

Site-Specific Ammonia Toxicity to Fish of the Red and Assiniboine Rivers and Implications for Manitoba Water Quality Objectives

by

Amy D. Partridge

A thesis submitted
to the Faculty of Graduate Studies
in partial fulfillment of the requirements
for the degree

MASTER OF SCIENCE

Department of Zoology
University of Manitoba
Winnipeg, MB

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Amy D. Partridge

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University
of Manitoba in partial fulfillment of the requirements of the degree

of

MASTER OF SCIENCE

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Abstract

Site-specific acute- and chronic-exposure toxicity tests were conducted on five fish species (i.e., channel catfish, fathead minnow, northern pike, walleye and white sucker) resident to the Red and Assiniboine Rivers to evaluate survival and growth effects of un-ionized ammonia (NH_3). Site-specificity was established using Red River water as the control and dilution water for all toxicity tests. 96-hour LC50 values ranged from 0.22 mg NH_3 /L for larval white sucker to >0.76 mg NH_3 /L for juvenile fathead minnows. End-of-Test LC20 values ranged from 0.13 mg NH_3 /L for larval northern pike to >0.58 mg NH_3 /L for juvenile fathead minnows. Growth was impaired in one group of juvenile fathead minnows at 0.52 mg NH_3 /L.

Results of these tests, expressed as total ammonia-nitrogen, were used to compare the sensitivities of resident fish species to ammonia concentrations meeting current Manitoba Surface Water Quality Objectives (MSWQOs). Acute criteria established by Manitoba Conservation for pHs and temperatures common in the Red and Assiniboine Rivers (i.e., pH = 7.8 - 8.4, temperature = 0°C - 25°C) range between 3.9 and 12.1 mg total ammonia-nitrogen/L. Results of this study suggest that these criteria are protective to only one of the five fish species tested (i.e., channel catfish). Any changes to acute criteria should reflect the heightened sensitivity of fish tested under site-specific conditions and should be lowered to provide an appropriate level of protection to cool water aquatic life.

Conversely, chronic criteria range between 1.6 and 12.9 mg total ammonia-nitrogen/L and are adequately protective or overprotective for all fish species tested except possibly northern pike at water temperatures below 15°C. Further testing or *in situ* monitoring of northern pike is recommended to ensure that Provincial chronic objectives support the maintenance and propagation of this species.

Finally, tests were conducted to determine whether NH₃ is the sole toxicant in treated effluent discharged into the Red and Assiniboine Rivers a municipal wastewater treatment facility in Winnipeg, Manitoba (i.e, the North End Water Pollution Control Centre). White sucker and fathead minnows were consistently more sensitive to NH₃ in the presence of effluent suggesting that another constituent of the effluent may increase ammonia toxicity or is itself toxic.

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List of Abbreviations

AMP = adenosine monophosphate

ASTM = American Society for Testing Materials

ATP = adenosine triphosphate

AV = acute value

BOT = beginning of test

CCC = continuous chronic criterion

CCME = Canadian Council of Ministers of the Environment

CEC = Clean Environment Commission

CI = confidence interval

CMC = criterion maximum concentration

CO₂ = carbon dioxide

CV = chronic value

DO = dissolved oxygen

EC_x = the effective concentration of a substance that induces a biological response, other than mortality, in x% of a test-population

ELS = early life stage

EOT = end of test

FAV = final acute value

GMAV = genus mean acute value

GMCV = genus mean chronic value

H⁺ = hydrogen ion

HgCl_2 = mercuric chloride

ICP = inhibitory concentration point estimate

ICPIN = name of program used to calculate the ICP

HCO_3^- = bicarbonate

IMP = inosine monophosphate

LC_x = the concentration of a substance that is lethal to x% of a test-population

LOEC = lowest observable effects concentration

MDNR = Michigan Department of Natural Resources

MSWQO = Manitoba Surface Water Quality Objectives

MWQSOG = Manitoba Water Quality Standards, Objectives and Guidelines

Na^+ = sodium ion

NEWPCC = North End Water Pollution Control Centre

NH_3 = un-ionized ammonia or ammonia

NH_4^+ = ionized ammonia or ammonium

$\text{NH}_3\text{-N}$ = un-ionized ammonia-nitrogen

$\text{NH}_4^+\text{-N}$ = ionized ammonia-nitrogen

NH_4Cl = ammonium chloride

$(\text{NH}_4)_2\text{SO}_4$ = ammonium sulphate

NOEC = no observable effects concentration

NPRI = National Pollutant Release Inventory

O-UC = Ornithine-Urea Cycle

P_{NH_3} = partial pressure of un-ionized ammonia

PNC = Purine Nucleotide Cycle

POTW = publicly-owned treatment works

REL = a parameter used by Erickson (1985) to describe the relative toxicity of

NH_3 versus NH_4^+

SERM = Saskatchewan Environment and Resource Management

SEWPCC = South End Water Pollution Control Centre

SMAV = species mean acute value

SMCV = species mean chronic value

SSWQO = site-specific water quality objectives

TDS = total dissolved solids

TSS = total suspended solids

US EPA = United States Environmental Protection Agency

WEWPCC = West End Water Pollution Control Centre

WPCC = water pollution control center

WQG = water quality guidelines

WQO = water quality objectives

WQS = water quality standards

WWTP = waste water treatment plant

YSI = Yellow Springs Instruments

1.0 INTRODUCTION

The Red and Assiniboine Rivers are valued resources in the Winnipeg area due to their aesthetic, ecological, economic and historic importance. The rivers were instrumental in guiding the development and growth of the City of Winnipeg and they continue to provide scenic waterways. An internationally valued sport-fishery and a host of recreational activities are supported by these river systems. They provide natural drainage of adjacent lands and have been used since the inception of the City of Winnipeg to assimilate urban wastewater (Wardrop/TetrES 1991). The Red River is the municipal drinking water supply for the City of Selkirk, located approximately 30 km downstream from the City of Winnipeg.

As a means of protecting regional water quality standards and establishing effluent limitations for dischargers, the City of Winnipeg initiated a pollution control program in the 1930s. This program has progressively improved over the past seventy years due to heightened public pressures for greater environmental protection and the City of Winnipeg's stewardship values towards continual improvement of river water conditions. Wastewater treatment for local dischargers has been one of the primary issues fueling the evolution of the pollution control program (Wardrop/TetrES 1991). Presently, the City of Winnipeg owns and operates three water pollution control centers (WPCCs) and

is investigating the costs and benefits associated with incorporating advanced wastewater treatment technologies into current treatment practices.

Until the late 1980s, the City of Winnipeg's pollution control program was the primary tool used to protect water quality of the Red and Assiniboine Rivers in the Winnipeg area. With the promulgation of the Manitoba Environment Act on March 31, 1988, the responsibility of regional water quality protection was shifted away, in part, from municipal authorities to Provincial authorities. Under the Manitoba Environment Act (1988), all projects with discharges to the environment that have the potential to create environmental impacts must possess a licence to operate in the province. In accordance with this act and by request of the Minister of the Environment, the City of Winnipeg applied for licences in February 1990 for its three WPCCs. Licences were to be issued following public hearings convened by the Clean Environment Commission (CEC) over nine days between November 1991 and January 1992. The hearings were intended to review the application of Manitoba Surface Water Quality Objectives (MSWQO) for the protection of important water uses of the Red and Assiniboine Rivers and the costs and benefits of applying additional protection to the local waterway (Wardrop/TetrES 1991).

MSWQO for un-ionized ammonia are designed to protect surface water uses including domestic, industrial and agricultural consumption, recreation activities and the propagation and maintenance of aquatic life and wildlife (Williamson

1988). The Routine Level of Protection for each water use should be achievable through the simultaneous application of a technology-based approach and a water quality-based approach. The technology-based approach ensures that pollutant concentrations in waste discharges are reduced or eliminated through practical and economically achievable treatment technologies. Stricter environmental controls can be established for sensitive water uses using a water quality-based approach which considers environmental regulatory activities, ambient water quality monitoring data, scientific toxicological information, stream characteristics, and public expectations concerning environmental quality (Manitoba Conservation 2001). Further modifications to water quality objectives can be made on a site-specific basis to better account for the unique characteristics of a water body provided that scientifically rigorous methods are followed (Manitoba Conservation 2001). Site-specific criteria may be developed in cases where resident species have a greater or lesser sensitivity to a particular toxicant than non-resident species; characteristics of a receiving water body ameliorate or enhance toxicity, etc. In combination, these approaches attempt to prevent the degradation of surface water quality without imposing restrictions that are overprotective for a particular water use (Williamson 1988).

During the 1991 and 1992 CEC hearings, concerns were raised regarding the protection of Cool Water Aquatic Life and Wildlife, a sensitive water use for the Winnipeg reaches of the Red and Assiniboine Rivers. Aquatic communities indigenous to a cool water habitat including bacteria, fungi, algae, aquatic plants,

aquatic insects, other aquatic invertebrates, reptiles, amphibians and fish are afforded a level of protection through Water Quality Objectives (WQO) defined for this water use. In addition, application of these objectives helps to maintain a healthy environment for aquatic and semi-aquatic wild animals such as waterfowl, shorebirds and fur-bearing mammals (Williamson 1988). At the time of the CEC hearings, representatives of Manitoba Environment reported that un-ionized ammonia levels were greater than recommended Manitoba objectives 7% to 59% of the time at various locations throughout the Winnipeg reaches of the Red and Assiniboine Rivers. Environmental un-ionized ammonia concentrations were elevated, in part, due to wastewater discharges from the City of Winnipeg's three WPCCs (CEC 1992).

Un-ionized ammonia elicits acute- and chronic- responses in fish populations (reviewed by US EPA 1985a, 1998, 1999) including biochemical and structural changes, convulsions and death (Randall and Wright 1987). The mechanisms of ammonia toxicity in fish are not fully understood, but disruptions of ion transport processes in the central nervous system are predominant (Walsh 1998). Sub-lethal ammonia exposure may reduce growth rates (Colt and Tchobanoglous 1978); reduce the oxygen carrying capacity of hemoglobin (Sousa and Meade 1977); increase oxygen consumption, respiratory rates and heart rates (Smart 1978); and increase urine output (Lloyd and Orr 1969). Reproductive effects include decreases in egg production or viability (Thurston et al. 1986), the number of normal larvae at hatch, and larval survival (Mayes et al. 1986).

Degenerative tissue damage to gills, livers (Hemanutz et al. 1987) and kidneys (reviewed by Tomasso et al. 1980) and the induction of brain lesions (Thurston et al. 1986) may also be incurred.

To minimize the risk of ammonia exposure to aquatic life within the Winnipeg reaches of the Red and Assiniboine Rivers, the incorporation of an additional wastewater treatment technology, nitrification, into current treatment practices was discussed at the hearings. The treatment of effluent through nitrification (i.e., the biological conversion of un-ionized ammonia to nitrate-nitrogen via nitrifying bacteria) has the potential to reduce river water un-ionized ammonia concentrations to levels that would meet Provincial criteria. However, a capital investment of \$120 to \$175 million and annual operating costs of \$2.3 to \$3.7 million would be required to achieve this; an expense deemed unjustifiable by the City of Winnipeg's consultants (CEC 1992).

Following the 1992 hearings, the CEC made the following recommendation:

"Detailed site-specific studies should be undertaken to determine both the acute toxic and chronic effects of un-ionized ammonia from wastewater effluent on the cool water aquatic life of the [Red and Assiniboine] rivers... The study results will be utilized to establish the un-ionized ammonia objective at a public hearing to be held within six months of the completion of the study."

In response, the City of Winnipeg initiated an Ammonia-Criteria Study. The development of site-specific objectives could better reflect the unique circumstances within the area under consideration and promote the establishment of appropriate long-term management practices.

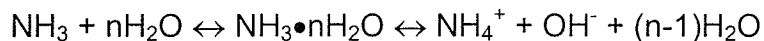
The prime contractor selected by the City of Winnipeg to conduct the Ammonia-Criteria Study was TetrES Consultants Inc. TetrES has designed a program that involves the integration of 13 related tasks - reviewing river water quality characteristics, monitoring in-stream fish behaviour, developing site-specific toxicological data for local fish and invertebrate species, etc. Completion of the Ammonia-Criteria Study will provide a framework from which regulatory decisions can be made.

All toxicological information derived during the Ammonia-Criteria Study for fish species native to the Red and/or Assiniboine Rivers is discussed in this thesis. *Two primary objectives were to compare acute- and chronic-exposure un-ionized ammonia sensitivities of local fish species tested under site-specific conditions with those reported in the public literature and to use the site-specific results to determine the protectiveness of current Provincial ammonia-criteria for resident fish species. A secondary objective was to determine whether un-ionized ammonia is the sole constituent of municipal wastewater effluent that produces toxic responses in local fish species.*

2.0 LITERATURE REVIEW OF AMMONIA TOXICOLOGY

2.1 Ammonia chemistry

Ammonia is a colourless, alkaline gas with a freezing point of -77.8°C and a boiling point of -33.35°C (reviewed by CCME 2000). It has an atomic mass of 17.03. In aqueous solution, ammonia exists in an un-ionized form (NH_3) and an ionized form (NH_4^+), which are in equilibrium according to the following expression:



(Thurston et al. 1981b, Alexander et al. 1986).

Total ammonia (or simply 'ammonia') refers to the sum of NH_3 and NH_4^+ . These terms may be expressed in their 'nitrogen' forms as un-ionized ammonia-nitrogen ($\text{NH}_3\text{-N}$), ionized ammonia-nitrogen ($\text{NH}_4^+\text{-N}$), and total ammonia-nitrogen (total ammonia-N) based on their slightly different molecular masses.

NH_3 is highly soluble in water (approximately 1000 times more soluble than CO_2) but has a low lipid to water partition coefficient of less than 0.1 (Wood 1993, Walsh 1998). Consequently, it diffuses readily through cell membranes via aqueous channels from compartments with high partial pressure of NH_3 (P_{NH_3}) to compartments with low P_{NH_3} . NH_4^+ , with its large, hydrated diameter and net

charge, is unable to permeate most biological membranes. NH_4^+ movement is primarily restricted to paracellular aqueous channels (i.e, the spaces between adjacent cells) (reviewed by Wood 1993).

In fresh water, ammonia speciation is highly influenced by pH and temperature and only negligibly by ionic strength (reviewed by US EPA 1999). The concentration of NH_3 in aqueous solution increases nearly tenfold for every increase by one pH unit; 40-50% increases occur with each 5°C rise in temperature (CCME 2000). Emerson et al. (1975) developed an equation for determining the equilibrium constant, pK_a , at different temperatures and another equation for determining the fraction of NH_3 , f_{NH_3} , in aqueous solution as a function of pH and temperature. The equations are:

$$\text{pK}_a = 0.09018 + 2729.92 / T \quad (\text{Eq 1})$$

Where:

$$\text{pK}_a = -\log_{10}K;$$

K = the acid dissociation constant of the NH_4^+ ion (i.e., 5.6×10^{-10}); and

T = temperature Kelvins or temperature in degrees Celsius + 273.15

And:

$$f_{\text{NH}_3} = 1 / (1 + 10^{\text{pK}_a - \text{pH}}) \quad (\text{Eq 2})$$

Where:

$$pK_a = -\log_{10}K;$$

K = the acid dissociation constant of the NH_4^+ ion (i.e., 5.6×10^{-10}); and

$$\text{pH} = -\log_{10}[\text{H}^+]$$

Similarly, the fraction of total ammonia that exists in its ionized form, $f_{\text{NH}_4^+}$, can be calculated with the equation:

$$f_{\text{NH}_4^+} = 1 / (1 + 10^{\text{pH}-\text{p}K_a}) \text{ (Eq 3).}$$

Where:

$$pK_a = -\log_{10}K;$$

K = the acid dissociation constant of the NH_4^+ ion (i.e., 5.6×10^{-10}); and

$$\text{pH} = -\log_{10}[\text{H}^+]$$

The sum of the fractions of NH_3 and NH_4^+ equals a value of one.

The concentration of NH_3 is negatively correlated with ionic strength and in freshwater systems with total dissolved solids (TDS) up to 200-300 mg/L, the effects of ionic strength may be negligible (CCME 2000). Table 2-1 summarizes percentages of total ammonia as NH_3 in low ionic strength water for temperatures ranging between 0°C and 30°C and pHs of 6 to 10.

Table 2-1. Percent of total ammonia as NH_3 in aqueous solution for temperatures between 0°C and 30°C and pHs of 6 to 10 (Emerson et al. 1975).

Temp ($^\circ\text{C}$)	pH								
	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
0	0.008	0.026	0.082	0.261	0.820	2.55	7.64	20.7	45.3
5	0.012	0.039	0.125	0.394	1.23	3.8	11.1	28.3	55.6
10	0.018	0.058	0.186	0.586	1.83	5.56	15.7	37.1	65.1
15	0.027	0.086	0.273	0.859	2.67	7.97	21.5	46.4	73.3
20	0.039	0.125	0.396	1.24	3.82	11.2	28.4	55.7	79.9
25	0.056	0.180	0.566	1.77	5.38	15.3	36.3	64.3	85.1
30	0.080	0.254	0.799	2.48	7.46	20.3	44.6	71.8	89.0

2.2 Ammonia production, distribution and excretion in fish

Ammonia is unique among regulated pollutants because it is produced endogenously by fish and other aquatic organisms, which have developed various physiological strategies for nitrogen excretion (US EPA 1999). Nitrogen turnover in fish is a continuous and unbalanced process. Consumed foodstuffs (e.g., carbohydrates, lipids and proteins) are digested into major components required by organisms for growth, metabolism and reproduction (Walsh 1998). Fish generally feed on high protein diets and excess proteins are degraded to carbon skeletons and incorporated into carbohydrates, lipids, etc., for growth and storage. Since proteins have high nitrogen contents relative to other storage compounds (e.g., carbohydrates and lipids), nitrogen is lost in the process (Walsh 1998). Even if the amount of ingested protein were to equal that required metabolically, interconversion of amino acids and synthesis of non-essential amino acids would result in net nitrogen loss. Furthermore, between meals existing proteins are continually renewed, a process that invariably results in lost nitrogen (Walsh 1998). During periods of starvation, protein reserves in muscle tissue are tapped to produce amino acids that can ultimately be used to make ATP to maintain regular metabolic activity (Moon and Foster 1995). Thus, despite the nutritional status of fish, they are almost always producing and excreting waste nitrogen (Walsh 1998).

