However, trace amounts are metabolized and excreted as either trimethylamine oxide, urea, uric acid, or creatine. The major process

thought to be responsible for this rapid excretion of ammonia is the nonionic diffusion of the un-ionized NH_3. Although this is thought to be the predominant mechanism of excretion, Goldstein *et al.* [21] has hypothesized that NH_4^+ crosses the gills via passive diffusion. They suggested that the gills of most fishes are slightly permeable to NH_4^+ which was also supported by Evans [22]. Since NH_4^+ concentrations in biological fluids are generally 10 to 100 times greater than NH_3 concentrations at most physiological pHs, even a relatively low permeability of NH_4^+ could have significant effects on the movement of ammonia across the gill cell membrane.

2.3.2 Modes of Action and Mechanisms of Toxicity

Once the un-ionized ammonia has entered the blood stream via diffusion through the cells at the gills, it is transported throughout the rest of the organism. Now that the NH_3 has entered the circulation system, it can exhibit its toxic effects on various organs. Upon examination of aquatic organisms exposed to lethal concentrations of ammonia, damage has been observed at the gills, kidney, liver, and blood. Although not all of the mechanisms of ammonia toxicity are known, it appears that ammonia affects each organ on the cellular level. However, it appears that once in the cell the un-ionized ammonia is not the primary toxicant since under approximate cellular pH of 6 the un-ionized form reverts to ionized ammonia.

On a cellular level, various modes of toxicity have been observed. Glacken [23] observed a disturbance of electrochemical gradients and intracellular pH changes in rainbow trout cell cultures. The disturbance of electrochemical gradients disrupts the transport of other nutrients across the cell membrane and the pH changes may lead to disturbance of proton gradients and inhibition of endocytosis and exocytosis as reported by Docherty and Snider [24]. Other studies by Glacken *et al.* [25] have observed the inhibition of enzymatic reactions with increased total ammonia concentration within the cell - e.g. the conversion of glutamate to α-ketoglutarate via glutamate dehydrogenase. This pathway

is used to degrade amino acids which produces ammonia. However, if ammonia concentrations within the cell are already elevated the glutamate dehydrogenase is inhibited. Also Martinelle and Haggstrom [20] have observed an increased demand for maintenance energy with elevated cellular ammonia concentrations. These effects have been observed at sub-lethal concentrations of ammonia as well as during chronic toxicity testing of ammonia to cell cultures.

The mechanisms for acute toxicity have been well documented. There are predominantly two mechanisms for acute toxicity in aquatic vertebrates and invertebrates.

1. The reduction of erythrocytes (red blood cells).

2. The increase in permeability of the organism to water.

The reduction of red blood cells interferes with the oxygen-carrying capacity of the blood. In cases of lethal acute toxicity testing the cause of death was asphyxia associated with the loss of red blood cells. Initially, when exposed to lethal concentrations of ammonia, fish exhibit signs of hyperexcitability and hyperventilation which is an indicator of the lack of oxygen to the brain. Another mechanism which is not completely understood is the increased permeability of the organism to water. This increase in water uptake is not directly attributed to death, but possibly accounts for the decrease in red blood cells. It also provides increased stress on the kidneys and affects the water balance of the organism. Lloyd and Orr [26] further suggested that any factor which affects the water balance in fish will also influence their susceptibility to ammonia poisoning.

The most studied mode of action for chronic ammonia toxicity is necrosis or decay of the gill tissue. This disease has most notably been documented in fish rearing ponds or tanks where the ammonia concentration in the pond and also the blood gradually increases due to nitrogen metabolism [2]. This autointoxication leads to toxic gill necrosis in a variety of fishes such as carp, trout, catfish, and largemouth bass.

2.3.3 Indicators of Acute and Chronic Toxicity

The physical symptoms of fish suffering from acute ammonia toxicity have been well documented, but little is available on these symptoms in invertebrate species. The first signs of ammonia toxicity in fish include a slight restlessness, and increased respiration; the fish congregate close to the water surface. In later stages, the fish gasp for air, their restlessness increases with rapid movements and respiration becomes irregular; then follows a stage of intense activity. Finally, the fish react violently to outside stimuli. Indicators of this stage are loss of balance, leaping out of the water, and muscle spasms seen in constant muscular twitches. Affected fish lie on their side and spasmodically open wide their mouths and gill opercula, which are the bony coverings protecting the gills. Then follows a short period of apparent recovery with a return to normal swimming. This stage is then replaced by another period of high activity; the body surface becomes pale and the fish die [2].

The skin of ammonia-poisoned fish is light in color and covered with a thick or excessive layer of mucus. In some cases small hemorrhages occur, mainly at the base of the pectoral fins and in the anterior part of the eye cavity. The gills are heavily congested and contain a considerable amount of mucus. Fish exposed to high ammonia concentrations may have slight to severe bleeding of the gills. The organs inside the body cavity are congested and show signs of degradation [2].

The predominant indicators for aquatic organisms exposed to chronic levels of ammonia are reduced weight gain and growth. These organisms exhibit varying degrees of gill necrosis depending on the concentration of un-ionized ammonia. Other invasive indicators are atrophy of the kidney and liver. However, within the literature there is conflicting evidence for the histopathology in gills, kidney and liver of fish exposed to ammonia concentrations which would be considered chronic levels.

2.4 Ammonia Discharge Regulations

2.4.1 Methods for Determining Discharge Limits

A national water quality criterion for ammonia and guidelines to derive 'site-specific' criteria [6] have been developed by the U.S. Environmental Protection Agency to protect aquatic life; these documents are intended to provide assistance in actuating statutory controls on the amount of ammonia released into the nation's surface waters. Both approaches are based on toxicological experiments with fish and aquatic invertebrates under laboratory test conditions [27]. The national criterion was derived from a large laboratory data base, whereas site-specific criteria are derived by subjecting representative species to laboratory acute tests with dilution water from a specific location. Both criteria have a two tiered structure:

1. a short-term concentration (1-hour average once every three years)
2. a long-term concentration (4-day average once every three years)

The 1-hour average is related to acute tests and the 4-day average is related to chronic tests. Each site must meet the ammonia criteria before the National Pollution Discharge Elimination System (NPDES) permit is granted.

The permit or regulation system in regard to ammonia is quite complicated and once again the criteria for ammonia are handled on a site-by-site basis with different criteria for freshwater and saltwater, cold and warmwater fishes, streams, lakes, and estuarine environments. The national criteria are based on un-ionized ammonia and the total ammonia discharged for each site is determined based on the 90th percentile pH and temperature, and for saltwater, the average salinity. Also upon appeal by the industry, the site-specific criteria may be established based upon indigenous species exposed to dilution water of the specific location. Table 2.5 lists the national criteria for un-ionized ammonia for salt and freshwater. The criteria listed in Table 2.5 state that the un-ionized

ammonia concentration can not exceed these limits more than once every three years on the average.

Table 2.5 - Un-ionized ammonia criteria.

Water Type	Fish Type	Un-ionized Ammonia (mg/L)	
		Acute	Chronic
Freshwater	Salmonids	0.160	0.0250
Freshwater	Cyprinids (Carp family)	unknown	0.050
Saltwater	All types	0.233	0.035

The acute criterion was developed based upon The Guidelines [6] and U.S. EPA 1985a [28] and used the 21 saltwater species which are listed in Chapter 3. The chronic criterion was developed based upon The Guidelines [6] and U.S. EPA 1985a [28]. The U.S. EPA 1985a uses acute-chronic ratios for freshwater species to derive the freshwater chronic criterion for ammonia; this document was used to establish saltwater chronic criterion for ammonia. The chronic criterion was based upon freshwater species as well as the two saltwater species (the mysid, *Mysidopsis bahia* and inland silverside, *Menidia beryllina*) due to the limited data on marine species. Based upon the three conclusions of U.S. EPA 1985a, the chronic criterion was derived. This document concludes that:

1. acute-chronic ratios of freshwater species appear to increase with decrease in pH.
2. data on temperature effects on the ratios are lacking.
3. acute-chronic ratios for the most acutely and chronically sensitive species are technically more applicable when trying to define concentrations chronically acceptable to acutely sensitive species.

Based upon these conclusions, four freshwater acute-chronic ratios along with the two saltwater acute-chronic ratios were chosen to determine the final chronic value. The four freshwater species along with their acute-chronic ratios were the channel catfish (10), bluegill (12), rainbow trout (14), and fathead minnow (20). The mysid and inland silverside had acute-chronic ratios of 7.2 and 21.3, respectively. Based upon these six values, the geometric mean was 13.1. The geometric mean was then divided by the final acute value of 0.465 mg NH_3 /L to yield the final chronic value of 0.035 mg NH_3 /L.

For each site the un-ionized ammonia is used along with the pH, temperature, and salinity to determine total ammonia concentration. Then depending on the type of receiving water (stream, lake, marine, etc.), different dilution effects are considered to determine the total ammonia as nitrogen concentration which can be discharged.

2.4.2 Potential Instream Limits

Based upon the national saltwater criteria, the potential instream limits for total ammonia are summarized in Table 2.6 and 2.7. These tables use the EPA criteria for un-ionized ammonia and the various pH, temperature, and salinity to yield a total ammonia concentration. However, this is not the discharge limit but rather the instream concentration. In order to determine the precise discharge limit the Guidelines for Waste Allocations Loads [29] must be consulted.

From Table 2.7 the approximate instream concentrations for the Chesapeake Bay are shown in bold print. These values suggest the possible 1-2 mg/L standards set by DEQ and described in Section 1.1.2 of this report.

Table 2.6 - Criteria maximum concentrations (one-hour average) for total ammonia (mg/L) [6].

	Temperature (°C)			
	0	10	20	30
		Salinity = 10 ppt		
pH				
7.4	110	52	25	12
7.6	69	33	16	7.7
7.8	44	21	10	5.0
8.0	27	13	6.4	3.1
8.2	18	8.5	4.2	2.1
8.4	11	5.4	2.7	1.4
		Salinity = 20 ppt		
7.4	116	54	27	12
7.6	73	35	17	7.9
7.8	46	23	11	5.2
8.0	29	14	6.7	3.3
8.2	19	8.9	4.4	2.1
8.4	12	5.6	2.9	1.5
		Salinity = 30 ppt		
7.4	125	58	27	13
7.6	79	37	21	8.5
7.8	50	23	11	5.4
8.0	31	15	7.3	3.5
8.2	20	9.6	4.6	2.3
8.4	13	6.0	2.9	1.6

Table 2.7 - Criteria continuous concentrations (four-day average) for total ammonia (mg/L) [6].

	Temperature (°C)			
	0	10	20	30
		Salinity = 10 ppt		
pH				
7.4	17	7.8	3.7	1.8
7.6	10	5.0	2.4	1.2
7.8	6.6	3.1	1.5	0.75
8.0	4.1	**2.0**	**0.97**	0.47
8.2	2.7	**1.3**	**0.62**	0.31
8.4	1.7	0.81	0.41	0.21
		Salinity = 20 ppt		
7.4	18	8.1	4.1	1.9
7.6	11	5.3	2.5	1.2
7.8	6.9	3.4	1.6	0.78
8.0	4.4	**2.1**	**1.0**	0.50
8.2	2.8	**1.3**	**0.66**	0.31
8.4	1.8	0.84	0.44	0.22
		Salinity = 30 ppt		
7.4	19	8.7	4.1	2.0
7.6	12	5.6	3.1	1.3
7.8	7.5	3.4	1.7	0.81
8.0	4.7	**2.2**	**1.1**	0.53
8.2	3.0	**1.4**	**0.69**	0.34
8.4	1.9	0.90	0.44	0.23

3

Toxicity of Ammonia to Aquatic Organisms

On the subject of this research, there were three areas in the literature that needed to be investigated.

- Acute toxicity of ammonia in freshwater and saltwater
- Chronic toxicity of ammonia in freshwater and saltwater
- Marine versus freshwater organisms

There has been some confusion about the terminology used to describe concentrations of ammonia in past literature [14]. In this review, the terms, "ionized ammonia" (NH_4^+) and "un-ionized ammonia" (NH_3), will be used to describe the two states of ammonia, and total ammonia or ammonia will refer to the combined concentrations of ionized and un-ionized ammonia ($NH_3 + NH_4^+$). Also, total ammonia will be expressed, on occasion, in terms of NH_3-N.

3.1 Acute Toxicity of Ammonia in Freshwater and Saltwater

The acute toxicity of ammonia in the aquatic environment has been studied since the late 1940's [9]. Initially, the research was conducted on salmonids, but since then, studies have been conducted on approximately 70 different organisms, both fresh and saltwater types, as well as vertebrate and invertebrate species. These organisms include aquatic insects, daphnids, crustaceans, mollusks, and fishes. The toxicity of ammonia has been more extensively studied with freshwater than with marine species. Since the research presented in this report deals with only saltwater species, this section will highlight the freshwater data and detail the saltwater data. The acute toxicity data will be summarized in the following manner:

1. Freshwater Vertebrates (Fishes) and Invertebrates
2. Saltwater Vertebrates (Fishes) and Invertebrates

One can make several generalizations when studying the acute toxicity of un-ionized ammonia to freshwater vertebrates (fishes). Salmonids are the most sensitive (96-hour LC_{50} for adults = 0.2 to 0.8 mg NH_3/L) with the cyprinid fish (includes carp, minnow, and dace) being the least sensitive (96-hour LC_{50} for adults = 1.0 to 1.5 mg NH_3/L). Sensitivity to NH_3 is greater in the egg and larval stages of fish and decreases as the fish reach maturity. Ninety-six hour LC_{50} for trout fry has been reported by Calamari and Marchetti [30] to be 0.16 mg NH_3/L, and Knoph [31] reported a 96-hour LC_{50} for Atlantic salmon par to be as low as 0.03 mg NH_3/L. However, there is great variability in the data caused by water characteristics and experimental procedure. Table 3.1 lists several 96-hour LC_{50} values for some of the more common freshwater fishes with the references listed in brackets. The 96-hour LC_{50} is for un-ionized ammonia. The pH ranged from 7.5 to 8.0.

Table 3.1 - 96-hour LC_{50} for various freshwater fish.

Freshwater Fish	96-hour LC_{50} (mg NH_3/L)
rainbow trout [32]	0.37-0.65
cutthroat trout [33]	0.52-0.80
Atlantic salmon [31]	0.03-0.15 (pH=6, juvenile)
fathead minnow [34]	0.70-1.20
walleye [34]	0.51-1.10
white sucker [34]	1.70-2.20
channel catfish [34]	1.00-1.30
carp [35]	1.00-1.50
bluegill sunfish [35]	0.95-1.18
smallmouth bass [35]	0.90-1.15

[ ] - Reference

Freshwater invertebrates are much less studied than fishes. The 96-hour LC_{50} of un-ionized ammonia is generally an order of magnitude greater than that of freshwater fishes. For this reason, regulations assume that if the fishes are protected from ammonia toxicity, then the invertebrates and benthic organisms will be protected as well. Arthur *et al.* [34] reported that mollusks were the most sensitive invertebrate group to ammonia. He determined that fingernail clams were twice as sensitive as the two snail species (*Physa gyrina* and *Helisoma trivolvis*) and that the most tolerant invertebrate species were the caddisfly and crayfish species. Table 3.2 shows the 96-hour LC_{50} of un-ionized ammonia for freshwater. The pH and temperature for all tests were not the same, so references should be checked for specifics.

Table 3.2 - 96-hour LC_{50} for various freshwater invertebrates.

Freshwater Invertebrate	96-hour LC_{50} (mg NH_3/L)
Daphnia magna [36]	2.94
Fingernail Clam [35]	1.10
Snail [34]	2.40
Amphipod [34]	3.12
Mayfly [34]	3.90
Isopod [34]	5.02
Caddisfly [34]	10.1
Crayfish [34]	18.3

[ ] - Reference

The acute toxicity of ammonia to saltwater organisms has been studied with a variety of test species. Twenty-one species of crustaceans, bivalve mollusks, and fishes have been studied. These include fishes, such as the winter flounder (*Pseudopleuronectes americanus*) [37], which is the most sensitive (excluding salmonids), to the three-spined stickleback (*Gasterosteus aculeatus*) [38], which is the least sensitive. Also, as in this

research, the sheepshead minnow has been tested by numerous authors, such as Poucher [39], Miller *et al.* [19], and E.A. Eng. [40]. These data were pooled together by the EPA resulting in a species mean acute value (SMAV) of 2.74 mg/L un-ionized ammonia [6]. The SMAV is the geometric mean of the LC_{50} data from both 48-hour and 96-hour tests. Another commonly tested marine fish is the inland silverside. Data reported by Poucher [39] and Fava *et al.* [41] yielded a SMAV of 1.32. Table 3.3 lists the 96-hour LC_{50} for a few of the more common saltwater fishes.

Table 3.3 - 96-hour un-ionized ammonia LC_{50} for various saltwater fish.

Saltwater Fish	96-hour LC_{50} (mg NH_3/L)
Atlantic salmon [18]	0.16-0.21 (juvenile, 30% seawater)
sheepshead minnow [19]	2.6-2.8 (larval, salinity = 30 g/kg)
inland silverside [19]	0.8-1.14 (larval, salinity = 30 g/kg)
killifish [42]	1.35-2.0 (48-hour LC_{50})

[ ] - Reference

In all cases, the larval stage of the marine fishes is more sensitive to ammonia than the adult. Juvenile striped bass (*Morone saxatilis*) (LC_{50} = 0.91 - 1.68 mg/L un-ionized ammonia) seem less sensitive than post yolk-sac larvae (LC_{50} = 0.33 - 0.58 mg/L un-ionized ammonia) [38]. This was also the case for the striped mullet (*Mugil cephalus*) [43]. The effect that pH, temperature, and salinity have on ammonia toxicity to marine fishes was presented in Section 2.2 of this report.

Generally, no direct correlation can be made between the sensitivity of invertebrates versus vertebrates in saltwater. An exception is the mollusks, which are the most tolerant, as detailed by Epifanio and Srna [44] for the Eastern oyster (*Crassostrea virginica*), SMAV = 19.1 mg/L un-ionized ammonia, and E.A. Eng. [40] for the brackish

water clam (*Rangia cuneata*), SMAV = 3.1 mg/L un-ionized ammonia. Other less tolerant invertebrates include the Sargassum shrimp (*Latreutes fucorum*) [43], the prawn (*Macrobrachium rosenbergii*) [45] and the mysid (*Mysidopsis bahia*) [19].

Similar to marine fishes, the marine invertebrates' tolerance to ammonia increases as the organism matures. Fava *et al.* [41] reported the un-ionized ammonia LC_{50} increased in larvae (1.1 mg/L) to adults (2.57 mg/L) for the grass shrimp (*Palaemonetes pugio*). This difference was also seen with the mysid [19]. The effect that pH, temperature, and salinity have on ammonia toxicity to marine invertebrates was presented in Section 2.2.

Table 3.4 lists the SMAV for the 21 saltwater species which have been tested. These species are listed in order of acutely sensitive to acutely tolerant. This information was taken from "Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989 [6]. The rank was based upon the genus mean acute value (the geometric mean of the SMAV within the genus).

Table 3.4 - Un-ionized ammonia species mean acute values for saltwater organisms [6].

Rank	Species	SMAV (mg/L)	pH Range	Temp. Range (^{O}C)	Salinity (ppt)
1	Winter flounder *Pseudopleuronectes americanus*	0.492	7.9-8.1	7.5	31
2	Red drum *Sciaenops ocellatus*	0.545	8.0-8.2	25-26	28-30
3	Sargassum shrimp *Latreutes fucorum*	0.773	8.07	23.4	28
4	Prawn *Macrobrachium rosenbergii*	0.777	6.8-8.3	28	12
5	Planehead filefish *Monocanthus hispidus*	0.826	8.07	23.4	28
6	Copepod *Eucalanus pileatus*	0.793	8.2	20.5	34
7	Copepod *Eucalanus elongatus*	0.867	8.0	20.3	34
8	White perch *Morone americana*	2.13	8.0	16	14
9	Striped bass *Morone saxatiois*	2.481	7.2-8.2	15-23	5-34
10	Mysid *Mysidopsis bahia*	1.021	7.0-9.2	19-26	10-31
11	Spot *Leiostomus xanthurus*	1.04	7.92	20.4	9.3
12	Atlantic silverside *Menidia menidia*	1.05	7.0-9.0	11-25	10-3

Table 3.4 - Un-ionized ammonia species mean acute values for saltwater organisms [6]. (Cont.)

13	Inland silverside *Menidia beryllina*	1.317	7.1-9.1	18-26	11-33
14	Striped mullet *Mugil cephalus*	1.544	7.99	21	10
15	Grass shrimp *Palamonetes pugio*	1.651	7.9-8.1	19-20	10-28
16	American lobster *Homarus americanus*	2.21	8.1	21.9	33.4
17	Sheepshead minnow *Cyprinodon variegatus*	2.737	7.6-8.1	10-33	10-33
18	Three-spined stickleback *Gasterosteus aculeatus*	2.932	7.6	15-23	11-34
19	Brackish water clam *Bangia cuneata*	3.08	7.95	20.2	9.2
20	Quahog clam *Mercenaria mercenaria*	5.36	7.7-8.2	20	27
21	Eastern oyster *Crassostrea virginica*	19.1	8.0	20	27

3.2 Chronic Toxicity of Ammonia in Freshwater and Saltwater

The chronic toxicity of ammonia is much less studied than its acute toxicity. Up until 1990, chronic data was available for only 11 freshwater species and no marine organisms [19]. Since then, one freshwater and two saltwater organisms have been tested. In this report only a few examples will be presented due to the specificity of the experiments. The fresh and saltwater fishes cited in this report include Chinook salmon, fathead minnow, and inland silverside. The fresh and saltwater invertebrates cited include *Daphnia Magna*, *Ceriodaphnia acanthina* and the mysid.

The effects of low levels of un-ionized ammonia have been seen in fresh and saltwater fishes. Chronic ammonia poisoning was the reputed cause of death for Chinook salmon living in water containing only 0.016 mg NH_3/L after only 6 weeks [46]. For the fathead minnow, the maximum acceptable toxicant concentration (MATC) was 0.21 mg NH_3/L which was based on survival of larvae [32]. Other MATC values were reported to be 0.49, 0.27, and 0.08 mg NH_3/L for the smallmouth bass [47], green sunfish [48], and bluegill [49], respectively. Weights of larval inland silverside were significantly reduced in un-ionized ammonia concentrations > 0.074 mg NH_3/L. Survival was reduced significantly only at "high" treatments of 0.38 mg NH_3/L. The acute:chronic ratio for inland silverside was determined to be 21.3 [19].

Research has been conducted on the chronic toxicity of ammonia to four aquatic invertebrates. The most studied invertebrate is *Daphnia magna.* Gersich and Hopkins [36] determined the 21-day chronic value for *D. magna*, presented as the MATC, to be 0.60 mg NH_3/L. Mount [50] determined the 7-day chronic NOEC for *D. magna* to be 0.527 mg/L un-ionized ammonia. Studies have also been conducted with one saltwater invertebrate, the mysid (*Mysidopsis bahia*). Miller *et al.* [19] determined that the survival of mysids during a 32-day life-cycle was not significantly reduced until an un-ionized ammonia concentration of 0.331 mg/L was reached. Also the male and female length

was affected at this concentration. The acute:chronic ratio for this mysid was 7.2, using the acute value of 1.70 mg NH_3/L from a flow-through 96-hour test conducted at 26.5 ^{o}C, 30.5 g/kg salinity, and 8.0 pH.

3.3 Marine Versus Freshwater Organisms

In considering toxicity of ammonia to aquatic life, one must study both freshwater and marine organisms. In most aquatic toxicity experiments, the freshwater organisms have been studied more in depth than marine fishes and macroinvertebrates. This would indicate that in the case of setting discharge standards for a specific toxicant in seawater, freshwater toxicity data was used if no data was available for marine organisms.

This section of the report compares the toxicity of freshwater organisms to saltwater organisms. These generalizations will be discussed in two categories: vertebrates (fishes) and macroinvertebrates.

In general, saltwater fishes are less sensitive to total ammonia than freshwater species due to the reduction of un-ionized ammonia by the increased ionic strength. The saltwater also biochemically reduces the toxicity of un-ionized ammonia. Both of these reasons are discussed in Section 2.2.3 of this report. For example Table 3.5 lists the 24-hour acute LC_{50} for Atlantic Salmon in both freshwater and saltwater since these are andronomous fish which can live in various salinities.

Table 3.5 - 24-hour LC_{50} for Atlantic salmon in salt and freshwater.

	Saltwater (30 ppt)	Freshwater
Atlantic Salmon	0.3 mg/L (juvenile)	0.15 mg/L (juvenile)

From Table 3.5 it is seen that the Atlantic Salmon is more tolerant to ammonia as the salinity increases. This is possibly due to reduced osmotic stress on the fish as discussed in Section 2.2.3.

Another comparison might be to discuss some of the most common fishes that are present in the marine environment as well as the freshwater ecosystem. For example Table 3.6 lists the 96-hour acute LC_{50} and 7-day chronic no-observed-effect-concentration (NOEC) in relation to weight gain for the most commonly tested marine and freshwater fishes, the sheepshead minnow and the fathead minnow, respectively.

Table 3.6 - Comparison of sheepshead and fathead minnow in relation to ammonia toxicity.

Organism	96-Hour Acute LC_{50} (mg NH_3/L)	7-Day Chronic NOEC (mg NH_3/L)
Sheepshead Minnow	2.6-2.8 [19]	0.34
Fathead Minnow [6]	0.70-1.20	0.15-0.34

[ ] - Reference

Data in Table 3.6 suggest that the sheepshead minnow can tolerate at least twice the un-ionized ammonia concentration when compared to the fathead minnow. Also, the 7-day

chronic NOEC for the sheepshead minnow is at least three times the concentration for the fathead minnow. These differences can not be attributed solely to the interactions of saltwater with ammonia, but also must be related to species differences.

A few generalizations can be made by comparing freshwater versus saltwater fishes. The salmonids are the most susceptible species in both salt and freshwater. Also the early life stages for both salt and freshwater fishes are more sensitive to un-ionized ammonia than the adults life stages. Finally, based upon the fishes tested to date, the guidelines set forth for NPDES permits using freshwater vertebrate toxicity data should protect marine fishes, as well.

Marine macroinvertebrates are much less studied than marine fishes; therefore, it is difficult to compare freshwater and marine macroinvertebrates due to the limited data. However, it appears from the few organisms tested, such as various shrimp and amphipods, that the marine macroinvertebrates are less tolerant to ammonia than their freshwater counterparts. However, when compared to marine fishes, there appears to be no trend. Therefore, in order to protect both marine vertebrates and invertebrates, both types must be taken into consideration. For example Table 3.7 lists acute toxicity data for a few marine and freshwater invertebrates.

From Table 3.7 it is shown that the most sensitive organisms from the limited acute toxicity data are the saltwater invertebrates. The freshwater fingernail clam is the only freshwater invertebrate which has a relatively low LC_{50} when compared to the saltwater organisms. Therefore, it would seem that the guidelines set forth for NPDES permits based upon freshwater vertebrate toxicity data would not protect marine invertebrates.

Table 3.7 - 96-hour LC_{50} for salt and freshwater invertebrates.

Freshwater Invertebrate	96-hour LC_{50} (mg NH_3/L)
Daphnia magna [36]	2.94
Fingernail Clam [35]	1.10
Snail [34]	2.40
Amphipod [34]	3.12
Mayfly [34]	3.90
Isopod [34]	5.02
Caddisfly [34]	10.1
Crayfish [34]	18.3
Saltwater Invertebrate	**96-hour LC_{50} (mg NH_3/L)**
marine amphipod [51]	0.83-3.5 (various species)
grass shrimp (juvenile) [42]	1.2
mysid [19]	1.33-1.94 (salinity = 30 g/kg)

[ ] - Reference

4

Research Test Organisms

In discussing any toxicity research, one cannot overlook the importance of the research test organisms. The reaction to a specific toxicant as well as the concentration which the organism can tolerate will be unique to each test species. Also, the reaction to a specific toxicant will be unique to the life stage of the particular organism. Other things which must be considered are the origin of the organisms and their relative health before toxicity testing. All of these items must be addressed in toxicity testing of each particular test species. This research was conducted with three test species: the sheepshead minnow (*Cyprinodon variegatus*), the mysid or opossum shrimp (*Mysidopsis bahia*), and the grass shrimp (*Palaemonetes pugio*). This chapter will discuss the concerns in reference to the three test organisms used in this research. The topics are covered in four sections:

4.1. Morphology, Taxonomy, and Life History

4.2. Mysid Culture Methods

4.3. Mysid Reference Testing

4.4. Specifics of Test Organisms

4.1 Morphology, Taxonomy, and Life History

Before the various test conditions for the three organisms are considered, it is of interest to give some background information of each of the species used in this research. A brief life history as well as taxonomy and morphology will be detailed in this section as follows:

-Sheepshead Minnow (*Cyprinodon variegatus*)
-Mysid (*Mysidopsis bahia*)
-Grass Shrimp (*Palaemonetes pugio*)

4.1.1 Sheepshead Minnow (*Cyprinodon variegatus*)

The sheepshead minnow (*Cyprinodon variegatus*) is widely distributed in the waters of the Gulf of Mexico and the Atlantic Ocean including the Chesapeake Bay. It belongs to the killifish family (Cyprinodonitidae) of which it is the only marine species. This family contains over 45 genera and 300 species worldwide excluding Australia.

The average size of an adult sheepshead minnow is 3-5 cm with a maximum length of just under 10 cm. The body of the sheepshead minnow is short and deep with depth increasing with age. Figure 4.1 shows the adult and juvenile sheepshead minnow.

The sheepshead minnow is characterized by a lack of lateral lines but the presence of numerous (24-29) lateral scale rows. The dorsal fin has nine to thirteen rays and the anal fin has nine to twelve rays. The males are characterized by a lustrous blue- to bluish-green area on the back from the nape to the dorsal fin. The males also have some dark bars on the sides which are poorly defined and a yellowish white to deep orange belly. The females are light olive, brown, or brassy with a yellowish or white belly [7].

The sheepshead minnow is a very tolerant marine fish. It is capable of flourishing in a wide range of temperatures (0-40^{o}C) and salinities (0.1 to 149 ppt) [52]. These minnows live in schools in calm, shallow estuarine bays, coves, and marshes. They also have adapted to live in some lakes with very high concentrations of dissolved salts. These omnivorous minnows are a vital link in the food chain, transferring energy from benthic plants and animals to the commercial valued fishes. They are fed upon by a variety of Chesapeake Bay fish such as the flounder (*Paralichthys dentatus*), striped bass (*Morone saxatilis*), spotted seatrout (*Cynoscion nebulosus*), and bluefish (*Pomatomus saxatilis*) [53].

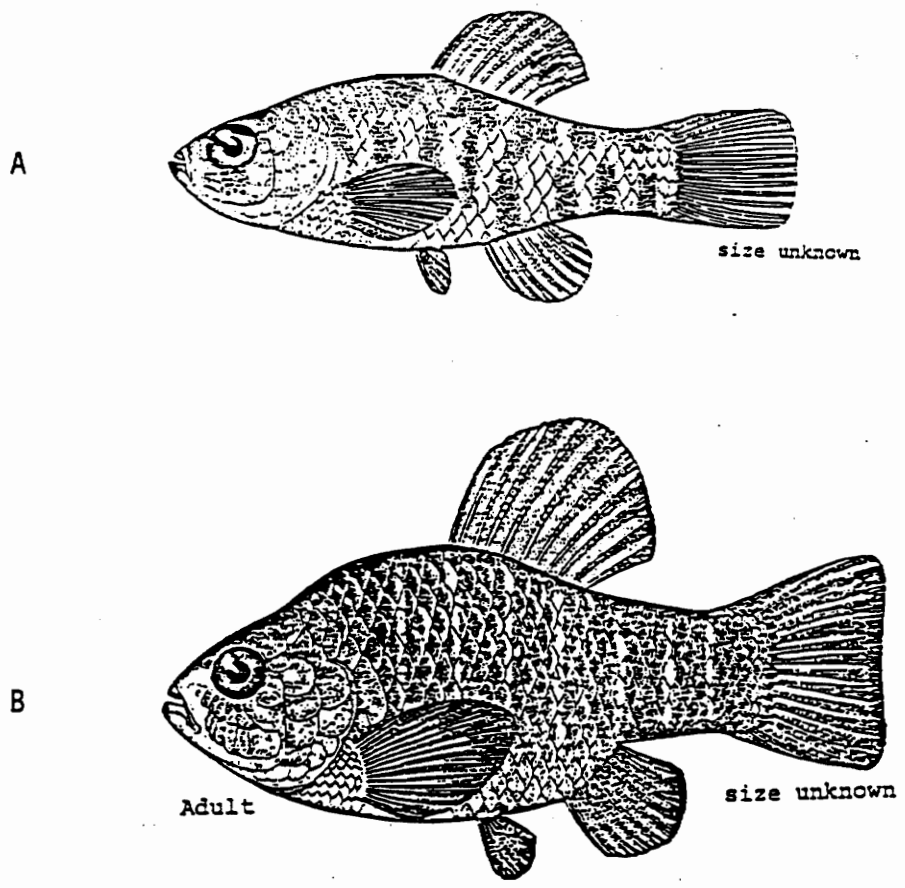

Figure 4.1 - Sheepshead minnow (*Cyprinodon variegatus*). A. juvenile; B. adult [7].

4.1.2 Mysid (*Mysidopsis bahia*)

The *Mysidopsis bahia* is the primary invertebrate in marine testing for the NPDES permitting and distributed throughout the Atlantic Ocean and the Gulf of Mexico as long as the salinity is greater than 15 ppt. They are one of three sympatric species including *Mysidopsis almyra* and *Mysidopsis bigelowi* which have been cultured successfully. The differences between these three species are minimal and the taxonomic key proposed by Heard [54] should be consulted for identification.

These small crustaceans are approximately 0.5 to 0.8 cm in length as adults, but can reach lengths of over 1.0 cm. Figure 4.2 shows a top and side view of the female, adult mysid and Figure 4.3 shows the morphological features which are useful in identifying the *Mysidopsis bahia*. They reach sexual maturity in laboratory cultures after 12 to 20 days with adequate temperature (20-25°C) and a healthy diet of *Artemia* [7].