Ammonia is a major metabolic waste product of fish, comprising more than 80% of nitrogen loss in most teleosts (Wood 1958). It is primarily produced in the liver through the transdeamination of excess amino acids not required for protein synthesis (Randall and Wright 1987, Walsh 1998), but may also be produced in the kidneys, gills, and skeletal muscle tissues (reviewed by Randall and Wright 1987, and Wood 1993). Transdeamination is a two-step process beginning with the transfer of the amino group of an L-amino acid to α -ketoglutarate (i.e., transamination) yielding glutamate and the corresponding α -keto acid (Randall and Wright 1987, Wood 1993). Glutamate is subsequently deaminated through the action of glutamate dehydrogenase generating NH_4^+ (Wood 1993, Walsh 1998). This pathway is illustrated in Figure 2-1. In the presence of specific enzymes, some individual amino acids (e.g., histidine, serine, and threonine) may liberate ammonia directly through deamination (Wood 1993).

The deamination of adenylates via the Purine Nucleotide Cycle (PNC) contributes a minor proportion of hepatic ammonia but is an important ammonia-producing reaction in fish muscle (Walsh 1998). The conversion of AMP to IMP through the action of AMP deaminase liberates ammonia in its un-ionized form (c.f., Figure 2-1). Some of the NH_3 enters the circulatory system and is excreted through the gills (Wood 1993). However, most is retained in muscle tissues and functions in stimulating phosphofructokinase, an enzyme that promotes glycolysis. Glycolysis (i.e., the splitting of glucose to two pyruvic acid molecules) helps meet the energy demands of contracting muscle by releasing units of ATP

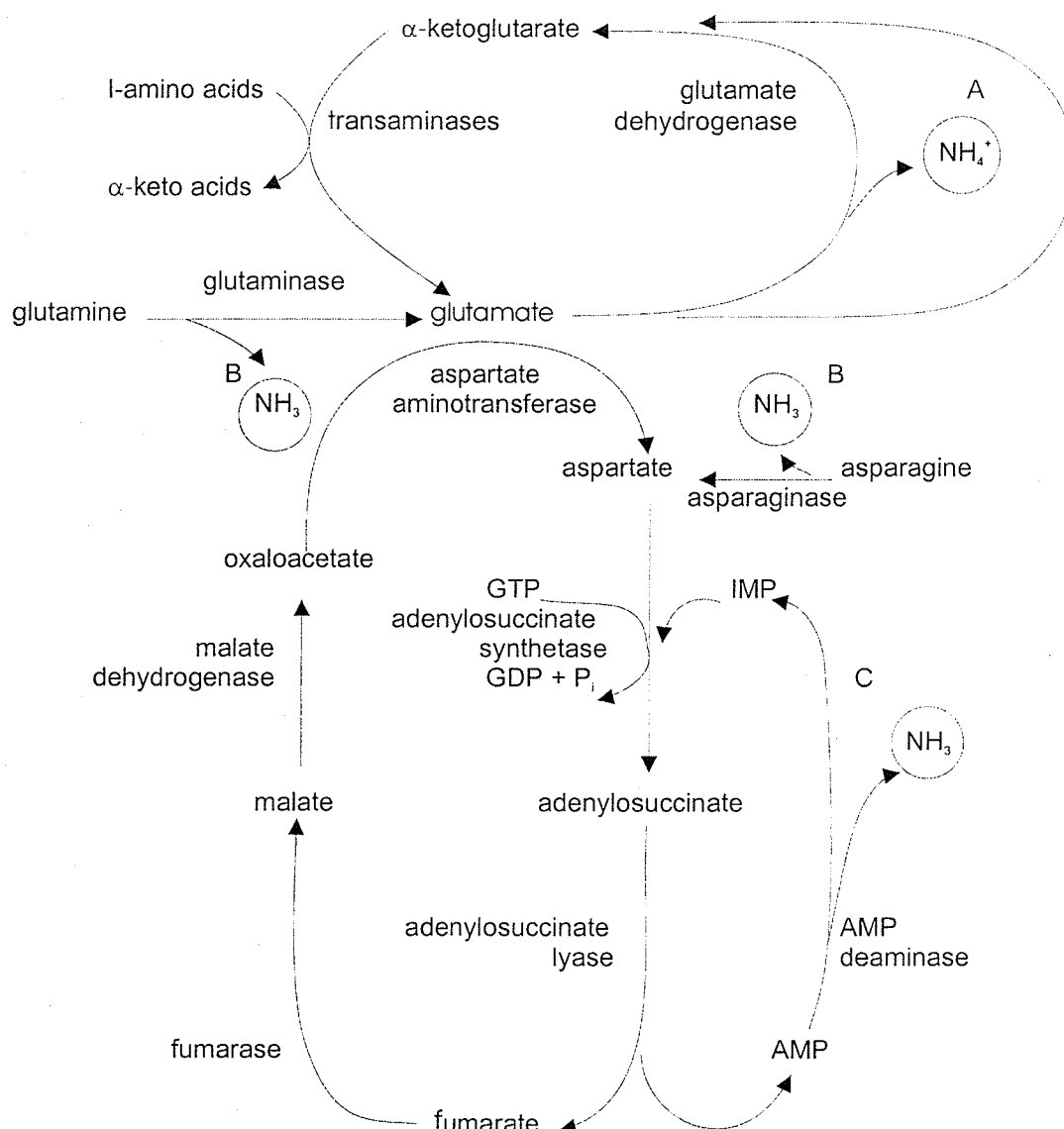


Figure 2-1. Biochemical pathways in fish for the production of ammonia from (A) l-amino acids (transdeamination), (B) amides (hydrolysis of amide groups), and (C) adenylates (purine nucleotide cycle), and their interrelationships (Wood 1993).

(reviewed by Wood 1993). Following exercise periods, AMP produced from ATP hydrolysis is scavenged and fed into one arm of the PNC thereby increasing the NH_3 load within muscle tissue (c.f., Figure 2-1). NH_3 helps restore low muscle-pH to resting levels by binding with free hydrogen ions (H^+) to form NH_4^+ (Walsh 1998). During burst exercise (i.e., anaerobic exercise) and under conditions of environmental hypoxia, adenylate deamination provides a significant contribution to total ammonia efflux from the body (reviewed by Wood 1993).

A third major pathway of ammonia generation is the deamination of glutamine and asparagine (i.e., amides) by glutaminase and asparaginase, respectively (Wood 1993, Walsh 1998). Glutaminase is present in muscle tissues and both enzymes occur in liver, kidney and gill tissues (reviewed by Wood 1993). NH_3 is released during amide hydrolysis (c.f., Figure 2-1) and may have a role in maintaining the acid/base balance in body tissues (Walsh 1998).

The dominant form of ammonia that is transferred between body tissues and blood plasma and the effects of physiological conditions on the distribution of internal ammonia are not known (Randall and Wright 1987, Walsh 1998). Some evidence suggests that NH_4^+ is the dominant species transported from muscle, but virtually nothing is known about ammonia transport from fish liver (reviewed in Walsh 1998).

At the pH of fish tissues, ammonia consists almost entirely of NH_4^+ as described by the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}_a + \log \left(\frac{[\text{base}]}{[\text{acid}]} \right) \text{ (Eq 4)}$$

Where:

$$\text{pK}_a = -\log_{10} K;$$

K = the dissociation constant; and

$$\text{pH} = -\log_{10} [\text{H}^+]$$

base = NH_3

acid = NH_4^+

Ammonia concentrations will be greatest in low pH compartments. When the pH of a body compartment drops, the concentration of NH_3 relative to NH_4^+ will be substantially lower therefore, NH_3 will influx into the compartment along its partial pressure gradient, bind with free H^+ ions and become trapped as NH_4^+ . If a membrane is semi-permeable, NH_4^+ will partition between compartments in proportion with H^+ concentrations to balance pH as the system approaches equilibrium. Under these conditions, the NH_4^+ flux is negligible compared to the NH_3 flux (reviewed by Randall and Wright 1987). Internal ammonia distributions between compartments can be described by the equation:

$$\frac{[\text{intracellular}]}{[\text{extracellular}]} = \frac{1 + 10^{(\text{pK}_a - \text{pH intracellular})}}{1 + 10^{(\text{pK}_a - \text{pH extracellular})}} \text{ (Eq 5)}$$

Due to low intracellular pHs relative to extracellular pHs, intracellular ammonia levels are greater. Literature reviewed by Randall and Wright (1987) found a pH-dependent relationship between blood plasma and erythrocyte ammonia concentrations; intracellular total ammonia concentrations were three times greater than plasma concentrations in the blood of a resting rainbow trout. A review by Wood (1993) also implicated membrane potential (i.e., the separation of charges across a membrane) as an important factor influencing NH_4^+ movements and, consequently, internal ammonia distribution.

During studies on ammonia distribution in lemon sole and rainbow trout (Wright et al. 1988a, 1988b and Wright and Wood 1988), Wright and coworkers found that pH gradients predominately influenced ammonia distribution across erythrocyte membranes in resting fish, but not under stressful conditions (e.g. exercise). They concluded that membrane potential was the primary factor governing ammonia distribution and proposed a model based on the dual permeability of NH_3 and NH_4^+ . This model suggests that NH_3 effluxes along (pH-dependent) partial pressure gradients would be balanced by NH_4^+ influxes along electrochemical gradients; a model that has both been supported and criticized by other researchers (reviewed by Wood 1993).

Significant quantities of ammonia are typically stored in the blood plasma of teleost fish at levels equaling 150 to 300 μM . However, some species are

capable of storing only trace amounts of ammonia while others (i.e., those capable of synthesizing urea) may have plasma concentrations as high as 6 mmol l⁻¹ (Walsh 1998). If internal ammonia concentrations accumulate to levels greater than basal storage-levels, ammonia can induce toxic effects. Therefore, ammonia must be excreted or converted to less toxic forms such as urea or glutamine to balance its production (Randall and Wright 1987).

Glutamine formation proceeds via the amidation of glutamate in the presence of glutamine synthetase. Glutamine synthetase is found throughout the body with higher activity levels in the brain where protection against ammonia toxicity is particularly important (reviewed by Wood 1993). Urea generation proceeds via uricolysis (i.e., purine degradation), the breakdown of arginine and the ornithine-urea cycle as described in Wood (1993). Uricolysis is the dominant urea-producing reaction in teleost fish (reviewed by Randall and Wright 1987).

At least 75% of excreted ammonia exits the body through the gills (Wood 1993), but smaller amounts may be eliminated through the kidneys (reviewed by Randall and Wright 1987) or the skin (Morii et al. 1978, Wood 1993). The mechanisms of branchial ammonia excretion are not fully understood but evidence supports three possible routes:

- (1) direct diffusion of NH₃ from blood to water (either through cells or between adjacent cells) (Wood 1993, Walsh 1998),

- (2) direct diffusion of NH_4^+ through paracellular channels (i.e., the spaces between adjacent cells) along its electrochemical gradient (Wood 1993, Walsh 1998),
- (3) carrier-mediated, active transport of NH_4^+ via $\text{Na}^+/\text{NH}_4^+$ or H^+/NH_4^+ exchangers located at apical membranes of gill cells; including Na^+/H^+ exchanger proteins and H^+ -ATPase/ Na^+ channel proteins (Randall and Wright 1987, Wood 1993, and Walsh 1998).

These pathways are illustrated in Figure 2-2.

In freshwater fish, NH_3 diffusion predominates, and is influenced by the water chemistry at the gill boundary layer. (NH_4^+ diffusion is only an effective means of eliminating ammonia from saltwater adapted fish since they have leakier junctions between adjacent gill-cells than freshwater fish). This boundary layer is typically acidic due to the exiting of CO_2 from blood plasma to the external environment and the subsequent hydration to HCO_3^- and H^+ . Consequently, NH_3 exiting the body across the gill surface is trapped in the boundary layer and hydrated to NH_4^+ to balance the pH. This conversion of ammonia to its ionized form maintains a relatively low external P_{NH_3} and an adequate diffusion gradient across the gill surface thus promoting NH_3 diffusion outward to the external environment (Randall and Wright 1987).

Excretion rates are variable depending on the state of the animal (e.g., resting versus active, fed versus starved), the species of the animal and environmental

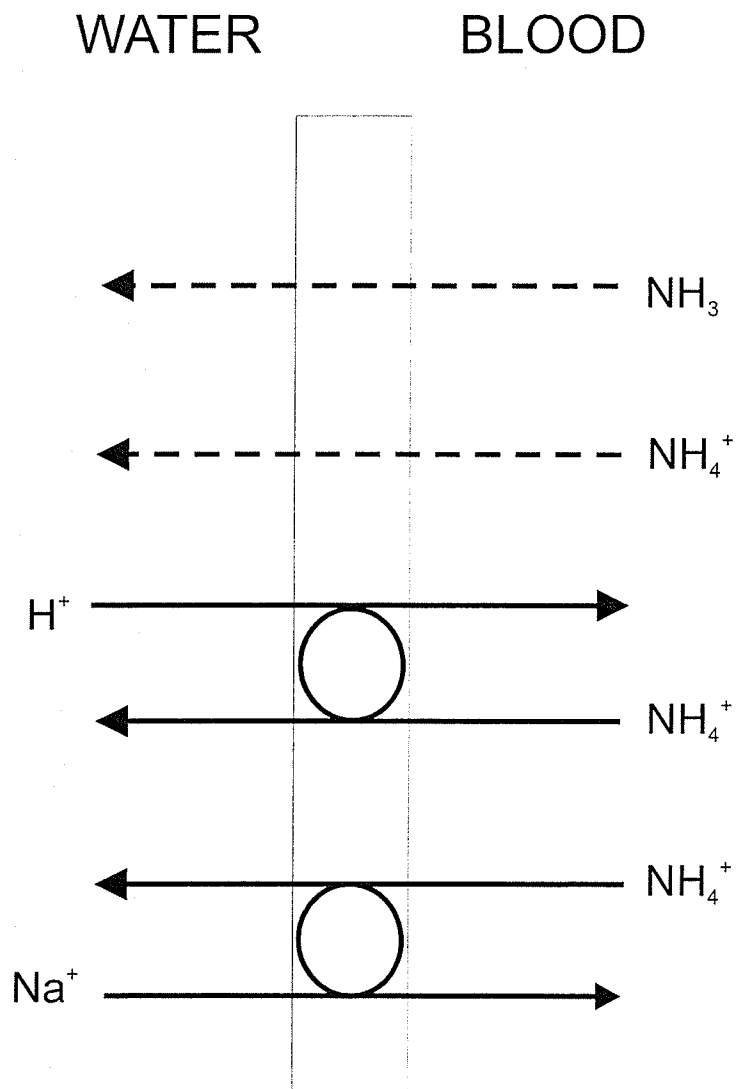


Figure 2-2. Summary of major pathways of NH_3 and NH_4^+ excretion across fish gill epithelium. Dashed arrows indicate diffusive pathways, solid arrows indicate carrier-mediated pathways (Walsh 1998, Wood 1993).

conditions (e.g., ambient water ammonia levels) (reviewed by Randall and Wright 1987). In general, ammonia is excreted in greater amounts in response to exercise, long-term acid exposure, hypercapnia (i.e., excess CO_2 in blood or other body tissues) and following food consumption. Elevated environmental ammonia and short-term exposure to acid or alkaline water decrease ammonia excretion rates (reviewed by Randall and Wright 1987). Changes in total ammonia production or body content in response to these variables may also influence excretion rates through physiological mechanisms that are not well understood (Randall and Wright 1987).

2.3 Mechanisms of ammonia toxicity in fish

Early research implicated NH_3 as the toxic form and NH_4^+ was considered to have little or no influence on ammonia toxicity (reviewed by Thurston et al. 1981b). However, more recent research suggests that although NH_4^+ may be inherently less toxic than its un-ionized counterpart, it is generally present in quantities great enough to contribute significantly to ammonia-toxicity (reviewed by US EPA 1999).

Because NH_3 is highly diffusive across gill membranes, internal and external ammonia concentrations equilibrate, and maintain equilibrium despite differences between ammonia tissue-concentrations and environmental-concentrations. Equilibrium is achieved through internal and external pH-effects on ammonia

speciation (Tomasso et al. 1980). Elevated environmental ammonia concentrations may reduce or reverse NH_3 -diffusion gradients from blood to ambient water causing total ammonia to accumulate in gill tissue and blood to potentially toxic levels (US EPA 1998). Furthermore, re-establishment of the NH_3 and NH_4^+ equilibrium within the fish will result in the conversion of some NH_3 to NH_4^+ , and additional NH_3 will diffuse inward. Therefore, small increases in environmental NH_3 levels may produce significant increases in internal total ammonia concentrations (Tomasso et al. 1980).

NH_4^+ has a lesser role in nitrogen excretion but several researchers have established mechanisms of NH_4^+ transport across gill epithelia in addition to limited diffusion (Randall and Wright 1987, Walsh 1998, Wood 1993; reviewed in Section 2.2 above). Increases in external NH_4^+ concentrations may hamper NH_4^+ excretion rates at the gill surface, as it does with NH_3 , thereby exacerbating the buildup of total ammonia tissue concentrations.

The primary toxic mechanism of ammonia has not been clearly demonstrated (Walsh 1998), but reductions in plasma sodium (Na^+) concentrations may play an important role by altering internal $\text{Na}^+/\text{NH}_4^+$ ratios (Tomasso et al. 1980). Elevated NH_4^+ concentrations in ambient water may restrict the operation of $\text{Na}^+/\text{NH}_4^+$ pumps in apical membranes of gill cells resulting in depleted internal Na^+ concentrations. Furthermore, because ammonia increases tissue permeability to water, additional Na^+ is lost in urine as urine output increases to

balance the water influx (reviewed by Tomasso et al. 1980). Na^+ has important functions in neuronal physiology, cardiac physiology, muscle physiology, and in the urinary system (Sherwood 1997).