The opossum shrimp is characterized by the females carrying the eggs and young in a marsupium pouch. Unlike *Daphnia* the eggs need to be fertilized, usually at night, in order for development to occur. The number of eggs produced by the female as well as the number of young per brood is directly related to body length as well as environmental conditions. A new brood is produced every four to seven days.

The young, once released, are vulnerable to predation even by the adult mysid because they are relatively immobile. Therefore, in culturing it is necessary to maintain *Artemia* available at all times to allow for recruitment. After 24 to 48 hours, the juveniles begin to pursue and feed on *Artemia* and are no longer susceptible to predation by the adults [7].

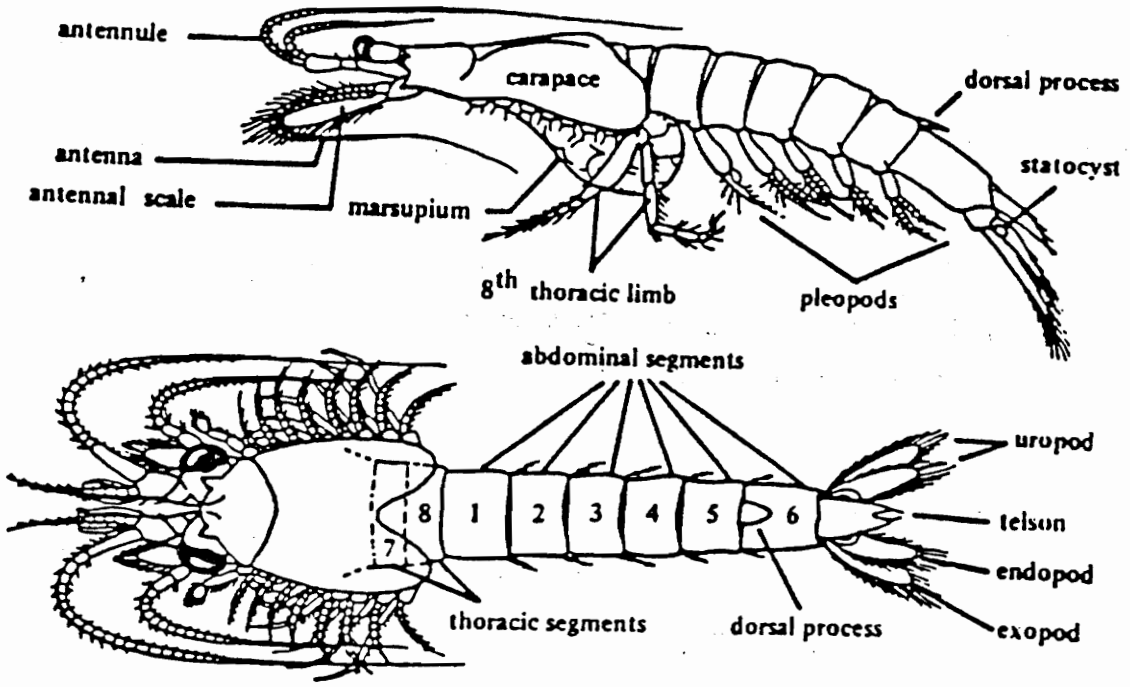

Figure 4.2 - Lateral and dorsal view of the female, adult mysid (*Mysidopsis bahia*) [7].

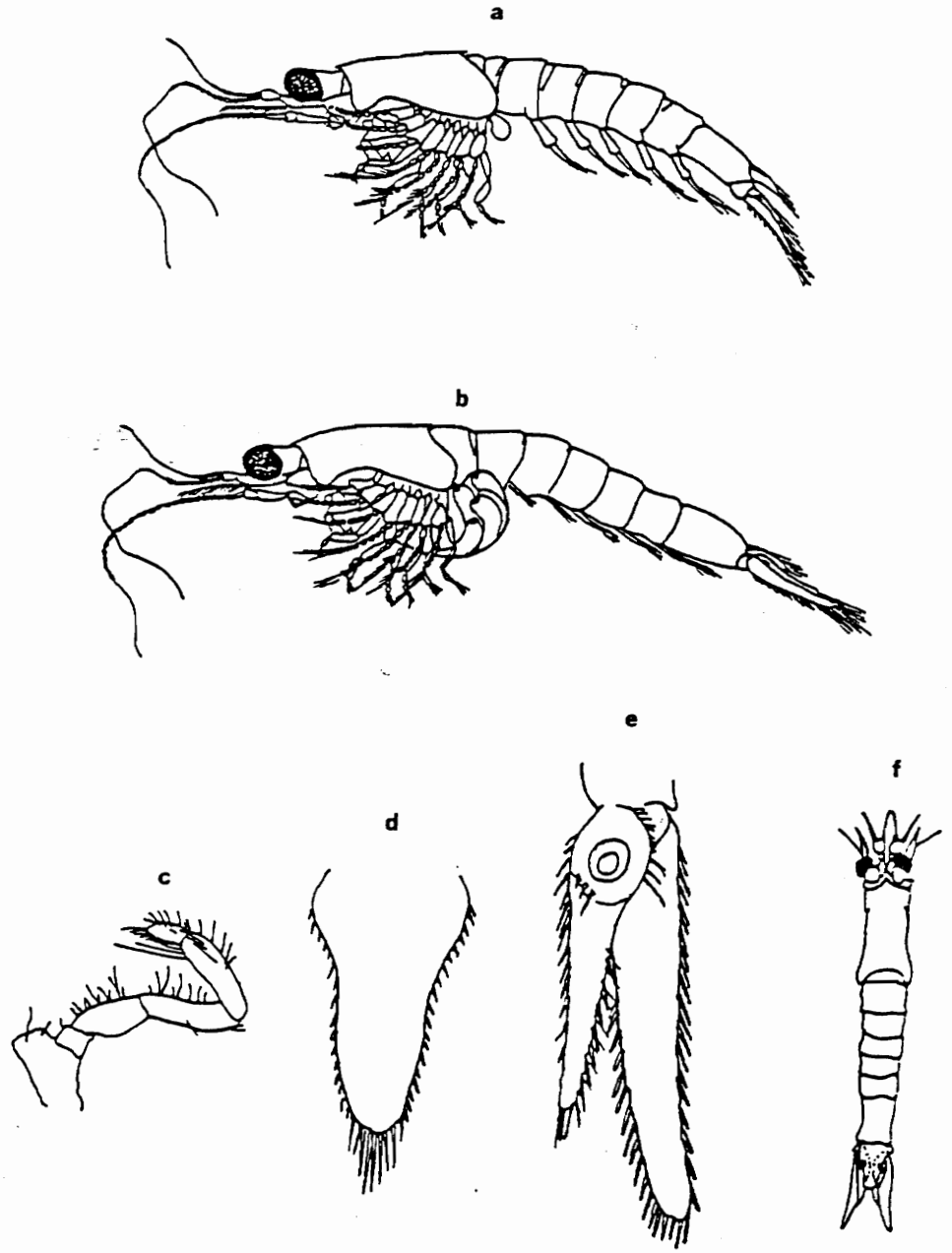

Figure 4.3 - Morphological features of the mysid (*Mysidopsis bahia*). a. male; b. female; c. thoracic leg; d. telson; e. right uropod, dorsal; f. male, dorsal [7].

4.1.3 Grass Shrimp (*Palaemonetes pugio*)

The grass shrimp is widely distributed throughout estuaries of the Atlantic Ocean and the Gulf of Mexico. It is found especially in beds of submerged vegetation. *Palaemonetes pugio* is very tolerant of a wide range of salinities from 0 ppt to as high as 43 ppt. However, its optimum salinity is in the range of 10 ppt to 20 ppt. *Palaemonetes pugio* belongs to the family Palaemonidae which includes several commercially harvested species. It is closely related to other types of grass shrimp including *Palaemonetes vulgaris* and *P. intermedius* of which both are used in marine toxicity testing. The differences between these three species are minimal and the taxonomic key proposed by Williams [55] should be consulted for identification. Figure 4.4 shows a side view of the adult female *Palaemonetes pugio*.

These transparent crustaceans measure approximately 3.3 cm for males with the ovigerous females measuring 3 to 5 cm. Males further differ with a more slender body and shorter legs with the juveniles resembling the males. They can be cultured in the laboratory provided they are fed a diet of algae and *Artemia*. However, the production of young is relatively slow with juveniles being produced by females at intervals between 5 to 6 weeks. Therefore, cultures to provide enough juveniles for toxicity testing must contain thousands of females to provide young on a continual basis. For this reason test organisms are for the most collected in the field. Growth to maturity takes two to three months under summer conditions (22 to 25°C) and four to six months under winter conditions (8 to 12°C) [55].

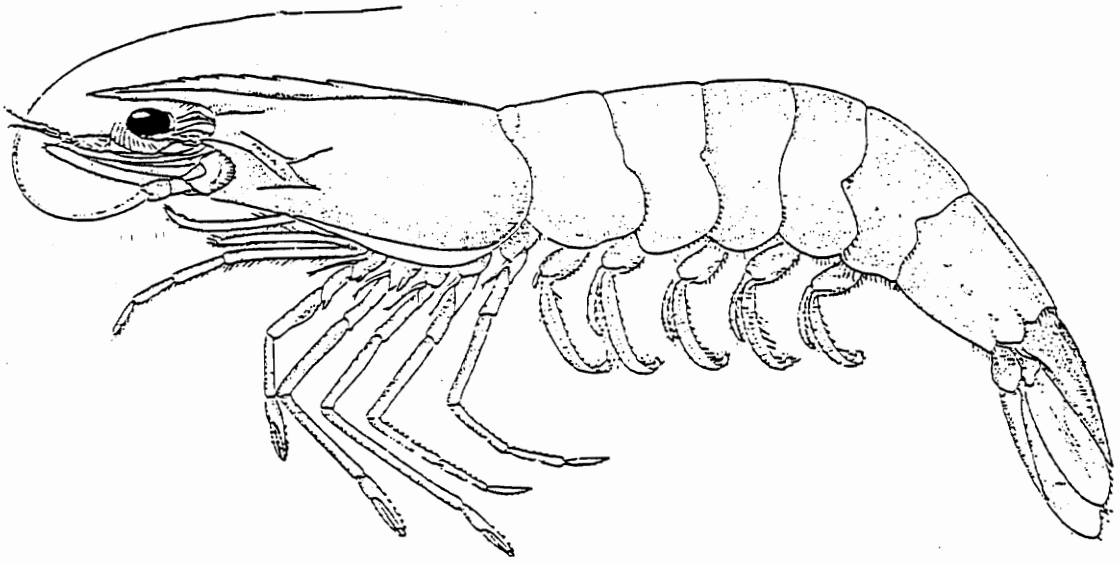

Figure 4.4 - Lateral view of the adult female grass shrimp (*Palaemonetes pugio*) [55].

4.2 Mysid Culture Methods

There are a variety of techniques to attain mysids, both juveniles and adults, for toxicity testing. These techniques include collection of the test species in the field, purchasing the mysids (test age) from an outside source, and in-house culturing of the mysids. For this research the in-house culturing of mysids was chosen due to the number of tests to be conducted and the minimal capital cost for setting up the culture. This section will describe the culturing process for mysids in the following order: culturing system, culture media and conditions, culture organisms, culture media and conditions, culture maintenance, and common mysid culturing problems.

4.2.1 Culturing System

Mysids can be cultured in either continuous-flow or closed recirculating systems. Due to the cost of a continuous-flow system, the culture for this research used closed systems consisting of 10 or 20-gallon tanks with an undergravel flow filtering system in each tank. It was determined that in order to be able to produce enough young per test (>200) on a daily basis, three 10-gallon and two 20-gallon tanks needed to be maintained with approximately 150 adult mysids per 10 gallons of seawater.

The undergravel flow system was established in each tank because a standard aquatic filtering pump creates a strong current and suction which the mysids cannot tolerate. These commercially available systems consist of two standpipes, two filter plates, two airstones, and need an external air supply for operation. A flow around 3 L/min. created by air forced through airstones within two upright standpipes was determined to produce enough current for the mysids to align themselves with the flow and actively feed on *Artemia*. Each aquarium had two filter plates, which formed a false bottom. Approximately two inches of crushed coral was placed on top of the filter plates. The air forced up standpipes via the airstones created a current which is drawn through the

crushed coral. Once the culture had been established, the crushed coral acted as a biological filtering system which will be discussed later in Section 4.2.2.

4.2.2 Culture Media and Conditions

In order to maintain a healthy, reproducing population of adult mysids, an adequate culture media and specific environmental conditions must be maintained. This section will attempt to address the specifics concerning the media and conditions used in this research to sustain a healthy culture.

The culture media was composed of three parts: seawater, gravel or coral, and the nitrifying community. The seawater can either be natural or artificially mixed using commercially available seasalts. For this research the artificial marine mix, Forty Fathoms®, was used with a salinity concentration between 25 and 28 ppt. Forty Fathoms® has been successfully used previously in culturing mysids [56].

The second component of the culture media was crushed gravel or coral. This component of the media provided a place for the nitrifying bacteria and algae to affix. The crushed media also provided a hiding place for the newly released mysid young in order to avoid predation. For this research crushed coral was purchased from a local pet supplier. Prior to tank setup, the crushed coral was autoclaved for 45 minutes and then allowed to cool gradually to ambient conditions.

Finally, the nitrifying community was established. This community ripened over time and could take as long as 1 month per 10 gallons of seawater. To shorten this ripening process approximately 20 mL of *Artemia* per 10 gallons was added daily for a week before the adult mysids were added. Also, in order to enhance the nitrifying community, a commercially available nitrifier known as Fritzyme® was added. Once the initial mysids were added, the ammonia, nitrite, and nitrate concentrations were monitored daily until the concentration spike for each was observed. During this monitoring process, the

ammonia spike was observe first, followed by the nitrite spike, and then the nitrate spike. After the nitrate spike was observed, the community was defined to be fully developed and the culture was ready to be used to produce young for testing.

In order to maintain a healthy mysid culture with maximum reproduction capacity, certain environmental conditions must be maintained. The most crucial conditions are temperature, salinity, daily light cycle, pH, dissolved oxygen, alkalinity, and ammonia, nitrite, and nitrate concentrations. Temperature, salinity, and dissolved oxygen were the most easily maintained. The temperature was stabilized at 25 to 27°C by an aquarium heater and the salinity range between 25 and 27 ppt was checked using a YSI portable salinity meter (model 30). This salinity range was maintained by the addition of deionized water or withdrawal of seawater replaced by deionized water. The daily light cycle was sixteen hours of illumination at 50 ft-c followed by 8 hours of darkness. The dissolved oxygen concentration was maintained at saturation through the airstones in the undergravel flow system. Table 4.1 lists the optimum conditions which were maintained in the five recirculating culture tanks.

Table 4.1 - Optimum environmental conditions for *Mysidopsis bahia*.

Condition	Range
Temperature (°C)	25-27
Salinity (ppt)	25-27
Daily Light Cycle (hr)	16 light, 8 dark
Dissolved Oxygen	>60% saturation
pH	8.0-8.2
Alkalinity (mg/L)	90-120
Ammonia (mg-N/L)	<1.0
Nitrite (mg-N/L)	<1.0
Nitrate (mg-N/L)	<18

Some of these conditions are interactive and therefore must be monitored closely. For instance the nitrifying community which converts ammonia to nitrite and then to nitrate, results in an increase in hydrogen ions, which causes a drop in pH and a loss in buffering capacity. However, it was found that the crushed coral provided enough buffering capacity to maintain the pH around 8.0 for approximately two months and then new sterilized coral needed to be added.

4.2.3 Culture Organisms

Once the tanks were conditioned as previously mentioned in Section 4.2.2, the mysids were added. These mysids (*Mysidopsis bahia*) were purchased from Cosper Environmental Services of Bohemia, New York. Appendix B lists several marine organism suppliers, along with their address and phone number, which were either used or contacted through this research. Initially, 20 mysids were added to the culture to test the health of the medium and after 96 hours more mysids were added such that the tanks contained 40 mysids per 10-gallons of seawater. Gradually the culture increased until there was approximately 150 adult mysids per 10 gallons of seawater. The tanks were stocked with mysids from several generations and various ages to ensure that young would be produced on a daily basis. Every month approximately 20 percent of the organisms (especially older males) were removed to ensure that the culture did not experience high mortality. If the cultures are correctly maintained, at least 20 percent of the adult population would consist of gravid females (i. e. have a visible brood pouch with young). Also, mysids were moved among the tank every month to ensure genetic diversity.

Before every toxicity test, young less than two days old needed to be collected for the appropriate test. These young were acquired by collecting the adult females bearing brood pouches and isolating them in a separate, 4-liter tank with similar water conditions outlined in Table 4.1. These females were placed in a large (10 cm by 15 cm) standard

fish transfer net for two days. The net allowed the juveniles to pass through the net isolating them from the females to avoid predation. A general rule-of-thumb was to collect one brood-carrying female per young needed in the test. These juveniles could then be used over the next three days.

Another time-consuming procedure for juvenile production was utilized in addition to the technique previously mentioned. This procedure involved removing juveniles from the stock tanks with a fine mesh net, placing them in a 1 L Pyrex® crystalline dish with seawater, and finally, removing those which were less than 2 mm long. A light table was utilized to distinguish the young which were less than 2 mm in length. These young were approximately less than 24 hours old, as outlined in EPA document no. 600/4-90-027 [7].

Both of these juvenile-collection methods were very time consuming, and an alternative method for collection is proposed in Section 4.2.5 of this report.

4.2.4 Culture Maintenance

In the maintenance of the cultures, there were a variety of daily, weekly, and monthly tasks which needed to be performed on each of the tanks. An outline of these procedures is presented in the next series of lists.

Daily Procedures

1. Feed each tank 3 mL of concentrated *Artemia* twice daily for every ten gallons of seawater.
2. Check airstones for appropriate airflow.
3. Maintain water level above standpipes for proper recirculation and aeration.
4. Check temperature of tanks and make appropriate adjustments.

Weekly Procedures

1. Check salinity and make appropriate adjustments.

2. Check pH and adjust with Na_2CO_3.

3. Check concentrations of NH_3, NO_2^{-1}, and NO_3^{-2}.

4. Remove algal growth from the internal surfaces of aquarium and turn over crushed coral.

Monthly Procedures

1. Remove 20 percent of the seawater and replace with new artificial seawater. The seawater should be removed by pumping from the bottom of the tank, disturbing the crushed coral bottom and removing some of the algae and any decaying matter from the bottom.

2. Once every two months remove most of the crushed coral and replaced with newly autoclaved crushed coral.

3. Remove and clean standpipes with deionized water.

4. Remove 20 percent of the mysid culture from each tank to prevent high mortality in the culture.

5. Move mysids among the five tanks to ensure genetic diversity.

6. Replace airstones.

4.2.5 Common Culturing Problems

In the mysid toxicity research, there were several problems associated with culturing which slowed completion of the research. These problems included:

1. Initial ammonia build-up in the stock tanks

2. Periodic reduced reproduction from individual tanks

3. Inefficient collection of females for juvenile production.

The first three 10-gallon culture tanks were initiated without nitrifying bacteria present. Therefore, it took approximately two months for the total ammonia concentration to reach

levels of less than 1 mg/L. Two weeks after the initial stocking of the tanks, total ammonia concentrations of 15 mg/L were measured in each of the three tanks. Therefore, there was a concern that the organisms in these three tanks might either have been weakened somewhat or have developed a tolerance to ammonia. Both of these possibilities would have adversely affected these experiments, since this research dealt with accessing the toxicity of ammonia. As a result, these initially stocked mysids were removed and replaced with healthy mysids before the research began.

To alleviate the initial ammonia build-up problem when the two 20-gallon tanks were setup, a small amount of crushed coral from the conditioned three tanks was added to the new tanks. This added the proper algae and nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) such that these two tanks did not experience a build-up of ammonia (<5 mg/L).

After two to three months, each stock tank experienced a reduced number of females carrying brood pouches. The subsequent lack of reproduction could have been related to several factors such as diminished pH, improper salinity or temperature, or overcrowding along with several others. However, the observed dominant factor was the age of the culture. This problem was avoided by removing 20 percent of the adults (predominantly the larger males) every month and replacing most of the coral with fresh coral every two months.

The final problem was mostly the inconvenience and time involved in transferring the ripe females from the stock tanks to the holding tank. The collection of enough females (200) for one toxicity test consisting of two replicates would take approximately 4 hours. This method of juvenile production for testing was obviously inefficient and cumbersome.

One should probably construct a mysid generator system similar to the one described by Reistsema and Neff [57]. In these systems the current in the tank is increased such that

the immobile juvenile mysids are swept by the current into a small chamber. The entrance to the chamber is covered by fine mesh net which allows only the newly hatched mysids to pass. Each morning the chamber is emptied from each tank, guaranteeing that the juveniles are less than 24 hours old. This technique for young collection would allow researchers to use their time more efficiently.

4.3 Mysid Reference Testing

In order to verify the health of the culture, standard 48-hour acute reference tests were conducted each month. The standard toxicant was an EPA-certified solution of cadmium ($CdCl_2$). The health of the culture was initially verified from existing mysid toxicity data for cadmium by conducting five 48-hour acute tests with two replicates in each test. Once the standard health of the culture was established with cadmium, one 48-hour acute test was conducted monthly to verify that the culture's health had been maintained.

The initial health of the mysid culture was established based upon the average LC_{50} (10 replicates) for cadmium. The average LC_{50} was determined to be 0.64 mg/L. This was the LC_{50} which was used to verify the health of the culture throughout the remainder of the mysid toxicity testing. This LC_{50} for cadmium was determined to be appropriate based upon the research conducted by Burton and Fisher [42]. They determined the LC_{50} for the juvenile mysid to be 0.85 mg/L. Figure 4.5 shows the acute reference toxicant (cadmium) control graph for the mysid culture. Also, Appendix C contains the bench sheets for the initial reference testing, as well as the monthly tests which were performed.

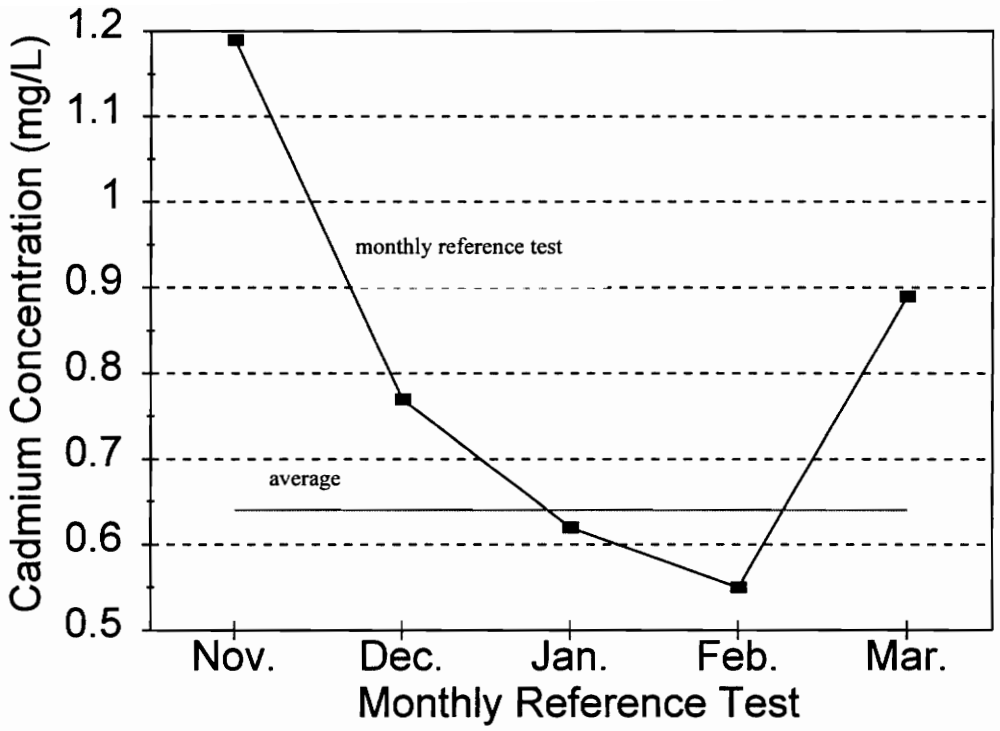

Figure 4.5 - Acute Control Graph for *Mysidopsis bahia*.

4.4 Specifics of Test Organisms

There were a multitude of specific details about the three test organisms which needed to be addressed. These specifics include fine points such as age, source, and acclimation procedures. These various items are presented in the following order:

-Sheepshead Minnow (*Cyprinodon variegatus*)
-Mysid (*Mysidopsis bahia*)
-Grass Shrimp (*Palaemonetes pugio*)

4.4.1 Sheepshead Minnow (*Cyprinodon variegatus*)

The sheepshead minnow is a relatively difficult organism to culture. It also requires several 20-gallon tanks in order to provide enough larvae on a continual basis for testing. Therefore, due to the capital cost, maintenance requirement, and space requirement, the test age minnows were acquired from an outside source. This would allow for toxicity testing to be initiated quickly and reduce the workload associated with culture maintenance.

The *Cyprinodon variegatus* larvae were purchased from Cosper Environmental Services Inc. of Bohemia, New York. Usually, enough minnows were ordered to conduct eight replicates of tests (approximately 500 minnows). The minnows arrived at less than five days old and all of the eight replicates were setup in less than two days after arrival. Before testing, the minnows were fed both *Artemia* and Tetrimin® flakes twice daily.

Upon arrival, the sheepshead minnow larvae were acclimated to the test dilution water slowly over the course of five hours. The shipping water's temperature, pH and salinity were checked to ensure that these conditions did not drastically differ from those of the dilution water. The temperature of the water was acclimated by placing the container in the constant temperature bath which was designated for the specific test (either 20°C or 25°C). The pH and salinity in the container were acclimated by removing approximately 20 percent of the container's water every hour and replacing it with dilution water. After five water changes, the holding tank's salinity and pH were essentially the same as the dilution water's salinity and pH. The dissolved oxygen was maintained at saturation in the holding tank by means of continual aeration.

To initiate testing, the larvae were removed from the holding tank individually with a pipet with an opening large enough not to harm them. Because they were previously acclimated to test conditions, the minnows did not experience any harmful effect other

than that caused by the toxicant. The specifics of the acute and chronic sheepshead minnow tests are detailed in Section 5.2.4 and Section 5.2.5 of this report.

4.4.2 Mysid (*Mysidopsis bahia*)

As discussed in Section 4.2 of this report, it was more cost-effective to culture the mysids in-house. Also, the method for juvenile collection was discussed in the aforementioned section. Therefore, approximately two days before a mysid acute test was to be initiated, the females were collected and allowed to release their young. The females were distributed throughout the stock tanks, and the healthy young were placed in a 600 mL beaker. The 600 mL beaker was placed in the 20°C constant temperature bath to acclimate to the standard acute toxicity temperature. The young mysids were released into standard dilution water at 25°C so there was no need to adjust the pH or salinity prior to testing. The dissolved oxygen was maintained above 5 mg/L in the 600 mL beaker by forcing air through a glass pipet within the beaker.

All young mysids which were used in the testing were less than 2 days old and were fed a Tetrimin® and yeast (TYC) and algae mixture 1 hour prior to testing. The mysids were placed in the test beaker using a standard 1 mL glass pipet. The specifics of the acute mysid tests are detailed in Section 5.2.4 of this report.

4.4.3 Grass Shrimp (*Palaemonetes pugio*)

The grass shrimp which were used in this research were not cultured in-house like the opossum shrimp but instead were purchased from an outside source which collected them directly from an estuary in Florida. The grass shrimp were purchased from Aquatic Research Organisms, Inc. in Massachusetts. Due to the time of year in which they were collected, it was possible to test grass shrimp which were 15-30 mm in length (approximately 2 months old). Juveniles can be collected only in the late summer months.

These organisms were acclimated to the dilution water in a similar manner as the *Cyprinodon variegatus* as detailed in Section 4.4.1. However, the grass shrimp were adjusted to the dilution water over a time of 2 days. This longer acclimation period was due to the variation between the pH of the shipping water (pH 7.5) and the dilution water (8.2). Once again, aeration was used to maintain the dissolved oxygen concentration at saturation.

When placed in close quarters, the molting grass shrimp will be preyed upon by other grass shrimp. To minimize this cannibalism, a collapsible mesh netting was placed throughout the holding tank to provide "hiding places", or to increase the surface area within the tank. This mesh reduced mortality within the holding tank.

As stated previously, the age of the grass shrimp was approximately 2 months. They were fed Tetrimin® flakes twice daily, as well as 1 hour prior to testing. The organisms were transferred to the test beakers using a standard fine mesh fish collection net. The specifics of the acute grass shrimp tests are detailed in Section 5.2.4 of this report.

5

Experimental Procedure and Setup

The literature search revealed that the marine invertebrates are more susceptible to ammonia than the marine fishes. Therefore, this research concentrated on two marine invertebrates and one marine fish. Some ammonia toxicity data already existed on these organisms, but also some new work was done as well. Both 48-hour static nonrenewal and 96-hour static renewal tests were performed with three organisms, and 7-day chronic renewal tests were done with one marine fish. In addition to the experiments, a method of pH control had to be developed due to the effect of pH on un-ionized ammonia. After the experiments were completed, the data were analyzed according to the EPA methods for measuring acute [7] and chronic [8] toxicity. This chapter describes the experimental procedures, as well as the methods for pH control and data analysis. These topics are covered in the following three sections:

5.1. pH Control in Ammonia Toxicity Experimentation

5.2. Experimental Setup and Procedure

5.3. Data Analysis

5.1 pH Control in Ammonia Toxicity Experimentation

In ammonia toxicity research, possibly the most important parameter is pH. Due to the dissociation of ammonia, small changes in pH can affect the NH_3 concentration (as discussed in Section 2.2.1) which the test organisms encounter. Therefore, inconsistency in the degree of control of test pH is a major source of variability in ammonia toxicity studies especially in seawater. This section addresses the importance of pH control as well as the acceptable pH variation established in previous research and by the EPA. It also discusses the procedure used to control the pH in these experiments and the three

common methods of pH control: acid/base addition, buffers, and the nitrogen headspace technique.

5.1.1 Importance of pH Control

The control of pH is difficult for most toxicity tests, especially in accessing the toxicity of ammonia. Ammonium salt solutions (in this research, NH_4Cl) are acidic, but are slow to reach equilibrium in seawater [6]. Consequently, pH typically declines during toxicity tests and the decline may be amplified by the metabolism of test organisms. Therefore, a proper pH control method must be chosen such that the pH does not decline significantly during testing.

This raises the question of what constitutes a significant pH change. As stated in EPA - Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989 [6], a significant pH change at a pH 8 would be $> \pm 0.1$ pH units. This type of error results in ±25% variation in the NH_3 exposure concentration. Research done by others such as Hansen [58], Schimmel [59], and Knoph [31] indicated that the pH variation should be no greater than ±0.1 pH units. Therefore, in order to keep in accordance with previous research, throughout the experiments presented in this report, the pH variations was kept to $\leq \pm 0.1$ pH units and in most experiments ≤ 0.05 pH units.

5.1.2 Methods of pH Control

As previously stated, there are three suitable methods of pH control for toxicity testing: acid/base addition, the nitrogen headspace technique, and buffers. These methods, if practiced properly, will reduce the variability of the NH_3 concentration during testing, therefore, predicting the un-ionized ammonia concentration more accurately.

Acid/base addition is a method which adjusts the pH either periodically or continuously through the addition of either a weak acid or weak base. This adjustment can either be

performed manually or automatically with a pH analyzer/controller and metering pump. The most common acid used is 0.5 to 1 N HCl and the most common base used is 0.5 to 1 N NaOH. Usually, if manual addition is used, treatments of acid or base are needed as frequently as every two hours.

The use of the nitrogen headspace technique is another effective method for pH control. The nitrogen headspace method entails either using a nitrogen cabinet or evacuating the headspace above the water level in the test beakers, replacing the air with nitrogen, then sealing the individual test beakers with cellophane. The nitrogen cabinet is by far the most time efficient method but requires considerable capital investment. The nitrogen cabinet allows for all test beakers to be placed within the space and relatively little monitoring of pH is needed while the beakers are within the cabinet. The sealing of the individual beakers is more labor intensive and requires the evacuation of air and replacement with nitrogen daily. Because the capital investment would be excessive, the nitrogen cabinet was not used in this research. The nitrogen sealing method was not used because the test beakers must be resealed each day.

The addition of buffers is the third method used to maintain the pH during NH_3 toxicity experiments. It requires the initial buffering of each test dilution to the appropriate pH and the subsequent buffering periodically during the test to maintain the pH. Several buffers are appropriate especially the ones of the carbonate system. For this research sodium carbonate (Na_2CO_3) was used to maintain the pH.

5.1.3 Procedure for pH Control using Na_2CO_3

The procedure for maintaining pH was developed by trial and error over a series of the first few toxicity tests. All test media were initiated at a pH of 8.2 and were adjusted to this value periodically. It was determined that the test dilutions needed to be adjusted at intervals of eight hours or less for a variation of less than ±0.1 pH units. However, for

most tests, the pH was measured every four hours except during the early morning hours (2-5 A.M.) The procedure was as follows:

1. Test dilutions were adjusted to pH 8.2 with Na_2CO_3 before exposing the test organisms

2. The pH was measured every four hours during daylight hours and adjusted to 8.2 with Na_2CO_3. (The final pH check was typically late in the evening such that eight hours elapsed before the next morning's pH adjustment.)

3. For tests with renewals, pH was adjusted as in Steps 1 and 2. However, for each daily renewal, the new dilution water was buffered to pH 8.2 prior to transferring into the test beakers. The pH was measured once the new dilution water was transferred to the test beakers. Adjustments were made if necessary.

4. $NaHCO_3$ was used to buffer the pH to 8.2 if it was overshot.

5.2 Experimental Setup and Procedure

In order to expand the existing saltwater ammonia toxicity data, 48-hour acute, 96-hour acute, and 7-day chronic experiments were performed with three saltwater organisms. Also, the 7-day test needed to be assessed for its effectiveness in measuring the chronic toxicity of ammonia to marine fishes. These experiments were conducted according to EPA guidelines; specifically the acute methods are presented in EPA [7] and the chronic methods are presented in EPA [8]. These experiments were performed at Olver Laboratories, Incorporated, which has the facilities (e.g. constant temperature baths, proper lighting, and measurement devices) to meet these experimental guidelines.

This section describes the experimental apparatus and procedures which were used throughout testing. It also describes the training which enabled the researcher to become proficient and experienced in aquatic toxicity testing before the work began.

5.2.1 Training in Toxicity Testing

In May, 1995, an agreement was made with a local company in Blacksburg, Virginia, to instruct and train a graduate student in the techniques required for toxicity testing in return for his volunteer services and the knowledge gained in culturing marine organisms. This company has a state-of-the-art bioassay laboratory and years of experience in toxicity testing. Techniques which were mastered by the student include acute and chronic bioassays for fathead minnow (*Pimephales promelas*) and two cladocerans (*Ceriodaphnia dubia* and *Daphnia magna*) outlined by the EPA [7 and 8]. These techniques included both static- and static-with-renewal test methods. Also, the procedures needed for culturing and the routine maintenance of test organisms was developed by the student through guidance provided by this company. All of the bioassays needed for this research were performed at the facilities of the company.

5.2.2 Organism and Dilution Preparation

Before each experiment, the organisms were collected, and specific dilutions for each test species were prepared. The sheepshead minnow and the grass shrimp were supplied from an outside source and acclimated to the appropriate conditions as described in Sections 4.4.1 and 4.4.3 of this report. The juvenile mysids were collected and acclimated as outlined in Section 4.4.2. The health of all organisms was verified through monthly toxicity experiments, either in-house (mysids) or at the outside sources (sheepshead minnow and grass shrimp).

The ammonia treatments for all tests were made from reagent grade NH_4Cl dissolved at 10,000 mg/L total ammonia, then diluted in synthetic saltwater (Forty Fathoms™) to provide the desired concentrations. Treatments were selected on the basis of range-find toxicity tests for each of the test species. Table 5.1 lists the dilutions used in all tests

conducted for each species. After the dilutions were prepared, the pH was buffered to 8.2 with Na_2CO_3 and then the organisms were added to each beaker.

Table 5.1 - Test dilutions for toxicity experiments.

	Test Dilutions (mg/L total ammonia)		
	48-hour	**96-hour**	**7-day**
Cyprinodon variegatus			
	control	control	control
	40	10	10
	50	20	20
	60	30	30
	70	40	40
	80	50	50
Mysidopsis bahia	control	control	--
	10	5	--
	20	15	--
	30	20	--
	40	25	--
	50	30	--
Palaemonetes pugio			
	control	--	--
	60	--	--
	70	--	--
	80	--	--
	90	--	--
	100	--	--

-- Not tested

5.2.3 Experimental Apparatus and Setup

The components in the experiments were as follows:

pH Meter:	Orion model 420A
Dissolved Oxygen Meter:	YSI model 51B
Salinity Meter:	YSI model 30/25 FT
Test Beakers:	600 mL (*Mysidopsis bahia* and *Cyprinodon variegatus*)
	1000 mL (*Palaemonetes pugio*)
Automatic Pipet:	Wheaton 100-1000 µL
Balance:	Metter AE240
Drying Oven:	Blue M single wall gravity convection oven

The pH, dissolved oxygen, and salinity meters were used to measure the initial, daily, and final conditions of the test solutions. The pH meter was used more frequently in order to maintain the pH and measure the temperature of the treatments. The dissolved oxygen meter was corrected based on the water salinity and temperature as instructed by the manufacturer. The automatic pipet was calibrated before each test.

Due to the size of the grass shrimp, 1 liter (L) beakers were used for those experiments; 600 mL beakers were used for all other tests. All tests included a control with two replicates for the 48-hour acute tests and four replicates for the 96-hour acute and 7-day chronic tests. The balance and the drying oven were used to evaluate the growth of the minnows for each treatment. Dry weights of the test organisms were measured at the conclusion of each 7-day chronic test.

5.2.4 Conditions and Experimental Procedure for Acute Toxicity Tests

Acute bioassays were performed with the mysid, grass shrimp, and sheepshead minnow. The young mysids were produced at Olver Laboratories, the young minnows were acquired from Cosper Environmental Services, Inc., and the grass shrimp were purchased from Charles River Aquatic Research Organisms. The 48-hour acute test conditions for

the sheepshead minnow, mysid, and grass shrimp are listed in Tables 5.2, 5.3, and 5.4, respectively. Using the test conditions listed in these tables, 16 replicates with sheepshead minnows, 16 replicates with mysids, and 16 replicates with grass shrimp were tested.

Table 5.2 - 48-hour acute bioassay conditions for *Cyprinodon variegatus*.

Test type:	Static non-renewal
Replicates per test:	2
Test duration:	48 h
Temperature:	20°C
Photoperiod:	16 h light, 8 h darkness
Test beaker size:	600 mL
Test volume:	250 mL
Age of organism:	<10 days; 24 h range
Feeding regime:	none
Toxicant:	Ammonium chloride
D. O.:	>4.0 mg/L
Dilution water:	25 ppt salinity, ±1 ppt
pH:	8.2, <±0.1 (adjusted every four hours during day)

Table 5.3 - 48-hour acute bioassay conditions for *Mysidopsis bahia*.

Test type:	Static non-renewal
Replicates per test:	2
Test duration:	48 h
Temperature:	20°C
Photoperiod:	16 h light, 8 h darkness
Test beaker size:	600 mL
Test volume:	250 mL
Age of organism:	<2 days; 24 h range
Feeding regime:	none
Toxicant:	Ammonium chloride
D. O.:	>4.0 mg/L
Dilution water:	25 ppt salinity, ±1 ppt
pH:	8.2, <±0.1 (adjusted every four hours during day)

Table 5.4 - 48-hour acute bioassay conditions for *Palaemonetes pugio*.

Test type:	Static non-renewal
Replicates per test:	2
Test duration:	48 h
Temperature:	20°C
Photoperiod:	16 h light, 8 h darkness
Test beaker size:	1 L
Test volume:	1 L
Age of organism:	1-2 months
Feeding regime:	none
Toxicant:	Ammonium chloride
D. O.:	>4.0 mg/L
Dilution water:	25 ppt salinity, ±1 ppt
pH:	8.2, <±0.1 (adjusted every four hours during day)

The 96-hour acute test conditions for the sheepshead minnow and mysid are listed in Tables 5.5 and 5.6, respectively. No 96-hour tests were conducted with the grass shrimp because there was sufficient data in the literature under similar experimental conditions. Using the test conditions listed in these tables, 16 replicates with sheepshead minnows and 16 replicates with mysids were tested.

Table 5.5 - 96-hour acute bioassay conditions for *Cyprinodon variegatus*.

Test type :	Static renewal
Replicates per test:	4
Test duration:	96 h
Temperature:	20°C
Photoperiod:	16 h light, 8 h darkness
Test beaker size:	600 mL
Test volume:	250 mL
Age of organism:	<10 days; 24 h range
Feeding regime:	<24 h *Artemia* nauplii twice daily
Toxicant:	Ammonium chloride
D. O.:	>4.0 mg/L
Dilution water:	25 ppt salinity, ±1 ppt
pH:	8.2, <±0.1 (adjusted every four hours during day)

Table 5.6 - 96-hour acute bioassay conditions for *Mysidopsis bahia*.

Test type:	Static renewal
Replicates per test:	4
Test duration:	96 h
Temperature:	20°C
Photoperiod:	16 h light, 8 h darkness
Test beaker size:	600 mL
Test volume:	250 mL
Age of organism:	<2 days; 24 h range
Feeding regime:	<24 h *Artemia* nauplii twice daily
Toxicant:	Ammonium chloride
D. O.:	>4.0 mg/L
Dilution water:	25 ppt salinity, ±1 ppt
pH:	8.2, <±0.1 (adjusted every four hours during day)

The experimental procedure for the 48-hour and 96-hour toxicity experiments was as follows:

1. The dilutions for the particular test species were prepared in clean test beakers as described in Section 5.2.2. Each dilution had two replicates. Each dilution was buffered to pH 8.2. Samples of each dilution were preserved for later testing in order to verify the ammonia concentration for each dilution.

2. Ten test organisms, which had been fed one hour before test initiation were transferred to each beaker individually in random order. The organisms were added in sequential order such that each beaker contained four, then eight, and finally ten organisms. The juvenile mysids and the sheepshead minnows were transferred via pipet and the grass shrimp were transferred via a standard fish net.

3. A nylon mesh netting was added to each of the grass shrimp test beakers to create "hiding place" for the molting shrimp.

4. Once all of the organisms were added, the beakers were placed in the 20°C constant temperature bath in random order.

5. The pH was checked and adjusted if necessary every four hours during the daytime and were not checked or adjusted for eight hours during the evening. The pH variation was always less than ±0.1 throughout the duration of the test and in most cases less than ±0.05.

6. Every 24 hours the pH, salinity, temperature, and dissolved oxygen were measured and recorded. The number of live organisms was counted and the dead were removed from the beakers.

7. The 48-hour grass shrimp tests were aerated to maintain the dissolved oxygen concentration above 4.0 mg/L.

8. The 96-hour tests were fed twice daily less than 24 hour *Artemia* nauplii. Every 24-hours approximately 200 mL of the test volume was removed and replaced with new solutions and buffered to pH 8.2. Also, daily the remaining *Artemia* was removed to reduce the oxygen demand. The 48-hour experiments were nonrenewal and were not fed.

9. At the end of the test period, final measurements of pH, temperature, dissolved oxygen, and salinity were taken and recorded. Also, the final

number of organisms alive was counted and recorded. A final water sample was taken to measure the ammonia concentration at the end of the test.

10. The final organism count was used to determine the LC_{50} and NOEC; this procedure is described in Section 5.3.

5.2.5 Experimental Procedure for Chronic Toxicity Tests

Chronic bioassays were performed with the sheepshead minnow (*Cyprinodon variegatus*). The young minnows were acquired from Cosper Environmental Services, Inc. The 7-day chronic tests were conducted according to the conditions listed in Table 5.7. Sixteen replicates were performed with sheepshead minnows according to the conditions listed in Table 5.7.

Table 5.7 - 7-day acute bioassay conditions for *Cyprinodon variegatus*.

Test type:	Static renewal
Replicates per test:	4
Test duration:	7 days
Temperature:	25°C
Photoperiod:	16 h light, 8 h darkness
Test beaker size:	600 mL
Test volume:	250 mL
Age of organism:	1-14 days; 24 h range
Feeding regime:	<24 h *Artemia* nauplii twice daily
Toxicant:	Ammonium chloride
D. O.:	>4.0 mg/L
Dilution water:	25 ppt salinity, ±1 ppt
pH:	8.2, <±0.1 (adjusted every four hours during day)

The experimental procedure for the 7-day toxicity experiments was as follows:

1. The dilutions for the *Cyprinodon variegatus* were prepared in clean test beakers as described in Section 5.2.2. Each dilution had four replicates. Each dilution was buffered to pH 8.2. Samples of each dilution were preserved for later testing in order to verify the ammonia concentration for each dilution.

2. Ten test organisms which had been fed one hour before test initiation were transferred to each beaker individually in random order. The organisms were added in sequential order such that each beaker contained four, then eight, and finally ten organisms. A pipet was used to transfer the sheepshead minnows to the test beakers.

3. Once all of the organisms were added, the beakers were placed in the 25°C constant temperature bath in random order.

4. The pH was checked and adjusted if necessary every four hours during the daytime and were not checked or adjusted for eight hours during the evening. The pH variation was always less than ±0.08 throughout the duration of the test.

5. Every 24 hours the pH, salinity, temperature, and dissolved oxygen were measured and recorded. The number of live sheepshead minnows was counted and the dead were removed from the beakers.

6. Each test beaker was fed twice daily less than 24 hour *Artemia* nauplii. Every 24-hours approximately 200 mL of the test volume was removed and replaced with new solutions and buffered to pH 8.2. Daily the remaining *Artemia* was removed to reduce the oxygen demand.

7. At the end of the 7-day test period, final measurements of pH, temperature, dissolved oxygen, and salinity were taken and recorded. Also, the final number of organisms alive was counted and recorded. A final water sample was taken to measure the ammonia concentration at the end of the test.

8. The final organism count was used to determine the LC_{50} and NOEC based upon statistical analysis; this procedure is described in Section 5.3.

9. Previous to the end of the test period, weighing pans were made and dried in an oven at 105°C for two hours, then placed in a dessicator for one hour. The dry mass of each of the pans was measured and recorded.

10. At the conclusion of the tests, the living minnows were removed from the test beakers and placed in the weighing pans. The pans were dried in the oven at 105°C for two hours, then placed in a dessicator for one hour. The dry mass of the fish was determined for the control plus the five dilutions. The weights of these six samples were compared in order to determine the LC_{50} and NOEC of ammonia which did not affect the weight gain of the fish. Section 5.3 describes the statistics involved in determining the LC_{50} and NOEC.

5.2.6 Experimental Verification of Ammonia Dose

In order to confirm the ammonia concentration of each of the dilutions, samples were taken, preserved with H_2SO_4, and tested for the ammonia concentration. Samples were taken at the beginning and end of each test and measured for the ammonia content as described in "*Standard Methods for the Examination of Water and Wastewater*" - Method - 4500 NH_3 B. and C. [13]. For the concentrations of ammonia involved in this research, it was determined that the proper technique for determining the ammonia concentration was a preliminary distillation step followed by titration.

Initially, samples were taken and measured every day for the 96-hour acute and 7-day chronic tests. It was determined that it was unnecessary to take samples every day because the ammonia concentrations did not vary more than 2 percent from the initial and final ammonia concentration. The average of the initial and final measured ammonia concentration was used as the exposure concentration. The values were used in determining the acute and chronic values. The un-ionized ammonia concentration was determined based upon the average pH, temperature, and salinity as described in Section 2.2.

The total ammonia values were exact to three significant figures based upon the accuracy of Method - 4500 NH_3 B. and C. The un-ionized ammonia concentrations were listed to three significant figures in Chapter 6 because these were calculated LC_{50} and NOEC values. One must remember that these un-ionized ammonia concentrations were

calculated values and were not accurate to three significant figures. These values were listed to three significant figures only to aid in establishing the national criteria.

5.3 Data Analysis

After the tests were completed, the number of mortalities from the acute testing, as well as the survival and growth data from the chronic experiments, was used to determine the LC_{50}, NOEC, and LOEC for each test species. The methods for determining these values are provided by the EPA in report no. 600/4-90/027 [7] and EPA in report no. 600/4-89/001 [8]. These methods include manual computational techniques as well as computer programs, such as Toxstat Version 3.3. However, manual computational techniques were not needed in this research based upon the EPA guidelines. These EPA documents were consulted throughout this research in reference to data analysis. The statistical techniques used in this research are outlined in the following two sections and are discussed as either acute toxicity analysis or chronic toxicity analysis. This section details the methods used in converting the LC_{50} and the NOEC from the individual tests into an average LC_{50} and NOEC for each of the species. Also, this section outlines the procedure for estimating the average pH and salinity for the series of tests. The average pH and salinity were used to determine the LC_{50} and NOEC in terms of un-ionized ammonia.

5.3.1 Acute Toxicity

In acute toxicity analysis, the most useful data are the LC_{50} and the NOEC. Specific methods are available for determining each of these parameters. In determining these values, each test could be analyzed separately and then averaged or all of the 16 replicates could be analyzed together such that for each organism there were 160 test organisms used in the data analysis yielding one value for the LC_{50} and another for the NOEC. Both of these methods were used and detailed in this report.

The Environmental Protection Agency report no. 600/4-90/027 [7] discusses four well-tested methods for determining the LC_{50} for multi-concentration acute toxicity tests. These methods are the Graphical Method, the Spearman-Karber Method, the Trimmed Spearman-Karber Method, and the Probit Method. The analysis scheme suggested in EPA report no. 600/4-90/027 is shown in Fig. 5.1. Based upon Fig. 5.1, the Probit Method was used to determine the LC_{50} for the sheepshead minnow, the mysid, and the grass shrimp. For details about this method, as well as the other three methods outlined in this report, refer to EPA report no. 600/4-90/027.

Determination of the NOEC is accomplished using hypothesis testing and is outlined in EPA report no. 600/4-90/027. The first step in determining the NOEC is to transform the mortality data by the arc-sine-square-root transformation. The mortality data must be stated as the proportion surviving in order to transform the data. This transformation is used to stabilize the variance and satisfy the normality requirement. The normality assumption is tested by the Shapiro-Wilk's test.

Once the normality assumption has been verified, the Bartlett's test for equality of variances was used to test the homogeneity of the variance assumption. If the homogeneity of variance assumption was met, then the modified T test or the Dunnett's test was used. However, if either the normality or the homogeneity of the variance assumption failed, the Steel's Many-One Rank Test or the Wilcoxon's Rank Sum Test was used to analyze the data. Figure 5.2 shows a flowchart of this procedure to determine the NOEC. This procedure was followed in determining the NOEC for the sheepshead minnow, mysid, and grass shrimp. The sheepshead minnow data were analyzed with the Steel's Many-One Rank Test to determine the NOEC. The mysid and the grass shrimp data were analyzed with both the Steel's Many-One Rank Test and the Dunnett's Test.

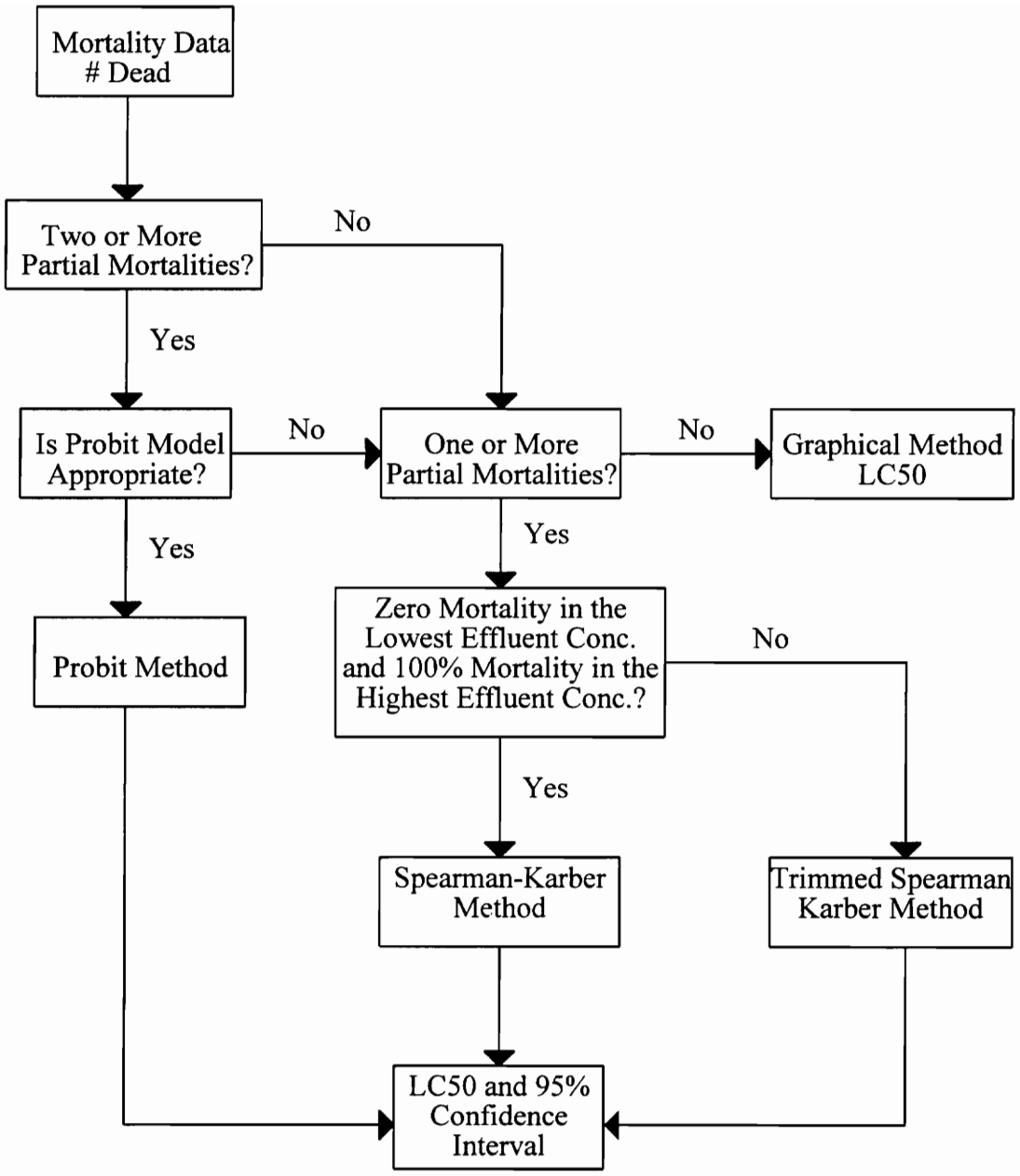

Figure 5.1 - Flowchart for determination of the LC_{50} for multi-concentration acute toxicity test [7].

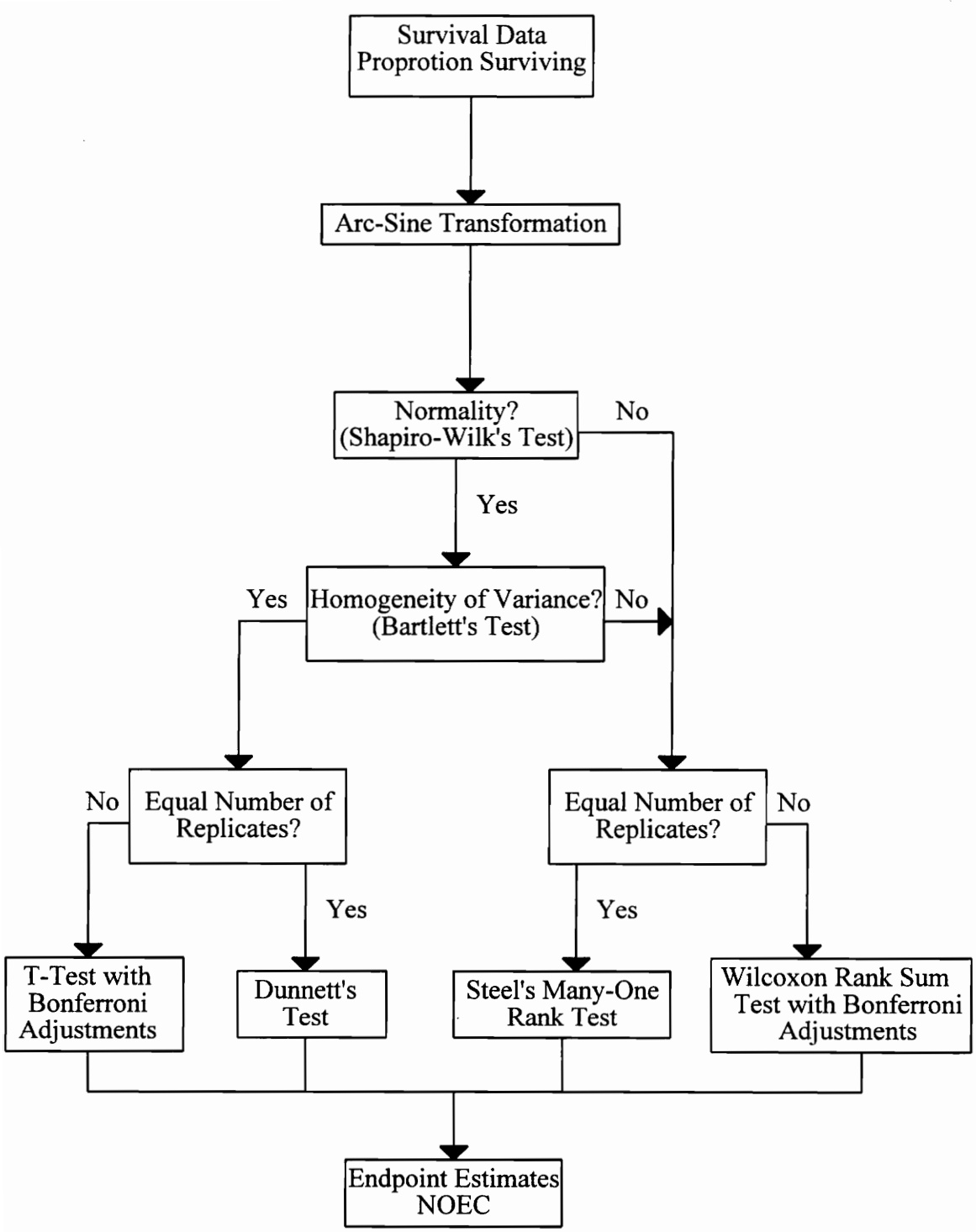

Figure 5.2 - Flowchart for analysis of mult-concentration test data [7].

5.3.2 Chronic Toxicity

The survival and growth data were used to determine 7-day LC_{50} and NOEC values based upon 16 replicates of the sheepshead minnow tests. The survival and growth data were analyzed with the statistical procedures outlined in EPA report no. 600/4-89/001 [8]. The data were analyzed using Toxstat Version 3.3. Statistical analysis was used to first analyze the survival data to determine the LC_{50} and the NOEC for the 7-day sheepshead minnow test. In order to find the NOEC, the normality of distribution and homogeneity of variance was determined by using Shapiro Wilk's and Bartlett's Tests, respectively. Depending on the test results, mortality was evaluated using Dunnett's Test or Steel's Many-One Rank Test. Also, dry weight data were used to determine the NOEC based upon growth. All weight data above the LC_{50} data were not used to determine the growth NOEC. The same procedure as described for the mortality NOEC was used for the growth data. Fig. 5.3 shows the EPA flowchart for determining the NOEC based on mortality or growth data.

For the sheepshead minnow tests, the Probit Method was used to calculate the LC_{50}, the mortality NOEC was determined using Steel's Many-One Rank Test, and the growth NOEC was determined using Dunnett's Test.

5.3.3 Methods for Determining Final LC_{50} and NOEC values

In this research, two methods were used in determining final LC_{50} and NOEC values resulting in two sets of data for each test species. These sets of data were presented on a total ammonia basis. Then, the un-ionized ammonia LC_{50} and NOEC were determined based upon the average pH, salinity, and temperature. One of the methods included simply analyzing the 16 replicates of data as one test using the statistical software package. This resulted in a LC_{50} with a 95 percent confidence interval (C. I.) and one value for the NOEC, both of which were listed on a total ammonia and un-ionized ammonia basis. These data were referred to as the 16 replicate values. The other method

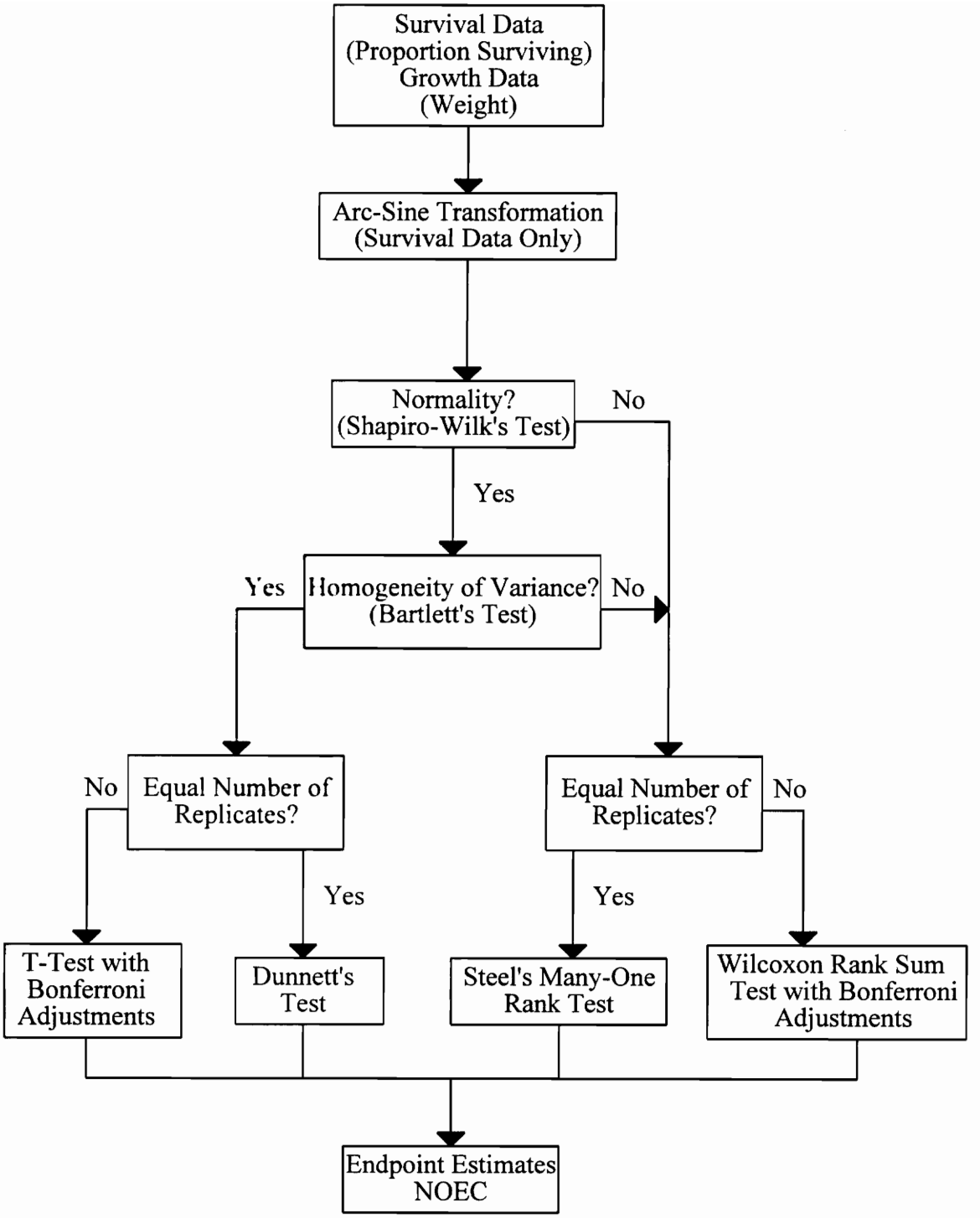

Figure 5.3 - Flowchart for analysis of sheepshead minnow survival and growth data [8].

involved analyzing each of the tests individually and then averaging the LC_{50}, the upper and lower limit of the 95 percent C. I., and the NOEC from each test resulting in the final set of values. These data were called the averaged LC_{50} and the averaged NOEC.

In order to determine the LC_{50} and the NOEC on an un-ionized NH_3 basis, an average pH was determined. Since the rate of pH change varied slightly from test to test, an average pH value was used to present the results on a un-ionized ammonia basis. The average pH was determined by averaging the upper and lower hydrogen ion concentration from each of the tests, and then averaging these hydrogen ion concentrations for a final average pH which was used in determining the un-ionized ammonia LC_{50}, 95 percent C. I., and NOEC for both the acute and chronic toxicity tests.

6

Results and Discussion

6.1 Acute Toxicity

The acute toxicity results include both 48-hour tests for the sheepshead minnow, mysid, and grass shrimp and 96-hour tests for the sheepshead minnow and mysid. The data were analyzed using Toxstat Version 3.3, as described in Section 5.3.1 to determine the LC_{50} and the NOEC in terms of total ammonia as well as un-ionized ammonia. Bench sheets with the actual mortality data are provided in Appendices D, E, and F for *Cyprinodon variegatus*, *Mysidopsis bahia*, and *Palaemonetes pugio*, respectively. From these data, the species mean acute values (SMAV) were determined based upon the geometric mean of the 48-hour and 96-hour LC_{50}. The SMAV were critical in evaluating a refined national criteria for ammonia based upon the research presented in this report.

6.1.1 Sheepshead Minnow (*Cyprinodon variegatus)*

A total of eight 48-hour static nonrenewal tests (2 replicates per test) and four 96-hour static renewal tests (4 replicates per test) were analyzed to determine the LC_{50} and NOEC for the sheepshead minnow. The final LC_{50} and NOEC for both the 48-hour and 96-hour tests were determined by two different methods (Section 5.3.3): the averaging method and the 16 replicate method . Both sets of LC_{50} and NOEC data are presented in this section and compared to previous work. This section lists the results from the sheepshead minnow tests in two sections:

1. Acute 48-hour static nonrenewal test results.
2. Acute 96-hour static renewal test results.

Table 6.1 lists the 48-hour LC_{50} (un-ionized ammonia) and the NOEC based upon analyzing the 16 replicates as one test with the Probit Method and the Steel's Many-One Rank Test, respectively. It also allows for a comparison of the data from this research to data provided in the literature.

Table 6.1 - 48-hour LC_{50} and NOEC for ammonia based upon 16 replicates for *Cyprinodon variegatus*.

Total LC_{50} (mg/L)	Un-ion. LC_{50} (mg/L)	Total NOEC (mg/L)	Un-ion. NOEC (mg/L)	Lit. Un-ion. LC_{50} [6] (mg/L)
56.0 (54.5 - 58.0)*	2.68 (2.61 - 2.77)*	40	1.9	2.1 -2.8

* 95% Confidence Interval

Table 6.1 shows that the LC_{50} for this research falls within the range presented in the literature for un-ionized NH_3. This un-ionized ammonia LC_{50} can then be converted to a total ammonia value based upon the temperature, pH, and salinity.

The averaging method for determining the LC_{50} was used as well. Table 6.2 lists the results from the eight tests (16 replicates) with sheepshead minnows. The bench sheets are given in Appendix D. This table gives the date of the test initiation, the total and un-ionized ammonia LC_{50} with the 95 percent C. I. in parentheses, the average pH value, and the total and un-ionized NOEC. All of the eight tests were analyzed using the Probit Method for the LC_{50} (see the flowchart in Fig. 5.1) and the Steel's Many-One Rank Test for the NOEC. It also lists the average LC_{50} based upon the averaging method described in Section 5.3.3.