Mechanisms of ammonia toxicity may parallel those experienced by mammals resulting in interference with brain metabolism, alterations in the morphology of brain and capillary cells, alterations in neurotransmitter levels, and alterations in electrophysical properties of neural tissue (reviewed by Walsh 1989). Some of these changes may occur due to reductions in phosphocreatine, glucose, glycogen, and ATP stocks in the brain brought about by interference with the Krebs Cycle. Elevated ammonia concentrations may reduce α -keto glutarate availability (an essential component of the Krebs Cycle) as it is coupled with ammonia to produce glutamine and lower tissue ammonia-concentrations (reviewed by Tomasso et al. 1980). In addition, NH_4^+ may directly inhibit ATP production by uncoupling phosphate groups from the rest of the molecule (reviewed by Tomasso et al. 1980). Most likely, several mechanisms act on the fish simultaneously and are governed by the concentration and species of ammonia present.

2.4 Sources of environmental ammonia

In 1996 and 1997, the total domestic demand for ammonia in Canada was approximately 3500 kt and an additional 1250 kt were exported. Applications of

ammonia are diverse and include: refrigeration, pulp and paper, mining, food processing and refining, fabrication of synthetic fibers, curing leather, manufacture of pharmaceuticals, vitamins, amino acids, lotions, cosmetics, cleansing agents, and detergents, production of explosives, rocket fuel, beer, plastics, and rubber; sugar purification, and the treatment and transformation of metals. Principally, ammonia is used in the production of nitrogenous fertilizers (i.e., ammonium nitrate, ammonium phosphate, and ammonium sulphate), but is also applied directly to agricultural fields in its anhydrous (i.e., dehydrated, crystallized) form. Ammonia is often incorporated in animal feed to increase its nutrient value (reviewed by CCME 2000).

Ammonia enters the environment via several anthropogenic and natural sources. Anthropogenic sources include sewage effluents, industrial wastes (from iron and steel mills, fertilizer plants, oil refineries and meat processing plants), coal gasification and liquefaction conversion process plants and agricultural discharges and runoff (Thurston et al. 1981b, CCME 2000). Natural sources include the decomposition of organic waste matter, atmospheric gas exchange, forest fires, wild and domestic animal waste, human and animal breath, and nitrogen fixation processes (i.e., the reduction of gaseous nitrogen to ammonia) (reviewed by CCME 2000).

Industrial emissions are significant point sources of ammonia as are the manufacturing of explosives and their subsequent use in mining and

construction. Sewage treatment plants are the largest non-industrial point sources of ammonia (reviewed by CCME 2000). Significant proportions also enter the Canadian environment via accidental anhydrous ammonia spills (Environment Canada 1999) and, consequently, ammonia has been included in the Environment Canada 1990 Canadian Chemical Spill Priority List (CCME 2000).

Four major non-point sources of ammonia are agricultural sources (e.g., areas of intensive farming and decomposition of livestock waste), residential and municipal sources (e.g., the use and disposal of ammonia-containing cleaning agents, and urban runoff) and atmospheric releases. All forms of mechanical transportation contribute to atmospheric ammonia concentrations as does the burning of municipal waste, emissions from sewage treatment plants, domestic heating, the decay of vegetation and the production and use of chemical fertilizers (reviewed by CCME 2000).

In 1996, 32 037 metric tonnes of ammonia were released into the Canadian Environment by industries reporting to the National Pollutant Release Inventory (NPRI); a value ranked second among all substances reported. 5 800 metric tonnes (i.e., 18%) were released to water. Municipal sewage treatment plants, transportation systems and animal husbandry systems were not required to report to the NPRI and consequently, their contributions are not included in the totals (NPRI 1996 cited in CCME 2000).

2.5 Environmental factors influencing ammonia toxicity

Temperature and pH are the two most important environmental factors influencing ammonia toxicity (US EPA 1985a). Although it is well-established that pH and temperature affect ammonia speciation with the fraction of NH_3 increasing with increasing pH and temperature (reviewed in Section 2.1), the effects of these two variables on ammonia toxicity are less well understood (US EPA 1999). In this thesis, literature pertaining to pH- and temperature-dependence of ammonia toxicity will be reviewed.

2.5.1 pH-dependence of ammonia toxicity

Research dating back to the 1930s identified a positive correlation between total ammonia toxicity to aquatic organisms and pH (reviewed by Erickson 1985, Russo 1985 and US EPA 1999; Broderius et al. 1985, Alexander 1986, Bergerhouse 1992). Because the fraction of total ammonia that exists in its un-ionized form increases with increasing pH, it was generally accepted that the toxic action of ammonia was due to NH_3 despite its small abundance compared with NH_4^+ (reviewed by Erickson 1985 and Russo 1985). Assuming that NH_3 is the primary source of toxicity in total ammonia with NH_4^+ being nontoxic or appreciably less toxic, one would expect lethal NH_3 concentrations for any given aquatic species to be reasonably constant regardless of the ambient pH and total

ammonia concentration. However, several investigations have found that the toxicity of NH_3 tends to increase as pH decreases (reviewed by Erickson 1985; Thurston et al. 1981b, Broderius et al. 1985 and Sheehan and Lewis 1986). Several explanations have been offered to qualify this relationship; none is definite.

Thurston et al. (1981b) suggested that NH_4^+ may contribute to the overall toxicity of total ammonia or that the toxicity of NH_3 may be heightened under conditions of more concentrated H^+ levels. The first of these two theories suggests that NH_3 and NH_4^+ are jointly toxic, a theory that was first modeled by Tabata (1962) and Armstrong et al. (1978) and later adopted by the US EPA (1985, 1998, 1999) as the predominate factor governing the pH-dependence of ammonia toxicity. The joint toxicity model described by the US EPA (1985, 1998, 1999) assumes that NH_4^+ is 100 times less toxic than NH_3 but suggests that at low pHs, NH_4^+ may be present in quantities great enough to contribute to the toxicity of total ammonia. At high pHs (i.e., $\text{pH} \geq 8.0$) the proportion of NH_3 is presumed to be great enough to dominate toxicity.

A review by Tomasso et al. (1980) suggested that elevated NH_4^+ concentrations might inhibit the $\text{Na}^+/\text{NH}_4^+$ pump at the gill surface and reduce Na^+ uptake. In addition, ammonia increases tissue permeability to water thereby stimulating urine production and excretion. Urinary losses of Na^+ coupled with a reduction of

Na^+ uptake may reduce plasma Na^+ concentrations to a level that contributes significantly to the toxicity of ammonia.

The theory [first proposed by Thurston et al. (1981b)] that the pH-dependence of ammonia toxicity is related to H^+ concentrations in the ambient water is supported by the work of Sheehan and Lewis (1986). Sheehan and Lewis (1986) exposed young channel catfish to two ammonia-salts [i.e., ammonium chloride, NH_4Cl and ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$] under several pH regimes. They found that the toxicity of NH_3 was significantly greater in NH_4Cl solutions than $(\text{NH}_4)_2\text{SO}_4$ solutions at each of four acidities (i.e., pH 6.0, 7.2, 8.0, and 8.8) and that toxicity decreased with increasing pH. They attributed osmotic concentration, rather than NH_4^+ concentration, as the variable governing pH-influences and ammonia salt-influences on ammonia toxicity. The rationale used to arrive at this conclusion was that as environmental osmotic concentrations increase due to reductions in pH or increases in ammonia salt concentrations (i.e., NH_4Cl , the more toxic ammonia salt has a higher osmotic concentration in solution than $(\text{NH}_4)_2\text{SO}_4$), hemoconcentration occurs, thereby concentrating blood ammonia levels and increasing the apparent toxicity of NH_3 . Since Sheehan and Lewis (1986) did not observe any changes in plasma osmolality or in the volume of individual erythrocytes, they deduced that hemoconcentration would result from isotonic dehydration. This theory is consistent with the work of Lloyd and Orr (1969) who found that elevated ammonia levels in ambient water increased urine flow in rainbow trout. Sheehan and Lewis (1986) concluded that

when osmotic effects are considered, the contribution of NH_4^+ to the toxicity of aqueous ammonia solutions is negligible or non-existent; the joint toxicity of NH_3 and osmotic concentration determine pH-effects on ammonia toxicity.

The US EPA (1999) reviewed the work done by Sheehan and Lewis (1986) and dismissed the implications of osmotic concentration on ammonia toxicity for several reasons, some of which are described here. First, Mount et al. (1997) reported inherently different toxicities of ammonia salts, therefore, the US EPA (1998, 1999) suggested that the greater toxicity of NH_4Cl than $(\text{NH}_4)_2\text{SO}_4$ observed by Sheehan and Lewis (1986) may have been due to factors other than osmotic effects. Second, the slope of the dose-response curve at low pH values determined by Sheehan and Lewis (1986) was inconsistent with that reported by Broderius et al. (1985) thereby discrediting interpretations of either dataset within that pH range. The US EPA (1998, 1999) cautioned that conclusions regarding the effects of NH_4^+ on ammonia toxicity for channel catfish be considered carefully since pH curves for this species are generally little affected by NH_4^+ .

Clearly, there is a general acceptance among researchers that the toxicity of NH_3 increases as pH decreases, however, the reason why this relationship exists has led to much disagreement and confusion.

2.5.2 Temperature-dependence of ammonia toxicity

Several researchers have evaluated the temperature-dependence of NH_3 toxicity and have consistently found a negative correlation between these two variables (Colt and Tchobanoglous 1976, Reinbold and Pescitelli, 1982, Thurston et al. 1983, Arthur et al. 1987, DeGraeve et al. 1987 and Nimmo et al. 1989). Although NH_3 concentrations (i.e., the most toxic species of total ammonia) are greater at warmer temperatures, these studies reflect a decrease rather than an increase in toxicity (Erickson 1985). When expressed in terms of total ammonia, temperature effects on acute and chronic ammonia toxicity are small in magnitude and variable (reviewed by the US EPA 1998, 1999). Only the temperature effects of NH_3 on ammonia toxicity will be discussed in this section.

Nimmo et al. (1989) evaluated the effects of NH_3 exposure on several species of fish (i.e., fathead minnows, johnny darters and white suckers) in each of laboratory (well) water, St. Vrain River water, and effluent from a water pollution control center under warm-water (i.e., 20°C) and cold-water (i.e., 6°C) conditions. All cold-water tests produced greater sensitivities to NH_3 for each species of fish tested suggesting that cold, low-flow conditions may be a critical period for warm-water fish.

Thurston et al. (1983) found that NH_3 toxicity to fathead minnows decreased as test-temperatures were warmed from 12°C to 22°C. Colt and Tchobanoglous

(1976) observed the same temperature-NH₃ toxicity relationship for channel catfish exposed to NH₃ in water temperatures ranging from 22°C to 30°C.

Arthur et al. (1987) tested five fish species' sensitivities to NH₃ (i.e., channel catfish, fathead minnow, white sucker, walleye, and rainbow trout) under ambient seasonal temperatures ranging from 3.5°C to 26.1°C. Although NH₃ toxicity to channel catfish, rainbow trout and white sucker tended to increase with decreasing temperature, Arthur et al. (1987) found no clear relationship between NH₃ toxicity and temperature for walleye and fathead minnow. They suggested that any apparent correlation between temperature and ammonia toxicity may be attributed to seasonal variations in water quality including dissolved oxygen and pH levels.

DeGraeve et al. (1987) evaluated the 96-hour acute- and 30-day chronic-effects of temperature on ammonia-toxicity for fathead minnow and channel catfish. Both fish species were nearly four times more sensitive to NH₃ at lower temperatures than at high temperatures (range: 6°C to 30°C) during the 96-hour tests. This relationship was upheld for the chronic-exposure tests conducted using fathead minnows, but not for those conducted with channel catfish. DeGraeve et al. (1987) offered two principle possible explanations for the inconsistency of the channel catfish data: (1) a temperature/NH₃ toxicity relationship may not exist for this species; and (2) the age and size of test

organisms varied considerably throughout the test-program and may have masked the effects of temperature.

To explain the relationship between temperature and ammonia toxicity, Erickson (1985) evaluated a joint toxicity model that assumed that both NH_3 and NH_4^+ contribute to the toxicity of total ammonia. Using rationale similar to that used to explain the pH-dependence of ammonia toxicity, the model suggested that although NH_4^+ is less toxic than NH_3 , it may be present at low temperatures in concentrations great enough to contribute significantly to the toxicity of total ammonia. Erickson (1985) concluded that joint toxicity could not explain temperature-dependent ammonia-toxicity because: (a) various parameters of the model suggested that the NH_4^+ is equally as toxic or more toxic than NH_3 , (b) the parameter used to describe the relative toxicity of NH_3 versus NH_4^+ (i.e., REL) was extremely variable, and (c) the REL fit well with pH-data and could not be used to describe ammonia dependence for both pH and temperature.

Temperature effects on various physiological processes including membrane permeability and endogenous ammonia production may influence ammonia-toxicity (US EPA 1999), however, with the present database, the mechanisms that account for this correlation remain unknown.

2.6 Acclimation effects on ammonia toxicity

Repeat exposure of fish to sub-lethal concentrations of ammonia may increase their tolerance to lethal levels via one of two mechanisms; a change in membrane permeability, or the detoxification of ammonia via its conversion to urea or glutamine (reviewed by Colt and Tchobanoglous 1976). Lloyd and Orr (1969) conducted a study to quantify the urine output of rainbow trout exposed to sub-lethal ammonia concentrations. During an unrelated study, they observed a diuretic response in rainbow trout following sub-lethal ammonia-exposure, and presumed that the increased urine flow was due to increases in fish-membrane permeability to water in the presence of the toxicant. In their subsequent study, Lloyd and Orr (1969) presented evidence that rainbow trout can acclimate to sub-lethal levels of environmental ammonia because repeat exposure reduces membrane permeability thereby enabling the fish to tolerate increased ammonia levels.

Olsen and Fromm (1971) found that goldfish (*Carassius auratus*) increased urea production and excretion in response to elevated ammonia exposure and maintained a normal rate of nitrogen excretion. Rainbow trout, however, were unable to increase urea excretion rates and experienced elevated blood ammonia levels consistent with those observed by Fromm and Gillette (1968). Only a handful of teleost fish are "ureogenic" and capable of *de novo* urea synthesis via the ornithine-urea cycle (O-UC) in the liver (reviewed by Walsh,

1998). Olsen and Fromm (1971) proposed that goldfish might generate urea from ammonia through purine synthesis and catabolism. This pathway requires several enzymes that are present in greater quantities following exposure to sub-lethal ammonia levels and may explain, in part, why some species are more resistant to ammonia than others.

Exposure of fish to fluctuating concentrations of ammonia at sub-lethal levels may also enable them to acclimate to environmental ammonia and tolerate higher levels than they would otherwise be able to (Thurston et al. 1981a). This concept will be evaluated in more detail below.

2.7 Pulsed-exposure effects on ammonia toxicity

Most laboratory experiments that have been conducted to evaluate the toxic effects of ammonia on fish exposed them to constant ammonia concentrations over short- and long-term periods (reviewed by Milne 2000). In nature, however, aquatic organisms are unlikely to receive constant ammonia exposure, in part due to changing environmental conditions that influence ammonia speciation and particularly in areas of intermittent pollution episodes. Pulsed-exposure effects are difficult to evaluate because the duration, frequency, and magnitude of the exposure all influence toxicity.

Thurston et al. (1981a) evaluated the responses of rainbow trout and cutthroat trout exposed to short-term cyclic fluctuations of ammonia. Several conclusions were derived by the authors: (1) fish can better tolerate a given average ammonia concentration when the concentration is fixed rather than fluctuating; (2) fish can tolerate ammonia levels above the 96-hour LC50 threshold if the toxicant-excursion is brief (e.g. a few hours) and the fish are able to recover in water containing ammonia below acutely toxic concentrations; and (3) fish exposed to short-term pulses of sub-lethal ammonia concentrations are better able to tolerate short-term pulses of otherwise lethal-ammonia concentrations.

Milne et al. (2000) performed laboratory experiments on rainbow trout and brown trout to determine the effects of ammonia-exposure duration and frequency. Rainbow trout were exposed to various NH_3 concentrations ranging between approximately 0.2 mg $\text{NH}_3\text{-N/L}$ and 1.30 mg $\text{NH}_3\text{-N/L}$ for 1-hour, 6-hour and 24-hour intervals. Survival rates were greater the shorter the exposure-period and Milne et al. (2000) suggested that during very short-term exposures, internal ammonia concentrations might not fully equilibrate with external concentrations thereby protecting the fish from toxic effects. Below concentrations of 0.5 mg $\text{NH}_3\text{-N/L}$, the fish were able to survive and recover from a 24-hour exposure, but at higher concentrations, fish only recovered when the stress-event was not longer than 1-hour. A model supported by the work of Milne et al. (2000) predicted that fish could recover from NH_3 concentrations below 0.5 mg $\text{NH}_3\text{-N/L}$ regardless of the duration of the pulse and full recovery was possible following

higher exposure-rates provided that fish were not exposed for longer than 200 minutes. This information suggests that 0.5 mg NH₃-N/L may be the critical concentration above which peak internal NH₃ concentrations may not be fully eliminated during subsequent recovery periods, provided that the stress event is sufficiently long (i.e., 200 minutes).

To determine the effects of exposure frequency on ammonia toxicity to brown trout, the fish were exposed to repeated sub-lethal ammonia pulses of different concentrations either once or three times weekly over a period of 53-days. Results of this effort indicated that brown trout can tolerate NH₃ concentrations up to 0.4 mg NH₃-N/L, and confirmed that, when exposed to lethal ammonia levels for short periods of time followed by compensatory respite periods, fish can withstand potentially lethal concentrations of ammonia (Milne et al. 2000). Although fish may survive short-term ammonia-exposure, repeated exposure may impair fish growth and induce histopathological effects. Milne et al. (2000) suggested that above the critical level of ammonia required to interfere with various physiological processes, the frequency of exposure might hamper growth rates to a greater extent than the actual concentration of ammonia producing the effect. Similarly, the severity of gill damage incurred by the brown trout seemed to be governed by the frequency of ammonia exposure rather than the ammonia-concentration. The work of Milne et al. (2000) and similar studies reviewed by Milne et al. (2000) have identified gill condition as a sensitive indicator of ammonia toxicity since some gill damage may be incurred and tolerated by fish

without significantly interfering with physiological processes affecting growth.

Finally, Milne et al. (2000) observed reductions in liver weights, possibly due to greater energy demands and uptake of stored liver-glycogen.