Table 6.2 - 48-hour individual test and average LC_{50} and NOEC for ammonia for *Cyprinodon variegatus*.

Date	Total LC_{50} (mg/L)	Total NOEC (mg/L)	Avg. pH	Un-ion. LC_{50} (mg/L)	Un-ion. NOEC (mg/L)
1/10/96	57.7 (55.0 - 60.6)*	40	8.17	2.70 (2.57 - 2.84)*	1.9
1/10/96	56.5 (52.4 - 61.0)*	50	8.16	2.59 (2.40 - 2.79)*	2.3
1/10/96	56.7 (53.3 - 60.3)*	50	8.17	2.65 (2.49 - 2.82)*	2.3
2/4/96	52.7 (46.6 - 59.5)*	50	8.18	2.52 (2.23 - 2.84)8	2.4
2/4/96	55.1 (50.3 - 60.4)*	50	8.18	2.63 (2.40 - 2.89)*	2.4
2/5/96	58.9 (56.4 - 61.4)*	50	8.19	2.87 (2.75 - 3.00)*	2.4
2/5/96	57.2 (53.8 - 60.9)*	50	8.19	2.79 (2.63 - 2.97)*	2.4
2/5/96	56.8 (54.3 - 59.4)*	50	8.19	2.77 (2.65 - 2.90)*	2.4
Average	**56.3 (52.8 - 60.4)***	**48.8**	**8.18**	**2.69 (2.52 - 2.89)***	**2.3**

* 95% Confidence Interval

The LC_{50} values calculated by both methods differed by < 1 percent. The 48-hour LC_{50} for the sheepshead minnow was calculated to be 56.0 mg/L total ammonia and 2.68 mg/L un-ionized ammonia based upon the results shown in Table 6.1. Also for the NOEC, the more stringent value should be used to protect the sheepshead minnow. The 48-hour NOEC was determined to be 40 mg/L total ammonia and 1.91 mg/L un-ionized ammonia.

Table 6.3 lists the 96-hour LC_{50} (un-ionized ammonia) and the NOEC based upon the analysis of 16 replicates as one test with the Probit Method and the Steel's Many-One Rank Test, respectively. It also lists 96-hour LC_{50} data found in the literature for the sheepshead minnow.

Table 6.3 - 96-hour LC_{50} and NOEC for ammonia based upon 16 replicates for *Cyprinodon variegatus*.

Total LC_{50} (mg/L)	Un-ion. LC_{50} (mg/L)	Total NOEC (mg/L)	Un-ion. NOEC (mg/L)	Lit. Un-ion. LC_{50} [19] (mg/L)
47.8 (44.9 - 51.0)*	2.09 (1.97 - 2.23)*	30	1.3	2.79

* 95% Confidence Interval

It is seen from Table 6.3 that the un-ionized LC_{50} determined by this research falls below the data presented by Miller *et al.* [19] for un-ionized NH_3. The data presented by Miller *et al.* was for sheepshead minnows under similar conditions except that they used a flow-through system at 25°C and 30 ppt salinity. Also Miller *et al.* conducted their test with only 4 replicates as compared to 16 replicates in this research.

The averaging method for determining the LC_{50} was also used for the 96-hour data. Table 6.4 lists the individual LC_{50} and NOEC values for each of the four tests (16 replicates) with sheepshead minnows. The bench sheets are given in Appendix D. This table gives the date of the test initiation, the total and un-ionized ammonia LC_{50} with the 95 percent C. I. in parentheses, the average pH value, and the total and un-ionized NOEC. All of the four tests were analyzed using the Probit Method for the LC_{50} (see Fig. 5.1) and the Steel's Many-One Rank Test for the NOEC (see Fig. 5.2). It also lists the average LC_{50} based upon the averaging method described in Section 5.3.3.

Table 6.4 - 96-hour individual test and average LC_{50} and NOEC for *Cyprinodon variegatus*.

Date	Total LC_{50} (mg/L)	Total NOEC (mg/L)	Avg. pH	Un-ion. LC_{50} (mg/L)	Un-ion. NOEC (mg/L)
12/8/95	53.0 (45.1 - 62.1)*	30	8.14	2.32 (1.98 - 2.72)*	1.3
1/10/96	47.4 (44.0 - 51.2)*	30	8.14	2.08 (1.93 - 2.24)*	1.3
2/7/96	47.1 (41.9 - 52.9)*	30	8.13	2.02 (1.79 - 2.26)*	1.3
2/8/96	41.3 (37.0 - 46.1)*	30	8.14	1.81 (1.62 - 2.02)*	1.3
Average	**47.2 (42.0 - 53.0)***	**30**	**8.14**	**2.07 (1.84 - 2.32)***	**1.3**

* 95% Confidence Interval

The 96-hour LC_{50} values calculated with both methods differed by approximately 1 percent. Therefore, the 96-hour LC_{50} for the sheepshead minnow was reported to be 47.8 mg/L total ammonia and 2.09 mg/L un-ionized ammonia based upon the statistical analysis of all 16 replicates (see Table 6.3). The 96-hour NOEC was determined to be 30 mg/L total ammonia and 1.31 mg/L un-ionized ammonia.

Finally, the SMAV for this work was determined to be 2.37 mg/L based upon the 48-hour and 96-hour LC_{50}. This value is reasonable given that the EPA [6] indicates that the SMAV for *Cyprinodon variegatus* is 2.74 mg/L.

6.1.2 Mysid (*Mysidopsis bahia*)

Eight 48-hour static nonrenewal tests (2 replicates per test) and four 96-hour static renewal tests (4 replicates per test) were analyzed to determine the LC_{50} and NOEC for *Mysidopsis bahia*. The final LC_{50} and NOEC for both the 48-hour and 96-hour tests were determined by the averaging and 16 replicate methods. Both sets of LC_{50} and NOEC values are presented in this section and compared to previous work. The bench sheets given in Appendix E show some mortality in the controls. This mortality appeared to be from the cannibalistic behavior of the juvenile mysids, since the carcasses in the controls had completely disappeared. Therefore, for future testing of the mysid, additional surface area (approximately 20 $in.^2$ of mesh netting) should be added to the test beakers to reduce mortality in the controls. This section lists the results from the mysid tests in two sections:

1. Acute 48-hour static nonrenewal test results.
2. Acute 96-hour static renewal test results.

Table 6.5 lists the 48-hour LC_{50} (un-ionized ammonia) and the 48-hour NOEC based upon analyzing the 16 replicates as one test with the Probit Method and the Steel's Many-

One Rank Test, respectively. The 48-hour LC_{50} value tabulated in this research is shown with an expected LC_{50} given in the literature.

Table 6.5 - 48-hour LC_{50} and NOEC for ammonia based upon 16 replicates for *Mysidopsis bahia*.

Total LC_{50} (mg/L)	Un-ion. LC_{50} (mg/L)	Total NOEC (mg/L)	Un-ion. NOEC (mg/L)	Lit. Un-ion. LC_{50} [19] (mg/L)
21.9 (20.5 - 23.5)*	1.00 (0.94 - 1.08)*	10	0.45	1.3 -1.9 (96-h)

* 95% Confidence Interval

Table 6.5 shows the 48-hour LC_{50} for this research is below the 96-hour range reported by Miller *et al.* [19] for un-ionized NH_3. One would expect that the 96-hour LC_{50} would be lower than the 48-hour LC_{50}. Experiments conducted by Miller *et al.* were at 25°C and 30 ppt salinity as compared to 20°C and 25 ppt for this research. However, these variations in the conditions do not explain the large discrepancies in the un-ionized NH_3 LC_{50}.

Table 6.6 lists the results from the eight tests (16 replicates) with the mysid. This table gives the date of the test initiation, the total and un-ionized ammonia 48-hour LC_{50} with the 95 percent C. I. in parentheses, the average pH values, and the total and un-ionized NOEC. All of the results from the eight tests were analyzed using the Probit Method for the LC_{50} (see flowchart in Fig. 5.1). The 48-hour NOEC was determined using the Dunnett's Test for the test initiated on 12/2/95, and the remaining NOECs were determined using Steel's Many-One Rank Test. The procedure for determining which

test to use for the NOEC was shown in Fig. 5.2. It also lists the average LC_{50} based upon the averaging method described in Section 5.3.3.

Table 6.6 - 48-hour individual test and average LC_{50} and NOEC for ammonia for *Mysidopsis bahia*.

Date	Total LC_{50} (mg/L)	Total NOEC (mg/L)	Avg. pH	Un-ion. LC_{50} (mg/L)	Un-ion. NOEC (mg/L)
12/2/95	24.0 (19.2 - 30.1)*	10	8.16	1.10 (0.88 - 1.38)*	0.46
12/8/95	21.7 (18.3 - 25.8)*	10	8.17	1.02 (0.86 - 1.21)*	0.47
1/14/96	21.7 (18.1 - 26.1)*	20	8.16	0.99 (0.83 - 1.20)*	0.92
1/18/96	24.4 (20.8 - 28.7)*	20	8.16	1.12 (0.95 - 1.31)*	0.92
1/31/96	20.8 (17.4 - 25.0)*	10	8.14	0.91 (0.76 - 1.10)*	0.44
2/10/96	21.6 (17.9 - 26.1)*	10	8.15	0.97 (0.80 - 1.17)*	0.45
2/18/96	20.6 (16.4 - 25.8)*	20	8.17	0.96 (0.77 - 1.21)*	0.94
2/29/96	24.3 (20.1 - 29.5)*	20	8.15	1.09 (0.90 - 1.32)*	0.90
Average	**22.4 (18.5 - 27.1)***	**15**	**8.16**	**1.03 (0.85 - 1.24)***	**0.69**

* 95% Confidence Interval

The LC_{50} values calculated by both methods differed by approximately 2 percent. The 48-hour LC_{50} for the mysid was reported to be 21.9 mg/L total ammonia and 1.00 mg/L un-ionized ammonia as given in Table 6.5. Also for the NOEC, the more stringent value should be used to protect the mysid. The 48-hour NOEC was determined to be 10 mg/L total ammonia and 0.46 mg/L un-ionized ammonia.

Table 6.7 lists the 96-hour LC_{50} (total and un-ionized ammonia) and the 96-hour NOEC based upon the 16 replicates as one test with the Probit Method and the Steel's Many-One Rank Test, respectively. The 96-hour LC_{50} found in the literature is also given.

Table 6.7 - 96-hour LC_{50} and NOEC for ammonia based upon 16 replicates for *Mysidopsis bahia.*

Total LC_{50} (mg/L)	Un-ion. LC_{50} (mg/L)	Total NOEC (mg/L)	Un-ion. NOEC (mg/L)	Lit. Un-ion. LC_{50} [19] (mg/L)
16.9 (15.3 - 18.6)*	0.76 (0.69 - 0.83)*	5.00	0.22	1.3 -1.9 (96-h)

* 95% Confidence Interval

Table 6.7 shows that the 96-hour LC_{50} derived from this research is below the 96-hour range presented by Miller *et al.* [19] for un-ionized NH_3. Experiments conducted by Miller *et al.* were at 25°C and 30 ppt salinity as compared to 20°C and 25 ppt for this research. This variation in temperature and salinity should affect only the total ammonia LC_{50}. However, some data presented by the EPA [6] suggest that the 96-hour LC_{50} is similar to the data reported in this document. The EPA lists 96-hour LC_{50}s ranging from 0.7 - 1.2 mg/L un-ionized ammonia.

Table 6.8 lists the results from the four tests (16 replicates) with the mysid. This table gives the date of the test initiation, the total and un-ionized ammonia 96-hour LC_{50} with the 95 percent C. I. in parentheses, the average pH values, and the 96-hour NOEC. All of the four tests were analyzed using the Probit Method for the LC_{50} (see the flowchart in Fig. 5.1) and the Steel's Many-One Rank Test (procedure shown in Fig. 5.2) for the 96-hour NOEC. It also lists the average LC_{50} based upon the averaging method described in Section 5.3.3.

Table 6.8 - 96-hour individual test and average LC_{50} and NOEC for ammonia for *Mysidopsis bahia.*

Date	Total LC_{50} (mg/L)	Total NOEC (mg/L)	Avg. pH	Un-ion. LC_{50} (mg/L)	Un-ion. NOEC (mg/L)
12/2/95	16.7 (13.5 - 20.6)*	5	8.15	0.75 (0.60 - 0.92)*	0.22
12/13/96	19.0 (15.6 - 23.2)*	5	8.14	0.83 (0.68 - 1.02)*	0.22
1/12/96	15.7 (13.0 - 18.9)*	5	8.15	0.70 (0.58 - 0.85)*	0.22
1/19/96	16.3 (13.5 - 19.7)*	5	8.15	0.73 (0.60 - 0.88)*	0.22
Average	**16.9 (13.9 - 20.6)8**	**5**	**8.15**	**0.76 (0.62 - 0.92)***	**0.22**

* 95% Confidence Interval

The 96-hour LC_{50} was identical using both methods; the only difference was the range of the 95 percent C. I. Therefore, the 96-hour LC_{50} for the mysid was reported to be 16.9 mg/L total ammonia and 0.76 mg/L un-ionized ammonia based upon the statistical analysis of all 16 replicates (see Table 6.7). The 96-hour NOEC was determined to be 5 mg/L total ammonia and 0.22 mg/L un-ionized ammonia. These results show that the mysid is very susceptible to low levels of ammonia when compared to the sheepshead minnow and the grass shrimp.

Finally the SMAV for this work was determined to be 0.87 mg/L. This value is reasonable when compared to EPA's SMAV [6] of 1.02 mg/L for *Mysidopsis bahia.*

6.1.3 Grass Shrimp (*Palaemonetes pugio*)

A total of eight 48-hour static nonrenewal tests (2 replicates per test) was analyzed to determine the LC_{50} and NOEC for the *Palaemonetes pugio.* The bench sheets for these experiments are given in Appendix F. The final LC_{50} and NOEC values were determined by the averaging and 16 replicate methods. Both of the sets of LC_{50} and NOEC are presented in this section and compared to previous work.

Table 6.9 lists the 48-hour LC_{50} and the NOEC (total and un-ionized ammonia) based upon analyzing the 16 replicates as one test. The LC_{50} was analyzed using the Probit Method (Fig. 5.1) and the NOEC used the Steel's Many-One Rank Test based upon the flowchart in Fig. 5.2. Table 6.9 also lists the 96-hour LC_{50} taken from the literature.

Table 6.9 - 48-hour LC_{50} and NOEC for ammonia based upon 16 replicates for *Palaemonetes pugio*.

Total LC_{50} (mg/L)	Un-ion. LC_{50} (mg/L)	Total NOEC (mg/L)	Un-ion. NOEC (mg/L)	Lit. Un-ion. LC_{50} [6] (mg/L)
74.4 (72.1 - 76.7)*	3.48 (3.37 - 3.59)*	50	2.34	2.57

* 95% Confidence Interval

Table 6.9 shows that the 48-hour LC_{50} for this research is above the value presented by the EPA [6] for un-ionized NH_3. The conditions for the 48-hour grass shrimp tests listed by the EPA [6] were similar to the conditions given in this report except for the size (age) of the shrimp. The size of the shrimp used in EPA testing was 10-20 mm but the size of the shrimp used in this research was 15-30 mm. This difference in size (age) of the test organisms was possibly the reason for the discrepancies in the test data. As the aquatic organisms mature, they become more tolerant to ammonia (see Chapter 3).

Table 6.10 - 48-hour individual test and average LC_{50} and NOEC for ammonia for *Palaemonetes pugio*.

Date	Total LC_{50} (mg/L)	Total NOEC (mg/L)	Avg. pH	Un-ion. LC_{50} (mg/L)	Un-ion. NOEC (mg/L)
3/4/96	71.1 (63.8 - 79.6)*	50	8.15	3.19 (2.86 - 3.57)*	2.2
3/6/96	75.3 (70.7 - 80.3)*	50	8.17	3.52 (3.31 - 3.76)*	2.3
3/6/96	77.7 (73.5 - 82.2)*	50	8.18	3.71 (3.51 - 3.93)*	2.4
3/6/96	78.2 (73.8 -82.9)*	50	8.18	3.74 (3.53 - 3.96)*	2.4
3/10/96	73.6 (64.6 - 83.9)*	70	8.17	3.44 (3.02 - 3.93)*	3.3
3/10/96	69.7 (63.5 - 76.5)*	70	8.16	3.19 (2.91 - 3.50)*	3.2
3/10/96	76.3 (69.2 - 84.1)*	60	8.16	3.49 (3.17 - 3.85)*	2.7
3/10/96	74.8 (70.3 - 79.5)*	60	8.16	3.43 (3.22 - 3.64)*	2.7
Average	**74.6 (68.7 - 81.1)***	**57.5**	**8.17**	**3.49 (3.22 - 3.80)***	**2.7**

* 95% Confidence Interval

Table 6.10 lists the results from the eight tests (16 replicates) with the grass shrimp. This table gives the date of the test initiation, the total and un-ionized ammonia LC_{50} with the 95 percent C. I. in parentheses, the average pH values, and the total and un-ionized NOEC. All of the eight tests were analyzed using the Probit Method for the LC_{50} (see flowchart in Fig. 5.1). The NOEC was determined based upon the Dunnett's Test for the final two tests listed in Table 6.10 and the Steel's Many-One Rank Test for the other six experiments. The table also lists the average LC_{50} based upon the averaging method described in Section 5.3.3.

The LC_{50} values calculated by both methods were determined to be less than 1% difference; however, the 95 percent C. I. was greater for the averaging method. Therefore, the 48-hour LC_{50} for the 1-2 month old grass shrimp was reported to be 74.4 mg/L total ammonia and 3.48 mg/L un-ionized ammonia (see Table 6.9). Also for the NOEC, the more stringent value should be used to protect the grass shrimp. The 48-hour NOEC was determined to be 50 mg/L total ammonia and 2.34 mg/L un-ionized ammonia. Since 96-hour tests were not conducted, the data (96-hour LC_{50} = 2.57 mg/L) presented by the EPA [6] was used along with the 48-hour LC_{50} to determine the SMAV (2.99 mg/L). This SMAV was considerably higher than the SMAV (1.65 mg/L) given by the EPA [6] which was probably due to the age of the test organisms.

6.2 Chronic Toxicity Results (Sheepshead Minnow)

Before this report, the chronic toxicity of ammonia had been tested with only two organisms, the Atlantic silverside and mysid. The 7-day chronic test was performed with the sheepshead minnow (*Cyprinodon variegatus*) in order to expand the data base for the chronic effects of ammonia to common saltwater organisms. These data are used herein to determine the acute-chronic ratio and to reevaluate the final chronic limits for marine organisms. This section lists the results from these 7-day chronic bioassays. It gives data

for both survival and growth of the *Cyprinodon variegatus*. This section details the data in the following order:

1. Survival Data (LC_{50} and NOEC based on mortality)
2. Growth Data (NOEC based on growth or weight)

The survival and growth data along with the initial and final conditions are listed in Appendix G.

Table 6.11 lists the mortality results from the four, 7-day chronic tests (16 replicates) for the sheepshead minnow. It gives the date of the test initiation, the total and un-ionized ammonia LC_{50} with the 95 percent C. I. in parentheses, the average pH values, and the total and un-ionized ammonia NOEC. The table also lists the averaged 7-day LC_{50} and 7-day NOEC as well as the 7-day LC_{50} and 7-day NOEC based upon the statistical analysis of the 16 replicates. The LC_{50} and NOEC values for both methods were identical. The 7-day LC_{50} was determined by the Probit Method and the 7-day NOEC based on mortality was determined by the Steel's Many-One Rank Test.

Table 6.11 - 7-day individual test and average LC_{50} and NOEC for ammonia for *Cyprinodon variegatus* based upon mortality.

Date	Total LC_{50} (mg/L)	Total NOEC (mg/L)	Avg. pH	Un-ion. LC_{50} (mg/L)	Un-ion. NOEC (mg/L)
12/15/95	39.5 (34.4 - 45.4)*	30	8.14	1.73 (1.51 - 1.99)*	1.3
2/2/96	39.9 (37.9 - 41.9)*	30	8.14	1.75 (1.66 - 1.84)*	1.3
2/2/96	37.7 (35.2 - 40.4)*	30	8.15	1.69 (1.58 - 1.81)*	1.3
2/7/96	38.6 (36.2 - 41.2)*	30	8.15	1.73 (1.62 - 1.85)*	1.3
Average	**38.9 (35.9 - 42.2)***	**30**	**8.15**	**1.74 (1.61 - 1.89)***	**1.3**
16 replicate method	**38.9 (37.0 - 40.8)***	**30**	**8.15**	**1.74 (1.66 - 1.83)***	**1.3**

* 95% Confidence Interval

The 7-day LC_{50} for the sheepshead minnow was determined to be 38.9 mg/L total ammonia and 1.74 mg/L un-ionized ammonia. The 7-day NOEC was determined to be 30 mg/L total ammonia and 1.34 mg/L un-ionized ammonia.

Table 6.12 lists the 7-day chronic NOEC based upon growth for the individual tests as well as the final NOEC values determined by both methods. All tests were analyzed using the Dunnett's Test (see flowchart in Fig. 5.3).

Table 6.12 - 7-day chronic NOEC for the *Cyprinodon variegatus* based upon growth.

Date	Tot. NH_3 (mg/L) NOEC	Un-ionized NH_3 (mg/L) NOEC
12/15/95	10	0.45
2/2/96	0	0.45
2/2/96	0	0.45
2/7/96	20	0.90
Average	**7.5**	**0.34**
16 replicate method	**10**	**0.45**

The 7-day NOEC was determined to be 7.5 mg/L total ammonia and 0.34 mg/L un-ionized ammonia. Since 7.5 mg/L was below the lowest dilution used in the experiments, the LOEC was defined as the lowest concentration which affected growth. Therefore, the 7-day LOEC was 10 mg/L total ammonia and 0.45 mg/L un-ionized ammonia. This results in an un-ionized ammonia chronic value of 0.39 mg/L. The chronic value was determined by calculating the geometric mean from the NOEC and LOEC. Based upon the acute value (LC_{50} = 2.68 mg/L) from Section 6.1.1, the acute-chronic ratio is 6.95. This procedure is described by the EPA [6]. The acute-chronic ratio of 6.95 compares to similar values of 21.3 for the inland silverside and 7.2 for the mysid. However, the chronic test for the inland silverside was conducted over a 28-day period, so it was expected that the acute-chronic ratio was a factor of three greater than that of the sheepshead minnow. This research shows that the 7-day test can be used to evaluate the chronic toxicity of ammonia for marine fishes, thus providing a short-term method for estimating the chronic toxicity of ammonia. The 7-day test can be used because growth

was inhibited in concentrations without significant mortality at these same concentrations.

6.3 Summary of Results

A summary of the acute and chronic results is given in Table 6.13.

Table 6.13 - Summary of acute and chronic results (un-ionized NH_3).

Property	***C. variegatus*** **(mg/L)**	***M. bahia*** **(mg/L)**	***P. pugio*** **(mg/L)**
48-hour LC_{50}	2.68	1.00	3.48
96-hour LC_{50}	2.09	0.76	**
SMAV	**2.37**	**0.87**	**2.99**
48-hour NOEC	1.91	0.46	2.34
96-hour NOEC	1.31	0.22	**
7-day LC_{50}	1.74	**	**
7-day NOEC (mortality)	1.34	**	**
7-day NOEC (growth)	0.34	**	**
7-day LOEC (growth)	0.45	**	**
Chronic Value	**0.39**	**0.23**	**
Acute-Chronic Ratio	**6.95**	**4.35**	**

** not tested

7

National Criteria Based Upon Results

The LC_{50} and NOEC data for the three organisms were used to refine the national criteria for ammonia. The national criteria for saltwater are separated into two standards: the one-hour average concentration (acute) and the four-day average concentration (chronic). The national criteria state that these concentrations cannot be exceeded more than once every three years. The current national criteria are listed as 0.233 mg/L un-ionized NH_3 for the acute criteria and 0.035 mg/L un-ionized NH_3 for the chronic criteria (see Table 2.5). These criteria were determined based upon saltwater and freshwater toxicity data and procedures outlined in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" [60].

The procedures used to calculate the national criteria for ammonia are outlined in this chapter. Also, the refined national criteria based upon the results in Chapter 6 are listed as well. These topics are covered in three sections:

1. Summary of Pertinent Toxicity Results
2. Summary of Procedure for Determining the National Criteria
3. Refined National Criteria

7.1 Summary of Pertinent Toxicity Results

The SMAV (geometric mean of 48-hour and 96-hour un-ionized ammonia LC_{50} for a specific organism), and the chronic value (geometric mean of chronic un-ionized ammonia NOEC and LOEC) were used to determine the final acute value and the final chronic value. Table 7.1 lists the results from this research which were used to derive the final acute and chronic values.

Table 7.1 - Summary of un-ionized NH_3 results used in calculating national criteria.

Property	*C. variegatus*	*M. bahia*	*P. pugio*
48-hour LC_{50}	2.68	1.00	3.48
96-hour LC_{50}	2.09	0.76	2.57*
SMAV	**2.37**	**0.87**	**2.99**
7-day NOEC (growth)	0.34	0.16*	**
7-day LOEC (growth)	0.45	0.33*	**
Chronic Value	**0.39**	**0.23***	**
Acute-Chronic Ratio	**6.95**	**4.35**	**

* EPA [6]
** unknown

7.2 Summary of Procedure for Determining the National Criteria

7.2.1 Acute Criterion

The final acute value (FAV) was calculated based upon the procedures outlined by Stephan *et al.* [60]. A summary of this procedure is as follows:

1. The species were ranked from most sensitive (R = 1) to most tolerant (R = N) based upon the GMAV (See Fig. 2.4). For ammonia, N was based upon the genus total number (N=18).

2. The GMAV from the top four species were used to calculate the FAV. For ammonia the four species were the winter flounder (*Pseudopleuronectes americanus*), the red drum (*Sciaenops ocellatus*), the Sargassum shrimp (*Latreutes fucorum*), and the prawn (*Macrobrachium rosenbergii*).

3. The FAV was calculated based upon these five equations and by using only the GMAV for the four species listed above.

$$P = R / (N + 1) \qquad (7.1)$$

$$S^2 = \frac{\sum((\ln(GMAV))^2) - ((\sum(\ln(GMAV)))^2 / 4)}{\sum(P) - ((\sum(\sqrt{P}))^2 / 4)} \quad (7.2)$$

$$L = \frac{\left(\sum(\ln(GMAV)) - S\left(\sum\left(\sqrt{P}\right)\right)\right)}{4} \quad (7.3)$$

$$A = S\left(\sqrt{0.05}\right) + L \quad (7.4)$$

$$FAV = e^A \quad (7.5)$$

4. The final acute criterion was FAV/2. Therefore, from Equations 7.1 through 7.5, the FAV = 0.465 mg NH_3/L and the final acute criterion was 0.233 mg NH_3/L.

7.2.2 Chronic Criterion

The final chronic criterion was calculated using the acute-chronic ratios from two saltwater and four freshwater organisms. This procedure was outlined in Section 2.4.1. The geometric mean of these six acute-chronic ratios was calculated and then divided into the FAV (0.465 mg NH_3/L) to yield a chronic criterion of 0.035 mg NH_3/L.

7.3 Refined National Criteria

The chronic criterion for ammonia was refined based upon the results from the toxicity experiments in this report. In this report the four freshwater species were excluded from the chronic calculation procedure. The acute criterion remained the same (0.233 mg NH_3/L) since the GMAV for the sheepshead minnow, mysid, and grass shrimp were not ranked among the top four acutely sensitive species.

Based upon the acute-chronic ratios for the mysid (4.35), inland silverside (21.3) and sheepshead minnow (6.95), the geometric mean of these three values was 8.64. By dividing the 8.64 into the FAV, the final chronic value was determined to be 0.054 mg NH_3/L. The total ammonia concentration can be determined based upon the temperature, pH, and salinity.

The refined national criteria are:

- Saltwater aquatic organisms should not be affected unacceptably if the one-hour average concentration of un-ionized ammonia does not exceed 0.233 mg/L more than once every three years on the average.

- Saltwater aquatic organisms should not be affected unacceptably if the four-day average concentration of un-ionized ammonia does not exceed 0.054 mg/L more than once every three years on the average.

8

Ammonia Toxicity Conclusions

Acute and chronic toxicity experiments are the most effective techniques to determine the effect ammonia has on aquatic marine life. Forty-eight hour, 96-hour, and 7-day toxicity tests are the basis for determining the one-hour average ammonia concentration (acute criterion) and the four-day average ammonia concentration (chronic criterion). It was essential to determine acute and chronic LC_{50} and NOEC data for organisms which inhabit the Chesapeake Bay watershed.

The acute LC_{50} and NOEC were determined for three common marine organisms. Also, the chronic toxicity of ammonia was evaluated for the sheepshead minnow (*Cyprinodon* variegatus) in order to determine an acute-chronic ratio for this species. These new data were used along with other toxicity data reported by the EPA [6] to calculate an updated national criteria for ammonia discharge into saltwater ecosystems. These national criteria should be updated as more research is conducted with additional marine organisms.

Based upon the research presented in Chapters 6 and 7, several conclusions were made and are presented in the following order:

- Acute Toxicity Conclusions
- Chronic Toxicity Conclusions
- National Criteria Conclusions

The conclusions are followed by a section which discusses the continuing research and the future work.

8.1 Acute Toxicity Conclusions

1. The rank of the three organisms tested with respect to their susceptibility to ammonia was as follows:
    1. mysid (48-hour LC_{50} = 1.00 mg/L).
    2. sheepshead minnow (48-hour LC_{50} = 2.68 mg/L).
    3. grass shrimp (48-hour LC_{50} = 3.48 mg/L).

2. The 48-hour LC_{50} (2.68 mg/L) for the sheepshead minnow was similar to the 48-hour LC_{50} reported in the literature under similar conditions. However, the 96-hour LC_{50} (2.09 mg/L) was 33% lower than the value reported by Miller *et al.* [19]. The 48-hour and 96-hour data in the literature for the juvenile sheepshead minnow were conflicting since the 96-hour LC_{50} (2.79 mg/L) was at the upper limit of the range suggested for the 48-hour LC_{50} (2.1 - 2.8 mg/L). However, the data determined in this study, obeyed the hypothesis that the LC_{50} decreases as time increases.

3. The mysid experiments indicated that the organism was more sensitive to ammonia than reported by Miller *et al* [19]. The 96-hour LC_{50} was determined to be 0.76 mg/L as compared to 1.3 - 1.9 mg/L from Miller *et al.* These differences could be related to pH control. The LC_{50} determined in this study was close to the values reported by the EPA [6].

4. The grass shrimp's tolerance (48-hour LC_{50} = 3.48 mg NH_3/L) may be because of the age of the test organisms (1-2 months). Work presented by Burton and Fisher [42] suggested that the 96-hour LC_{50} for juvenile *Palaemonetes pugio* (<1 week old) was 1.2 mg/L un-ionized NH_3 under similar conditions.

5. There appeared to be no experiments in the literature which were conducted under the conditions of this study. Therefore, the SMAV found in the literature were compared with the SMAV calculated from the 48-hour and 96-hour LC_{50}s in this study. The SMAV for the sheepshead minnow was determined to be 2.37 mg/L as compared to a reported SMAV of 2.74 mg/L (13.5% difference). The SMAV for the mysid was determined to 0.87 mg/L as compared to a reported SMAV of 1.02 (14.7% difference). The SMAV for the grass shrimp was determined to be 2.99 mg/L as compared to a reported SMAV of 1.65 mg/L (81.2% difference) [6].

8.2 Chronic Toxicity Conclusions

To date, the chronic toxicity of ammonia has been determined for only three saltwater species. The organisms which have been tested are: the inland silverside (*Menidia beryllina*) - 28-day early life-stage test; the mysid (*Mysidopsis bahia*) - 32-day life cycle test; and the sheepshead minnow (*Cyprinodon variegatus*) - 7-day early life-stage test. This study contributed chronic toxicity data for the sheepshead minnow to the literature. The conclusions from this study were as follows:

1. The 7-day NOEC of 0.45 mg/L based on mortality along with the acute data indicate that the sheepshead minnow was the most ammonia tolerant of the three species tested in this research.

2. The NOEC was determined to be 7.5 mg/L total ammonia (0.34 mg NH_3/L) and high mortality was not seen until the 40 mg/L total ammonia (1.79 mg NH_3/L) treatment. Therefore, this research indicated that the 7-day test has the potential to be a more time efficient and effective means for assessing the chronic toxicity of ammonia to marine fishes.

3. The acute-chronic ratio for the *Cyprinodon variegatus* was 6.95 as compared to 21.3 for the *Menidia beryllina* and 7.2 [19] for the *Mysidopsis bahia* (acute-chronic ratio was determined to be 4.35 for this research). The acute-chronic ratio for *Cyprinodon variegatus* indicated that this organism was not as chronically sensitive as *Menidia beryllina*. However, the acute-chronic ratio for the sheepshead minnow was based upon a 7-day chronic test as compared to a 28-day chronic test for the *Menidia beryllina*.

8.3 National Criteria Conclusions

1. The final acute criterion remained at 0.233 mg NH_3/L since none of the species tested in this research ranked among the top four acutely sensitive organisms.

2. The final chronic criterion was determined to be 0.054 mg NH_3/L based upon saltwater organisms only.

8.4 Continuing Research and Future Work

It is apparent from the limited data on the toxicity of ammonia to saltwater species that further testing is necessary to gain more confidence in acute and chronic toxicity values. Research is continuing at Virginia Polytechnic Institute and State University to expand both the acute and chronic toxicity data base. The work is focused on 48-hour and 96-hour acute, as well as 7-day chronic tests, for marine fishes, and 14-day or 28-day chronic tests for marine invertebrates. The following list summarizes the current research, as well as the future work, which needs to be performed in order to better understand the impact that ammonia has on marine vertebrates and invertebrates. This work will allow more appropriate ammonia discharge limits to be established in order to maintain the health of marine ecosystems.