3.0 CRITERIA DEVELOPMENT APPROACH USED BY MANITOBA CONSERVATION FOR REGULATING AMMONIA IN THE AQUATIC ENVIRONMENT.

3.1 Overview

Manitoba Conservation recently published a technical draft of *Manitoba Water Quality Standards, Objectives and Guidelines* (MWQSOG) (February 1, 2001) for review and comment. When finalized, this document will supercede two previous publications that regulate pollutants in the aquatic environment, *Manitoba Surface Water Quality Objectives* (Williamson 1988) and *The Development and Use of Water Quality Objectives in Manitoba* (Williamson 1990).

Two principal management strategies employed by Manitoba Conservation for the protection, maintenance and rehabilitation of water quality in Manitoba are (a) a "technology-based" approach and (b) a "water quality-based" approach. The "technology-based" approach sets practical and economically achievable goals for pollution minimization for all activities and waste discharges affecting a water body. The "water quality-based" approach provides additional environmental limits when more stringent controls are required to protect important water uses. As discussed in Section 1.0, these water uses include domestic, industrial and agricultural consumption, recreational activities and the propagation and maintenance of aquatic life and wildlife (Williamson 1988). The water quality-based approach helps to protect these water uses by providing a fundamental

link between environmental regulatory activities, ambient water quality monitoring data, scientific toxicological information, stream characteristics, and public expectations concerning environmental quality. Using both the technology-based and water quality-based approaches, water quality standards, objectives and guidelines (described below) are determined by Manitoba Conservation as part of a three-tiered system aimed at protecting water quality in Manitoba.

Tier I - Water Quality Standards (WQS) offer guidance on acceptable levels of treatment that must be achieved by all dischargers in Manitoba using the technology-based approach. Tier I - WQS are consistent with standards developed by the Canadian Council of Ministers of the Environment (CCME) under the Canada-Wide Accord on Environmental Harmonization (Manitoba Conservation 2001). For municipal wastewater effluents, secondary treatment technologies must be employed to meet minimum standards for fecal coliform organisms, biochemical oxygen demand and total suspended sediments in effluent (Manitoba Conservation 2001). Ammonia, however, is regulated under Manitoba's Tier II - Water Quality Objectives (WQO) because it is a common pollutant in the province that is routinely controlled through a licencing process imposed under the Manitoba Environment Act (Manitoba Conservation 2001).

Tier II - WQO are derived using a water quality-based approach and offer a higher degree of protection to important ground or surface water uses than Tier I - WQS. These objectives integrate scientific information such as ecological,

toxicological, hydrological and water chemistry data with socially based information such as water uses and public expectations of environmental quality. Manitoba Conservation also supports the modification of existing objectives on a site-specific basis to better reflect the unique conditions of a particular area, provided that scientifically rigorous methods are followed (Manitoba Conservation 2001).

Tier III - Water Quality Guidelines (WQG) are broader in scope than either Standards or Objectives and are applied to a large number of variables across Canada, consistent with those recommended by other national efforts. For example, numerical environmental quality guidelines for water, lake and river bottom sediments, and residues in fish tissues or the tissues of other aquatic life are recommended by the CCME and incorporated in Manitoba's Tier III - WQG for the protection of wildlife consumers. Additional numerical guidelines recommended by Health Canada for the protection of human consumers of aquatic life are also included. Finally, narrative guidelines are described in Manitoba's Tier III - WQG since it is not practical to develop quantitative guidelines for every possible chemical, physical or biological variable. When appropriate, Tier III - WQG are elevated to Tier II - WQO to enable regulators to address a particular pollutant more specifically and offer more adequate protection to human and non-human life. Such is the case for ammonia (Manitoba Conservation 2001).

3.2 Implementation of Tier II - Water Quality Objectives

In Manitoba Conservation's draft *Manitoba Water Quality Standards, Objectives and Guidelines* document (2001) several variables were defined or discussed in order to ensure the appropriate implementation of Tier II - WQO. These variables include the level of protection necessary to maintain or improve the health of a particular water body, the acceptable frequency and duration of guideline exceedances, the water quality and flow characteristics of a particular water body, and the effects of mixing zones. Each of these variables is discussed below.

The level of protection applied to a water body in Manitoba depends on its water quality designation as defined by Manitoba Conservation. Most ground and surface waters are managed through "routine levels of protection" provided by Tier I – WQS and, when more restrictive requirements are necessary, Tier II – WQO. The Red and Assiniboine Rivers fall into this category. Routine levels of protection offer protection to 95% of genera represented in an aquatic system from a measurable effect at the ambient water quality criteria concentration. This principle was adopted from the US EPA and reflects the philosophy that a healthy aquatic community can be maintained if most of the genera are protected from unacceptable impacts most of the time. Higher levels of protection are provided under circumstances where an ecologically, recreationally, or commercially important species falls within the 5% margin of sensitive genera.

Additionally, endangered or rare species are given special consideration as are water bodies deemed High Quality or Exceptional Value (Manitoba Conservation 2001).

High Quality Waters or Exceptional Value Waters require subsequently higher levels of protection because they have biological, chemical and physical qualities that exceed the established standards, objectives and guidelines and because they have social or environmental characteristics that elevate their value. Presently, the Upper Burntwood, Upper Grass River, and Clearwater Lake watersheds are designated as High Quality Waters and receive a level of protection that ensures that all species will be protected in all places at all times. Exceptional Value Waters will receive a level of protection that will provide a near zero risk of unanticipated impacts and will be restricted from development opportunities. In Manitoba, there are currently no water bodies that have received this level of classification (Manitoba Conservation 2001).

Manitoba Conservation recognizes that natural characteristics of a water body may exceed certain water quality objectives and compromise the health of the aquatic community. In such cases, appropriate objectives may be unattainable and the natural conditions should not be regarded as violations of the regulatory guidance-regime. Under these circumstances, further impairments to the aquatic system due to anthropogenic activities should be restricted or prevented unless it

can be proven that the activity does not exacerbate the problem (Manitoba Conservation 2001).

An important consideration, particularly for provinces that see substantial fluctuations in water flows throughout the year as Manitoba does, is whether or not Tier II - WQO should be met continuously. Extreme low flow events are infrequent and criteria exceedances under such conditions have substantial cost-implications for pollution dischargers. Furthermore, healthy aquatic systems are capable of withstanding and recovering from some degrees of stress. Consequently, specific low flow levels have been selected and provide a lower limit below which compliance with Tier II – WQO is not required. For rivers and streams, one-hour average ammonia concentrations must not exceed the acute criterion more than once every three years. The four-day average and 30-day average ammonia concentrations also must not exceed the chronic criteria more than once every three years. The exceedance frequency employed by Manitoba Conservation is recommended by the US EPA (1985a, 1998, 1999) as the amount of time required by most aquatic communities to completely recover from a stress-event.

The minimum "design flow" below which Tier II – Water Quality Objectives do not apply incorporates two factors, the duration of the averaging period during which flow rates of a river or stream are measured and the frequency of an extreme low flow event. The design flow employed by Manitoba Conservation to prevent

unacceptable acute effects is the 1-Day, 3-Year Biological Flow or the 1Q10 Hydrological Flow. The 1-Day, 3-Year Biological Flow is an estimate of low flows that occur, on average once every three years based on 24-hour running harmonic average measurements of water flows (Rossman 1990). The 1Q10 Hydrological Flow is an estimate of minimum water flows that occur, on average, once every ten years. It is calculated using a three-step procedure described by Rossman (1990).

Similarly, the design flows employed by Manitoba Conservation to prevent unacceptable chronic effects are the 4-Day, 3-Year Biological Flow and the 30-Day, 3-Year Biological Flow which are equivalent to the 7Q10 Hydrological Flow, and the 30Q10 Hydrological Flow, respectively. If either the biological or hydrological method of calculating the minimum design flow yields a value of $0.003 \text{ m}^3/\text{s}$ or less, Manitoba Conservation provides guidance for the implementation of water quality criteria under the classification of "Intermittent Streams" (Manitoba Conservation 2001). Neither the Red River nor the Assiniboine River falls into this category.

Two final factors that influence the implementation of water quality criteria are the presence and characteristics of mixing zones. The influence of mixing zones on the distribution and dilution of pollutants in an aquatic system is beyond the scope of this report, but guidance for the minimization of mixing zone impacts is provided by Manitoba Conservation (2001).

3.3 Calculations of Manitoba Water Quality Objectives (WQO) for ammonia

Manitoba Conservation has adopted, without modification, the procedure developed and advanced by the US EPA (1999) for calculating acute (i.e. maximum) and chronic (i.e., continuous) criteria for ammonia. This section and Sections 3.3.1 and 3.3.2 will describe the criteria-development procedure utilized by the US EPA (1999); application to Manitoba's WQO will be discussed in Section 3.4.

Acute criteria were developed by the US EPA (1999) using LC50 values generated for 48 species representing 34 genera whereas chronic criteria were derived from EC20 values generated for 12 species of 9 genera. LC50 values are the concentrations of ammonia that are lethal to 50% of a test-population over a specific time interval (e.g., 96-hours). EC20 values are the concentrations of ammonia required to produce a biological response, other than mortality, in 20% of the test-organisms over a specific time interval (e.g., 96-hours). EC20 values used by the US EPA (1999) were based on a variety of endpoints including: (a) survival, embryo production, and embryo hatchability for invertebrates and aquatic insects subjected to life-cycle and partial life-cycle tests and, (b) embryo hatchability, fry survival and fry growth for early life stage (ELS) tests conducted on fish. If reductions in both survival and growth were observed

in a particular test, biomass (the product of these two variables) was used for analysis (US EPA 1999).

To develop acute or chronic criteria, the US EPA (1999) first converted all endpoint values to total ammonia nitrogen values using test-temperatures, test-pHs and Equations 1 and 2 (Section 2.1; US EPA 1999). Next, the endpoint values were standardized to a pH of 8.0 using the equations:

$$AV_t = (AV_{t,8}) (0.0489 / (1+10^{7.204-pH}) + 6.95 / (1+10^{pH-7.204})) \text{ (Eq 6);}$$

$$CV_t = (CV_{t,8}) (0.0676 / (1+10^{7.688-pH}) + 2.91 / (1+10^{pH-7.688})) \text{ (Eq 7).}$$

Where:

AV_t = the acute value expressed in terms of total ammonia nitrogen at a particular temperature, t, and a pH of 8.0.

CV_t = the chronic value expressed in terms of total ammonia nitrogen at a particular temperature, t, and a pH of 8.0.

Invertebrate data used to derive chronic criteria were also standardized to a temperature of 25°C using the following equation:

$$\log(LC50_T) = \log(LC50_{25}) + S \cdot (T-25) \text{ (Eq 8)}$$

Where:

$LC50_T$ = the total ammonia LC50 at temperature T,

$LC50_{25}$ = the estimated total ammonia LC50 at 25°C, and

S = the slope of log LC50 versus temperature. (The US EPA (1999) used a slope of -0.028 determined with regression analysis of several invertebrate data.)

No temperature adjustments were made for fish data used in developing acute or chronic criteria because available data reviewed by the US EPA (1999) suggested that ammonia toxicity to fish is minimally dependent on temperature.

3.3.1 Formulation of the acute criterion by the US EPA (1999)

The protocol followed by the US EPA (1999) to obtain the acute criterion or the Criterion Maximum Concentration (CMC) is outlined below:

- a) Calculate the Species Mean Acute Values (SMAV) from the pH-adjusted total ammonia nitrogen values using the geometric mean of all LC50s reported for each species tested.
- b) Calculate the Genus Mean Acute Values (GMAV) using the geometric means of all SMAVs within each genus. For steps (a) and (b), geometric means are used rather than arithmetic means because the sensitivities to a toxicant of individual organisms within a species and of

species within a genus are more likely to follow a log-normal distribution pattern than a normal distribution pattern (US EPA 1994).

- c) Rank all GMAVs in order from most sensitive (i.e., Rank = 1) to least sensitive (i.e., Rank = n, the number of genera in the list).
- d) Calculate, using regression analysis of the lowest four GMAVs (i.e., Ranks 1-4), the 5th percentile of genera most sensitive to ammonia or the Final Acute Value (FAV).
- e) Compare the FAV with the lowest SMAV and if the SMAV is the lesser of the two values, use it in place of the FAV for step (f).
- f) Divide the FAV (or lowest SMAV) by two (no rationale given by US EPA (1999) regarding magnitude of this safety factor) to obtain the Criterion Maximum Concentration (CMC).

Using this method, a CMC can be calculated for waters having a pH of 8.0. By factoring the CMC into Equation 6 (i.e., the pH-adjusted equation for acute values), criteria objectives can be fit to all pHs between the values of 6.0 and 9.0.

For example:

The US EPA (1999) calculated a FAV of 14.32 mg total ammonia-N/L, a value greater than the lowest SMAV for rainbow trout of 11.23 mg total ammonia-N/L. Consequently, the FAV was lowered to 11.23 mg total ammonia-N/L and the CMC was calculated to be 5.615 mg total ammonia-

N/L. Substitution of the CMC into Equation 6 to express the CMC as a function of pH resulted in the following criteria-developing equation:

$$\text{CMC} = 0.275 / (1 + 10^{7.204-\text{pH}}) + 39.0 / (1 + 10^{\text{pH}-7.204}) \text{ (Eq 9)}$$

The US EPA (1999) also calculated the CMC excluding data from the family Salmonidae because this family consists of fish species that are highly sensitive to ammonia and are typically present in cold water systems. Excluding salmonids, the US EPA-derived CMC was 8.4 mg/L. Substitution of the CMC into Equation 6 to express the CMC as a function of pH resulted in the following criteria-developing equation for cold water fisheries:

$$\text{CMC} = 0.411 / (1 + 10^{7.204-\text{pH}}) + 58.4 / (1 + 10^{\text{pH}-7.204}) \text{ (Eq 10)}$$

3.3.2 Formulation of the chronic criterion by the US EPA (1999)

The protocol followed by the US EPA (1999) to calculate the chronic criterion was first developed for a temperature of 25°C and a pH of 8.0 and later factored into general criteria-developing equations (defined below) for the full range of temperature and pH conditions common to aquatic systems.

To begin, the US EPA (1999) pooled and ranked pH- and temperature-adjusted Genus Mean Chronic Values (GMCVs) representing EC20 endpoints. (Species

Mean Chronic Values (SMCV) and GMCVs were determined using geometric means as described in Section 3.3.1 for developing acute criteria). The Continuous Chronic Criterion (CCC) for the 5th percentile of sensitive genera was determined via regression analysis of the four lowest GMCVs. At a temperature of 25°C and a pH of 8.0, this value was 1.24 mg total ammonia-N/L. Because the chronic dataset was small (N=10), the CCC was determined via extrapolation below the lowest GMCV (i.e., 1.45 mg total ammonia-N/L for *Hyaella* at 25°C). For datasets containing fewer genera than 20, the most sensitive genus represents more than 5% of the aquatic community and the degree of extrapolation below the lowest GMCV depends on the slope of the regression line. The CCC of 1.24 mg total ammonia-N/L was 15.6 percent lower than the lowest GMCV of 1.45 mg total ammonia-N/L; a degree of extrapolation considered modest and reasonable by the US EPA (1999) given the low number of tested genera.

The US EPA (1999) defined the CCC for the full range of pH and temperature conditions as a value that is 85.4 percent of either the temperature-adjusted GMCV for *Hyaella* (i.e., $1.45 * 10^{0.028(25-\text{Temperature})}$) or the lowest fish GMCV (i.e., 2.85 for the genus *Lepomis*). Two equations were developed depending on the presence or absence of ELS fish because ammonia toxicity to fish increases with decreasing temperature, but the most sensitive life stages of fish (i.e., ELS) are typically absent during cold-water seasons. When ELS fish are present, chronic criteria can be developed using the following equation:

$$CCC = ([0.0577 / (1 + 10^{7.688-pH})] + [2.487 / (1 + 10^{pH-7.688})]) * a \quad (\text{Eq 11})$$

Where: $a = 2.85$ or

$$= 1.45 * 10^{0.028(25-\text{Temperature})}$$

whichever is less.

When ELS fish are absent:

$$CCC = ([0.0577 / (1 + 10^{7.688-pH})] + [2.487 / (1 + 10^{pH-7.688})]) * b \quad (\text{Eq 12})$$

Where: $b = 1.45 * 10^{0.028(25-c)}$

and $c = \text{maximum temperature or } 7^{\circ}\text{C}$, whichever is greater.

When ELS fish are present, the GMCV of 2.85 for *Lepomis* drives the CCC unless the temperature adjusted GMCV for *Hyaella* is lower than 2.85 (c.f., Equation 11). The critical temperature at which this occurs is 14.5°C , above which the CCC is driven by the sensitivity of *Hyaella* to ammonia. At a pH of 8.0, the minimum and maximum CCC ranges between 1.24 mg total ammonia-N/L (temp. = 25°C) to 2.43 mg total ammonia-N/L (temp. < 14.5°C).

When ELS fish are absent, the CCC is based exclusively on the sensitivity of *Hyaella* to ammonia regardless of temperature. Using Equation 12, the CCC

increases as temperature decreases to a minimum of 7°C. Below this value, the temperature-dependence to ammonia toxicity plateaus thereby establishing the upper CCC limit at 7°C. At a pH of 8.0, this upper limit equals 3.95 mg total ammonia-N/L, a value 1.6 times greater than the upper limit determined using Equation 11. Therefore, in the absence of ELS fish, the CCC has the potential to yield a greater value (i.e., less restrictive criteria) than if ELS fish were present. These adjustments are especially important to ammonia dischargers that treat effluent with biological agents because cold water temperatures reduce the efficiency of this process making it difficult for dischargers to meet criteria objectives during the winter months (US EPA 1999).

3.4 Application of the Criterion Maximum Concentration (CMC) and the Continuous Chronic Criterion (CCC) within the framework provided by Manitoba Conservation

Manitoba Conservation's Provincial Water Quality Objectives (WQO) are based on the CMC and CCC equations described above and published in the *US EPA 1999 Update of Ambient Water Quality Criteria for Ammonia*. The criteria-developing equations appropriate for the protection of cool water aquatic life and wildlife (i.e., the most sensitive water use classification for the Winnipeg reaches of the Red and Assiniboine Rivers) are provided in Appendix A. These equations will be referred to in this thesis using the designation given by Manitoba

Conservation followed by the '*' symbol. For example, 'Eq. 1' denoted by Manitoba Conservation will be referred to here as Equation 1*.