1. Currently, work is ongoing with the inland silverside (*Menidia beryllina*). Both 48-hour static acute and 7-day static chronic tests are being performed under test conditions similar to those described in this report.

2. Chronic studies with the grass shrimp (*Palaemonetes pugio*) will be undertaken to expand the database and assess an acute-chronic ratio.

3. Plans are underway to study the acute and chronic effects of ammonia to the post-larval stage of the blue crab (*Callinectes sapidus*). This work should prove valuable to not only the seafood industry, but others who are discharging ammonia to the Chesapeake Bay region.

4. Other vertebrate test species which are being considered for testing, especially in chronic experiments, include:

    - Winter flounder (*Pseudopleuronectes americanus*)
    - Summer flounder (*Paralichthys dentatus*)
    - Killifish (*Fundulus simillis*)
    - Mummichog (*Fundulus heteroclitus*)
    - Spot (*Leostomus xanthurus*)

5. Other invertebrate test species which are being considered for testing, especially in chronic experiments, include:

    - White shrimp (*Penaeus setiferus*)

- Pink shrimp (*Penaeus duoarum*)
- Mysid (*Mysidopsis amyra*)
- Mysid (*Metamysidopsis elongata*)
- American oyster (*Crassostrea virginica*)

6. Acute results along with the 7-day chronic test results will be used to refine the national criteria values according to the guidelines referenced in this report.

References

1. Metcalf and Eddy, Inc. (1991) Wastewater Engineering. McGraw-Hill, Inc., New York, chap. 3.

2. Svobodova, Z., R. Lloyd, J. Machova, and B. Vykusova (1993) Water Quality and Fish Health - a EIFAC Technical Paper. Food and Agriculture Organization, Rome, pp. 11-15.

3. McVeigh, P.J. (1995) "Treatment of Blue Crab Processing Wastewater Using Upflow Anaerobic Sludge Blanket (UASB) and Air Stripping Technologies." M.S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virgina.

4. Diz, H.R. (1994) "Anaerobic/Aerobic Pretretment of Blue Crab (*Callinectes sapidus*) Cooker Wastewater." M.S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virgina.

5. Wolfe, C.L. (1993) "Biological and Physical Treatment of Crab Processing Industry Wastewater." M.S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virgina.

6. US EPA (1989) Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989. EPA 440/5-88-004. Office of Water Regulations and Standards, Criteria and Standards Division, Washington, DC, USA.

7..US EPA (1991) Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA 600/4-90/027. Office of Research and Development, U.S. EPA, Washington, DC, USA.

8. US EPA (1989) Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA 600/4-89/001. Office of Research and Development, U.S. EPA, Cincinnati, OH, USA.

9. Wuhrmann, K. and H. Woker (1948) "Experimentelle Untersuchungen uber die Zyanidlosungen mit verschiedener Sauerstoffspannung und Temperaturauf Fische." (an English interpretation) Schweiz Z. Hudrol. Vol. 15, pp. 235-260.

10. Jacobs, M.H. (1940) "Some aspects of cell permeability to weak electolytes." Cold Spring Harbor Symposium of Quantitative Biology. Vol. 8, pp. 30-39.

11. Milne, M.D., B.H. Scribner, and M.A. Crawford (1958) "Non-ionic diffusion and the excretion of weak acids and bases." American Journal of Medicine. Vol. 24, pp. 709-729.

12. Bower, C.E. and J.P. Bidwell (1978) "Ionization of ammonia in seawater: effects of temperature, pH, and salinity." Journal of the Fisheries Research Board of Canada. Vol. 35, pp. 1012-1016.

13. American Public Health Association, American Water Works Association and Water Polution Control Federation. (1995) Standard Methods for the Examination of Water and Wastewater. 19th ed., American Public Health Association, Washington, D.C., pp. 4-75 - 4-78.

14. Alabaster, J.S. (1980) Water Quality Criteria for Freshwater Fish. Butterworths, London, chap. 4.

15. Emerson, K., R.C. Russo, R.E. Lund, and R.V. Thurston (1975) "Aqueous ammonia equilibrium calculations: effect of pH and temperature." Journal of the Fisheries Research Board of Canada. Vol. 32, pp. 2379-2383.

16. Burrows, R.E. (1964) "Effects of accumulated excretory products on hatchery-reared salmonids. Research Replication of the U.S. Fish and Wildlife Service. Vol. 66, pp.1-12.

17. Brown, V.M. (1968) "The calculation of the acute toxicity of mixtures of poisons to rainbow trout." Water Research. Vol. 2, pp. 723-733.

18. Alabaster, J.S., D.G. Shurben, and G. Knowles (1979) "The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo salar*." Journal of Fish Biology. Vol. 15, pp. 705-712.

19. Miller, D.C., S. Poucher, J.A. Cardin, and D. Hansen (1990) "The acute and chronic toxicity of ammonia to marine fish and a mysid." Archives of Environmental Contamination and Toxicology. Vol. 19, PP. 40-48.

20. Martinelle, K., and L. Haggstrom (1993) "Mechanisms of ammonia and ammonium ion toxicity in animal cells: transport across cell membranes." Journal of Biotechnology. Vol. 30, pp. 339-350.

21. Goldstein, L., J.B. Claiborne, D.E. Evans (1982) "Rapid communication - ammonia excretion by the gills of two marine teleost fish: the importance of NH_4^+ permeance." The Journal of Experimental Zoology. Vol. 219, pp. 395-397.

22. Evans, D.H. (1980) "Kinetic studies of ion transport by fish gill epithelium." American Journal of Physiology. Vol. 238, pp R224-R230.

23. Glacken, M.W (1988) "Catabolic control of fish cell culture." Biotechnology. Vol.6, pp. 1041-1050.

24. Docherty, P.A. and M.D. Snider (1991) "Effects of hypertonic and sodium-free medium on transport of a membrane glycoprotein along the secretory pathway in cultured mammalian cells." Journal of Cellular Physiology. Vol. 146, pp. 34-42.

25. Glacken, M.W., E. Adema, and A.J. Sinskey (1988) "Mathematical description of hybridoma culture kinetics: I. Initial metabolic rates." Biotechnology and Bioengineering. Vol. 32, pp. 491-506.

26. Lloyd, R. and L.D. Orr (1969) "The diuretic response by rainbow trout to sub-lethal concentrations of ammonia." Water Research. Vol. 3, pp. 335-344.

27. Hermanutz, R.O., S.F. Hedtke, J.W. Arthur, R.W. Andrew, D.N. Allen (1987) "Ammonia effects on microinvertebrates and fish in outdoor experimental streams." Environmental Pollution. Vol. 47, pp. 249-283.

28. U.S. EPA (1985a) "Ambient water quality criteria for ammonia-1984." NTIS# PB85-227114. National Technical Information Service, Springfield, VA 22161.

29. U.S. EPA (1986) Chapter 1 - Stream Design Flow for Steady-State Modeling. in: Book VI - Design Conditions. Technical Guidance Manual for Performing Waste Load Allocations. Office of Water, U.S. EPA, Washington, D.C.

30. Calamari, D. and R. Marchetti (1975) "Predicted and observed acute toxicity of copper and ammonia to rainbow trout (*Salmo gairdneri* Rich.)." Progess in Water Technology. Vol. 7, pp. 569-577.

31. Knoph, M.B. (1992) "Acute toxicity of ammonia to atlantic salmon (*Salmo salar*) parr." Comprehensive Biochemistry and Physiology. Vol. 101C, no. 2, pp. 275-282.

32. Thurston, R.V., R.C. Russo, and G.A. Vinogradov (1981) "Ammonia toxicity to fishes. effect of pH on the toxicity of the un-ionized ammonia species." Environmental Science and Technology. Vol. 15, no. 7, pp. 837-840.

33. Thurston, R.V., R.C. Russo, and C.E. Smith (1978) "Acute toxicity of ammonia and nitrite to cutthroat trout fry." Transactions of the American Fisheries Society. Vol. 107, no. 2, pp. 361-368.

34. Arthur, J.W., C.W. West, K.N. Allen, and S.F. Hedtke (1987) "Seasonal toxicityof ammonia to five fish and nine invertebrate species." Bulletin of Environmental Contamination and Toxicology. Vol. 38, pp. 324-331.

35. Mayes, M.A., H.C. Alexander, and D.L. Hopkins (1986) "Acute and chronic oxicity of ammonia to freshwater fish: a site-specific study." Environmental Toxicology and Chemistry. Vol. 5, pp. 437-442.

36. Gersich, F.M. and D.L. Hopkins (1986) "Site-specific acute and chronic toxicity of ammonia to*Daphnia magna* straus." Environmental Toxicology and Chemistry. Vol. 5, pp. 443-447.

37. Cardin, J.A. (1986) "Memorandum to David Hansen." U.S. EPA, Narragansett, Rhode Island.

38. Hazel, C.R., W. Thomsen, and S.J. Meith (1971) "Sensitivity of striped bass and stickleback to ammonia in relation to temperature and salinity." California Fish and Game. Vol. 57, no. 3, pp. 154-161.

39. Poucher, S. (1986) "Memorandum to David Hansen." U.S. EPA, Narragansett, Rhode Island.

40. E.A. Eng. (EA Engineering, Science, and Technology, Inc.). (1986) "Proposed modified effluent limitations for ammonia." EA Report BET54E. Prepared for Bethlehem Steel Corporation, Sparrows Point, MD 21219.

41. Fava, J.A., J.J. Gift, A.F. Maciorowski, W.L. McCulloch, and H.J. Reisinger II. (1984) "Comparitive toxicity of whole and liquid phase sewage sludges to marine organisms." Pages 229-252 in: Aquatic Toxicology and Hazard Assessment: Seventh Symposium, R. Cardwell, R. Purdy, and R. Banner (Eds.). ASTM STP 854, ASTM, Philadelphia, PA.

42. Burton, D.T. and D.J. Fisher (1990) "Acute toxicity of cadmium, copper, zinc, ammonia, 3,3'-dichlorobenzidine, 2,6-dichloro-4-nitroaniline, methylene chloride, and 2,4,6-trichlorophenol to juvenile grass shrimp and killifish." Bulletin of Environmental Contamination and Toxicology. Vol. 44, pp. 776-783.

43. Venkataraamiah, A., G.J. Lakshmi, C. Best, G. Gunter, E. Hartwig, and P Wilde. (1981) "Studies on toxicity of OTEC plant components on marine animals from the Gulf of Mexico." MTIS# DE82012327, National Technical Information Service, Springfield, VA.

44. Epifano, E.C. and R.F. Srna (1975) "Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*." Marine Biology. Vol. 33, no. 3, pp. 241-246.

45. Armstrong, D.A., D. Chippendale, A. W. Knight, and J.E. Colt (1978) "Interaction of ionized and un-ionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii*." Biological Bulletin. Vol. 150, no. 1, pp. 1-14.

46. Hampson, B.L. (1976) "Ammonia concentration in relation to ammonia toxicity during a rainbow trout rearing experiment in a closed freshwater-seawater system." Aquaculture. Vol. 9, pp. 61-70.

47. Broderius, S.J., R.A. Drummond, J.T. Fiandt, and C.L. Russom (1985) "Toxicity of ammonia to early life stages of the smallmouth bass at four pH values." Environmental Toxicology and Chemistry. Vol. 4, pp. 87-96.

48. McCormick, J.H., S.J. Broderius, and J.T. Fiandt (1984) "Toxicity of ammonia to early life stages of the green sunfish *Lepomis cyanellus*." Environmental Pollution Series A. Vol. 36, pp. 147-163.

49. Smith, W.E., T.H. Roush, and J.T. Fiandt (1984) "Toxicity of ammonia to early life stages of bluegill (*Lepomis macrochirus*)." Internal Report, EPA-600/X-84-175, U.S. EPA, Diluth, MN.

50. Mount, D.I. (1982) "Memorandum to R.C. Russo." U.S. EPA, Narragansett, Rhode Island.

51. Kohn, N.P., J.Q. Word, D.K. Niyogi (1994) "Acute toxicity of ammonia to four species of marine amphipod." Marine Environmental Research. Vol. 38, pp. 1-15.

52. Nordlie, F.G. (1987) "Plasma osmotic Na^+ and Cl^- regulation under euryhaline conditions in *Cyprinodon variegatus* Lacepede." Comprehensive Biochemical Physiology. Vol. 86A, no. 1, pp. 57-61.

53. Carter, M.A., L.A. Burns, T.R. Cavinder, K.R. Dugger, P.L. Fore, D.B. Hicks, H.L. Revells, and T.W. Schmidt (1973) "Ecosystem analysis of the Big Cypress Swamp and estuaries." EPA 904/9-74-002, Surveillance and Analysis Division, U.S. EPA, Athens, GA.

54. Heard, R.W. (1982) "Guide to the common tidal marsh invertebrates of the northeastern Gulf of Mexico." Publication No. MASGP-79-004, Mississippi-Alabama Sea Grant Consortium ,Ocean Springs, Mississippi.

55. Williams, A.B. (1984) Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida. Smithsonian Institution Press, Washington, D.C., p. 76.

56. Ward, S.H. (1991b) "A comparison of natural and artificial seawater for culturing and toxicity testing with *Mysidopsis bahia*." In: W. Landis, M.A Lewis, and J.S. Hughes, eds., First Symposium on Environmental Toxicology and Risk Assessment, ASTM, April 14-16, Atlantic City, New Jersey.

57. Reistsema, L.A. and J.M. Neff (1980) "A recirclating artificial seawater system for the laboratory culture of *Mysidopsis bahia*." Estuaries. Vol. 3, pp. 321-323.

58. Hansen, D.J. (1984) "Utility of toxicity tests to measure effects of substances on marine organisms." Pages 33-56 in: Concepts in Marine Pollution Measurements. H. White (Ed.), Maryland Sea Grant College, University of Maryland, College Park, Maryland.

59. Schimmel, S.C. (1986) "Memorandum to David J. Hansen." U.S. EPA, Narragansett, Rhode Island.

60. Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs (1985) "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses." NTIS #PB85-227049. National Technical Information Service, Springfield, VA.

Bibliography

Landis, W.G. and M. Yu (1995) Introduction to Environmental Toxicology: Impacts of Chemicals Upon Ecological Systems. Lewis Publishers, Boca Raton.

National Research Council (1979) Ammonia. University Park Press, Baltimore.

Slack, A.V. and G.R. James (1973) Ammonia - Part I. Marcel Dekker, Inc., New York.

Snoeyink, V.L. and D. Jenkins (1980) Water Chemistry. John Wiley & Sons, New York.

Starr, C. and R. Taggart (1989) Biology: The Unity and Diversity of Life. Wadsworth Publishing Company, Belmont.

Appendix A

% Un-ionized Ammonia as a Function of pH, Temperature, and Salinity

Salinity = 18-22 ppt

		pH								
Temp. (°C)	pK_a	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4
0	10.10	0.32	0.40	0.50	0.63	0.79	0.99	1.24	1.56	1.96
5	9.94	0.46	0.58	0.72	0.91	1.14	1.43	1.80	2.25	2.82
10	9.78	0.66	0.83	1.05	1.31	1.65	2.07	2.59	3.23	4.04
15	9.61	0.96	1.20	1.51	1.90	2.37	2.97	3.71	4.63	5.76
20	9.45	1.39	1.74	2.18	2.73	3.41	4.26	5.30	6.58	8.15
25	9.29	2.00	2.51	3.14	3.91	4.88	6.07	7.52	9.28	11.4

Salinity = 23-27 ppt

		pH								
Temp. (°C)	pK_a	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4
0	10.13	0.29	0.37	0.47	0.59	0.74	0.93	1.16	1.46	1.83
5	9.97	0.43	0.54	0.68	0.85	1.07	1.34	1.68	2.10	2.63
10	9.81	0.62	0.78	0.98	1.23	1.54	1.93	2.42	3.03	3.78
15	9.64	0.90	1.13	1.41	1.77	2.22	2.78	3.47	4.33	5.39
20	9.48	1.30	1.63	2.04	2.55	3.19	3.98	4.97	6.17	7.65
25	9.32	1.87	2.34	2.93	3.66	4.57	5.68	7.05	8.72	10.7

Salinity = 28-31 ppt

		pH								
Temp. (°C)	pK_a	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4
0	10.14	0.29	0.36	0.46	0.57	0.72	0.90	1.14	1.43	1.79
5	9.98	0.42	0.52	0.66	0.83	1.04	1.31	1.64	2.06	2.57
10	9.82	0.60	0.76	0.96	1.20	1.51	1.89	2.36	2.96	3.70
15	9.65	0.88	1.10	1.38	1.73	2.17	2.72	3.40	4.24	5.28
20	9.49	1.27	1.59	1.99	2.50	3.12	3.90	4.86	6.04	7.49
25	9.33	1.83	2.29	2.87	3.58	4.47	5.56	6.90	8.54	10.5

Salinity = 32-40 ppt

		pH								
Temp. (°C)	**pK_a**	**7.6**	**7.7**	**7.8**	**7.9**	**8.0**	**8.1**	**8.2**	**8.3**	**8.4**
0	10.16	0.28	0.35	0.44	0.55	0.69	0.86	1.09	1.36	1.71
5	10.00	0.40	0.50	0.63	0.79	1.00	1.25	1.57	1.97	2.46
10	9.84	0.58	0.73	0.91	1.15	1.44	1.80	2.26	2.83	3.54
15	9.67	0.84	1.05	1.32	1.66	2.07	2.60	3.25	4.06	5.05
20	9.51	1.21	1.52	1.90	2.39	2.98	3.73	4.65	5.78	7.17
25	9.35	1.75	2.19	2.74	3.43	4.28	5.32	6.61	8.18	10.1

Appendix B

Suppliers of Marine Toxicity Testing Organisms

Aquatic Indicators
710 Holmes Boulevard
St. Augustine, FL 32084
(904) 829-1194

Charles River Aquatic Research Organisms
251 Ballardvale Street
Wilmington, MA 01887
(800) 927-1650

Cosper Environmental Services, Incorporated
83 Carlough Road
Bohemia, NY 11716
(516) 563-8899

Florida Bioasssay Supplies
2809 NW 161 CL
Gainesville, FL 32609
(904) 984-5297

Gulf Specimens
P.O. Box 237
Panacea, FL 32346
(904) 984-5297

Marinco
7524 Castle Drive
Sarasota, FL 34240
(813) 377-5219

Appendix C

Mysid Reference Bench Sheets

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: Reference Test
PERMIT NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: AM/PM 1/1 (DATE)
COMPOSITE COLLECTED:
FROM AM/PM 1/1 (DATE)
TO AM/PM 1/1 (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.1
INITIAL D.O.: 6.9
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysid
CULTURE NO.:
AGE: 72 days
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48-hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 8:00 AM/PM 11/10/95 (DATE)
END OF TEST 8:00 AM/PM 11/12/95 (DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
1/1 1/1 1/1
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	Salinity (ppt) ~~CONDUCTIVITY (umhos/cm)~~ INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	9			6.9	6.8	6.7			8.1	8.0	8.0			20	20	20					25.2/25.3
	B	10	9	8																				
0.25	A	10	10	8			6.9	6.6	6.7			8.1	8.0	8.0			20	20	20					25.2/25.4
mg/L	B	10	9	7																				
0.50	A	10	10	8			6.9	6.8	6.6			8.1	7.9	7.8			20	20	20					25.2/25.5
mg/L	B	10	8	6																				
0.75	A	10	10	6			6.9	6.8	6.7			8.1	8.0	8.0			20	20	20					25.2/25.3
mg/L	B	10	9	6																				
1.0	A	10	10	3			6.9	7.0	6.6			8.1	8.0	8.0			20	20	20					25.2/25.3
mg/L	B	10	8	2																				
1.25	A	10	7	1			6.9	6.8	6.7			8.0	7.9	7.9			20	20	20					25.2/25.4
mg/L	B	10	6	0																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: Reference Toxt
PERMIT NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.1
FROM AM/PM / / (DATE) INITIAL D.O.: 7.1
TO AM/PM / / (DATE) ADJUSTMENTS:
JOB NO.:
SAMPLE NO.:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysid
CULTURE NO.:
AGE: 2-2days
LENGTH(X±SD):
WEIGHT(X±SD):
TEST MODE: 48-hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 9:00 AM/PM 11/6/95 (DATE)
END OF TEST 8:00 AM/PM 11/8/95 (DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	Salinity (ppt) ~~CONDUCTIVITY (umhos/cm)~~ INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	9			7.1	6.8	6.6			8.1	7.9	8.0			20	20	20					25.0/25.2
	B	10	10	9																				
0.25	A	10	10	9			7.1	6.9	7.0			8.1	7.9	8.1			20	20	20					25.0/25.3
mg/L	B	10	8	8																				
0.50	A	10	9	7			7.1	6.6	6.7			8.1	7.8	7.9			20	20	20					25.0/25.3
mg/L	B	10	10	6																				
0.75	A	10	9	4			7.1	6.7	6.8			8.1	7.8	8.0			20	20	20					25.0/25.3
mg/L	B	10	9	3																				
1.0	A	10	9	4			7.1	7.0	6.8			8.1	7.9	7.9			20	20	20					25.0/25.4
mg/L	B	10	8	2																				
1.25	A	10	8	2			7.1	6.9	6.7			8.1	7.9	8.0			20	20	20					25.0/25.3
mg/L	B	10	8	1																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: Reference Toxt
PERMIT NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: ____ AM/PM / / (DATE)
COMPOSITE COLLECTED:
FROM ____ AM/PM / / (DATE)
TO ____ AM/PM / / (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.0
INITIAL D.O.: 7.0
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysid
CULTURE NO.:
AGE: <2days
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48-hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 8:00 AM/PM 11/6/95 (DATE)
END OF TEST 8:00 AM/PM 11/8/95 (DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	Salinity(ppt) ~~CONDUCTIVITY (umhos/cm)~~ INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.0	6.8	6.7			8.0	7.8	7.8			20	20	20					24.6/25.0
	B	10	9	8																				
0.25	A	10	10	7			7.0	6.8	6.8			8.0	7.8	7.8			20	20	20					24.6/25.1
mg/L	B	10	10	9																				
0.5	A	10	9	7			7.0	6.7	6.7			8.0	7.8	7.9			20	20	20					24.6/24.9
mg/L	B	10	9	8																				
0.75	A	10	8	4			7.0	6.6	6.7			8.0	7.8	8.0			20	20	20					24.6/24.9
mg/L	B	10	10	4																				
1.0	A	10	10	3			7.0	6.6	6.8			8.0	7.8	7.9			20	20	20					24.6/25.0
mg/L	B	10	9	2																				
1.25	A	10	8	0			7.0	6.9	6.8			8.0	7.9	7.9			20	20	20					24.6/24.9
mg/L	B	10	8	0																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: Reference Test
PERMIT NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / (DATE)
COMPOSITE COLLECTED:
FROM AM/PM / / (DATE)
TO AM/PM / / (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: ~~7.2~~ 8.0
INITIAL D.O.: 7.2
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysid
CULTURE NO.:
AGE: 2-2days
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48-hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 8:00 (AM)/PM 11/3/95(DATE)
END OF TEST 8:00 (AM)/PM 11/~~4~~5/95(DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	Salinity (ppt) CONDUCTIVITY (~~umhos/cm~~) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	9	9			7.2	6.8	6.9			8.0	7.9	8.0			21	20	20					25.2/25.6
	B	10	10	10																				
0.25 mg/L	A	10	10	8			7.2	7.0	6.8			8.0	7.9	8.0			21	20	20					25.2/25.5
	B	10	10	8																				
0.5 mg/L	A	10	10	7			7.2	7.0	6.9			8.0	7.9	8.0			21	20	20					25.2/25.5
	B	10	10	8																				
0.75 mg/L	A	10	8	6			7.2	6.9	6.8			8.0	7.9	8.0			21	20	20					25.2/25.6
	B	10	9	6																				
1.00 mg/L	A	10	10	5			7.2	7.0	6.9			8.0	7.9	8.0			21	20	20					25.2/25.5
	B	10	9	4																				
1.25 mg/L	A	10	9	1			7.2	7.0	6.8			8.0	7.9	8.0			21	20	20					25.2/25.5
	B	10	9	1																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: Reference Test
PERMIT NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / (DATE) T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.0
FROM AM/PM / / (DATE) INITIAL D.O.: 7.1
TO AM/PM / / (DATE) ADJUSTMENTS:
JOB NO.:
SAMPLE NO.:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysid
CULTURE NO.:
AGE: 6-2days
LENGTH(X±SD)
WEIGHT(X±SD)
TEST MODE: 48-hour static
DILUTION WATER: RSW
PHOTOPERIOD 16hr DAY/8hr NIGHT

START OF TEST 9:00 AM/PM 11/1/95 (DATE)
END OF TEST 9:00 AM/PM 11/3/95 (DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	~~CONDUCTIVITY (umhos/cm)~~ Salinity (ppt) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	9	9			7.1	6.9	6.7			8.0	7.8	7.9			21	20	20					25.0 / 25.3
	B	10	10	10																				
0.25 mg/L	A	10	9	8			7.1	6.8	6.6			8.0	7.8	7.9			21	20	20					25.0 / 25.4
	B	10	9	9																				
0.5 mg/L	A	10	9	6			7.1	6.6	6.8			8.0	7.8	7.9			21	20	20					25.0 / 25.2
	B	10	8	8																				
0.75 mg/L	A	10	8	5			7.1	6.9	7.0			8.0	7.8	7.9			21	20	20					25.0 / 25.3
	B	10	9	4																				
1.00 mg/L	A	10	9	5			7.1	6.6	6.7			8.0	7.8	7.9			21	20	20					25.0 / 25.2
	B	10	10	3																				
1.25 mg/L	A	10	10	2			7.1	6.8	6.8			8.0	7.8	7.9			21	20	20					25.0 / 25.3
	B	10	8	3																				
																								/
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: Reference Test / Mar. JOB NO.:
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.0
FROM AM/PM / / (DATE) INITIAL D.O.: 7.0
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 11:00 AM/PM 3/4/96 (DATE)
TEST SPECIES: Mysids END OF TEST 11:30 AM/PM 3/6/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600mL
AGE: <2days TEST SOLUTION VOLUME: 250mL
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): / / / / / /
TEST MODE: 48-hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	9	9			7.0	6.8	6.6			8.0	8.0	8.0			20	20	20					24.5 / 25.0
	B	10	10	10																				
0.25	A	10	10	9			7.0	6.7	6.7			8.0	8.0	8.1			20	20	20					24.5 / 24.9
mg/L	B	10	9	9																				
0.50	A	10	10	9			7.0	6.6	6.6			8.0	7.9	8.0			20	20	20					24.5 / 24.8
mg/L	B	10	10	9																				
0.75	A	10	8	6			7.0	6.8	6.8			8.0	7.8	8.0			20	20	20					24.5 / 25.0
mg/L	B	10	7	7																				
1.0	A	10	9	2			7.0	6.9	6.8			8.0	8.0	8.0			20	20	20					24.5 / 24.9
mg/L	B	10	9	6																				
1.25	A	10	8	1			7.0	6.9	6.7			8.0	7.9	8.0			20	20	20					24.5 / 24.8
mg/L	B	10	9	4																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I: /DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:
CLIENT: Reference Test / Feb
PERMIT NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: ____ AM/PM ____ (DATE)
COMPOSITE COLLECTED:
FROM ____ AM/PM ____ (DATE)
TO ____ AM/PM ____ (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.0
INITIAL D.O.: 7.2
ADJUSTMENTS:

TEST INFORMATION:
ANALYST(S): DBH
TEST SPECIES: Mysids
CULTURE NO.:
AGE: <2 days
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48-hour static
DILUTION WATER: ESW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 9:30 AM/PM 2/19/96 (DATE)
END OF TEST 9:00 AM/PM 2/16/96 (DATE)
TEST CONTAINER SIZE: 600 mL
TEST SOLUTION VOLUME: 250 mL
DISSOLVED OXYGEN: (TIME / TEST SOLN / CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	8	7			7.2	6.9	6.7			8.0	7.8	7.9			20	20	20					25.0 / 25.2
	B	10	9	9																				
0.25	A	10	9	8			7.2	7.0	6.8			8.0	7.9	8.0			20	20	20					25.0 / 25.3
mg/L	B	10	9	9																				
0.50	A	10	9	7			7.2	7.0	6.7			8.0	7.8	8.0			20	20	20					25.0 / 25.3
mg/L	B	10	10	7																				
0.75	A	10	8	5			7.2	7.1	7.0			8.0	7.7	7.8			20	20	20					25.0 / 25.2
mg/L	B	10	9	3																				
1.0	A	10	9	2			7.2	7.1	6.9			8.0	7.8	8.0			20	20	20					25.0 / 25.2
mg/L	B	10	9	4																				
1.25	A	10	8	1			7.2	7.1	6.8			8.0	7.9	8.0			20	20	20					25.0 / 25.2
mg/L	B	10	6	1																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I: /DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:
CLIENT: Reference Tox / Jan JOB NO.:
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.0
FROM AM/PM / / (DATE) INITIAL D.O.: 6.9
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:
ANALYST(S): DBH START OF TEST 9:00 AM/PM 1/18/96 (DATE)
TEST SPECIES: Mysid END OF TEST 9:00 AM/PM 1/20/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600mL
AGE: <2days TEST SOLUTION VOLUME: 250mL
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): / / / / / /
TEST MODE: 48-hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS 0	24	48	72	96	DISSOLVED OXYGEN (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	TEMPERATURE (°C) 0	24	48	72	96	INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
Con	A	10	10	10			6.9	6.8	6.7			8.0	8.0	7.9			20	20	20					25.2/25.4
	B	10	10	10																				
0.25	A	10	9	8			6.9	6.9	6.8			8.0	7.9	7.9			20	20	20					25.2/25.5
mg/L	B	10	10	9																				
0.50	A	10	9	7			6.9	6.8	6.7			8.0	7.8	7.8			20	20	20					25.2/25.4
mg/L	B	10	9	8																				
0.75	A	10	8	3			6.9	6.7	6.6			8.0	7.9	8.0			20	20	20					25.2/25.4
mg/L	B	10	7	5																				
1.00	A	10	8	2			6.9	6.6	6.6			8.0	8.0	8.0			20	20	20					25.2/25.3
mg/L	B	10	9	3																				
1.25	A	10	8	1			6.9	6.7	6.6			8.0	7.9	8.0			20	20	20					25.3/25.5
mg/L	B	10	7	0																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: Reference Test / Doc. JOB NO.:
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 7.9
FROM AM/PM / / (DATE) INITIAL D.O.: 7.0
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysid
CULTURE NO.:
AGE: 2-2days
LENGTH(X±SD):
WEIGHT(X±SD):
TEST MODE: 48-hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

START OF TEST 9:00 AM/PM 12/16/95 (DATE)
END OF TEST 9:00 AM/PM 12/18/95 (DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	~~CONDUCTIVITY (umhos/cm)~~ Salinity (ppt) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	9			7.0	6.9	6.8			7.9	7.9	7.9			20	20	20					29.8 / 24.9
	B	10	10	10																				
0.25	A	10	9	9			7.0	7.0	6.9			7.9	7.9	7.9			20	20	20					24.8 / 25.1
mg/L	B	10	10	9																				
0.50	A	10	10	8			7.0	6.7	6.9			7.9	7.8	7.8			20	20	20					24.8 / 25.0
mg/L	B	10	10	9																				
0.75	A	10	8	7			7.0	7.0	6.9			7.9	7.8	7.8			20	20	20					24.8 / 25.0
mg/L	B	10	8	6																				
1.0	A	10	9	4			7.0	7.0	6.8			7.9	7.8	7.9			20	20	20					24.8 / 25.0
mg/L	B	10	8	3																				
1.25	A	10	9	2			7.0	6.9	6.8			7.9	7.8	7.7			20	20	20					24.8 / 25.0
mg/L	B	10	7	0																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:
CLIENT: Reference Test / Nov. JOB NO.:
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: Cadmium
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.0
FROM AM/PM / / (DATE) INITIAL D.O.: 7.0
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:
ANALYST(S): DBH START OF TEST 9:00 AM/PM 11/16/95 (DATE)
TEST SPECIES: Mysid END OF TEST 9:00 AM/PM 11/18/95 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600mL
AGE: 22days TEST SOLUTION VOLUME: 250mL
LENGTH(X̄±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X̄±SD): / / / / / /
TEST MODE: 48-hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	~~CONDUCTIVITY (umhos/cm)~~ Salinity (ppt) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.0	6.8	6.8			8.0	7.8	8.0			20	20	20					24.6 / 24.8
	B	10	8	8																				
0.25	A	10	10	10			7.0	6.8	6.7			8.0	7.8	7.9			20	20	20					24.6 / 24.8
mg/L	B	10	9	9																				
0.50	A	10	9	8			7.0	6.7	6.7			8.0	7.9	8.0			20	20	20					24.6 / 24.4
mg/L	B	10	9	8																				
0.75	A	10	9	9			7.0	6.8	6.8			8.0	7.8	7.9			20	20	20					24.6 / 24.4
mg/L	B	10	8	8																				
1.0	A	10	6	4			7.0	6.6	6.7			8.0	7.9	8.0			20	20	20					24.6 / 24.8
mg/L	B	10	9	5																				
1.25	A	10	9	4			7.0	6.9	6.8			8.0	8.0	8.0			20	20	20					24.6 / 24.4
mg/L	B	10	7	4																				
ANALYST'S INITIALS																						NOTES:		

COMMENTS:
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I: /DWG/LAB/ACUTETOX

Appendix D

Acute Toxicity Bench Sheets for the Sheepshead Minnow (*Cyprinodon variegatus*)

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study JOB NO.: 2
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 6.9
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 8:00 AM/PM 1/10/96 (DATE)
TEST SPECIES: sheepshead min. END OF TEST 8:00 AM/PM 1/12/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600 mL
AGE: 8 days TEST SOLUTION VOLUME: 250 mL
LENGTH(X±SD) DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD) / / / / / /
TEST MODE 48 hour static AERATION:
DILUTION WATER RSW BEGINNING DATE/TIME:
PHOTOPERIOD 16hr DAY/8hr NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	9	9			6.9	6.8	6.7			8.20	8.16	8.15			20	20	19					23.8 / 24.1
	B	10	10	10								8.20	8.15	8.14										
40	A	10	10	10			6.9	6.7	6.6			8.21	8.18	8.13			20	20	19					23.8 / 24.1
	B	10	10	10								8.21	8.17	8.16										
50	A	10	8	8			6.9	6.6	6.4			8.20	8.15	8.15			20	20	19					23.8 / 24.1
	B	10	9	9								8.20	8.13	8.14										
60	A	10	9	1			6.9	6.6	6.5			8.21	8.12	8.12			20	20	19					23.8 / 24.1
	B	10	8	6								8.21	8.11	8.13										
70	A	10	4	2			6.9	6.7	6.6			8.20	8.13	8.14			20	20	19					23.8 / 24.1
	B	10	6	2								8.20	8.14	8.14										
80	A	10	2	0			6.9	6.8	6.6			8.21	8.16	8.13			20	20	19					23.8 / 24.2
	B	10	1	0								8.21	8.14	8.12										
ANALYST'S INITIALS																								

NOTES: pH adjusted to 8.2 every 4 hours during daytime

COMMENTS: Concentration (mg NH_3 total / L
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWC/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study JOB NO.: 1
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 6.9
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 8:00 AM/PM 1/10/96 (DATE)
TEST SPECIES: sheepshead min END OF TEST 8:00 AM/PM 1/12/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600mL
AGE: 8days TEST SOLUTION VOLUME: 250mL
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): / / / / / /
TEST MODE: 48 hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			6.9	6.7	6.7			8.21	8.19	8.16			20	20	20					24.2/24.6
	B	10	10	10								8.21	8.17	8.14										
40	A	10	10	10			6.9	6.7	6.6			8.21	8.18	8.17			20	20	20					24.2/24.7
	B	10	10	10								8.21	8.18	8.17										
50	A	10	10	9			6.9	6.8	6.8			8.22	8.16	8.18			20	20	20					24.1/24.5
	B	10	9	9								8.22	8.15	8.16										
60	A	10	7	4			6.9	6.8	6.7			8.20	8.13	8.14			20	20	20					24.2/24.6
	B	10	7	2								8.20	8.13	8.16										
70	A	10	3	2			6.9	6.9	6.6			8.20	8.14	8.16			20	20	20					24.2/24.6
	B	10	3	0								8.20	8.14	8.15										
80	A	10	1	0			6.9	6.6	6.6			8.20	8.12	8.14			20	20	20					24.2/24.6
	B	10	2	0								8.20	8.12	8.14										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total /L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH3 Study JOB NO.: 3
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 6.9
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 8:00 AM/PM 1/10/96 (DATE)
TEST SPECIES: sheeps head min. END OF TEST 8:00 AM/PM 1/12/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600 mL
AGE: 8 days TEST SOLUTION VOLUME: 250 mL
LENGTH(X̄±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X̄±SD): / / / / / /
TEST MODE: 48 hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			6.9	6.8	6.7			8.20	8.18	8.15			20	20	20					23.9/24.3
	B	10	10	10								8.20	8.18	8.16										
40	A	10	9	9			6.9	6.7	6.6			8.21	8.17	8.18			20	20	20					24.0/24.2
	B	10	10	9								8.21	8.16	8.17										
50	A	10	9	8			6.9	6.7	6.6			8.20	8.17	8.16			20	20	19					24.0/24.3
	B	10	9	9								8.20	8.17	8.15										
60	A	10	5	3			6.9	6.8	6.6			8.21	8.15	8.13			20	20	19					23.8/23.9
	B	10	7	5								8.21	8.16	8.12										
70	A	10	4	2			6.9	6.6	6.5			8.20	8.15	8.10			20	20	20					23.8/24.2
	B	10	2	1								8.20	8.15	8.11										
80	A	10	4	0			6.9	6.9	6.7			8.20	8.16	8.09			20	20	19					23.9/24.3
	B	10	2	0								8.20	8.15	8.10										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total /L
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study JOB NO.: 1
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 7.0
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 9:00 AM/PM 2/4/96 (DATE)
TEST SPECIES: sheepshead min. END OF TEST 8:30 AM/PM 2/6/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600mL
AGE: 9 days TEST SOLUTION VOLUME: 250 mL
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): / / / / / /
TEST MODE: 48 hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	9			7.0	6.9	6.6			8.21	8.16	8.14			20	20	20					24.2 / 24.5
	B	10	10	9								8.21	8.18	8.13										
40	A	10	9	8			7.0	6.8	6.7			8.20	8.15	8.12			20	20	20					24.2 / 24.6
	B	10	10	10								8.20	8.13	8.13										
50	A	10	10	10			7.0	6.9	6.7			8.20	8.14	8.14			20	20	20					24.2 / 24.6
	B	10	10	8								8.20	8.15	8.17										
60	A	10	8	5			7.0	6.7	6.6			8.21	8.14	8.14			20	20	20					24.2 / 24.6
	B	10	7	2								8.21	8.16	8.16										
70	A	10	4	2			7.0	6.8	6.6			8.20	8.17	8.17			20	20	20					24.1 / 24.4
	B	10	3	1								8.20	8.15	8.16										
80	A	10	2	0			7.0	6.8	6.6			8.21	8.14	8.15			20	20	20					24.1 / 24.5
	B	10	3	0								8.21	8.12	8.15										
ANALYST'S INITIALS																								

NOTES: pH adjusted to 8.2 every 4 hours during daytime

COMMENTS: Concentration (mg NH_3 total / L
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE ____ OF ____

SAMPLE INFORMATION:

CLIENT: NH_3 Study JOB NO.: 2
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 6.9
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: sheepshead min.
CULTURE NO.:
AGE: 10 days
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

START OF TEST 9:00 (AM)/PM 2/4/96 (DATE)
END OF TEST 10:00 AM/PM 2/6/96 (DATE)
TEST CONTAINER SIZE: 600 mL
TEST SOLUTION VOLUME: 250 mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			6.9	6.7	6.6			8.21	8.18	8.15			20	20	20					24.1 / 24.4
	B	10	9	9								8.21	8.18	8.16										
40	A	10	9	9			6.9	6.7	6.5			8.21	8.15	8.16			20	20	20					24.3 / 24.6
	B	10	10	10								8.21	8.16	8.16										
50	A	10	10	10			6.9	6.8	6.4			8.21	8.15	8.17			20	20	20					24.2 / 24.4
	B	10	9	7								8.22	8.14	8.16										
60	A	10	8	6			6.9	6.7	6.5			8.22	8.16	8.14			20	20	20					24.0 / 24.2
	B	10	5	3								8.21	8.17	8.15										
70	A	10	4	2			6.9	6.6	6.6			8.22	8.18	8.15			20	20	20					24.3 / 24.6
	B	10	3	0								8.21	8.17	—										
80	A	10	1	0			6.9	6.7	6.6			8.22	8.15	—			20	20	—					24.1 / 24.3
	B	10	2	0								8.23	8.14	—										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I: /DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study JOB NO.: 1
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 6.8
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 9:30 (AM)/PM 2/5/96 (DATE)
TEST SPECIES: sheepshead min. END OF TEST 9:30 (AM)/PM 2/7/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600 mL
AGE: 10 days TEST SOLUTION VOLUME: 250 mL
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): / / / / / /
TEST MODE: 48 hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			6.8	6.8	6.4			8.22	8.22	8.22			20	20	20					23.7 / 24.1
	B	10	10	10								8.22	8.22	8.22										
40	A	10	10	10			6.8	6.8	6.6			8.23	8.16	8.20			20	20	20					23.7 / 24.2
	B	10	10	10								8.23	8.14	8.16										
50	A	10	10	10			6.8	6.8	6.4			8.23	8.18	8.17			20	20	20					23.7 / 24.0
	B	10	9	8								8.23	8.16	8.15										
60	A	10	9	8			6.8	6.8	6.6			8.23	8.16	8.18			20	20	20					23.7 / 24.1
	B	10	6	3								8.23	8.18	8.20										
70	A	10	2	0			6.8	6.8	6.4			8.23	8.18	8.23			20	20	20					23.7 / 24.1
	B	10	3	0								8.23	8.19	8.23										
80	A	10	0	0			6.8	6.8	—			8.23	8.18	—			20	20	DBH 2/7 ~~20~~					23.7 / 24.0
	B	10	0	0								8.23	8.17	—										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: ____ AM/PM ____ / ____ T.R.C. ____
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM ____ AM/PM ____ / ____ (DATE) INITIAL D.O.: 6.8
TO ____ AM/PM ____ / ____ (DATE) ADJUSTMENTS: ____

JOB NO.: 2
SAMPLE NO.:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: sheepshead min.
CULTURE NO.:
AGE: 10 days
LENGTH(X±SD):
WEIGHT(X±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

START OF TEST 9:30 (AM)/PM 2/15/96 (DATE)
END OF TEST 9:30 (AM)/PM 2/17/96 (DATE)
TEST CONTAINER SIZE: 600 mL
TEST SOLUTION VOLUME: 250 mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
__/__ __/__ __/__
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			6.8	6.8	6.2			8.22	8.20	8.19			20	20	20					23.7/24.1
	B	10	10	10								8.22	8.21	8.22										
40	A	10	9	8			6.8	6.8	6.6			8.23	8.40	8.22			20	20	20					23.7/24.2
	B	10	10	9								8.23	8.23	8.40										
50	A	10	9	9			6.8	6.8	6.2			8.23	8.20	8.19			20	20	20					23.7/24.0
	B	10	10	10								8.23	8.18	8.16										
60	A	10	7	5			6.8	6.8	6.6			8.22	8.20	8.18			20	20	20					23.7/24.1
	B	10	10	2								8.22	8.18	8.18										
70	A	10	5	4			6.8	6.8	6.7			8.23	8.18	8.13			20	20	20					23.7/24.1
	B	10	5	0								8.23	8.20	8.16										
80	A	10	0	0			6.8	6.8	6.7			8.23	8.20	8.17			20	20	20					23.7/24.1
	B	10	2	0								8.23	8.18	8.15										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study JOB NO.: 3
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / (DATE) T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 6.8
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 9:30 (AM)/PM 2/5/96 (DATE)
TEST SPECIES: sheepshead min. END OF TEST 9:30 (AM)/PM 2/7/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600 mL
AGE: 10 days TEST SOLUTION VOLUME: 250 mL
LENGTH(X±SD) DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD) / / / / / /
TEST MODE 48 hour static AERATION:
DILUTION WATER RSW BEGINNING DATE/TIME:
PHOTOPERIOD 16hr DAY/8hr NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS 0	24	48	72	96	DISSOLVED OXYGEN (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	TEMPERATURE (°C) 0	24	48	72	96	INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
Con	A	10	10	10			6.8	6.8	6.6			8.22	8.20	8.22			20	20	20					23.7 / 24.1
	B	10	10	10								8.22	8.20	8.22										
40	A	10	10	10			6.8	6.8	6.7			8.23	8.19	8.23			20	20	20					23.7 / 24.2
	B	10	10	10	DBH 2/7							8.23	8.14	8.21										
50	A	10	9	~~10~~ 9			6.8	6.8	6.5			8.23	8.19	8.16			20	20	20					23.7 / 24.0
	B	10	10	9								8.23	8.19	8.18										
60	A	10	7	~~10~~ 5	DBH 2/7		6.8	6.8	6.6			8.23	8.18	8.20			20	20	20					23.7 / 24.1
	B	10	6	0								8.23	8.16	8.19										
70	A	10	2	1			6.8	6.8	6.6			8.23	8.16	8.19			20	20	20					23.7 / 24.1
	B	10	3	0								8.23	8.19	—										
80	A	10	0	0			6.8	6.8	6.6			8.23	8.19	—			20	20	—					23.7 / 24.1
	B	10	4	0								8.23	8.16	—										
ANALYST'S INITIALS																								

NOTES: pH adjusted to 8.2 every 4 hours during daytime

COMMENTS: Concentration (mg NH_3 total /L
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

STATIC RENEWAL ACUTE TOXICITY TEST DATASHEET

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED ___ AM/PM __/__/__
COMPOSITE COLLECTED (DATE)
FROM ___ AM/PM __/__/__ (DATE)
TO ___ AM/PM __/__/__ (DATE)
JOB NO.
SAMPLE NO.:
T.R.C.:
INITIAL pH: 8.2
INITIAL D.O.: 7.1
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST ORGANISM:
SPECIES Sheepshead min.
CULTURE NO.
AGE 0 days
LENGTH($\bar{x}\pm SD$)
WEIGHT($\bar{x}\pm SD$)
TEST MODE 96 hour static
DILUTION WATER RSW
START OF TEST 8:00 (AM)/PM 12/08/95 (DAT
END OF TEST 8:00 (AM)/PM 12/12/95 (DAT
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
TEST SOLUTION RENEWAL SCHEDULE: daily
D.O. CHECK (100%) / / /
TEST SOLUTION AERATION:
PHOTOPERIOD: 16HR. DAY/8HR. NIGHT

CONCENTRATION Control

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/10

INITIAL	DAY					
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.1	6.3	6.4	6.6	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.16	8.18	8.15	8.19
	D.O.(mg/l)		6.0	5.6	5.4	5.6
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 10mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/10

INITIAL	DAY					
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.1	6.2	6.5	6.4	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.16	8.18	8.19	8.17
	D.O.(mg/l)		6.2	6.0	5.8	5.6
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 20mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/9	10/8	10/8	10/8
C/D	10/10	10/10	10/10	10/10	10/10

INITIAL	DAY					
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.1	6.2	5.9	6.3	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.18	8.15	8.16	8.14
	D.O.(mg/l)		6.0	5.6	5.2	5.5
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 30mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	9/9

INITIAL	DAY					
	pH	8.20	8.20	8.21	8.20	
	D.O. (mg/l)	7.1	6.1	6.4	6.5	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.14	8.16	8.12	8.13
	D.O.(mg/l)		5.9	5.6	5.8	5.5
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 40mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	9/10	9/10
C/D	10/10	8/10	8/10	8/9	7/9

INITIAL	DAY					
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.1	6.2	6.4	6.6	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.12	8.10	8.08	8.09
	D.O.(mg/l)		5.6	5.7	5.8	5.5
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 50mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/8	6/8	7/5	4/5
C/D	10/10	9/10	9/10	6/9	6/2

INITIAL	DAY					
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.1	6.2	6.5	6.6	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.10	8.08	8.11	8.11
	D.O.(mg/l)		5.6	5.9	5.8	6.0
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

LAB\DATASHT

OLVER LABORATORIES INCORPORATED
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

STATIC RENEWAL ACUTE TOXICITY TEST DATASHEET

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED ___ AM/PM __/__/__
COMPOSITE COLLECTED (DATE)
FROM ___ AM/PM __/__/__ (DATE)
TO ___ AM/PM __/__/__ (DATE)

JOB NO.
SAMPLE NO.:
T.R.C.:
INITIAL pH: 8.2
INITIAL D.O.: 7.1
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST ORGANISM:
SPECIES Sheepshead min.
CULTURE NO.
AGE 8 days
LENGTH(X±SD)
WEIGHT(X±SD)
TEST MODE 96-hour static
DILUTION WATER RSW

START OF TEST 9:00 (AM)/PM 1/10/96 (DAT
END OF TEST 9:00 (AM)/PM 1/14/96 (DAT
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
TEST SOLUTION RENEWAL SCHEDULE: daily
D.O. CHECK (100%) __/__ __/__ __/__
TEST SOLUTION AERATION:
PHOTOPERIOD: 16HR. DAY/8HR. NIGHT

CONCENTRATION Control

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/10

		INITIAL DAY				
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.1	6.6	6.3	6.4	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	24	24	24	24	
	~~CONDUCTIVITY~~ (mg/l as CaCO3) (PPT)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.17	8.16	8.18	8.19
	D.O.(mg/l)		6.2	5.9	6.0	5.9
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 10mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/10

		INITIAL DAY				
	pH	8.20	8.21	8.21	8.21	
	D.O. (mg/l)	7.1	6.4	6.2	6.0	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	24	24	24	24	
	~~CONDUCTIVITY~~ (mg/l as CaCO3) (PPT)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.15	8.16	8.13	8.16
	D.O.(mg/l)		6.0	6.0	5.8	5.7
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 20mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/10

		INITIAL DAY				
	pH	8.21	8.21	8.20	8.20	
	D.O. (mg/l)	7.1	6.2	6.1	6.4	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	24	24	24	24	
	~~CONDUCTIVITY~~ (mg/l as CaCO3) (PPT)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.16	8.19	8.12	8.11
	D.O.(mg/l)		6.2	5.8	5.9	6.1
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 30mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/9	10/9	10/9
C/D	10/10	9/10	9/10	9/10	9/10

		INITIAL DAY				
	pH	8.20	8.21	8.20	8.21	
	D.O. (mg/l)	7.1	6.5	6.3	6.1	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	24	24	24	24	
	~~CONDUCTIVITY~~ (mg/l as CaCO3) (PPT)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.14	8.13	8.16	8.10
	D.O.(mg/l)		6.0	6.0	5.8	5.9
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 40mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/8	10/7	8/7	8/7	8/7
C/D	9/10	9/10	9/10	9/9	9/9

		INITIAL DAY				
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	6.9	5.8	6.1	6.3	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	24	24	24	24	
	~~CONDUCTIVITY~~ (mg/l as CaCO3) (PPT)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~		25	25	25	25
	pH		8.13	8.10	8.08	8.10
	D.O.(mg/l)		5.9	5.5	6.0	6.0
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 50mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	8/8	6/6	6/5	6/2
C/D	10/10	9/9	9/9	7/7	4/3

		INITIAL DAY				
	pH	8.20	8.21	8.20	8.20	
	D.O. (mg/l)	6.9	6.0	6.2	6.3	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	24	24	24	24	
	~~CONDUCTIVITY~~ (mg/l as CaCO3) (PPT)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	~~pH~~ D.O. (mg/L)		6.0	5.8	6.1	6.2
	~~D.O.(mg/l)~~ pH		8.12	8.10	8.08	8.10
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

OLVER LABORATORIES INCORPORATED
1110 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

STATIC RENEWAL ACUTE TOXICITY TEST DATASHEE

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED ___ AM/PM __/__/__
COMPOSITE COLLECTED (DATE)
FROM ___ AM/PM __/__/__ (DATE)
TO ___ AM/PM __/__/__ (DATE)

JOB NO.
SAMPLE NO.:
T.R.C.:
INITIAL pH: 8.2
INITIAL D.O.: 7.0
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST ORGANISM:
SPECIES Sheepshead min.
CULTURE NO.
AGE 5 days
LENGTH($\bar{X}\pm SD$)
WEIGHT($\bar{X}\pm SD$)
TEST MODE 96 hour static
DILUTION WATER RSW

START OF TEST 8:00 (AM)/PM 2/7/96(DAT
END OF TEST 8:00 (AM)/PM 2/11/96(DAT
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
TEST SOLUTION RENEWAL SCHEDULE: daily
D.O. CHECK (100%) / / / /
TEST SOLUTION AERATION:
PHOTOPERIOD: 16HR. DAY/8HR. NIGHT

CONCENTRATION Control

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/10

INITIAL DAY						
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.0	6.2	5.9	5.7	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	29	29	29	29	
	~~SALINITY~~ (PPT)					
	~~(mg/l as CaCO3)~~ ~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.16	8.17	8.18	8.20
	D.O.(mg/l)		6.2	5.9	5.8	5.5
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 10 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	9/10	9/10	9/10
C/D	10/10	10/10	10/10	10/10	10/10

INITIAL DAY						
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.0	6.3	6.9	6.6	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	29	29	24	24	
	~~SALINITY~~ (PPT)					
	~~(mg/l as CaCO3)~~ ~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.16	8.15	8.16	8.14
	D.O.(mg/l)		6.0	5.8	5.6	5.7
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 20 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/10

INITIAL DAY						
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.0	6.0	5.6	6.2	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	29	29	29	29	
	~~SALINITY~~ (PPT)					
	~~(mg/l as CaCO3)~~ ~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.15	8.13	8.12	8.11
	D.O.(mg/l)		6.0	5.6	5.2	5.7
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 30 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/9

INITIAL DAY						
	pH	8.20	8.21	8.21	8.20	
	D.O. (mg/l)	6.9	6.4	6.0	6.2	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	29	29	29	29	
	~~SALINITY~~ (PPT)					
	~~(mg/l as CaCO3)~~ ~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.12	8.13	8.10	8.11
	D.O.(mg/l)		5.9	5.8	5.6	5.7
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 40 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	8/10	8/9	6/8	5/7
C/D	10/10	9/10	7/9	6/7	4/7

INITIAL DAY						
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.0	6.1	6.0	5.8	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	29	29	29	29	
	~~SALINITY~~ (PPT)					
	~~(mg/l as CaCO3)~~ ~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.12	8.09	8.10	8.12
	D.O.(mg/l)		5.9	6.6	5.4	5.2
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 50 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	7/9	7/9	6/8	4/6
C/D	10/10	10/10	10/10	5/6	4/3

INITIAL DAY						
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.0	6.2	6.4	6.3	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity	29	29	29	29	
	~~SALINITY~~ (PPT)					
	~~(mg/l as CaCO3)~~ ~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.10	8.11	8.06	8.09
	D.O.(mg/l)		5.9	5.6	5.4	5.7
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY
1110 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

STATIC RENEWAL ACUTE TOXICITY TEST DATASHEET

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED ___ AM/PM __/__/__ (DATE)
COMPOSITE COLLECTED (DATE)
FROM ___ AM/PM __/__/__ (DATE)
TO ___ AM/PM __/__/__ (DATE)

JOB NO.
SAMPLE NO.:
T.R.C.:
INITIAL pH: 8.2
INITIAL D.O.: 6.9
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST ORGANISM:
SPECIES sheepshead min.
CULTURE NO.
AGE 6 days
LENGTH(X±SD)
WEIGHT(X±SD)
TEST MODE 96 hour static
DILUTION WATER RSW

START OF TEST 8:00 AM/PM 2/8/96 (DAT
END OF TEST 8:00 AM/PM 2/12/96 (DAT
TEST CONTAINER SIZE: 600 mL
TEST SOLUTION VOLUME: 250 mL
TEST SOLUTION RENEWAL SCHEDULE:
D.O. CHECK (100%) / / / /
TEST SOLUTION AERATION:
PHOTOPERIOD: 16HR. DAY/8HR. NIGHT

CONCENTRATION Control

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/10	10/10	10/10	10/10

INITIAL DAY					
pH	8.20	8.20	8.20	8.20	
D.O. (mg/l)	6.9	6.3	6.1	6.2	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.18	8.17	8.16	8.19
D.O.(mg/l)		5.9	6.0	5.8	5.9
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 10 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	10/10
C/D	10/10	10/9	10/9	10/9	10/9

INITIAL DAY					
pH	8.20	8.20	8.20	8.20	
D.O. (mg/l)	6.9	6.2	6.9	6.2	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal		25	25	25	25
pH		8.16	8.15	8.12	8.13
D.O.(mg/l)		5.9	6.0	5.8	5.7
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 20 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/10	10/10	10/10	9/10
C/D	10/10	9/10	8/10	8/10	8/10

INITIAL DAY					
pH	8.20	8.20	8.20	8.20	
D.O. (mg/l)	6.9	6.0	6.2	6.3	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.16	8.12	8.11	8.10
D.O.(mg/l)		5.9	5.7	5.8	6.0
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 30 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/9	10/9	10/9	10/9
C/D	10/10	10/8	10/8	10/8	10/8

INITIAL DAY					
pH	8.20	8.20	8.20	8.20	
D.O. (mg/l)	6.9	6.0	6.3	6.5	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.14	8.11	8.12	8.10
D.O.(mg/l)		6.0	5.8	5.7	6.0
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 40 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	9/9	8/7	6/5	6/5
C/D	10/10	10/8	9/8	8/7	7/7

INITIAL DAY					
pH	8.20	8.20	8.20	8.20	
D.O. (mg/l)	6.9	6.0	5.9	6.2	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.11	8.08	8.09	8.10
D.O.(mg/l)		6.0	5.5	5.7	5.9
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 50 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	8/7	5/6	4/2	1/0
C/D	10/10	9/7	9/6	3/2	3/2

INITIAL DAY					
pH	8.20	8.20	8.20	8.20	
D.O. (mg/l)	6.9	5.9	6.1	6.2	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.10	8.11	8.08	8.11
D.O.(mg/l)		6.0	5.5	5.7	5.9
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

Appendix E

Acute Toxicity Bench Sheets for the Mysid (*Mysidopsis bahia*)

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:
CLIENT: NH_3 Study JOB NO.:
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH:
FROM AM/PM / / (DATE) INITIAL D.O.: 8.2
TO AM/PM / / (DATE) ADJUSTMENTS: 7.3

TEST INFORMATION:
ANALYST(S): DBH START OF TEST 8:00 (AM)/PM 12/2/96 (DATE)
TEST SPECIES: Mysids END OF TEST AM/PM 12/4/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 600mL
AGE: 2 2days TEST SOLUTION VOLUME: 250mL
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): / / / / / /
TEST MODE: 48 hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	9	9			7.3	7.1	6.8			8.21	8.19	8.18			21	20	20					25.0/25.3
	B	10	9	9								8.21	8.18	8.18										
10	A	10	10	9			7.3	7.0	6.7			8.20	8.16	8.17			21	20	20					25.0/25.4
	B	10	9	9								8.20	8.16	8.17										
20	A	10	6	5			7.3	6.9	6.9			8.21	8.13	8.12			21	20	20					25.0/25.3
	B	10	7	7								8.21	8.14	8.14										
30	A	10	7	7			7.3	6.9	6.9			8.20	8.16	8.14			20	20	20					25.0/25.4
	B	10	8	4								8.20	8.12	8.12										
40	A	10	6	2			7.3	7.0	6.8			8.20	8.11	6.08			20	20	20					25.0/25.3
	B	10	4	4								8.21	8.11	8.07										
50	A	10	1	1			7.3	7.0	6.8			8.21	8.12	8.03			20	20	20					25.0/25.3
	B	10	4	0								8.21	8.12	8.09										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study JOB NO.:
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 7.2
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 8:00 AM/PM 12/8/93 (DATE)
TEST SPECIES: Mysids END OF TEST 8:00 AM/PM 12/10/93 (DATE)
CULTURE NO: TEST CONTAINER SIZE: 600 mL
AGE: 2-2 days TEST SOLUTION VOLUME: 250 mL
LENGTH(X±SD) DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD) / / / / / /
TEST MODE: 48 hour static AERATION:
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD 16hr DAY/8hr NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	7.0	6.6			8.20	8.19	8.16			21	20	20					25.0 / 25.3
	B	10	10	10								8.20	8.18	8.15										
10	A	10	10	9			7.2	7.0	6.8			8.20	8.18	8.17			21	20	20					25.0 / 25.3
	B	10	10	8								8.20	8.16	8.16										
20	A	10	9	7			7.2	7.1	6.8			8.20	8.15	8.14			21	20	20					25.0 / 25.4
	B	10	8	4								8.20	8.15	8.14										
30	A	10	7	5			7.2	6.9	6.9			8.20	8.16	8.12			21	20	20					25.0 / 25.4
	B	10	9	5								8.21	8.16	8.12										
40	A	10	5	0			7.2	6.9	7.0			8.21	8.11	8.12			21	20	20					25.0 / 25.4
	B	10	6	2								8.21	8.10	8.09										
50	A	10	1	0			7.2	6.9	6.9			8.21	8.10	8.08			21	20	20					25.0 / 25.3
	B	10	5	0								8.21	8.10	8.07										
ANALYST'S INITIALS																								

NOTES: pH adjusted to 8.2 every 4 hours during daytime

COMMENTS: Concentration (mg NH_3 total/L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy/controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 7.2
TO AM/PM / / (DATE) ADJUSTMENTS:
JOB NO.:
SAMPLE NO.:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysids
CULTURE NO.:
AGE: < 2 days
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 8:30 (AM)/PM 1/14/96 (DATE)
END OF TEST 9:00 (AM)/PM 1/16/96 (DATE)
TEST CONTAINER SIZE: 600 mL
TEST SOLUTION VOLUME: 250 mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS 0	24	48	72	96	DISSOLVED OXYGEN (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	TEMPERATURE (°C) 0	24	48	72	96	INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
Con	A	10	10	10			7.2	6.6	6.7			8.21	8.20	8.16			20	20	20					24.3 / 24.7
	B	10	10	9								8.21	8.20	8.16										
10	A	10	9	9			7.2	6.6	6.7			8.20	8.18	8.13			20	20	20					24.3 / 24.7
	B	10	9	9								8.20	8.18	8.12										
20	A	10	8	5			7.2	6.7	6.8			8.20	8.17	8.16			20	20	20					24.3 / 24.8
	B	10	9	7								8.20	8.17	8.11										
30	A	10	8	5			7.2	6.8	6.8			8.21	8.13	8.10			20	20	20					24.5 / 24.8
	B	10	7	5								8.21	8.12	8.10										
40	A	10	5	2			7.2	6.6	6.7			8.20	8.10	8.06			20	20	20					24.6 / 24.7
	B	10	6	0								8.20	8.09	8.07										
50	A	10	3	0			7.2	6.5	6.6			8.21	8.06	8.03			20	20	20					24.5 / 24.8
	B	10	4	0								8.21	8.05	8.04										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / (DATE)
COMPOSITE COLLECTED:
FROM AM/PM / / (DATE)
TO AM/PM / / (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.2
INITIAL D.O.: 7.3
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysids
CULTURE NO.:
AGE: < 2 days
LENGTH($\bar{X}$±SD):
WEIGHT($\bar{X}$±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 8:00 AM/PM 1/18/96 (DATE)
END OF TEST 8:30 AM/PM 1/20/96 (DATE)
TEST CONTAINER SIZE: 600 mL
TEST SOLUTION VOLUME: 250 mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.3	7.0	6.8			8.20	8.16	8.16			20	20	20					25.0 / 25.4
	B	10	9	9								8.20	8.18	8.15										
10	A	10	9	9			7.3	7.0	7.0			8.21	8.14	8.17			20	20	20					25.0 / 25.2
	B	10	10	10								8.21	8.16	8.15										
20	A	10	9	9			7.3	7.1	7.0			8.21	8.15	8.15			20	20	20					25.0 / 25.2
	B	10	9	7								8.21	8.14	8.13										
30	A	10	7	6			7.3	7.1	7.0			8.20	8.12	8.13			20	20	20					25.0 / 25.3
	B	10	9	5								8.20	8.11	8.13										
40	A	10	6	1			7.3	6.9	6.9			8.22	8.13	8.15			20	20	20					25.0 / 25.4
	B	10	6	0								8.22	8.10	8.09										
50	A	10	0	0			7.3	6.9	—			8.21	8.06	~~8.09~~	DBH 1/20/96		20	20	20					25.0 / 25.3
	B	10	3	0					6.8			8.21	8.05	8.10										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / (DATE)
COMPOSITE COLLECTED:
FROM AM/PM / / (DATE)
TO AM/PM / / (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.2
INITIAL D.O.: 7.1
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysids
CULTURE NO.:
AGE: <2 days
LENGTH(X±SD):
WEIGHT(X±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 9:00 (AM)/PM 1/31/96(DATE)
END OF TEST 9:30 AM/(PM) 2/2/96(DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS 0	24	48	72	96	DISSOLVED OXYGEN (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	TEMPERATURE (°C) 0	24	48	72	96	INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
Con	A	10	10	10			7.1	6.5	6.6			8.22	8.20	8.12			21	20	20					24.5 / 25.0
	B	10	10	9								8.22	8.20	8.12										
10	A	10	10	9			7.1	6.6	6.6			8.21	8.20	8.13			21	20	20					24.6 / 25.0
	B	10	9	9								8.21	8.20	8.03										
20	A	10	9	6			7.1	6.6	6.6			8.22	8.18	8.10			21	20	20					24.5 / 25.1
	B	10	8	5								8.22	8.14	8.04										
30	A	10	8	4			7.1	6.6	6.6			8.22	8.14	8.04			21	20	20					24.5 / 25.1
	B	10	9	5								8.22	8.16	8.07										
40	A	10	4	1			7.1	6.4	6.6			8.21	8.14	8.06			21	20	20					24.5 / 25.0
	B	10	7	1								8.21	8.15	8.05										
50	A	10	4	0			7.1	6.4	6.6			8.22	8.12	8.02			21	20	20					24.6 / 25.0
	B	10	2	0								8.22	8.18	8.06										
ANALYST'S INITIALS																								