Equation 1* (equivalent to Equation 11 in Section 3.3.2) is used to determine chronic WQO when ELS fish are present or when surface water temperatures are greater than 5°C. Average 30-day water quality measurements for ammonia must not exceed the WQO determined with Equation 1* more than once every three years, on average, for the continued protection of aquatic organisms and the water quality uses of a particular system. In addition, the highest 4-day average ammonia concentrations within the 30-day period should be less than 2.5 times the WQO, or should not exceed the WQO determined with Equation 2*. (Equation 2* is simply Equation 1* times 2.5). Equations 1* and 2* are appropriate for surface waters having pHs greater than or equal to 6.5 and less than or equal to 9.0 (Manitoba Conservation 2001).

Equation 4* (equivalent to Equation 12 in Section 3.3.2) is used to determine chronic WQO when ELS fish are absent or when surface water temperatures are less than or equal to 5°C. Average 30-day water quality measurements for ammonia must not exceed the WQO determined with Equation 4* more than once every three years, on average, for the continued protection of aquatic organisms and the water quality uses of a particular system. In addition, the highest 4-day average ammonia concentrations within the 30-day period should be less than 2.5 times the WQO, or should not exceed the WQO determined with

Equation 5*. (Equation 5* is simply Equation 4* times 2.5). Equations 4* and 5* are appropriate for surface waters having pHs greater than or equal to 6.5 and less than or equal to 9.0 (Manitoba Conservation 2001).

Equations 3* and 6* (equivalent to Equation 10 in Section 3.3.1) are identical and apply to all periods regardless of water temperature and the presence or absence of ELS fish. Total ammonia-N concentrations averaged over one-hour intervals should not exceed the recommended acute criterion more than once every three years (Manitoba Conservation 2001).

4.0 MATERIALS AND METHODS

4.1 Study area

The Assiniboine-Red drainage basin occupies an area exceeding 270,000 km² (MacLaren 1986) and drains the prairie regions of southern Manitoba, southeastern Saskatchewan, North Dakota, and northwestern Minnesota (TetrES 2001). The study area comprises a small fraction of the total Assiniboine-Red drainage area and is located in southern Manitoba within margins surrounding the City of Winnipeg as shown in Figure 4-1. The study area extends north along the Red River from St. Adolphe to Selkirk and east along the Assiniboine from Headingly to the point where it merges with the Red River. The main tributaries in the study area are La Salle River, Cook's Creek, and Sturgeon Creek (c.f., Figure 4-1).

River flows are dominated by spring runoff generated by snowmelt and spring rains, and decrease steadily throughout the summer. Minimum monthly flow rates often occur during January or February. Annual flow rates average 162 m³/s upstream of Winnipeg and 225 m³/s downstream, with the flow-contribution from the Assiniboine River accounting for the increase in downstream flow rates (TetrES 2001). Over 15 control structures including the Shellmouth Dam and Portage Diversion on the Assiniboine River and Winnipeg Floodway and St. Andrews Lock on the Red River regulate river flows and levels throughout the drainage basin (Wardrop/TetrES 1991).

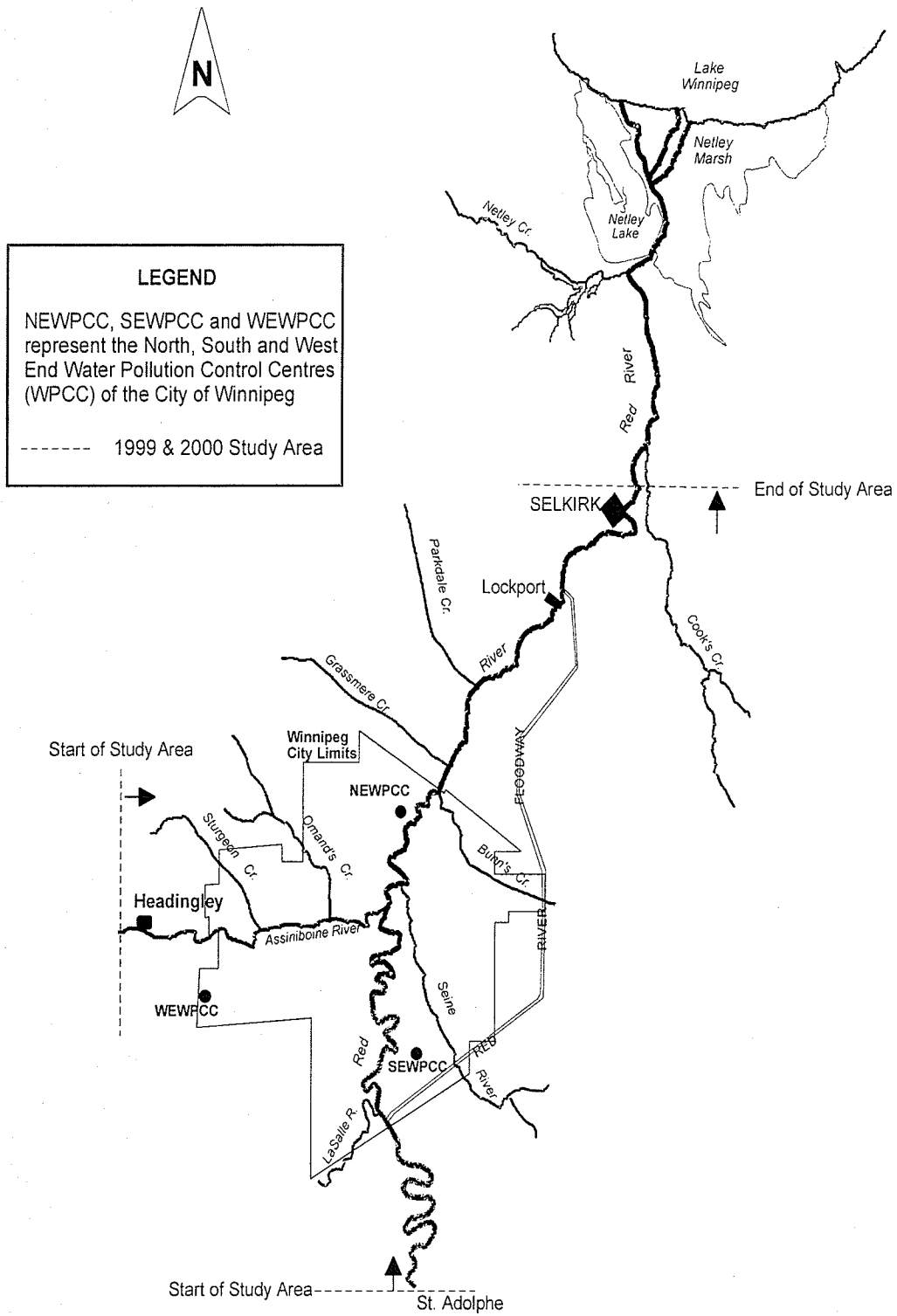


Figure 4-1. Study area (TetrES 2001).

Since 1977, the City of Winnipeg has collected biweekly, year-round water samples (conditions permitting) at several sampling stations throughout the study area (Figure 4-2) in an effort to monitor water quality characteristics for the Red and Assiniboine Rivers. Minimum and maximum monthly averages for key parameters relating to ammonia toxicity were calculated by TetrES Consultants (2001) using the long-term record for 1977 to 1997 and are presented in this thesis in Table 4-1.

River water pH values typically range between 7.8 and 8.4 (c.f., Table 4-1), with fluctuations attributed primarily to algae activity or the impacts of runoff (TetrES 2001). Photosynthetic activity of algae consumes dissolved CO₂ in the water column thereby increasing the overall river water pH. Temperatures vary widely throughout the year obtaining average minimum values of 1°C and average maximum values of 23°C (c.f., Table 4-1). Sufficient dissolved oxygen concentrations for the support of aquatic life have been maintained in the rivers, even under conditions of low flows and high temperatures and are typically near or above 8.05 mg/L year-round (TetrES 2001). Total suspended solids in the study area are typically high (up to 243 mg/L, c.f., Table 4-1) due to the underlying clay within the streambeds. Runoff from rural and urban land also contributes significantly to the sediment load and consequently suspended solid concentrations are often highest in April (following spring runoff or flooding) and decrease throughout the summer and into the fall (TetrES 2001). Average

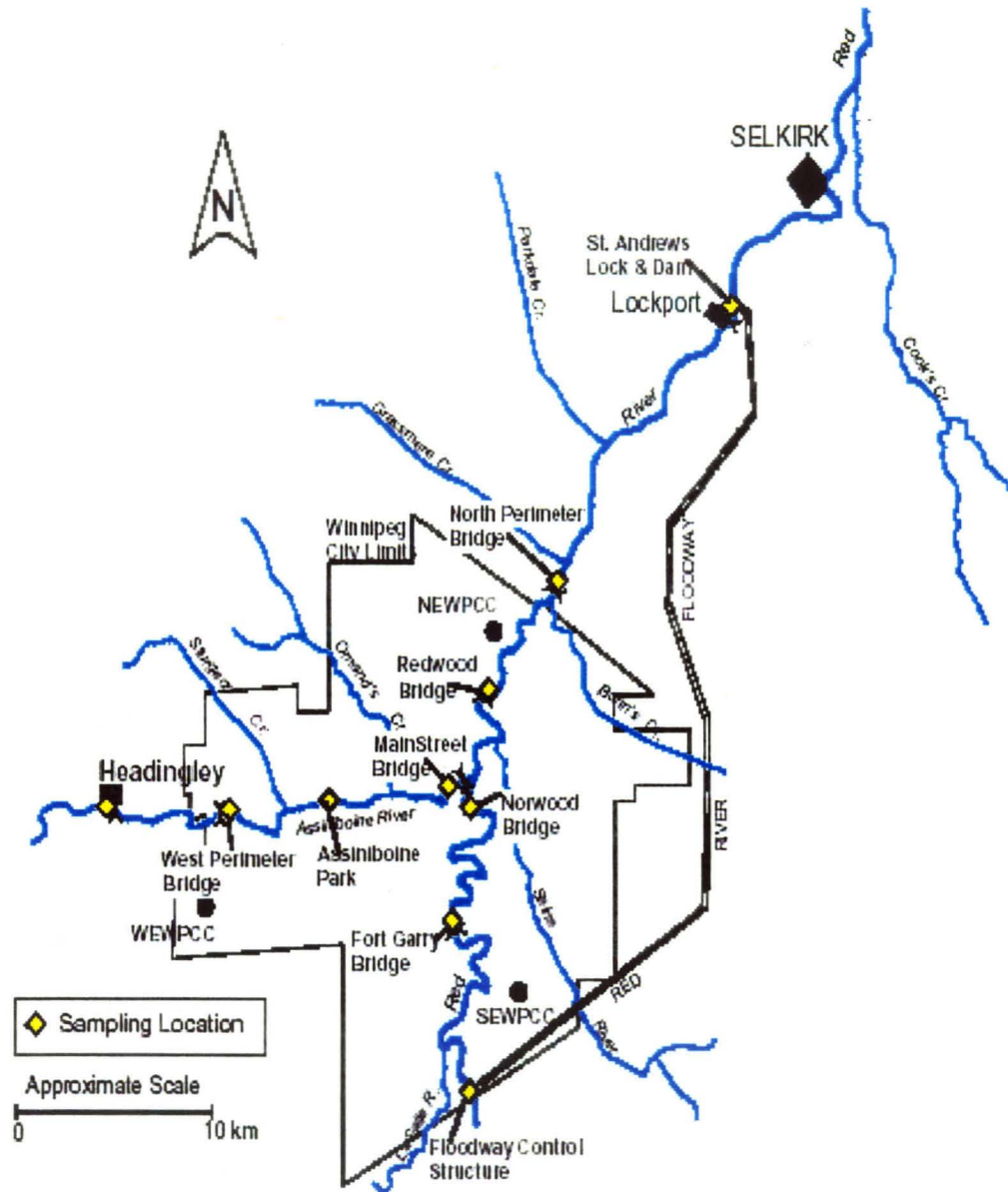


Figure 4-2. Locations of the City of Winnipeg's sampling stations for water chemistry analysis (TetrES 2001).

Table 4-1. Long-term water quality parameters from the City of Winnipeg's database (TetrES 2001).

Parameter	Range of long-term monthly averages ^a	
	1977-1997	
	Minimum	Maximum
Temperature (°C)	1.3	22.8
Dissolved Oxygen (mg/L)	7.5	12.0
Total Organic Carbon (mg/L)	14.4	17.9
pH	7.77	8.36
Suspended Solids (mg/L)	21	243
Turbidity (N.T.U.) ^b	12	75
Total Phosphorus (mg/L)	0.24	0.44
Total Kjeldahl Nitrogen (mg/L)	1.65	2.21
Total Ammonia (mg/L)	0.16	0.80
Nitrate and Nitrite (mg/L)	0.15	0.90
Chlorophyll_a (µg/L)	10	40

Notes:

- a Water samples were collected biweekly, year-round (conditions permitting) from several sampling sites located within the study area (Figure 4-2).
- b Nephelometry = light scattering by suspended particles; measured by means of a turbidity meter giving nephelometric turbidity units (N.T.U.).

ammonia concentrations also show a high degree of variance with lower monthly averages of 0.16 mg/L and upper monthly averages of 0.80 mg/L (c.f., Table 4-1). The highest concentrations are typically measured in late fall and winter when river water flows are low (TetrES 2001).

The Red and Assiniboine Rivers receive treated effluents from three city-owned and operated WPCCs shown in Figure 4-1. The largest of the three facilities is the North End WPCC (NEWPCC), followed by the South End WPCC (SEWPCC) and the West End WPCC (WEWPCC). These treat approximately 70%, 20% and 10% of the wastewater generated by the City of Winnipeg, respectively. All three facilities provide primary and secondary treatment to the wastewater. Effluent quality (as measured through a variety of parameters such as pH, suspended solids, grease, ammonia, etc.) varies throughout the year and from one year to the next. Average total ammonia-nitrogen concentrations in treated effluent vary between treatment facilities with the lowest values realized at the WEWPCC (i.e., 6.2 mg/L), followed by the SEWPCC (i.e., 17.4 mg/L) and the NEWPCC (i.e., 19.0 mg/L) (TetrES 2001).

4.2 Fish species selection

Test-species were selected on the basis of residency in the Red and Assiniboine Rivers, their importance economically (i.e., as sport species) or as key members of the aquatic community, and their availability. As much as possible, test-organisms representative of early life-stages were used to develop a data matrix

for criteria development that would be protective of fish in their most sensitive life stages. Five species used during toxicity testing that met the selection criteria include walleye (*Stizostedion vitreum*), white sucker (*Catostomus commersoni*), fathead minnow (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), and northern pike (*Esox lucius*).

Approximately 50 fish species inhabit the waters of the Red and Assiniboine rivers; channel catfish, walleye, sauger, northern pike, goldeye, mooneye, freshwater drum and yellow perch have been identified as key sport species (TetrES 2000). Based on a 1999 angler survey conducted by TetrES (2000), results indicate that the majority of anglers do not target a particular species of fish. However, for those who do, walleye and channel catfish are the preferred species. This result was consistent with results from on-site angler surveys completed by Kitch (1994). Among the key forage species resident to the Red and Assiniboine rivers are various minnows and shiners as well as young channel catfish, bullheads, carp, and white sucker (TetrES 2000). All five of the species tested in the present study represent either a key sport or forage species (or both). In addition, the fathead minnow is recognized as a standard test-species by Environment Canada (1992) and provided valuable data regarding the quality of test-conditions relative to similar studies reported in the public literature.

4.3 Fish collection/sources and holding conditions

Test-fish were captured locally or obtained from a Provincial hatchery in Saskatchewan or commercial hatcheries in Canada or the United States (Table 4-2). Collection and Handling Permits obtained from Manitoba Conservation (formerly Manitoba Department of Natural Resources) are provided in Appendix B.

All fish obtained from hatcheries were transported in oxygen-saturated, ice-chilled carbuoys to: (a) on-site continuous-flow holding tanks receiving Red River water (i.e., Tests 6, 8, 9, 10), (b) off-site continuous-flow holding-tanks receiving La Salle River water (i.e., Test 1), or (c) on-site static, aerated holding tanks containing Red River water (i.e., Test 5) (c.f., Table 4-2). Acclimation of all test fish to the holding conditions followed guidance provided by the American Society for Testing and Materials (ASTM), designation E 729-88a (ASTM 1996). Specifically:

- The transport-water was slowly replaced with increasingly greater volumes of holding-water over a 6 to 12 hour period.
- Fish were not subjected to rapid changes in water temperature (i.e., $<3^{\circ}\text{C}$ per 12-hour period).
- Dissolved oxygen concentrations were maintained between 60% and 100% saturation in all holding tanks except those containing locally collected fathead minnows (discussed below).

Table 4-2. Holding conditions of fish prior to test-start.

Test no.	Species	Age (BOT ^a)	Source/ Collection Site	Date Acquired	Test start	Holding time	Holding water	Holding condition
1	Walleye	8 days	SERM ^p Fish Culture Station (Lake Diefenbaker, SK)	May 31, 1999	June 5, 1999	5 days	La Salle River	Flow-through
2	White sucker	18 days	Cedar Lake (Grand Rapids, MB)	May 17, 1999 (hatched ~8 days later)	June 5, 1999	11 days	La Salle River	Flow-through
3A&B	White sucker	24 days	Cedar Lake (Grand Rapids, MB)	May 17, 1999 (hatched ~8 days later)	June 10, 1999	16 days	La Salle River	Flow-through
4A&B	White sucker	29 days	Cedar Lake (Grand Rapids, MB)	May 17, 1999 (hatched ~8 days later)	June 15, 1999	21 days	La Salle River	Flow-through
5A&B	Fathead minnow	3 days	Aquatic Biosystems Inc. (CO, USA)	August 12, 1999	August 13, 1999	1 day	Red River	Static
6	Channel catfish	117 days	Aquatic Resource Organisms (NH, USA)	August 13 and 14, 1999 ^c	October 23, 1999	9-10 days	Red River	Flow-through
7	Fathead minnow	~90 days ^d	Local drainage pond ^e (MB)	August and September, 1999	October 23, 1999	23 days (min)	La Salle River/ Red River	Flow-through

Test no.	Species	Age (BOT ^a)	Source/ Collection Site	Date Acquired	Test start	Holding time	Holding water	Holding condition
8	Fathead minnow	35-45 days	Aquatic Resources (CA, USA)	Oct. 21, 1999	October 27, 1999	6 days	Red River	Flow-through
9	Northern pike	10 days	SERM Fish Culture Station (Lake Diefenbaker, SK)	May 20, 2000	May 24, 2000	4 days	Red River	Flow-through
10	Walleye	39 days	Leonard's Walleye Hatchery (ON)	June 5, 2000	June 8, 2000	3 days	Red River	Flow-through

Notes:

a BOT = beginning of test

b SERM = Saskatchewan Environment and Resource Management

c fish were sent in two shipments, one day apart

d exact age of fish unknown

e Site of local drainage pond – within city limits of Winnipeg; south east corner of Kenaston at Scurfield

- Un-ionized ammonia concentrations were maintained at low levels (i.e., $<35 \mu\text{g/L}$).
- Fish were handled as little as possible.
- Fish were observed for signs of stress such as physical damage to external body structures, mortality, disease, external parasites, erratic swimming behaviour, lack of or loss of appetite, gasping at the surface, and abnormal colouring.