NOTES: pH adjusted to 8.2 every 4 hours during daytime

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM (DATE)
COMPOSITE COLLECTED: (DATE)
FROM AM/PM (DATE)
TO AM/PM (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.2
INITIAL D.O.: 7.2
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysids
CULTURE NO.:
AGE: < 2 days
LENGTH($\bar{X}$±SD):
WEIGHT($\bar{X}$±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 8:00 (AM)/PM 2/10/96(DATE)
END OF TEST 8:30 (AM)/PM 2/12/96(DATE)
TEST CONTAINER SIZE: 600 mL
TEST SOLUTION VOLUME: 250 mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	6.6	6.6			8.21	8.18	8.12			21	20	20					24.3 / 24.8
	B	10	9	9								8.21	8.18	8.14										
10	A	10	9	9			7.2	6.7	6.5			8.20	8.16	8.15			21	20	20					24.3 / 24.7
	B	10	9	8								8.20	8.16	8.13										
20	A	10	9	8			7.2	6.8	6.5			8.21	8.17	8.10			21	20	20					24.3 / 24.8
	B	10	9	5								8.21	8.17	8.11										
30	A	10	7	5			7.2	6.8	6.4			8.20	8.16	8.12			21	20	20					24.4 / 24.8
	B	10	9	4								8.20	8.16	8.13										
40	A	10	5	2			7.2	6.8	6.6			8.21	8.14	8.11			21	20	20					24.5 / 24.8
	B	10	7	1								8.21	8.19	8.11										
50	A	10	3	0			7.2	6.8	6.6			8.21	8.13	8.10			21	20	20					24.5 / 24.8
	B	10	3	0								8.21	8.14	8.10										
																								/
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: ____ AM/PM __/__
COMPOSITE COLLECTED: (DATE)
FROM ____ AM/PM __/__ (DATE)
TO ____ AM/PM __/__ (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.2
INITIAL D.O.: 7.2
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Mysids
CULTURE NO.:
AGE: <2days
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 9:00 (AM)/PM 2/18/96 (DATE)
END OF TEST 9:00 (AM)/PM 2/20/96 (DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
__/__ __/__ __/__
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	6.9	6.7			8.22	8.18	8.16			20	20	20					24.7 / 24.9
	B	10	8	8								8.22	8.17	8.19										
10	A	10	9	8			7.2	6.9	6.8			8.21	8.19	8.19			20	20	20					24.7 / 25.0
	B	10	7	6								8.21	8.20	8.20										
20	A	10	9	8			7.2	6.9	6.8			8.22	8.16	8.14			20	20	20					24.7 / 25.0
	B	10	8	8								8.22	8.19	8.16										
30	A	10	6	4			7.2	6.8	6.7			8.21	8.13	8.12			20	20	20					24.7 / 24.9
	B	10	7	3								8.21	8.12	8.11										
40	A	10	3	2			7.2	6.8	6.6			8.21	8.11	8.10			20	20	20					24.7 / 25.0
	B	10	2	1								8.21	8.06	8.06										
50	A	10	4	1			7.2	6.7	6.9			8.21	8.11	8.11			20	20	20					24.7 / 25.6
	B	10	4	1								8.21	8.10	8.16										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM 1/1
COMPOSITE COLLECTED: (DATE)
FROM AM/PM 1/1 (DATE)
TO AM/PM 1/1 (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.2
INITIAL D.O.: 7.0
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: mysids
CULTURE NO.:
AGE: 2 2days
LENGTH(X±SD):
WEIGHT(X±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 9:00 AM/PM 2/29/96(DATE)
END OF TEST 9:30 AM/PM 3/2/96(DATE)
TEST CONTAINER SIZE: 600 mL
TEST SOLUTION VOLUME: 250 mL
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
1/1 1/1 1/1
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.0	7.0	6.4			8.20	8.19	8.15			20	20	20					24.4/25.0
	B	10	10	10								8.20	8.19	8.15										
10	A	10	9	8			7.0	6.9	6.7			8.21	8.21	8.15			20	20	20					24.4/25.0
	B	10	9	8								8.21	8.21	8.16										
20	A	10	8	7			7.0	6.9	6.8			8.20	8.20	8.13			20	20	20					24.4/24.9
	B	10	6	6								8.20	8.18	8.14										
30	A	10	7	5			7.0	6.9	6.8			8.20	8.18	8.11			20	20	20					24.4/24.9
	B	10	8	6								8.20	8.18	8.11										
40	A	10	5	3			7.0	7.0	6.9			8.20	8.20	8.13			20	20	20					24.4/25.0
	B	10	5	3								8.20	8.21	8.14										
50	A	10	2	0			7.0	7.0	6.8			8.20	8.17	8.10			20	20	20					24.4/25.0
	B	10	3	0								8.20	8.20	8.12										
ANALYST'S INITIALS																						NOTES: pH adjust to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY
1110 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

STATIC RENEWAL ACUTE TOXICITY TEST DATASHEE

SAMPLE INFORMATION:

CLIENT: NH_3 study
JOB NO.
PERMIT NO.:
SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED ___ AM/PM __/__/__
COMPOSITE COLLECTED (DATE)
FROM ___ AM/PM __/__/__ (DATE)
TO ___ AM/PM __/__/__ (DATE)
T.R.C.:
INITIAL pH: 8.2
INITIAL D.O.: 7.1
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST ORGANISM:
SPECIES Mysids
CULTURE NO.
AGE < 2 days
LENGTH(X±SD)
WEIGHT(X±SD)
TEST MODE 96 hour static
DILUTION WATER RSW
START OF TEST 9:00 (AM)/PM 1/12/96 (DAT
END OF TEST 9:00 (AM)/PM 1/16/96 (DAT
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
TEST SOLUTION RENEWAL SCHEDULE:
D.O. CHECK (100%) __/__/__/__
TEST SOLUTION AERATION:
PHOTOPERIOD: 16HR. DAY/8HR. NIGHT

CONCENTRATION Control

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	9/8	8/8	8/8	8/8
	C/D	10/10	10/9	10/9	10/9	9/9

	DAY	INITIAL	24	48	72	96
	pH	8.20	8.21	8.21	8.40	
	D.O. (mg/l)	7.1	6.8	6.9	6.5	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
	~~SALINITY~~ (mg/l as CaCO3)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ sal.	→	25	25	25	25
	pH		9.12	8.88	8.10	8.08
	D.O.(mg/l)		5.9	6.0	5.7	5.9
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 5mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	10/9	9/9	9/9	9/9
	C/D	10/10	10/9	10/9	10/9	10/9

	DAY	INITIAL	24	48	72	96
	pH	8.20	8.20	8.20	9.20	
	D.O. (mg/l)	7.1	6.7	6.3	6.0	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	29	24	24	24	
	~~SALINITY~~ (mg/l as CaCO3)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ sal.	→	25	25	25	25
	pH		8.16	8.08	8.10	8.11
	D.O.(mg/l)		5.8	5.6	5.4	5.8
	TEMPERATURE(°C)					
	ANALYST'S INITIALS					

CONCENTRATION 15mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	10/9	9/8	6/5	6/5
	C/D	10/10	8/8	8/7	8/6	8/6

	DAY	INITIAL	24	48	72	96
	pH	8.20	8.20	8.41	8.21	
	D.O. (mg/l)	7.1	6.3	6.5	6.6	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	29	29	29	24	
	~~SALINITY~~ (mg/l as CaCO3)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ sal.		25	25	25	25
	pH		8.15	8.14	8.10	8.08
	D.O.(mg/l)		5.9	5.7	5.5	5.9
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 20mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	9/9	6/6	5/4	4/4
	C/D	10/10	8/7	7/3	6/3	5/3

	DAY	INITIAL	24	48	72	96
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.1	6.6	6.4	6.3	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
	~~SALINITY~~ (mg/l as CaCO3)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.11	8.08	8.08	8.11
	D.O.(mg/l)		5.6	5.4	5.7	5.8
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 25 mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	8/7	6/5	5/4	3/2
	C/D	10/10	9/10	6/7	6/4	5/3

	DAY	INITIAL	24	48	72	96
	pH	8.20	8.21	8.21	8.20	
	D.O. (mg/l)	7.1	6.4	6.7	6.3	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	29	24	24	24	
	~~SALINITY~~ (mg/l as CaCO3)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.14	8.10	8.10	8.06
	D.O.(mg/l)		5.7	5.9	6.0	6.1
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 30mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	8/9	6/4	3/3	1/2
	C/D	10/10	8/7	5/4	2/2	0/3

	DAY	INITIAL	24	48	72	96
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.1	6.3	6.4	6.6	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPT)	29	29	29	24	
	~~SALINITY~~ (mg/l as CaCO3)					
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.16	8.12	8.10	8.10
	D.O.(mg/l)		5.9	6.2	6.0	6.0
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY
1110 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

STATIC RENEWAL ACUTE TOXICITY TEST DATASHEE

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED ___ AM/PM __/__/__
COMPOSITE COLLECTED (DATE)
FROM ___ AM/PM __/__/__ (DATE)
TO ___ AM/PM __/__/__ (DATE)

JOB NO.
SAMPLE NO.:
T.R.C.:
INITIAL pH: 8.2
INITIAL D.O.: 7.1
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST ORGANISM:
SPECIES Mysids
CULTURE NO.
AGE 2 2days
LENGTH(x̄±SD)
WEIGHT(x̄±SD)
TEST MODE 96 hour static
DILUTION WATER RSW

START OF TEST 8:00 (AM)/PM 1/19/96 (DATI
END OF TEST 9:00 (AM)/PM 1/23/96 (DAT
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
TEST SOLUTION RENEWAL SCHEDULE:
D.O. CHECK (100%) __/__/__/__
TEST SOLUTION AERATION:
PHOTOPERIOD: 16HR. DAY/8HR. NIGHT

CONCENTRATION Control

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	9/8	9/8	9/8	9/8
C/D	10/10	10/10	10/9	10/9	10/9

INITIAL DAY	0	24	48	72	96
pH	8.20	8.20	8.14	8.21	
D.O. (mg/l)	7.1	6.3	6.4	6.6	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~ALKALINITY~~ HARDNESS (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.	~~25~~	25	25	25	25
pH		8.18	8.16	8.17	8.20
D.O.(mg/l)		6.0	5.8	5.7	5.5
TEMPERATURE(°C)					
ANALYST'S INITIALS					

CONCENTRATION 5 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	9/10	8/9	8/9	8/9
C/D	10/10	9/10	9/10	9/10	9/9

INITIAL DAY	0	24	48	72	96
pH	8.21	8.20	8.21	8.20	
D.O. (mg/l)	7.1	6.4	6.6	6.5	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~ALKALINITY~~ HARDNESS (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.18	8.14	8.13	8.10
D.O.(mg/l)		6.0	5.8	5.6	5.7
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 15 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	9/10	9/9	6/8	3/8
C/D	10/10	9/8	9/8	9/8	9/6

INITIAL DAY	0	24	48	72	96
pH	8.21	8.20	8.20	8.21	
D.O. (mg/l)	7.1	6.3	6.5	6.2	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~ALKALINITY~~ HARDNESS (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.15	8.11	8.10	8.08
D.O.(mg/l)		6.1	5.8	5.7	5.9
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 20 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/8	8/6	6/5	3/5
C/D	10/10	8/7	7/6	5/6	5/6

INITIAL DAY	0	24	48	72	96
pH	8.20	8.20	8.20	8.20	
D.O. (mg/l)	7.1	6.5	6.6	6.7	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~ALKALINITY~~ HARDNESS (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.15	8.11	8.12	8.10
D.O.(mg/l)		6.2	5.9	6.0	6.2
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 25 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	8/9	7/4	7/2	4/1
C/D	10/10	9/7	8/5	6/5	6/5

INITIAL DAY	0	24	48	72	96
pH	8.20	8.20	8.20	8.20	
D.O. (mg/l)	7.1	6.4	6.7	6.8	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~ALKALINITY~~ HARDNESS (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.12	8.11	8.12	8.09
D.O.(mg/l)		6.1	6.2	5.9	6.0
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 30 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	7/6	6/4	3/1	2/0
C/D	10/9	8/3	5/2	4/0	1/0

INITIAL DAY	0	24	48	72	96
pH	8.20	8.21	8.20	8.21	
D.O. (mg/l)	7.1	6.6	6.7	6.3	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
~~ALKALINITY~~ HARDNESS (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL DAY					
~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.12	8.14	8.10	8.09
D.O.(mg/l)		6.0	6.2	6.0	5.9
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY
1110 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

STATIC RENEWAL ACUTE TOXICITY TEST DATASHEET

SAMPLE INFORMATION:

CLIENT: NH3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED ___ AM/PM __/__/__
COMPOSITE COLLECTED (DATE)
FROM ___ AM/PM __/__/__ (DATE)
TO ___ AM/PM __/__/__ (DATE)

JOB NO.
SAMPLE NO.:
T.R.C.:
INITIAL pH: 8.2
INITIAL D.O.: 7.2
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST ORGANISM:
SPECIES Mysids
CULTURE NO.
AGE < 2 days
LENGTH(x̄±SD)
WEIGHT(x̄±SD)
TEST MODE 96 hour static
DILUTION WATER RSW

START OF TEST 8:00 AM/PM 12/02/95 (DAT
END OF TEST 8:00 AM/PM 12/06/95 (DAT
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
TEST SOLUTION RENEWAL SCHEDULE:
D.O. CHECK (100%) __/__/__/__
TEST SOLUTION AERATION:
PHOTOPERIOD: 16HR. DAY/8HR. NIGHT

CONCENTRATION Control

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	9/10	8/10	8/10	8/10
C/D	10/10	10/9	9/8	9/8	9/8

INITIAL DAY					
pH	8.21	8.21	8.20	8.21	
D.O. (mg/l)	7.2	6.8	6.6	6.6	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL ~~CONDUCTIVITY~~ Sal.	~~65~~	25	25	25	25
pH		8.12	8.10	8.08	8.12
D.O.(mg/l)		5.6	5.9	5.9	5.7
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 5 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	10/9	9/9	9/9	9/9
C/D	10/10	10/8	10/7	9/7	9/7

INITIAL DAY					
pH	8.21	8.20	8.21	8.21	
D.O. (mg/l)	7.2	6.6	6.9	6.4	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL ~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.13	8.08	8.06	8.10
D.O.(mg/l)		6.2	5.9	5.9	6.1
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 15 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	9/8	8/8	7/8	6/8
C/D	10/10	10/8	9/8	8/8	8/7

INITIAL DAY					
pH	8.21	8.21	8.20	8.20	
D.O. (mg/l)	7.2	6.3	6.4	6.7	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL ~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.12	8.13	8.09	8.09
D.O.(mg/l)		6.6	6.0	5.9	5.8
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 20 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	9/9	7/7	6/7	5/4
C/D	10/10	10/9	9/7	8/7	6/6

INITIAL DAY					
pH	8.21	8.20	8.20	8.20	
D.O. (mg/l)	7.2	6.3	6.6	6.7	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL ~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.16	8.13	8.10	8.10
D.O.(mg/l)		5.9	6.0	5.7	5.9
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 25 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	7/8	6/6	5/4	3/2
C/D	10/10	7/9	5/8	5/7	5/4

INITIAL DAY					
pH	8.21	8.20	8.20	8.20	
D.O. (mg/l)	7.2	6.3	6.4	5.9	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL ~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.11	8.10	8.12	8.08
D.O.(mg/l)		6.2	6.3	6.6	5.6
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

CONCENTRATION 30 mg/L

NUMBER OF LIVE ORGANISMS	TIME(HOURS) 0	24	48	72	96
A/B	10/10	5/7	5/4	2/3	0/2
C/D	10/10	6/8	5/6	3/1	3/0

INITIAL DAY					
pH	8.21	8.20	8.21	8.21	
D.O. (mg/l)	7.2	6.2	6.4	6.5	
TEMPERATURE(°C)	20	20	20	20	
~~CONDUCTIVITY~~ Salinity (PPT)	24	24	24	24	
~~HARDNESS~~ (mg/l as CaCO3)					
~~ALKALINITY~~ (mg/l as CaCO3)					
ANALYST'S INITIALS					
FINAL ~~CONDUCTIVITY~~ Sal.		25	25	25	25
pH		8.11	8.13	8.10	8.08
D.O.(mg/l)		6.3	5.9	5.7	6.0
TEMPERATURE(°C)		20	20	20	20
ANALYST'S INITIALS					

LAB DATASHEET

OLVER LABORATORIES INCORPORATED
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

STATIC RENEWAL ACUTE TOXICITY TEST DATASHEET

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED ___ AM/PM __/__/__
COMPOSITE COLLECTED (DATE)
FROM ___ AM/PM __/__/__ (DATE)
TO ___ AM/PM __/__/__ (DATE)
JOB NO.
SAMPLE NO.:
T.R.C.:
INITIAL pH: 8.2
INITIAL D.O.: 7.2
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST ORGANISM:
SPECIES Mysids
CULTURE NO.
AGE 22days
LENGTH($\bar{x}\pm SD$)
WEIGHT($\bar{x}\pm SD$)
TEST MODE 96 hour static
DILUTION WATER RSW
START OF TEST 8:20 (AM)/PM 12/13/95 (DATE)
END OF TEST 8:20 (AM)/PM 12/17/95 (DATE)
TEST CONTAINER SIZE: 600mL
TEST SOLUTION VOLUME: 250mL
TEST SOLUTION RENEWAL SCHEDULE:
D.O. CHECK (100%) / / / /
TEST SOLUTION AERATION:
PHOTOPERIOD: 16HR. DAY/8HR. NIGHT

CONCENTRATION Control

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	10/9	10/9	10/8	9/8
	C/D	10/10	10/9	10/9	10/9	10/9

		INITIAL	24	48	72	96
INITIAL	DAY					
	pH	8.20	8.21	8.21	8.21	
	D.O. (mg/l)	7.2	6.3	6.6	6.2	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPt)	24.0	24.3	24.5	24.3	
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		24.5	24.6	24.6	24.8
	pH		8.14	8.11	8.12	8.10
	D.O.(mg/l)		6.1	6.3	6.4	6.0
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 5mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	10/9	10/9	9/9	9/9
	C/D	10/10	9/10	9/10	9/10	9/10

		INITIAL	24	48	72	96
INITIAL	DAY					
	pH	8.21	8.20	8.20	8.21	
	D.O. (mg/l)	7.2	6.5	6.6	6.2	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPt)	24.0	24.2	24.1	24.1	
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		24.2	24.3	24.2	24.2
	pH		8.15	8.12	8.10	8.10
	D.O.(mg/l)		6.0	6.2	5.9	5.8
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 15mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	10/9	9/8	9/7	8/7
	C/D	10/10	10/9	9/9	9/9	9/9

		INITIAL	24	48	72	96
INITIAL	DAY					
	pH	8.21	8.21	8.21	8.21	
	D.O. (mg/l)	7.2	6.0	6.2	6.9	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPt)	24.0	24	24	24	
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.	~~24~~	24	24	24	24
	pH		8.13	8.11	8.15	8.10
	D.O.(mg/l)		5.8	5.3	5.5	5.9
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 20mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	9/9	8/7	7/5	5/4
	C/D	10/10	9/9	9/8	7/6	6/6

		INITIAL	24	48	72	96
INITIAL	DAY					
	pH	8.20	8.21	8.21	8.21	
	D.O. (mg/l)	7.2	6.6	6.4	6.3	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		25	25	25	25
	pH		8.11	8.13	8.10	8.08
	D.O.(mg/l)		5.4	5.3	5.5	5.7
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 25mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	8/9	7/6	4/5	3/5
	C/D	10/10	8/10	7/5	6/2	4/1

		INITIAL	24	48	72	96
INITIAL	DAY					
	pH	8.20	8.20	8.20	8.20	
	D.O. (mg/l)	7.2	6.5	6.6	6.3	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	~~CONDUCTIVITY~~ Sal.		24	24	24	24
	pH		8.16	8.11	8.10	8.08
	D.O.(mg/l)		5.6	5.7	5.9	6.0
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

CONCENTRATION 30mg/L

NUMBER OF LIVE ORGANISMS		TIME(HOURS) 0	24	48	72	96
	A/B	10/10	6/6	5/4	4/2	4/1
	C/D	10/10	8/5	6/3	5/1	4/1

		INITIAL	24	48	72	96
INITIAL	DAY					
	pH	8.20	8.11	8.21	8.21	
	D.O. (mg/l)	7.2	6.3	6.6	6.5	
	TEMPERATURE(°C)	20	20	20	20	
	~~CONDUCTIVITY~~ Salinity (PPt)	24	24	24	24	
	~~ALKALINITY~~ (mg/l as CaCO3)					
	ANALYST'S INITIALS					
FINAL	CONDUCTIVITY		24	24	24	24
	pH		8.12	8.09	8.08	8.08
	D.O.(mg/l)		5.9	5.6	5.8	6.0
	TEMPERATURE(°C)		20	20	20	20
	ANALYST'S INITIALS					

Appendix F

Acute Toxicity Bench Sheets for the Grass Shrimp (*Palaemonetes pugio*)

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 7.2
TO AM/PM / / (DATE) ADJUSTMENTS:
JOB NO.:
SAMPLE NO.:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Grass Shrimp
CULTURE NO.:
AGE: 1-2 months
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 8:00 (AM)/PM 3/4/96(DATE)
END OF TEST 8:00 (AM)/PM 3/6/96(DATE)
TEST CONTAINER SIZE: 1 liter
TEST SOLUTION VOLUME: 1 liter
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC) / / / / / /
AERATION:
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	5.4	5.6			8.20	8.16	8.17			20	20	20					25.0/25.3
	B	10	10	9								8.20	8.13	8.18										
60	A	10	10	9			7.2	5.5	5.3			8.20	8.15	8.16			20	20	20					25.0/25.4
	B	10	9	7								8.20	8.13	8.12										
70	A	10	8	6			7.2	5.8	6.1			8.20	8.16	8.13			20	20	20					25.0/25.3
	B	10	9	6								8.20	8.14	8.16										
80	A	10	7	5			7.2	5.9	6.2			8.20	8.12	8.14			20	20	20					24.9/25.3
	B	10	9	5								8.20	8.15	8.10										
90	A	10	3	1			7.2	5.9	6.3			8.20	8.13	8.11			20	20	20					25.0/25.3
	B	10	6	4								8.20	8.14	8.09										
100	A	10	3	0			7.2	5.7	6.0			8.20	8.12	8.06			20	20	20					25.0/25.3
	B	10	2	0								8.20	8.09	8.06										
ANALYST'S INITIALS																								

NOTES: pH adjusted to 8.2 every 4 hours during daytime

COMMENTS: Concentration (mg NH_3 total/L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 7.1
TO AM/PM / / (DATE) ADJUSTMENTS:
JOB NO.:
SAMPLE NO.:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Grass shrimp
CULTURE NO.:
AGE: 1-2 months
LENGTH(X±SD):
WEIGHT(X±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 4:00 AM/PM 3/6/96(DATE)
END OF TEST 4:00 AM/PM 3/8/96(DATE)
TEST CONTAINER SIZE: 1 liter
TEST SOLUTION VOLUME: 1 liter
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC) / / / / / /
AERATION: yes
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	~~7.1~~ 5.1	DBH 3/8/96			8.22	8.17	8.15			21	20	20					24.8 / 25.3
	B	10	10	10					5.3			8.22	8.20	8.14										
60	A	10	9	8			7.0	~~7.0~~ 5.2	DBH 3/8/96			8.21	8.14	8.15			21	20	20					24.9 / 25.3
	B	10	9	8					5.0			8.21	8.16	8.13										
70	A	10	10	7			7.0	~~6.9~~ 5.3	DBH 3/8/96			8.20	8.14	8.20			21	20	20					24.8 / 25.3
	B	10	10	6					5.2			8.20	8.13	8.17										
80	A	10	6	5			7.0	5.0	5.6			8.21	8.15	8.17			21	20	20					24.8 / 25.3
	B	10	10	4								8.21	8.16	8.13										
90	A	10	4	3			6.9	5.5	5.8			8.21	8.17	8.18			21	20	20					24.8 / 25.3
	B	10	6	2								8.21	8.13	8.14										
100	A	10	5	0			7.0	5.8	6.2			8.20	8.14	8.17			21	20	20					24.8 / 25.2
	B	10	4	1								8.20	8.14	8.16										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: ____ AM/PM ____ / ____ T.R.C. ____
COMPOSITE COLLECTED: ____ (DATE) INITIAL pH: 8.2
FROM ____ AM/PM ____ / ____ (DATE) INITIAL D.O.: 7.1
TO ____ AM/PM ____ / ____ (DATE) ADJUSTMENTS: ____

JOB NO.:
SAMPLE NO.:

TEST INFORMATION:

ANALYST(S): D BH
TEST SPECIES: Grass shrimp
CULTURE NO.:
AGE: 1-2 months
LENGTH(X±SD)
WEIGHT(X±SD)
TEST MODE: 48 hr. static
DILUTION WATER: RSW
PHOTOPERIOD 16hr DAY/8hr. NIGHT

START OF TEST 5:00 AM/PM 3/6/96 (DATE)
END OF TEST 5:00 AM/PM 3/8/96 (DATE)
TEST CONTAINER SIZE: 1 liter
TEST SOLUTION VOLUME: 1 liter
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION: moderate
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	5.3	5.1			8.22	8.18	8.16			21	20	20					24.7 / 25.2
	B	10	10	10								8.21	8.18	8.15										
60	A	10	10	9			7.2	5.3	5.2			8.20	8.15	8.18			21	20	20					24.8 / 25.1
	B	10	10	8								8.20	8.16	8.16										
70	A	10	10	8			7.1	5.4	5.0			8.20	8.17	8.18			21	20	20					24.7 / 25.2
	B	10	9	7								8.20	8.16	8.16										
80	A	10	8	7			7.0	5.6	5.3			8.21	8.17	8.18			21	20	20					24.7 / 25.2
	B	10	7	3								8.21	8.16	8.14										
90	A	10	7	2			7.0	5.5	5.7			8.21	8.14	8.19			21	20	20					24.8 / 25.2
	B	10	7	3								8.21	8.13	8.14										
100	A	10	3	0			7.0	5.8	6.1			8.21	8.16	8.16			21	20	20					24.8 / 25.2
	B	10	4	1								8.21	8.14	8.18										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study
PERMIT NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / (DATE)
COMPOSITE COLLECTED:
FROM AM/PM / / (DATE)
TO AM/PM / / (DATE)
JOB NO.:
SAMPLE NO.:
T.R.C.
INITIAL pH: 8.2
INITIAL D.O.: 7.2
ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Grass shrimp
CULTURE NO.:
AGE: 1-2 months
LENGTH(X±SD):
WEIGHT(X±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT
START OF TEST 6:00 AM/PM 3/6/96 (DATE)
END OF TEST 6:00 AM/PM 3/8/96 (DATE)
TEST CONTAINER SIZE: 2 liter
TEST SOLUTION VOLUME: 1 liter
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION: moderate
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	5.0	4.8			8.21	8.14	8.13			21	20	20					25.0 / 25.3
	B	10	10	10								8.21	8.15	8.15										
60	A	10	10	8			7.2	5.1	5.2			8.21	8.18	8.17			21	20	20					25.0 / 25.3
	B	10	10	9								8.21	8.19	8.16										
70	A	10	9	6			7.2	5.2	5.5			8.21	8.17	8.15			21	20	20					25.0 / 24 25.2 DBH 3/8/96
	B	10	10	8								8.21	8.16	8.14										
80	A	10	6	5			7.2	5.4	5.8			8.20	8.15	8.16			21	20	20					25.0 / 25.3
	B	10	10	7								8.20	8.16	8.16										
90	A	10	7	2			7.2	5.5	5.7			8.21	8.14	8.13			21	20	20					24.9 / 25.3
	B	10	4	3								8.21	8.12	8.11										
100	A	10	3	1			7.2	5.7	6.0			8.20	8.10	8.10			21	20	20					25.6 / 25.3
	B	10	5	0								8.20	8.09	8.06										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I: /DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 Study JOB NO.:
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM 1/1 T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM 1/1 (DATE) INITIAL D.O.: 7.1
TO AM/PM 1/1 (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 8:00 AM/PM 3/10/96 (DATE)
TEST SPECIES: Grass shrimp END OF TEST 8:00 AM/PM 3/12/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 1 liter
AGE: 1-2 months TEST SOLUTION VOLUME: 1 liter
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): 1/1 1/1 1/1
TEST MODE: 48hstatic AERATION: moderate
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	9	8			7.1	7.0	5.3			8.20	8.16	8.14			20	20	20					25.0/25.3
	B	10	10	10								8.20	8.16	8.13										
60	A	10	9	8			7.1	5.9	6.1			8.20	8.11	8.12			20	20	20					25.0/25.3
	B	10	10	9								8.20	8.15	8.16										
70	A	10	7	7			7.1	6.0	6.2			8.20	8.13	8.16			20	20	20					25.0/25.3
	B	10	10	9								8.20	8.15	8.17										
80	A	10	8	3			7.1	5.8	6.4			8.20	8.14	8.13			20	20	20					25.0/25.2
	B	10	8	6								8.20	8.14	8.16										
90	A	10	7	5			7.1	5.9	6.5			8.20	8.12	8.11			20	20	20					25.0/25.4
	B	10	9	1								8.20	8.15	8.09										
100	A	10	1	0			7.1	6.3	6.3			8.20	8.16	8.09			20	20	20					25.0/25.3
	B	10	4	0																				
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total/L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy/controls healthy

I:/DWC/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study JOB NO.: 1
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / (DATE) T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 7.2
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH
TEST SPECIES: Grass shrimp
CULTURE NO.:
AGE: 1-2 months
LENGTH(X̄±SD):
WEIGHT(X̄±SD):
TEST MODE: 48 hour static
DILUTION WATER: RSW
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

START OF TEST 9:00 (AM)/PM 3/10/96 (DATE)
END OF TEST 9:00 AM/PM 3/12/96 (DATE)
TEST CONTAINER SIZE: 1 liter
TEST SOLUTION VOLUME: 1 liter
DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
/ / / / / /
AERATION: moderate
BEGINNING DATE/TIME:

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	5.8	6.0			8.21	8.16	8.15			20	20	20					25.0/25.3
	B	10	9	9								8.11	8.14	8.16										
60	A	10	10	10			7.2	6.0	6.1			8.20	8.17	8.15			20	20	20					25.0/25.3
	B	10	9	8								8.20	8.18	8.15										
70	A	10	8	7			7.2	6.1	6.5			8.21	8.16	8.14			20	20	20					25.0/25.3
	B	10	7	5								8.21	8.14	8.13										
80	A	10	8	4			7.2	6.6	6.7			8.20	8.14	8.13			20	20	20					25.0/25.3
	B	10	8	4								8.20	8.12	8.13										
90	A	10	6	2			7.2	6.8	6.8			8.21	8.11	8.10			20	20	20					25.0/25.3
	B	10	6	0								8.21	8.12	8.11										
100	A	10	7	0			7.2	6.6	6.9			8.20	8.13	8.09			20	20	20					25.0/25.3
	B	10	3	0								8.20	8.12	8.08										
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study JOB NO.: 2
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 7.3
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 9:00 AM/PM 3/10/96 (DATE)
TEST SPECIES: Grass shrimp END OF TEST 9:00 AM/PM 3/12/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 1 liter
AGE: 1-2 months TEST SOLUTION VOLUME: 1 liter
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): / / / / / /
TEST MODE: 48 hour static AERATION: moderate
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS 0	24	48	72	96	DISSOLVED OXYGEN (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	TEMPERATURE (°C) 0	24	48	72	96	INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
Con	A	10	9	9			7.3	6.8	6.3			8.21	8.18	8.15			20	20	20					24.8 / 25.1
	B	10	10	10								8.21	8.17	8.16										
60	A	10	10	9			7.3	6.4	6.4			8.21	8.16	8.15			20	20	20					24.8 / 25.2
	B	10	10	10								8.21	8.15	8.14										
70	A	10	9	7			7.3	6.2	6.1			8.20	8.16	8.13			20	20	20					24.8 / 25.3
	B	10	9	7								8.20	8.14	8.15										
80	A	10	8	6			7.3	6.3	6.1			8.21	8.16	8.12			20	20	20					24.8 / 25.2
	B	10	8	4								8.21	8.15	8.14										
90	A	10	6	2			7.3	6.3	6.2			8.20	8.10	8.11			20	20	20					24.8 / 25.2
	B	10	7	2								8.20	8.12	8.10										
100	A	10	2	1			7.3	5.9	6.2			8.20	8.09	8.08			20	20	20					24.8 / 25.2
	B	10	4	1								8.20	8.10	8.07										
																								/
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWG/LAB/ACUTETOX

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

ACUTE TOXICITY TEST

DATA SHEET PAGE 1 OF 1

SAMPLE INFORMATION:

CLIENT: NH_3 study JOB NO.: 3
PERMIT NO.: SAMPLE NO.:
TOXICANT/EFFLUENT: NH_4Cl
SAMPLE TYPE:
GRAB COLLECTED: AM/PM / / T.R.C.
COMPOSITE COLLECTED: (DATE) INITIAL pH: 8.2
FROM AM/PM / / (DATE) INITIAL D.O.: 7.2
TO AM/PM / / (DATE) ADJUSTMENTS:

TEST INFORMATION:

ANALYST(S): DBH START OF TEST 9:00 AM/PM 3/10/96 (DATE)
TEST SPECIES: Grass shrimp END OF TEST 9:00 AM/PM 3/12/96 (DATE)
CULTURE NO.: TEST CONTAINER SIZE: 2 liter
AGE: 1-2 months TEST SOLUTION VOLUME: 1 liter
LENGTH(X±SD): DISSOLVED OXYGEN: (TIME/TEST SOLN/CONC)
WEIGHT(X±SD): / / / / / /
TEST MODE: 48 hour static AERATION: moderate
DILUTION WATER: RSW BEGINNING DATE/TIME:
PHOTOPERIOD: 16hr.DAY/8hr.NIGHT

CONC OR %	REP	NUMBER OF LIVE ORGANISMS					DISSOLVED OXYGEN (mg/l)					pH					TEMPERATURE (°C)					INITIAL ALKALINITY (mg/l as $CaCO_3$)	INITIAL HARDNESS (mg/l as $CaCO_3$)	CONDUCTIVITY (umhos/cm) INITIAL/FINAL
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96			
Con	A	10	10	10			7.2	6.0	6.1			8.21	8.18	8.17			20	20	20					24.8 / 24.9
	B	10	10	10								8.20	8.18	8.15										
60	A	10	10	10			7.2	5.9	5.7			8.21	8.16	8.15			20	20	20					24.8 / 25.2
	B	10	7	6								8.21	8.14	8.18										
70	A	10	10	5			7.2	6.0	6.6			8.20	8.16	8.16			20	20	20					24.8 / 25.1
	B	10	9	7								8.20	8.11	8.13										
80	A	10	6	3			7.2	6.3	6.4			8.21	8.13	8.16			20	20	20					24.8 / 25.2
	B	10	9	7								8.21	8.17	8.16										
90	A	10	6	2			7.2	6.5	6.5			8.20	8.16	8.10			20	20	20					24.8 / 25.2
	B	10	6	3								8.20	8.12	8.12										
100	A	10	2	0			7.2	6.6	6.7			8.21	8.20	8.09			20	20	20					24.8 / 25.2
	B	10	1	0								8.21	8.18	8.10										
																								/
ANALYST'S INITIALS																						NOTES: pH adjusted to 8.2 every 4 hours during daytime		

COMMENTS: Concentration (mg NH_3 total / L)
CONDITION OF ORGANISMS AT TEST INITIATION/END: healthy / controls healthy

I:/DWC/LAB/ACUTETOX

Appendix G

Chronic Toxicity Bench Sheets for the Sheepshead Minnow (*Cyprinodon variegatus*)