Fathead minnows used in Test 7 were captured in set cages in a local drainage pond within Winnipeg city-limits. The cages were emptied at least twice per week and captured fish (including minnows or other species) were held in the flow-through facility receiving La Salle River water. The fish were later transported to the laboratory where they were sorted by species and held for a minimum of nine days in Red River water. Some difficulties were encountered while holding these fish in the laboratory and on several occasions, river water flows to the holding tanks became temporarily blocked with debris resulting in a reduction in DO concentrations to lethal levels (i.e., $<2 \text{ mg/L}$). Once flows were restored to the holding tanks, the fish were left, undisturbed, for a recovery period of approximately one to two hours after which time all dead fish were removed and discarded. Surviving fish were closely monitored for several days prior to testing; the majority appeared to make a full recovery. Surviving fish were

deemed acceptable for testing fish are able to tolerate low DO levels for brief periods of time (US EPA 1999).

White sucker used in Tests 2, 3, and 4, were reared from locally collected eggs held in a flow-through system receiving La Salle River water for an incubation period of approximately eight days. The procedure used to obtain fertilized fish eggs is described in Appendix C. The newly hatched fish were held in La Salle water for a minimum of 11 days before they were transported to the laboratory for testing. Since the hatching success was high (i.e., estimated 99%), three consecutive tests were completed with different batches of fish from the same stock. Consequently, the holding times of the white sucker fry increased between tests from 11 to 21 days and the age of the organisms increased from 18 to 29 days, as indicated in Table 4-2.

All fish were held for a minimum of 24 hours prior to test-initiation and, with the exception of those used in Tests 5A and 5B, fish were held for at least 72 hours (c.f., Table 4-2). ASTM (1996) recommends, in standard guide E 729-88a, that test organisms be held in the dilution water at the test temperature for at least 48 hours before introducing them to the test system in order to allow them to fully acclimate to the test conditions. Due to time constraints for laboratory availability, fish used in Tests 5A and 5B were not held for the recommended 48 hours prior to testing.



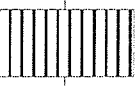



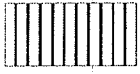

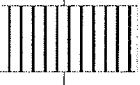




4.4 Overview of toxicity-testing program

Static, semi-static and flow-through tests ranging in duration from 72-hours to 30-days were conducted over two time intervals - May 1999 to November 1999 and May 2000 to July 2000 (Figure 4-3). For each test, at least 150 fish were exposed to a minimum of five NH_3 concentrations in ambient water according to US EPA (1993) and/or ASTM (1996) standards methods. Test-fish were monitored for survival and/or growth effects. A summary of the test-conditions is provided in Appendix D.

All tests were conducted at the NEWPCC in Winnipeg in temporary laboratories designed and constructed specifically for the Ammonia-Criteria Study.

Reagent grade ammonium chloride (NH_4Cl) (purity = 99.5%) was the sole source of ammonia used in all but three tests. During the first test-season, three pairs of semi-static tests were run in parallel; one used NH_4Cl only as a NH_3 source, the other used both NH_4Cl and treated effluent. Effluent was obtained from an on-site outfall as it emptied into a storm-sewer.

Red River water was used as control-water and dilution water for all tests. It was collected from a site approximately 0.2 km upstream of the NEWPCC discharge point and, within practical limits, was unaltered from its natural state in the laboratories. The collection site was downstream of the point of convergence of

Test Conditions	1999						2000		
	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	May	Jun.	Jul.
NH ₃ -spiked Red River water (no effluent)									
									
									
									
NH ₃ -spiked Red River water plus effluent									
									

Legend:





-  - acute-exposure, static test
-  - acute-exposure, semi-static test
-  - chronic-exposure; semi-static test
-  - chronic-exposure; flow-through test

Figure 4-3. Test-program.

the Red and Assiniboine Rivers and therefore contained water representative of both rivers. Background NH_3 levels ranged from <0.01 to 0.06 mg/L.

96-hour LC50s or 72-hour LC50s were calculated for all acute-exposure tests (i.e., ≤ 96 -hours) whereas End-of-Test (EOT) LC20s and EC20s were calculated for all chronic-exposure tests (i.e., >96 -hours). EC20s were based on growth responses measured using EOT dry weight differences between control- and exposed-organisms as the growth metric.

The goals of the toxicity program were: (a) to expose local fish species to NH_3 under site-specific conditions and evaluate their responses after acute- and chronic-exposure and (b) to determine whether the toxicity of NH_3 from dissolved NH_4Cl is different from the toxicity of NH_3 -containing effluent.

4.4.1 Static and semi-static testing

Two static tests and six semi-static tests were conducted on newly hatched (i.e., 72 hours) or young (i.e., 8 to 29 day) fathead minnow, white sucker and walleye fry. Each test-population was exposed to a range of NH_3 concentrations in Red River water with and without treated effluent for a period of 72-hours, 96-hours or 10-days (c.f. Appendix D). Exposure-solutions were prepared once at the beginning of the static tests (i.e. T1 and T2) and daily for all semi-static tests (i.e. T3A&B, T4A&B and T5A&B). Grab samples of river water and effluent were collected, as needed, and transported to the laboratory in 20-L carbuoys.

Appendix E describes the protocol used to prepare exposure-solutions. Briefly, a measured amount of ammonia-stock (i.e., NH_4Cl dissolved in de-ionized laboratory water) was added to either river water or effluent to elevate the total ammonia-N concentration to the highest desired nominal-concentration (e.g. 32 mg/L). Initial total ammonia-N concentrations in the effluent were determined using a CHEMet® Ammonia Kit. If the background total ammonia-N concentration was higher than the highest desired nominal-concentration, the effluent was diluted with river water until the desired nominal-concentration was obtained. Subsequent exposure-concentrations were obtained by serially diluting the solutions with river water by a factor of 0.5.

Test-solutions were distributed among 1-L polyethylene test-chambers in equal volumes of 500 ml or 750 ml (c.f., Appendix D). For all semi-static tests, test-chambers consisted of 1-L polyethylene containers into which a second container with a screened bottom was inserted. The diameter of the mesh was large enough to allow water to pass through the membrane but small enough to retain all fish in the 'insert-container'. During daily replacement of the test-solutions, the insert-containers (plus the fish) were slowly withdrawn from the old test-solution and lowered into fresh stock. Exchanges were made only after the temperatures equilibrated.

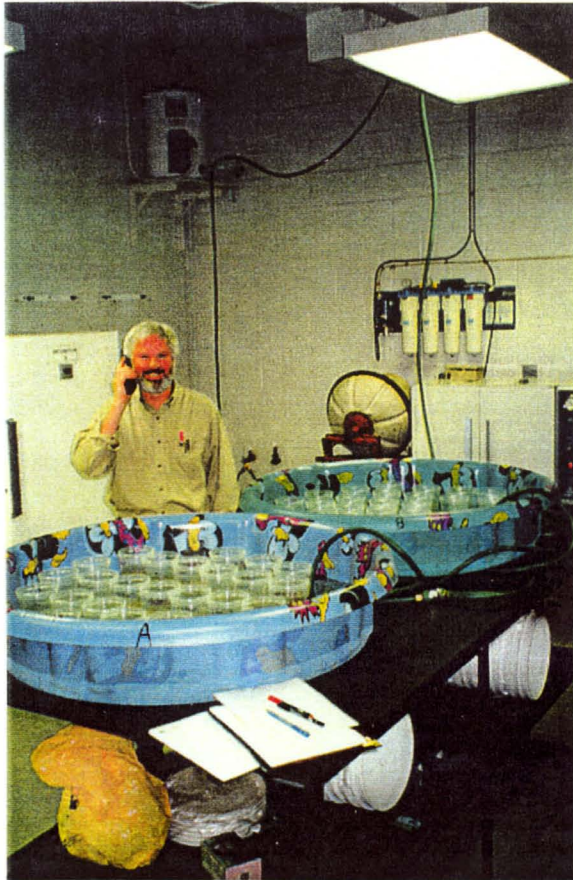
Three to four replicates were prepared for each exposure-concentration with the number of replicates being determined by the supply of fish available for testing.

Labelled test-chambers were stationed in flow-through water baths (Figures 4-4 to 4-5) receiving City of Winnipeg tap water. Prior to the start of each test, test-fish were also held in the water baths. They were sorted into groups of ten and added to the test-chambers once the temperatures of the test-solutions matched that of the water bath to eliminate the potential for thermal shock.

Fish used during testing were withdrawn from the centre of their holding tanks using 1-L polyethylene cups. They were sorted into groups of ten by pouring the fish in holding water back and forth between cups until each cup contained ten fish. Alternately, the fish and holding water were poured into an open tray and fish were captured individually with a wide-mouthed pipette and re-released into a 1-L cup containing river water. If, during either process, a fish appeared unhealthy or dead, it was removed from the sample, discarded and replaced with a healthy individual. For the static tests, ten fish were transferred to the test-chambers in 100 ml of river water thus increasing the volume of test-solutions from 500 ml to 600 ml and diluting the nominal test-concentration by approximately 17%. For all semi-static tests, test-fish were initially held in insert containers submerged in river water and were transferred to the test-solutions by lifting the insert containers (plus the fish) from the river water and slowly lowering them into the test-solutions. This procedure allowed the fish to be transferred from one aquatic medium to another with minimal exchange of the media.



Figure 4-4. Test chambers and water baths used during static and semi-static testing.



Test chambers for used during static and semi-static testing



Larval white sucker

Figure 4-5. Static and semi-static toxicity testing laboratory.

Modifications to the test-system were made as relative size differences and age classes among test-fish changed thus altering space requirements and rates of DO consumption. For example, loading of fish in test chambers (i.e., the mass of biological tissue per volume of test solution) should not be so high as to: (1) reduce dissolved oxygen concentrations below recommended limits, (2) increase concentrations of metabolic waste products above recommended limits or (3) stress organisms due to aggression or crowding (ASTM 1996). Accordingly, test-solution volumes were altered and the continuous aeration was added to the test chambers of some tests depending on the requirements for each group of fish (c.f., Appendix D). The addition of continuous aeration did not alter the NH_3 concentrations, as NH_3 concentrations were periodically sampled and measured over 24-hour periods.

Temperature, pH, and DO measurements were obtained daily using mercury thermometers, a Hach pH meter (calibrated to standard pHs of 4.0, 7.0 and 10.0), and a Yellow Springs Instruments (YSI) dissolved oxygen meter (model 50). Randomly selected test-chambers at high, medium and low exposure-concentrations were sampled under the assumption that they were representative of all test-conditions across the exposure-gradient. Mortality-checks were performed once daily for all test-chambers and dead fish were recorded and removed from the exposure-system. During semi-static testing, mortality checks were conducted prior to replenishing test-solutions and water

chemistry measurements were collected within four hours after test-solution exchange.

Total ammonia-N concentrations were determined from 5- to 30-ml samples of test-solution collected daily from one replicate of each exposure-concentration per test. Analyses were done on-site at the NEWPCC chemistry laboratory using a Technicon Auto Analyser II (Figure 4-6) and the phenate colourimetric method (Clesceri et al. 1998). All samples not analysed within 24 hours, were preserved with 0.5- to 1.0-ml of mercuric chloride (HgCl_2) and stored in a refrigerator at 4°C. Results reported by the NEWPCC lab are documented in Appendix F.

During the first four tests (i.e., T1, T2, T3A and T3B), the fry retained yolk sacs and were not actively feeding. On the third day of T1 and T2, one drop of Wardley Liquid-Baby-Fish Food was placed in each test-chamber to ensure that food would be available should the fish fully consume their yolk sacs and begin feeding. During subsequent tests (i.e., T4A, T4B, T5A and T5B), fish were fed approximately 4.4 mg (dry weight) of newly hatched, live, brine shrimp twice daily for all but the final day of the each test (c.f., Appendix D). Fish chronically exposed to NH_3 (i.e., T4A and T4B, 10-day exposure) were not fed during the last 24-hours of each test to ensure that the contents of their digestive tracts were completely eliminated prior to making whole-body dry-weight measurements.



Figure 4-6. Technicon Auto Analyzer II used to determine $\text{NH}_3\text{-N}$ concentrations.

Surviving fish of the chronic-exposure tests, (i.e., T4A and T4B), were removed from the exposure-chambers, counted, placed on labeled and tared aluminium foil sheets and dried at 103°C for approximately 5 hours. Dry-fish were weighed to the nearest 10^{-4} grams. Fish used during all other static and semi-static tests were removed from the exposure-chambers, counted and discarded.

4.4.2 Flow-through testing

Five flow-through tests were conducted on four species of larval or juvenile fish (i.e., fathead minnows, channel catfish, walleye, and northern pike). Each test-population was exposed to elevated NH_3 concentrations for 12 to 30 days. NH_4Cl was the only source of ammonia (c.f., Appendix D).

The flow-through tests were conducted in a laboratory constructed in the dewatering building of the NEWPCC (Figure 4-7). The laboratory was continuously supplied with Red River water pumped through a 900-m underground pipeline (Figure 4-8) to a 2500-L holding-tank (Figure 4-9). The holding tank served as a primary settling chamber for suspended solids and provided a two-hour water reserve necessary for maintaining water supplies within the laboratory during cleaning operations of the pump-intake.

From the holding tank, water was pumped through a 20-micron filter and into an 80-liter container secured near the top of a 3.66-m heavy-duty shelving unit (Figure 4-10). From this chamber, water was distributed to a series of testing-



Figure 4-7. Site of flow-through laboratory at the dewatering building of the NEWPCC.



Figure 4-8. Red River water-supply pump.

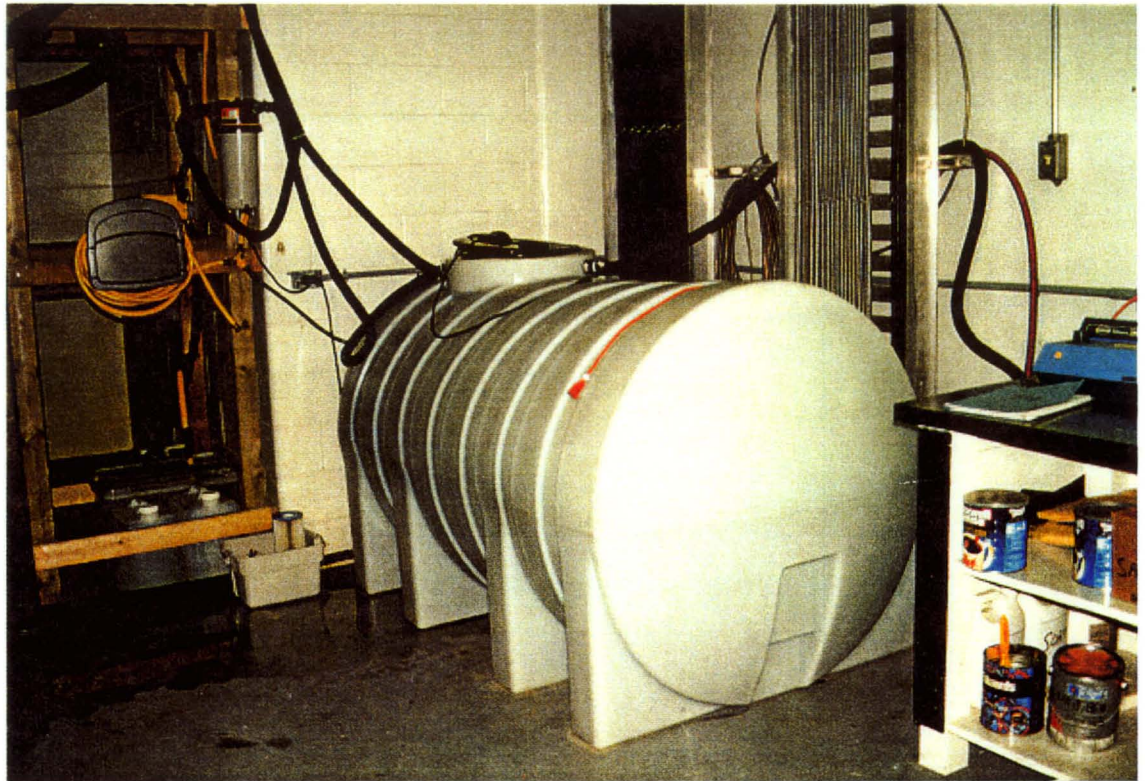


Figure 4-9. River water holding tank.



Figure 4-10. Shelving unit with river water and ammonia supplies.

tables (Figures 4-11 and 4-12) and holding-tanks (Figure 4-13) through two gravity-fed, 3.2-cm (i.e., 1 1/4-inch) ABS pipes (Figure 4-14). River water was also directed through (a) a PVC gate valve for tests conducted in 1999 (Figure 4-15) and (b) a 'swing arm' device for tests conducted in 2000 (Figure 4-16). These devices controlled the flow of water into an 80-liter 'ammonia mixing-chamber' located approximately 1-m beneath the river water container (c.f., Figure 4-10). Flow rates through the swing arm were controlled by adjusting the height of an extension of tubing directed at 90° angles from the side of a bucket. This motion altered the head-pressure differential between the overflow-drain near the top of the bucket and the mouth of the tubing thus controlling the flow of water through the arm. The swing arm system replaced the gate valve in the second year of testing since it allowed the passage of water at a more stable rate and could be adjusted more accurately.