OLVER LABORATORIES INCORPORATED

ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

Sheepshead
LARVAL ~~FATHEAD~~ MINNOW
SURVIVAL AND GROWTH TEST
SURVIVAL DATA SHEET

CLIENT: NH_3 study JOB NO.: _____ PAGE 1 OF 5

PERMIT NO. : _____ TEST ORGANISM AGE: 5 days

TOXICANT/EFFLUENT: NH_4Cl START OF TEST: 9:00 (AM)/PM 12/15/95 (DATE)

DILUTION WATER: Reconstituted Seawater END OF TEST: 9:00 (AM)/PM 12/22/95 (DATE)

NUMBER SURVIVING PER DAY

CONC	REP	START	1	2	3	4	5	6	7	COMMENTS
Control	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	9	9	9	9	9	9	
10 mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
20 mg/L total NH_3	A	10	10	9	9	9	9	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
30 mg/L total NH_3	A	10	10	10	10	9	9	9	9	
	B	10	10	10	10	10	10	10	10	
	C	10	10	9	9	9	8	8	8	
	D	10	10	10	10	10	10	10	10	
40 mg/L total NH_3	A	10	9	7	7	7	4	3	3	
	B	10	9	6	6	6	5	4	4	
	C	10	10	9	8	7	7	7	7	
	D	10	10	9	9	6	6	6	6	
50 mg/L total NH_3	A	10	6	3	2	0	0	0	0	
	B	10	9	6	5	1	0	0	0	
	C	10	9	7	7	4	1	1	0	
	D	10	8	7	4	1	0	0	0	
ANALYST'S INITIALS										

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\FATHDSUR

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ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study
JOB NO.: _____ PAGE 2 OF 5
PERMIT NO.: _____
TEST ORGANISM: sheepshead minnow
TOXICANT/EFFLUENT: NH_4Cl
DILUTION WATER: RSW
DATE TEST INITIATED: 12/15/95

CONCENTRATION 10mg/L

INITIAL	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.21	8.21	8.20	8.21	8.20	8.21	
D.O. (mg/l)	6.4	6.5	6.6	6.7	6.9	6.4	6.6	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal.(ppt)	25.0	25.3	25.2	25.0	24.8	24.9	25.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL								
pH	8.14	8.12	8.15	8.10	8.08	8.09	8.10	
D.O. (mg/l)	5.6	5.5	5.9	6.0	5.7	5.3	5.2	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal.(ppt)	25.2	25.4	25.4	25.2	25.1	25.1	25.4	
ANALYST'S INITIALS								

CONCENTRATION Control

INITIAL	1	2	3	4	5	6	7	REMARKS
pH	8.21	8.21	8.20	8.21	8.20	8.21	8.22	
D.O. (mg/l)	6.6	6.8	6.7	6.5	6.2	6.4	6.3	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal.(ppt)	25.0	25.2	25.3	25.0	25.0	25.1	25.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL								
pH	8.16	8.12	8.16	8.10	8.08	8.08	8.09	
D.O. (mg/l)	5.7	5.6	5.9	6.0	5.7	5.6	5.9	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal.(ppt)	25.3	25.4	25.5	25.2	25.2	25.3	25.3	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study — JOB NO.: _____ PAGE 3 OF 5

PERMIT NO.: _____ TEST ORGANISM: sheepshead minnow

TOXICANT/EFFLUENT: NH_4Cl - DILUTION WATER: RSW

DATE TEST INITIATED: 12/15/95

CONCENTRATION 30mg/L

INITIAL	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.21	8.20	8.21	8.20	8.20	8.20	8.20	
D.O. (mg/l)	6.9	6.6	6.7	6.4	6.2	6.2	6.4	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.0	25.3	25.2	25.4	25.0	25.2	25.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.16	8.12	8.10	8.16	8.10	8.08	8.06	
D.O. (mg/l)	5.8	5.9	6.0	6.1	5.5	5.6	5.8	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.3	25.5	25.4	25.6	25.2	25.3	25.3	
ANALYST'S INITIALS								

CONCENTRATION 20mg/L

INITIAL	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.20	8.21	8.20	8.21	8.20	8.21	
D.O. (mg/l)	6.8	6.5	6.6	6.7	6.3	6.4	6.7	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.0	25.2	25.4	25.3	25.1	25.2	25.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.15	8.13	8.14	8.10	8.07	8.06	8.10	
D.O. (mg/l)	5.7	5.9	6.2	6.0	6.0	6.1	5.9	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.2	25.3	25.6	25.4	25.3	25.2	25.3	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study — JOB NO.: _____ PAGE 4 OF 5

PERMIT NO.: _____ TEST ORGANISM: sheepshead minnow

TOXICANT/EFFLUENT: NH_4Cl. DILUTION WATER: RSW

DATE TEST INITIATED: 12/15/95

CONCENTRATION 50 mg/L

INITIAL

DAY	1	2	3	4	5	6	7	REMARKS
pH	8.21	8.20	8.21	8.21	8.20	8.20	8.21	
D.O. (mg/l)	6.9	6.6	6.4	6.5	6.6	6.3	6.6	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.0	25.5	25.1	25.0	25.0	25.1	25.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.17	8.13	8.10	8.09	8.10	8.09	8.07	
D.O. (mg/l)	5.9	6.0	5.8	5.5	5.7	5.9	6.0	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.3	25.6	25.3	25.2	25.2	25.3	25.3	
ANALYST'S INITIALS								

CONCENTRATION 40 mg/L

INITIAL

	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.20	8.21	8.21	8.22	8.21	8.20	
D.O. (mg/l)	6.9	6.7	6.4	6.6	6.3	6.0	6.2	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.0	25.3	25.2	25.2	25.0	24.9	25.0	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.12	8.16	8.10	8.08	8.06	8.11	8.12	
D.O. (mg/l)	5.8	6.0	5.7	5.6	5.4	5.2	5.8	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.2	25.2	25.3	25.3	25.2	25.0	25.0	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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LARVAL ~~FATHEAD~~ Sheepshead MINNOW SURVIVAL AND GROWTH TEST
GROWTH DATA SHEET

CLIENT: NH_3 study JOB NO.: PAGE 5 OF 5
PERMIT NO: ANALYST(S): DBH
TOXICANT/EFFLUENT: NH_4Cl DATE WEIGHED: 12/22/95
BALANCE CALIBRATION CHECK: EXPECTED: g/ACTUAL: g

CONC	REP	FOIL WEIGHT (mg)	FOIL WEIGHT +ORGANISMS (mg)	WEIGHT OF ORGANISMS (mg)	NUMBER OF ORGANISMS	AVERAGE WEIGHT PER ORGANISMS	PERCENT SURVIVAL
Control	A	76.82	88.88	12.06	10	1.206	100%
	B	63.63	75.95	12.32	10	1.232	100%
	C	69.78	81.04	11.26	10	1.126	100%
	D	62.21	72.53	10.32	9	1.147	90%
10 mg/L total NH_3	A	55.63	66.76	11.13	10	1.113	100%
	B	57.92	69.38	11.46	10	1.146	100%
	C	62.31	72.99	10.68	10	1.068	100%
	D	48.82	60.78	11.96	10	1.196	100%
20 mg/L total NH_3	A	60.62	71.29	10.67	10	1.067	100%
	B	61.34	71.28	9.94	10	0.994	100%
	C	70.36	80.57	10.21	10	1.021	100%
	D	56.32	66.08	9.76	10	0.976	100%
30 mg/L total NH_3	A	70.46	77.77	7.31	9	0.812	90%
	B	72.53	80.16	7.63	10	0.763	100%
	C	64.48	70.27	5.79	8	0.724	80%
	D	60.32	68.88	8.56	10	0.856	100%
40 mg/L total NH_3	A	59.96	60.92	0.96	3	0.321	30%
	B	52.33	54.94	2.61	4	0.652	40%
	C	46.76	50.83	4.07	7	0.582	70%
	D	49.76	52.72	2.96	6	0.493	60%
	A						
	B						
	C						
	D						

BALANCE CALIBRATION CHECK: EXPECTED: g/ACTUAL: g
COMMENTS:

OLVER LABORATORIES INCORPORATED
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Sheepshead
LARVAL ~~FATHEAD~~ MINNOW
SURVIVAL AND GROWTH TEST
SURVIVAL DATA SHEET

CLIENT : NH_3 study JOB NO.: _____ PAGE 1 OF 5

PERMIT NO. : _____ TEST ORGANISM AGE: 4 days

TOXICANT/EFFLUENT: NH_4Cl START OF TEST: 8:00 (AM)/PM 2/12/96 (DATE)

DILUTION WATER: Reconstituted Seawater END OF TEST: 8:00 (AM)/PM 2/19/96 (DATE)

NUMBER SURVIVING PER DAY

CONC	REP	START	1	2	3	4	5	6	7	COMMENTS
Control	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
10mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	9	9	9	9	
	D	10	10	10	10	10	10	10	10	
20mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
30mg/L total NH_3	A	10	10	9	9	9	9	9	9	
	B	10	10	10	10	10	10	10	10	
	C	10	9	9	9	9	9	9	9	
	D	10	10	10	10	10	10	10	10	
40mg/L total NH_3	A	10	10	10	9	6	5	5	5	
	B	10	10	8	8	8	7	7	7	
	C	10	9	7	7	5	5	4	4	
	D	10	9	9	8	8	6	6	6	
50mg/L total NH_3	A	10	8	5	4	3	1	0	0	
	B	10	7	4	2	0	0	0	0	
	C	10	7	5	0	0	0	0	0	
	D	10	9	8	6	2	0	0	0	
ANALYST'S INITIALS										

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\FATHDSUR

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CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study
JOB NO.: _____ PAGE 2 OF 5
PERMIT NO.: _____
TEST ORGANISM: sheepshead minnow
TOXICANT/EFFLUENT: NH_4Cl
DILUTION WATER: RSW
DATE TEST INITIATED: 2/2/96

CONCENTRATION 10 mg/L

INITIAL

	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.21	8.20	8.21	8.21	8.21	8.20	
D.O. (mg/l)	6.9	6.7	6.2	6.4	6.3	6.5	6.7	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY~~ (~~umhos/cm~~) sal. (ppt)	25.4	25.3	24.9	25.0	25.1	24.5	24.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.13	8.10	8.12	8.15	8.10	8.08	8.08	
D.O. (mg/l)	5.6	5.8	5.2	5.5	6.0	6.0	6.0	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (~~umhos/cm~~) sal. (ppt)	25.6	25.5	25.3	25.3	25.2	24.9	24.5	
ANALYST'S INITIALS								

CONCENTRATION Control

INITIAL

	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.21	8.21	8.20	8.21	8.20	8.20	
D.O. (mg/l)	6.7	6.6	6.2	6.7	6.6	6.5	6.3	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY~~ (~~umhos/cm~~) sal. (ppt)	25.0	25.3	24.9	25.0	25.1	24.5	24.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.12	8.10	8.13	8.10	8.09	8.08	8.10	
D.O. (mg/l)	5.4	5.6	5.7	6.0	6.1	5.9	6.0	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (~~umhos/cm~~) sal. (ppt)	25.5	25.4	25.3	25.4	25.1	25.0	24.6	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study
JOB NO.: _____ PAGE 3 OF 5
PERMIT NO.: _____
TEST ORGANISM: sheepshead minnow
TOXICANT/EFFLUENT: NH_4Cl
DILUTION WATER: RSW
DATE TEST INITIATED: 2/2/96

CONCENTRATION 30 mg/L

INITIAL	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.20	8.20	8.20	8.20	8.20	8.20	
D.O. (mg/l)	6.9	6.7	6.2	6.3	6.3	6.6	6.8	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY~~ (umhos/cm) sal. (ppt)	25.4	25.3	25.0	25.1	25.1	24.6	24.3	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.11	8.10	8.14	8.15	8.11	8.09	8.09	
D.O. (mg/l)	5.6	5.8	6.0	6.1	5.5	6.0	6.1	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) sal. (ppt)	25.6	25.5	25.1	25.4	25.4	25.0	24.7	
ANALYST'S INITIALS								

CONCENTRATION 20 mg/L

INITIAL	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.21	8.20	8.21	8.20	8.20	
D.O. (mg/l)	6.8	6.7	6.3	6.3	6.3	6.5	6.7	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY~~ (umhos/cm) sal. (ppt)	25.4	25.4	25.2	25.1	25.1	24.7	24.4	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.14	8.10	8.15	8.12	8.13	8.14	8.09	
D.O. (mg/l)	5.6	6.0	5.5	5.7	5.8	6.0	6.4	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) sal. (ppt)	25.5	25.7	25.3	25.2	25.3	25.0	24.7	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study — JOB NO.: _____ PAGE 4 OF 5

PERMIT NO.: _____ TEST ORGANISM: sheepshead minnow

TOXICANT/EFFLUENT: NH_4Cl — DILUTION WATER: RSW

DATE TEST INITIATED: 2/2/96

CONCENTRATION 50 mg/L

INITIAL	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.20	8.21	8.20	8.20	8.21	8.20	
D.O. (mg/l)	6.9	6.6	6.3	6.5	6.6	6.6	6.6	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.4	25.3	25.0	25.0	25.1	24.6	24.4	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.12	8.15	8.11	8.16	8.08	8.08	8.09	
D.O. (mg/l)	5.7	5.6	5.9	6.0	6.1	5.8	5.9	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.6	25.5	25.2	25.2	25.3	24.9	24.6	
ANALYST'S INITIALS								

CONCENTRATION 40 mg/L

INITIAL	1	2	3	4	5	6	7	REMARKS
pH	8.21	8.20	8.20	8.21	8.20	8.20	8.20	
D.O. (mg/l)	6.9	6.3	6.2	6.6	6.4	6.5	6.3	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.4	25.2	25.2	25.2	25.1	24.9	24.6	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.12	8.06	8.10	8.14	8.07	8.10	8.10	
D.O. (mg/l)	5.5	5.6	5.8	5.9	6.0	6.0	6.0	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
CONDUCTIVITY (umhos/cm)	25.6	25.3	25.4	25.4	25.3	25.1	24.9	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

Sheepshead
LARVAL ~~FATHEAD~~ MINNOW SURVIVAL AND GROWTH TEST
GROWTH DATA SHEET

CLIENT: NH_3 study JOB NO.: PAGE 5 OF 5
PERMIT NO: ANALYST(S): DBH
TOXICANT/EFFLUENT: NH_4Cl DATE WEIGHED: 2/9/96
BALANCE CALIBRATION CHECK: EXPECTED: g/ACTUAL: g

CONC	REP	FOIL WEIGHT (mg)	FOIL WEIGHT +ORGANISMS (mg)	WEIGHT OF ORGANISMS (mg)	NUMBER OF ORGANISMS	AVERAGE WEIGHT PER ORGANISMS	PERCENT SURVIVAL
Control	A	60.24	71.38	11.14	10	1.114	100%
	B	55.56	67.72	12.16	10	1.216	100%
	C	64.31	75.29	10.98	10	1.098	100%
	D	69.66	81.70	12.04	10	1.204	100%
10mg/L total NH_3	A	44.12	54.78	10.66	10	1.066	100%
	B	50.16	61.19	11.03	10	1.103	100%
	C	52.87	62.49	9.62	9	1.069	90%
	D	62.81	73.26	10.45	10	1.045	100%
20mg/L total NH_3	A	50.92	59.57	8.65	10	0.865	100%
	B	55.68	64.46	8.78	10	0.878	100%
	C	62.23	71.34	9.11	10	0.911	100%
	D	67.81	75.89	8.08	10	0.808	100%
30mg/L total NH_3	A	50.24	56.64	6.40	9	0.711	90%
	B	49.67	57.74	8.07	10	0.807	100%
	C	60.66	67.46	6.80	9	0.756	90%
	D	52.98	60.75	7.77	10	0.777	100%
40mg/L total NH_3	A	61.33	64.09	2.76	5	0.551	50%
	B	64.69	66.80	2.11	7	0.301	70%
	C	57.83	59.47	1.64	4	0.411	40%
	D	59.61	61.96	2.35	6	0.392	60%
50mg/L total NH_3	A	—	—		0		
	B	—	—		0		
	C	—	—		0		
	D	—	—		0		

BALANCE CALIBRATION CHECK: EXPECTED: g/ACTUAL: g
COMMENTS:

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1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

sheepshead
LARVAL ~~FATHEAD~~ MINNOW
SURVIVAL AND GROWTH TEST
SURVIVAL DATA SHEET

CLIENT: NH_3 Study JOB NO.: _____ PAGE 1 OF 5

PERMIT NO. : _____ TEST ORGANISM AGE: 4 days

TOXICANT/EFFLUENT: NH_4Cl START OF TEST: 9:00 (AM)/PM 2/2/96 (DATE)

DILUTION WATER: Reconstituted Seawater END OF TEST: 9:00 (AM)/PM 2/9/96 (DATE)

NUMBER SURVIVING PER DAY

CONC	REP	START	1	2	3	4	5	6	7	COMMENTS
Con	A	10	10	10	10	10	10	10	9	
	B	10	10	10	9	9	8	8	8	
	C	10	10	10	10	10	10	10	10	
	D	10	9	9	9	9	9	9	9	
10 mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	9	9	9	9	9	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	9	9	
20 mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
30 mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
40 mg/L total NH_3	A	10	10	8	7	7	6	5	5	
	B	10	9	6	4	4	4	4	4	
	C	10	10	7	7	7	7	6	6	
	D	10	9	8	8	8	8	8	8	
50 mg/L total NH_3	A	10	2	0	—	—	—	—	—	
	B	10	0	0	—	—	—	—	—	
	C	10	0	0	—	—	—	—	—	
	D	10	3	0	—	—	—	—	—	
ANALYST'S INITIALS										

COMMENTS: pH adjusted to 8.2 every 4 hours during day time

LAB\FATHDSUR

OLVER LABORATORIES INCORPORATED
ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study — JOB NO.: _____ PAGE 2 OF 5

PERMIT NO.: _____ — TEST ORGANISM: sheepshead minnow

TOXICANT/EFFLUENT: NH_4Cl - — DILUTION WATER: RSW

DATE TEST INITIATED: 2/2/96

CONCENTRATION 10mg/L

INITIAL

DAY	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.22	8.22	8.21	8.22	8.22	
D.O. (mg/l)	7.2	6.2	6.5	6.3	6.5	6.2	6.3	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) Salinity	25.7	25.6	25.0	25.1	24.5	24.8	25.2	
HARDNESS (ppt) (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.10	8.15	8.15	8.14	8.10	8.06	8.10	
D.O. (mg/l)	4.8	5.8	5.9	5.8	6.5	5.5	5.9	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) Salinity (ppt)	25.9	25.9	26.0	25.2	24.3	24.8	25.4	
ANALYST'S INITIALS								

CONCENTRATION Con

INITIAL

	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.18	8.22	8.22	8.23	8.21	8.22	
D.O. (mg/l)	7.1	5.8	6.5	6.3	6.3	6.1	6.2	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) Sal. (ppt)	25.7	25.6	25.0	25.1	24.5	24.8	25.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.08	8.18	8.14	8.18	8.09	8.10	8.13	
D.O. (mg/l)	4.5	5.8	5.9	5.9	6.4	5.6	6.4	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) Sal (ppt)	26.0	26.1	26.1	25.2	25.0	24.1	25.4	
ANALYST'S INITIALS								

COMMENTS: pH adjusted every 4 hours to 8.2 during daytime

LAB\CHRONTOX

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ENVIRONMENTAL BIOLOGY LABORATORY
1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study
JOB NO.: _____ PAGE 3 OF 5
PERMIT NO.: _____
TEST ORGANISM: sheepshead minnow
TOXICANT/EFFLUENT: NH_4Cl
DILUTION WATER: RSW
DATE TEST INITIATED: 2/2/96

CONCENTRATION 30 mg/L

INITIAL	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.23	8.23	8.22	8.23	8.23	
D.O. (mg/l)	7.2	6.2	6.2	6.3	6.5	6.1	6.5	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) Salinity	25.8	26.0	25.0	25.1	24.5	25.0	25.4	
HARDNESS (ppt) (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.10	8.20	8.08	8.10	8.09	8.09	8.18	
D.O. (mg/l)	4.5	5.9	6.1	6.0	5.7	5.8	6.4	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) Sal.(ppt)	26.1	26.1	25.0	24.8	24.6	25.6	25.7	
ANALYST'S INITIALS								

CONCENTRATION 20 mg/L

INITIAL	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.22	8.23	8.23	8.23	8.22	
D.O. (mg/l)	7.1	6.2	6.4	6.3	6.5	6.2	6.4	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) Sal.(ppt)	25.8	26.0	25.0	25.1	24.3	25.0	25.4	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.12	8.14	8.13	8.08	8.09	8.11	8.15	
D.O. (mg/l)	4.5	6.0	6.0	6.1	6.6	5.3	6.0	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
CONDUCTIVITY (umhos/cm)	26.0	26.1	25.4	26.1	25.0	25.3	25.5	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study — JOB NO.: _____ PAGE 4 OF 5

PERMIT NO.: _____ — TEST ORGANISM: sheepshead minnow

TOXICANT/EFFLUENT: NH_4Cl - — DILUTION WATER: RSW

DATE TEST INITIATED: 2/2/96

CONCENTRATION 50 mg/L

INITIAL	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22						
D.O. (mg/l)	7.1	6.2						
TEMPERATURE (°C)	25	25						
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.8	26.0						
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL								
pH	8.05	8.17						
D.O. (mg/l)	4.5	6.1						
TEMPERATURE (°C)	25	25						
CONDUCTIVITY (umhos/cm)	25.9	26.1						
ANALYST'S INITIALS								

CONCENTRATION 40mg/L

INITIAL	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.22	8.23	8.23	8.23	8.23	
D.O. (mg/l)	7.1	6.2	6.4	6.5	6.6	6.3	6.5	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.8	26.0	25.1	25.1	24.8	25.0	25.4	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL								
pH	8.08	8.09	8.14	8.18	8.14	8.12	8.20	
D.O. (mg/l)	4.6	6.1	6.1	6.2	5.9	6.0	6.5	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
CONDUCTIVITY (umhos/cm)	26.1	26.1	25.2	252	25.0	25.4	25.5	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

LARVAL ~~FATHEAD~~ Sheepshead MINNOW SURVIVAL AND GROWTH TEST
GROWTH DATA SHEET

CLIENT: NH_3 Study JOB NO.: PAGE 5 OF 5
PERMIT NO: ANALYST(S): DBH
TOXICANT/EFFLUENT: NH_4Cl DATE WEIGHED: 2/9/96
BALANCE CALIBRATION CHECK: EXPECTED: g/ACTUAL: g

CONC	REP	FOIL WEIGHT (mg)	FOIL WEIGHT +ORGANISMS (mg)	WEIGHT OF ORGANISMS (mg)	NUMBER OF ORGANISMS	AVERAGE WEIGHT PER ORGANISMS	PERCENT SURVIVAL
Con	A	56.47	67.34	10.87	9	1.208	90%
	B	60.02	70.30	10.28	8	1.285	80%
	C	59.71	70.62	10.91	10	1.091	100%
	D	55.96	66.81	10.85	9	1.206	90%
10 mg/L	A	65.77	77.12	11.35	10	1.135	100%
	B	65.26	74.37	9.17	9	1.019	90%
	C	60.68	71.61	10.93	10	1.093	100%
	D	64.66	73.54	8.88	9	0.987	90%
20 mg/L	A	48.16	55.81	7.65	10	0.765	100%
	B	58.73	68.29	9.56	10	0.956	100%
	C	58.42	66.74	8.32	10	0.832	100%
	D	65.28	73.77	8.49	10	0.849	100%
30 mg/L	A	68.37	74.60	6.23	10	0.623	100%
	B	48.77	56.42	7.65	10	0.765	100%
	C	60.82	68.08	7.26	10	0.726	100%
	D	54.89	62.26	7.37	10	0.737	100%
40 mg/L	A	67.63	69.55	1.92	5	0.384	50%
	B	82.22	83.36	1.14	4	0.285	40%
	C	56.05	58.32	2.27	6	0.378	60%
	D	67.71	71.58	3.87	8	0.484	80%
	A						
	B						
	C						
	D						

BALANCE CALIBRATION CHECK: EXPECTED: g/ACTUAL: g
COMMENTS:

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Sheepshead
LARVAL ~~FATHEAD~~ MINNOW
SURVIVAL AND GROWTH TEST
SURVIVAL DATA SHEET

CLIENT: NH_3 study JOB NO.: _____ PAGE 1 OF 5

PERMIT NO. : _____ TEST ORGANISM AGE: 3 days

TOXICANT/EFFLUENT: NH_4Cl START OF TEST: 9:00 (AM)/PM 2/7/96 (DATE)

DILUTION WATER: Reconstituted Seawater END OF TEST: 9:00 (AM)/PM 2/14/96 (DATE)

NUMBER SURVIVING PER DAY

CONC	REP	START	1	2	3	4	5	6	7	COMMENTS
Control	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
10 mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
20 mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	9	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
30 mg/L total NH_3	A	10	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	10	
40 mg/L total NH_3	A	10	9	9	9	9	9	8	6	
	B	10	10	10	10	10	10	8	8	
	C	10	10	10	10	10	10	6	5	
	D	10	10	10	10	10	10	1	1	
50 mg/L total NH_3	A	10	10	8	8	1	0	0	0	
	B	10	10	8	7	2	1	1	1	
	C	10	10	8	6	3	1	1	1	
	D	10	10	10	10	9	1	1	1	
ANALYST'S INITIALS										

COMMENTS: pH adjusted every 4 hours during daytime

LAB\FATHDSUR

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CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study
JOB NO.: _____ PAGE 2 OF 5
PERMIT NO.: _____
TEST ORGANISM: sheepshead minnow
TOXICANT/EFFLUENT: NH_4Cl
DILUTION WATER: RSW
DATE TEST INITIATED: 2/7/96

CONCENTRATION 10 mg/L

INITIAL	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.20	8.22	8.22	8.22	8.22	8.22	
D.O. (mg/l)	6.8	6.5	6.2	6.4	6.1	6.8	6.2	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ salinity (PPT)	25.4	25.5	25.5	24.5	24.0	24.0	24.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.15	8.20	8.14	8.08	8.09	8.09	8.10	
D.O. (mg/l)	5.4	5.2	5.6	5.6	6.2	5.0	5.4	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.7	25.6	25.5	24.5	24.1	24.3	24.9	
ANALYST'S INITIALS								

CONCENTRATION Control

INITIAL	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.22	8.22	8.22	8.22	8.22	
D.O. (mg/l)	6.8	6.4	6.2	6.3	6.0	6.8	6.2	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.4	25.5	25.5	24.6	24.0	24.0	24.1	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.11	8.20	8.12	8.10	8.10	8.10	8.10	
D.O. (mg/l)	5.2	5.4	5.6	5.8	6.3	5.2	5.0	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal. (ppt)	25.4	25.6	25.5	24.2	24.3	24.2	24.6	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

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CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 study
JOB NO.: _____ PAGE 3 OF 5
PERMIT NO.: _____
TEST ORGANISM: sheepshead minnow
TOXICANT/EFFLUENT: NH_4Cl
DILUTION WATER: RSW
DATE TEST INITIATED: 2/7/96

CONCENTRATION 30 mg/L

INITIAL

	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.22	8.22	8.22	8.22	8.22	
D.O. (mg/l)	6.8	6.6	6.3	6.3	6.3	6.8	6.2	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY~~ (umhos/cm) sal. (ppt)	25.4	25.5	24.4	24.1	23.9	24.0	25.2	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.14	8.20	8.12	8.09	8.12	8.08	8.08	
D.O. (mg/l)	5.6	5.3	5.6	5.8	5.8	5.5	5.2	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) sal. (ppt)	25.9	25.7	24.2	23.7	24.1	24.6	24.8	
ANALYST'S INITIALS								

CONCENTRATION 20 mg/L

INITIAL

	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.21	8.21	8.21	8.22	8.22	8.22	
D.O. (mg/l)	6.8	6.5	6.2	6.2	6.2	6.6	6.2	
TEMPERATURE (°C)	24	24	24	24	24	24	24.0	
~~CONDUCTIVITY~~ (umhos/cm) sal. (ppt)	25A	25.5	24.4	24.1	23.9	24.0	25.1	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.14	8.21	8.11	8.10	8.22	8.10	8.10	
D.O. (mg/l)	5.4	5.2	5.6	5.6	5.9	5.1	5.2	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY~~ (umhos/cm) sal. (ppt)	25.5	25.9	24.3	24.0	24.1	24.4	24.3	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

CHRONIC TOXICITY TEST
CHEMICAL ANALYSIS DATA SHEET

CLIENT: NH_3 Study
JOB NO.: _____ PAGE 4 OF 5
PERMIT NO.: _____
TEST ORGANISM: sheepshead minnow
TOXICANT/EFFLUENT: NH_4Cl
DILUTION WATER: RSW
DATE TEST INITIATED: 2/7/96

CONCENTRATION 50 mg/L

INITIAL

	DAY 1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.21	8.22	8.22	8.21	8.22	
D.O. (mg/l)	6.8	6.4	6.4	6.4	6.4	6.8	6.4	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal.(ppt)	25.4	25.5	24.4	24.2	23.9	24.3	25.0	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.14	8.20	8.12	8.09	8.09	8.10	8.10	
D.O. (mg/l)	5.7	5.3	5.7	5.6	6.2	6.0	5.4	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal.(ppt)	25.4	25.8	24.2	23.7	24.6	24.6	25.2	
ANALYST'S INITIALS								

CONCENTRATION 40 mg/L

INITIAL

	1	2	3	4	5	6	7	REMARKS
pH	8.20	8.22	8.22	8.22	8.22	8.22	8.22	
D.O. (mg/l)	6.8	6.5	6.3	6.3	6.2	6.8	6.3	
TEMPERATURE (°C)	24	24	24	24	24	24	24	
~~CONDUCTIVITY (umhos/cm)~~ sal.(ppt)	25.4	25.5	24.4	24.3	23.9	24.1	25.1	
HARDNESS (mg/l as CaCO3)								
ALKALINITY (mg/l as CaCO3)								
ANALYST'S INITIALS								

FINAL

pH	8.12	8.18	8.13	8.08	8.17	8.08	8.08	
D.O. (mg/l)	5.7	5.6	5.7	5.8	6.3	5.6	5.4	
TEMPERATURE (°C)	25	25	25	25	25	25	25	
~~CONDUCTIVITY (umhos/cm)~~ sal.(ppt)	25.7	25.6	25.0	23.7	24.4	24.2	25.0	
ANALYST'S INITIALS								

COMMENTS: pH adjusted to 8.2 every 4 hours during daytime

LAB\CHRONTOX

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1116 SOUTH MAIN STREET BLACKSBURG, VIRGINIA 24060

LARVAL FATHEAD MINNOW SURVIVAL AND GROWTH TEST
GROWTH DATA SHEET

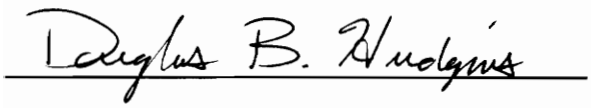
CLIENT: NH_3 study JOB NO.: PAGE 5 OF 5
PERMIT NO: ANALYST(S): DBH
TOXICANT/EFFLUENT: NH_4Cl DATE WEIGHED: 2/14/96
BALANCE CALIBRATION CHECK: EXPECTED: g/ACTUAL: g

CONC	REP	FOIL WEIGHT (mg)	FOIL WEIGHT +ORGANISMS (mg)	WEIGHT OF ORGANISMS (mg)	NUMBER OF ORGANISMS	AVERAGE WEIGHT PER ORGANISMS	PERCENT SURVIVAL
Control	A	71.74	82.88	11.14	10	1.114	100%
	B	76.04	88.06	12.02	10	1.202	100%
	C	67.76	79.13	11.37	10	1.137	100%
	D	89.53	100.94	11.41	10	1.141	100%
10mg/L total NH_3	A	59.46	70.02	10.56	10	1.056	100%
	B	52.93	63.95	11.02	10	1.102	100%
	C	57.02	68.18	11.16	10	1.116	100%
	D	62.30	73.05	10.75	10	1.075	100%
20mg/L total NH_3	A	60.59	71.90	11.31	10	1.131	100%
	B	65.87	76.97	11.10	9	1.233	90%
	C	59.79	69.81	10.02	10	1.002	100%
	D	58.94	69.69	10.75	10	1.075	100%
~~tota~~ 30mg/L total NH_3	A	55.61	63.68	8.07	10	~~0.807~~ 0.0807	100%
	B	51.59	60.61	9.02	10	0.902	100%
	C	47.27	56.59	9.32	10	0.932	100%
	D	50.28	59.16	8.88	10	0.888	100%
40mg/L total NH_3	A	55.99	59.31	3.32	6	0.553	60%
	B	55.36	59.83	4.47	8	0.559	80%
	C	56.76	59.18	2.42	5	0.484	50%
	D	45.96	46.31	0.350	1	0.350	10%
50mg/L total NH_3	A	—	—	—	0	—	—
	B	40.68	41.13	0.45	1	0.45	10%
	C	49.25	49.91	0.66	1	0.66	10%
	D	57.04	57.30	0.26	1	0.26	10%

BALANCE CALIBRATION CHECK: EXPECTED: g/ACTUAL: g
COMMENTS:

Vita

Douglas Bland Hudgins was born 15 October 1969 in Richmond, Virginia. He lived in Mathews County, Virginia where he graduated from high school. Douglas attended Virginia Polytechnic Institute and State University in Blacksburg from 1987 to 1992 and received a Bacheor of Science degree in Mechanical Engineering. During his undergraduate studies, Douglas worked for E. I. DuPont in Richmond, Virginia under the Cooperative Education Program and also worked for DuPont as an engineer during the summer following graduation. Following the summer of 1992, Douglas attended the graduate school of Virginia Polytechnic Institute and State University and received two Master of Science degrees: one in Mechanical Enigeering in May of 1994 and one in Environmental Engineering in July of 1996. Upon Graduation, Douglas will pursue an environmental engineering consultant career.



Douglas B. Hudgins