Ammonia was introduced to the system through a LMI™ dosing pump that supplied the ammonia mixing-chamber with a highly concentrated solution of dissolved NH_4Cl (Figure 4-17). The NH_4Cl stock was prepared according to the protocol described in Appendix G. From the mixing-chamber, an ammonia-containing solution was distributed to the testing-tables through a second set of ABS piping arranged in a similar configuration as that used to distribute the river water.

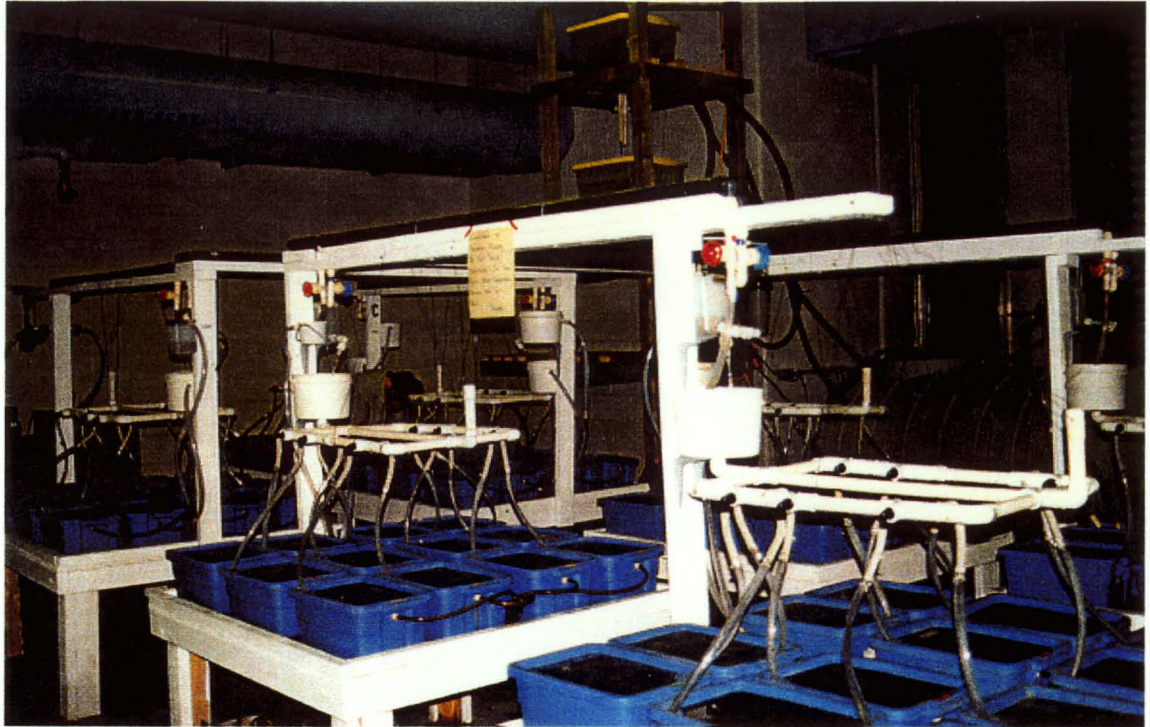


Figure 4-11. Testing-tables in flow-through laboratory.



Figure 4-12. Flow-through laboratory from another perspective.

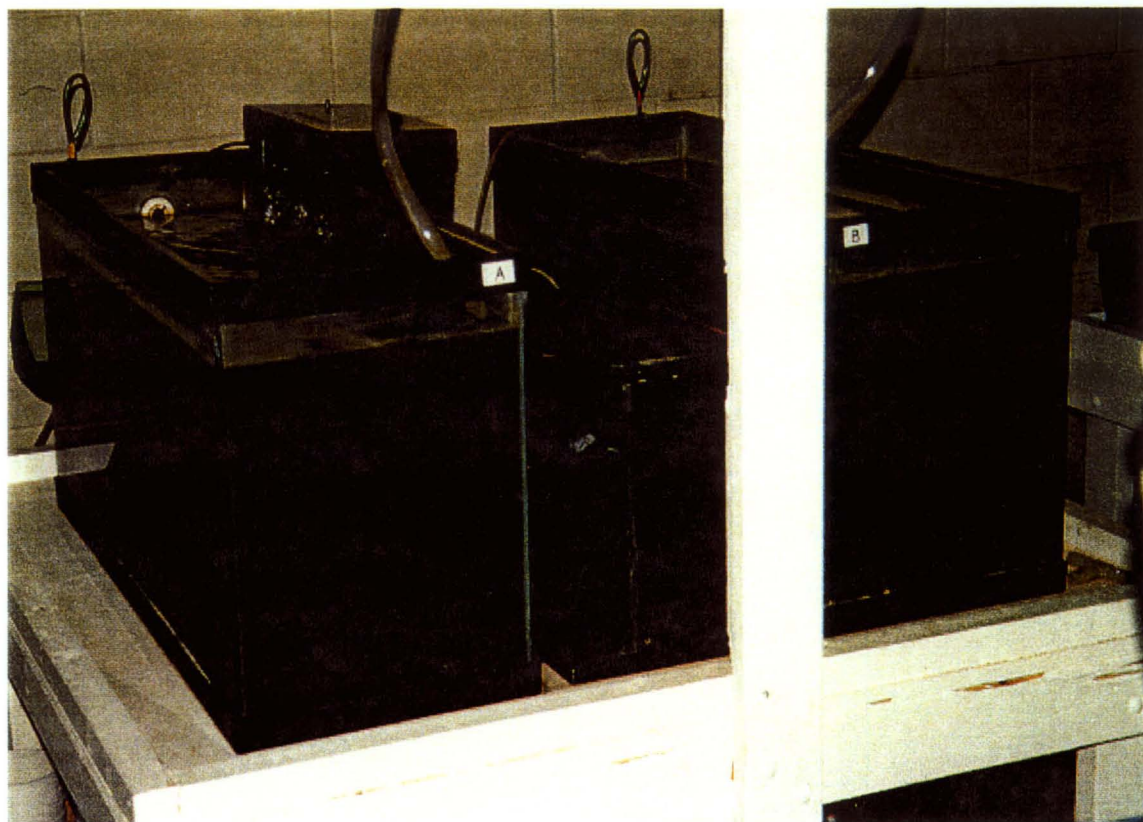


Figure 4-13. Holding tanks.

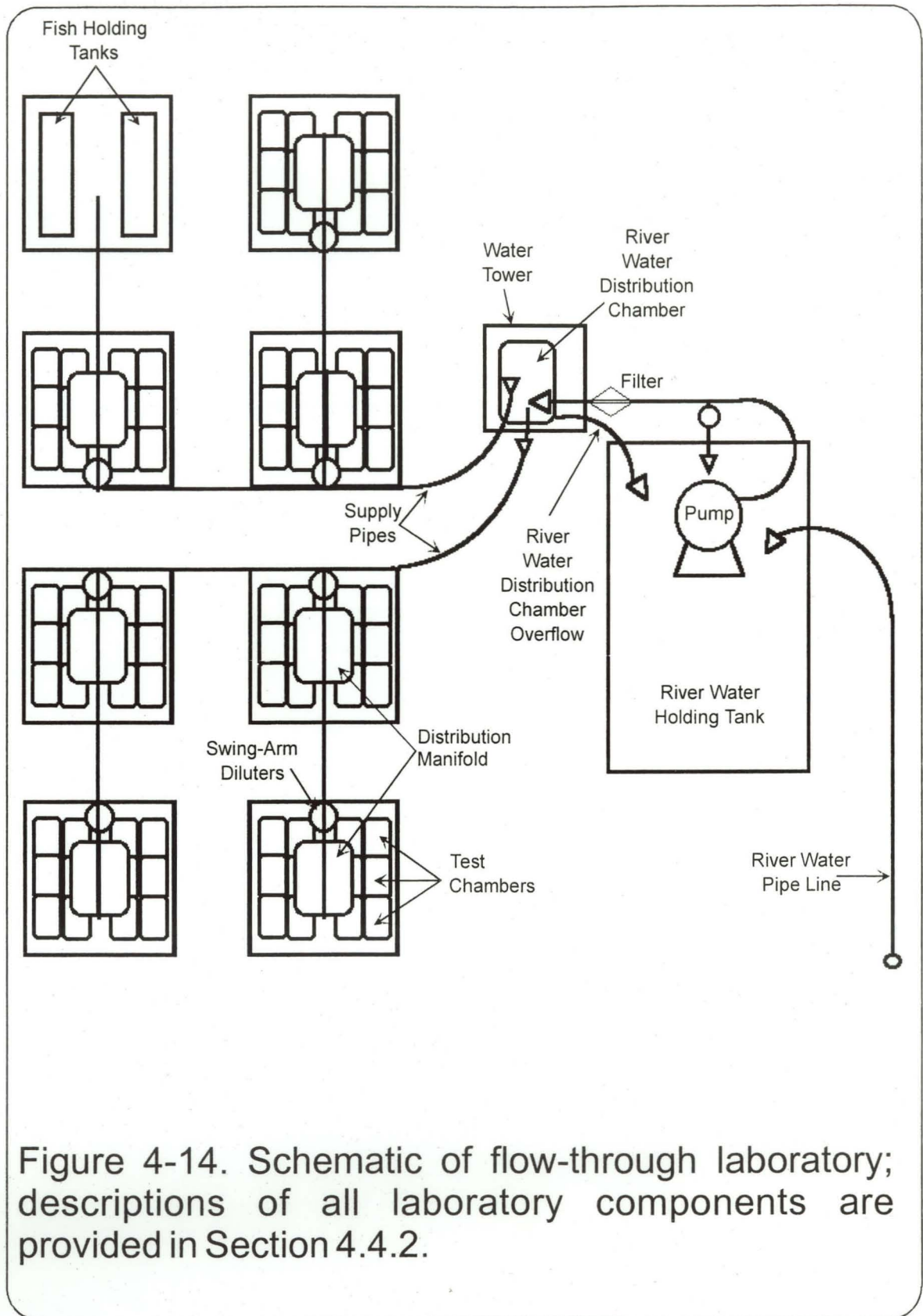


Figure 4-14. Schematic of flow-through laboratory; descriptions of all laboratory components are provided in Section 4.4.2.

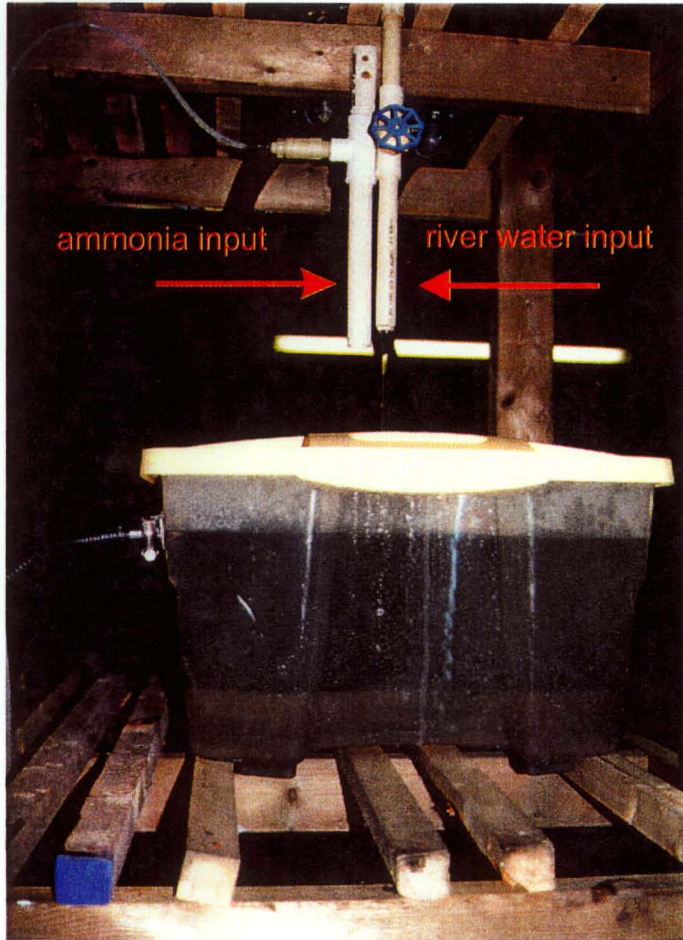


Figure 4-15. Ammonia mixing-chamber; river water input controlled via gate valve.

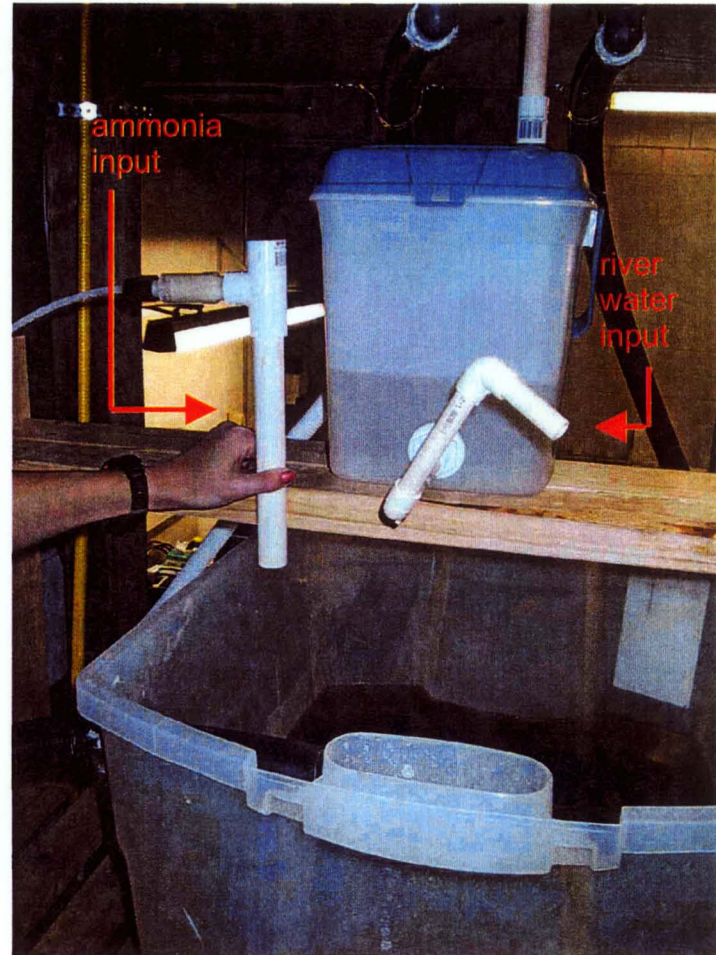


Figure 4-16. Ammonia mixing chamber; river water input controlled via swing arm (described in Section 4.4.2).

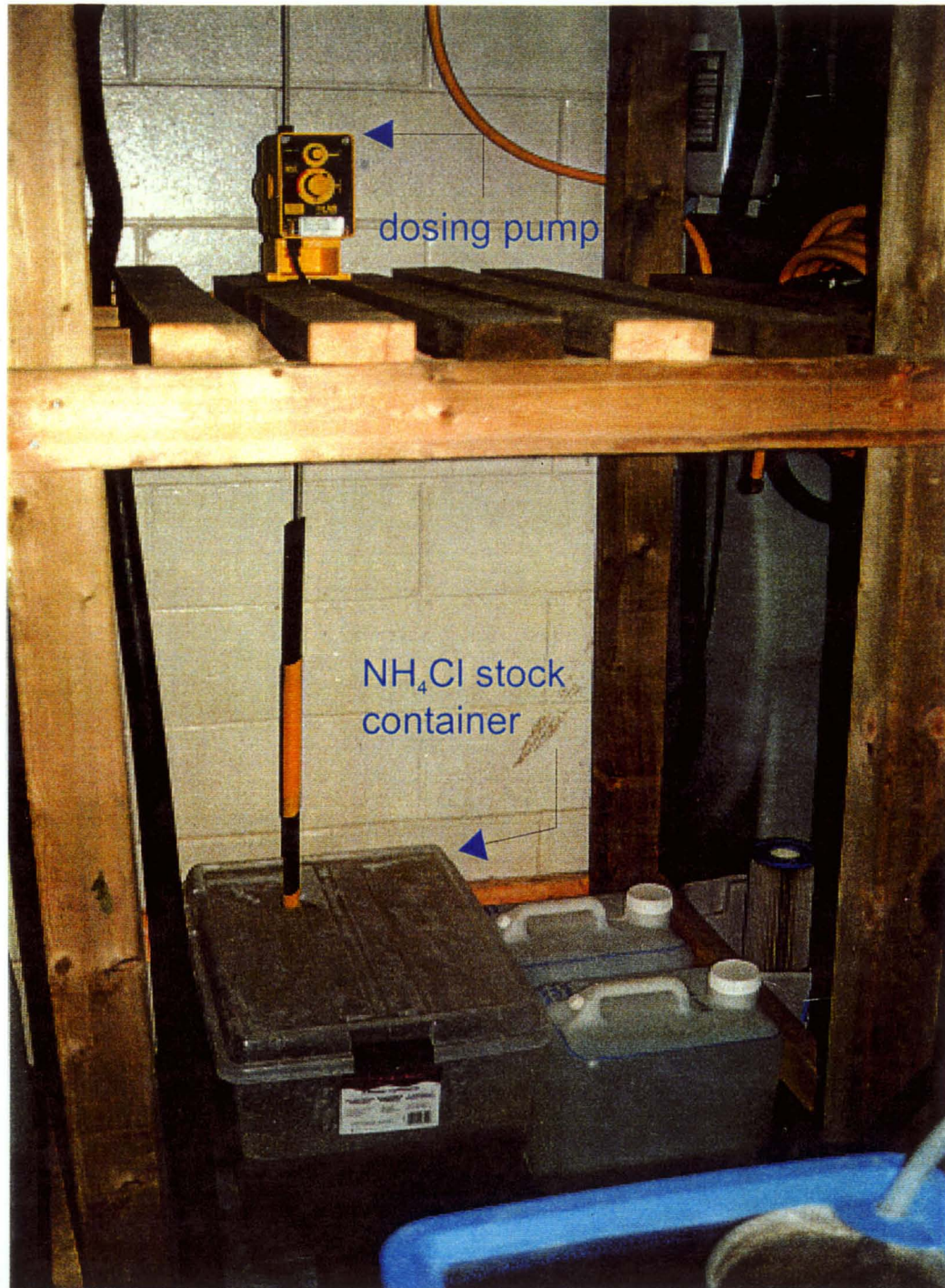


Figure 4-17. NH₄Cl stock container and dosing pump.

At each testing-table, exposure-solutions were prepared using a diluter system that was fed a continuous supply of ammonia-solution and river water. Exposure-concentrations were controlled by adjusting the flow rates in the diluter-system with a series of PVC gate valves and swing arms (Figures 4-18 and 4-19) (c.f., Appendix G). In other words, the flow rates of toxicant-solution and dilution water entering a mixing bucket at each testing-table were controlled so that the test-chambers each received the same level of toxicant. A flow-splitting device (a.k.a., the 'distribution-manifold') at each testing-table directed the exposure-solution to each test-chamber (Figure 4-20). All test-chambers were equipped with overflows and designed for continuous test-solution replenishment. The minimum replacement rate was approximately 30 times per day. Up to six testing-tables (i.e., exposure-concentrations) were used per test with a capacity to supply up to 12 test-chambers. Four replicate test chambers were used for all tests except one (i.e., T10), which used only three because fewer fish were available for testing.

The number of organisms distributed to each flow-through test chamber was a function of animal size and quantity at test-start - between 15 and 20 organisms were used. Fish were fed daily with food recommended by hatcheries (c.f., Appendix D) for all but the last day of each test. Fish were not fed within 24 hours of test-termination to ensure that consumed food had been completely digested and eliminated from the gut prior to whole-body dry-weight measurements.

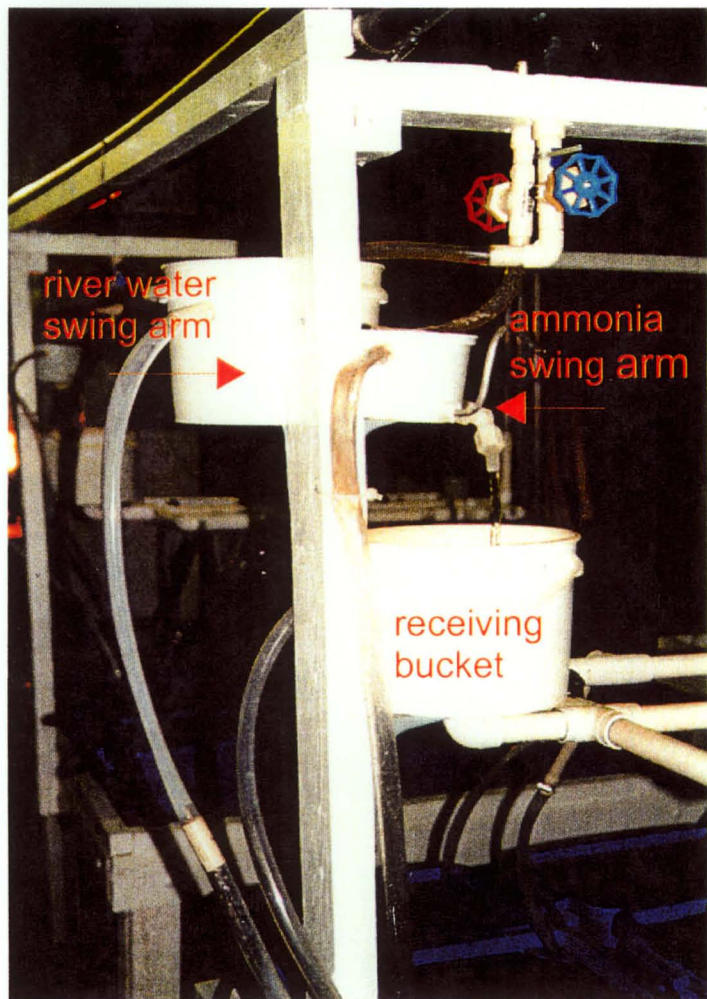


Figure 4-18. River water and ammonia swing arm diluters.

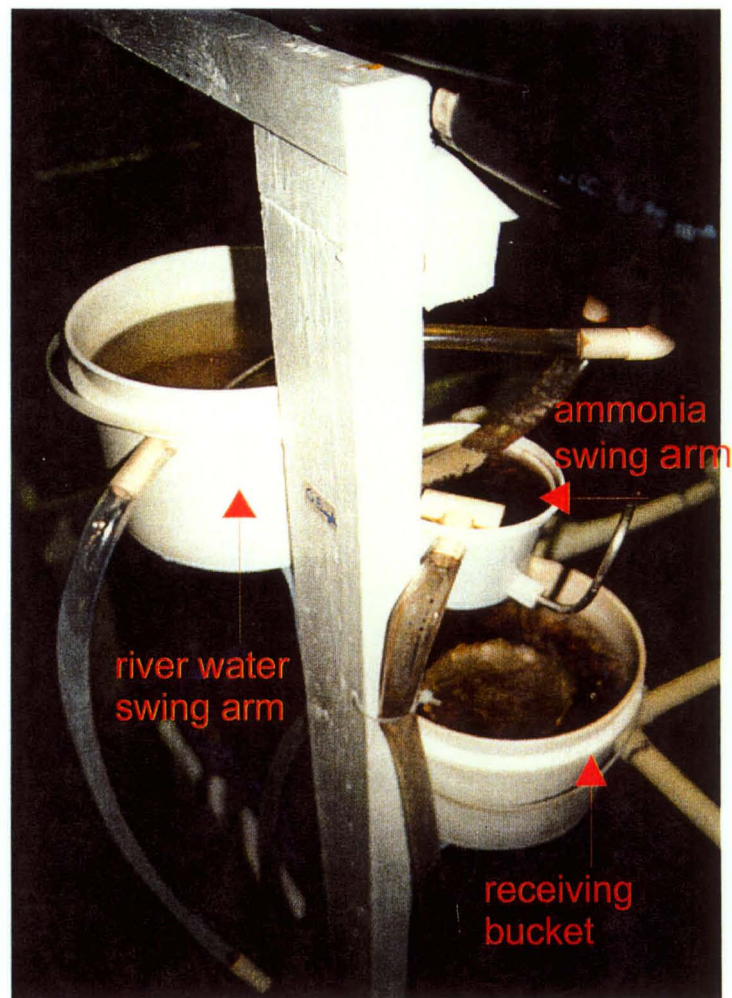


Figure 4-19. Top view of the river water and ammonia swing arm diluters.

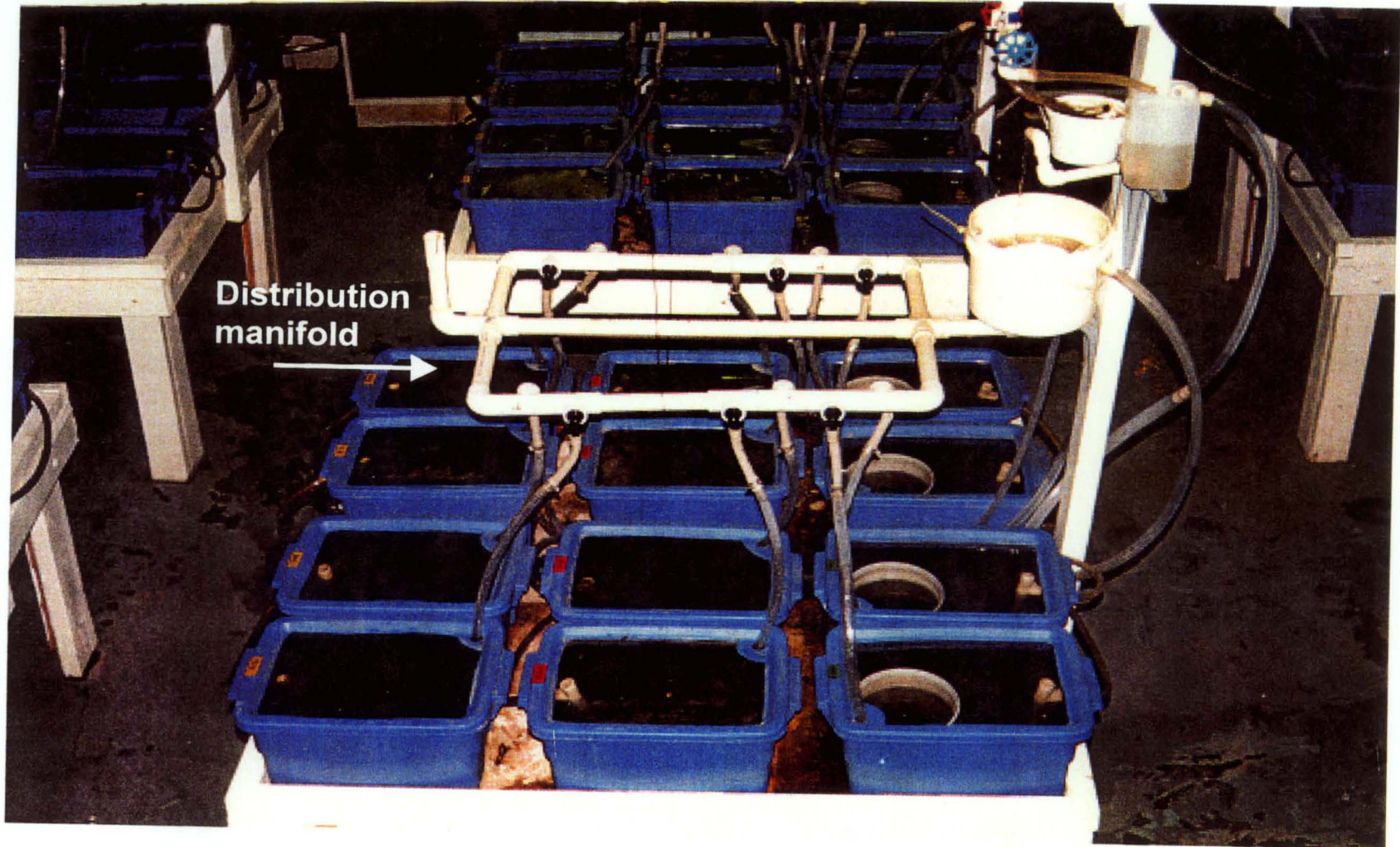


Figure 4-20. Distribution manifold (i.e., Flow-splitting device).

The dilution system was checked daily to ensure that nominal total ammonia-N concentrations were maintained. Adjustments were made to the flow rates, if necessary, to achieve the desired nominal concentrations. Once the system stabilized, water temperature, pH and DO measurements were obtained from a representative sample of chambers for each species using conventional instruments described in Section 4.4.1.

Total ammonia-N exposure-concentrations were measured from 60-ml samples collected prior to and following calibration using a Chemet® Ammonia Kit and the NEWPCC's Technicon Auto Analyzer II, respectively during 1999 testing. Measurements were taken twice daily to account for variability in exposure-concentrations due to changes in flow rates in the diluter systems resulting from sediment build-up. Graphical analysis of the level of agreement between results of the Chemet® and Auto Analyzer analytical methods for determining total ammonia-N concentrations yielded an R^2 value of 0.9911. Therefore, temporal differences in total ammonia-N concentrations in the laboratory were likely due to conditions of the exposure system rather than differences in the mode of analysis.

For tests conducted in 2000, total ammonia-N concentrations were measured using both methods analysis for the first 10 days of testing, but improved stability of the test-system due to changes made to the primary and secondary diluters eliminated the necessity of collecting duplicate samples. For the remainder of

the 2000 test-program, water samples were collected following calibration and analyzed with the Auto Analyzer. All water samples analyzed by the NEWPCC lab were obtained, preserved, and stored according to the protocol described for the static and semi-static tests. Results are reported in Appendix F.

Fish-mortality for each test was checked daily using one of two methods of inspection: (a) visual-inspection method and (b) net-collection method. Visual inspections of larval fish were possible because they were held in small test-chambers with screened bottoms that could be lifted from a second set of chambers containing test-solution. As the inner containers were lifted out of solution, the volume of water surrounding the fish was reduced to a level at which the fish could be observed and counted. Since the turbidity of water was high (i.e., 40-155 mg/L TSS), the larval fish could only be counted at depths up to approximately 10-cm. Juvenile fish were held in larger containers without screened inserts and could not be visually inspected. Instead, the bottom of each test-chamber was swiped with a net to collect and remove any dead fish. Dead fish were counted and recorded, then discarded.

At the end of each test, surviving fish were removed from the test-chambers, counted and terminated using either a lethal dose of MS-222 (i.e., tricaine or Finquel), or thermal shock with hot tap water. The fish were then placed on labelled and tared aluminium-foil trays, dried at 103°C for 24 to 48 hours then weighed to the nearest 10^{-4} gram on a Mettler PM600 balance.

4.5 Statistical analysis of toxicological data

Point estimates of the NH_3 concentrations that caused a 50% and 20% reduction in the survival and/or growth of the test-fish were calculated via the linear interpolation method (i.e., the inhibitory concentration (ICP) approach) and a statistical software program, ICPIN version 2.0. The linear interpolation method is suitable for analysis of a wide-range of data because the data need not fit standard parametric regression models, and a 'smoothing' technique supports the assumption that responses will increase with increasing toxicant concentration (Norberg-King 1993). The US EPA (1989, 1991) encouraged the use of this analytical method for calculating sub-lethal toxicity endpoints in addition to other standard methods of analysis previously recommended in *Short-term methods for estimating the chronic toxicity of effluents and surface waters to freshwater organisms* (US EPA 1985b). Although the linear interpolation method was primarily intended to analyse sub-lethal toxicity effects, it is also a good estimator of lethal toxicity effects.

For this thesis, toxicological data were organized into suitable input metrics for the ICPIN program, which included average NH_3 concentrations and endpoint measurements expressed quantitatively (i.e., as percent survival for lethal endpoints and dry weight per live fish at EOT for growth endpoints). Average NH_3 concentrations for all replicate test-chambers at a particular exposure-concentration were calculated for a specified time-interval (i.e., 96 hour, 10 day,

or EOT) using average total ammonia-N concentrations, average test-pHs (i.e., calculated as arithmetic means of the H^+ concentrations in replicate test-chambers), average test-temperatures and Equations 1 and 2. A sample calculation for determining average NH_3 concentrations is provided in Appendix H.

The ICPIN program calculates lethal or inhibitory-concentrations (i.e., LC or IC) of a stressor affecting x% of the test-population by comparing pairs of adjacent toxicant concentrations with their corresponding response-means as described in US EPA (1994). In brief, the pairs of exposure-concentrations and mean responses that bracket the expected response (i.e., 20% or 50% of the test-populations) are used to estimate the lethal or inhibitory toxicant concentration according to the following equation:

$$ICP = C_J + [M_i(1-p/100)-M_J] \frac{(C_{J+1} - C_J)}{(M_{J+1} - M_J)} \quad (Eq 13)$$

- Where: ICP = inhibitory (or lethal) estimated concentration of a toxicant that produces a percent-reduction in growth or survival that is quantitatively different from the control group
- C_J = exposure-concentration whose observed mean response is $> M_i(1-p/100)$
- C_{J+1} = exposure-concentration whose observed mean response is $< M_i(1-p/100)$
- M_i = 'smoothed' (defined below) mean response for the control
- M_J = 'smoothed' (defined below) mean response for concentration J
- M_{J+1} = 'smoothed' (defined below) mean response for concentration J+1
- p = percent reduction in growth or survival response of exposed organisms relative to the response of control organisms

The ICPIN program assumes that the mean responses for all replicate exposure data are 'monotonically non-increasing', where the mean response of organisms at successively higher exposure-concentrations is less than or equal to the mean response at the previous concentration (Norberg-King 1993). If the data are not monotonically non-increasing, they are 'smoothed' by replacing adjacent response means with their average. A worked-example of the smoothing technique follows:

Assume three groups of 10 fish each were held for 96 hours in aquaria containing (a) river water only (i.e., control group), (b) 2.0 mg NH₃/L river water, (c) 4.0 mg NH₃/L river water, or (d) 8.0 mg NH₃/L river water. A possible data matrix using survival as an endpoint is:

Ammonia conc. (mg NH ₃ /L)	Percent Mortality			Mean Response (Y)	Smoothed Response (M)
	Replicate A	Replicate B	Replicate C		
Control group	5	15	10	10	7.8
2.0	10	5	10	8.3	7.8
4.0	5	5	5	5	7.8
8.0	10	15	20	15	15

To smooth this dataset, the mean response of the control group (Y_1) of 10% is first compared with the mean response of the group exposed to 2.0

mg NH₃/L (Y₂) of 8.3%. Since Y₂ is less than Y₁ (i.e., 8.3% < 10%), the data are not 'monotonically non-increasing' and the average of the two values is calculated and used as the smoothed mean response for both the control group (i.e., M₁) and the lowest exposure-concentration group (i.e., M₂). In this case, M₁ and M₂ would equal 9.2% (i.e., the average of 10% and 8.3%). However, the newly calculated mean response (i.e., 9.2%) is greater than the mean response of the next lowest exposure-concentration (Y₃ at 4.0 mg NH₃/L) so the average of Y₁, Y₂, and Y₃ becomes the smoothed mean response (M) for all three groups (i.e., M₁=M₂=M₃). For the above dataset, this value equals 7.8%. This process is repeated until each subsequent response mean in a series is compared with the rest of the series (Norberg-King 1993). Since M₁, M₂ and M₃ are less than the mean response for the fish exposed to 8.0 mg NH₃/L (i.e., 7.8% < 15%), further smoothing is not required for the above dataset. The response mean for the fourth group (Y₄) retains its original value of 15% (i.e., M₄ = Y₄).

A second assumption of the linear interpolation method is that responses of test-organisms follow a piecewise (i.e., segmented) linear response function. This assumption is met for all toxicity tests conducted where responses are measured along a gradient of increasing toxicant concentration (Norberg-King 1993).

Finally, the linear interpolation method assumes that the data are from a random, independent and representative sample of the test data (Norberg-King 1993).

The inhibitory (or lethal) percent estimate can be obtained easily with a computer or hand-held calculator using Equation 13. However, computation of 95% confidence is accomplished using the Bootstrap method, a computationally intensive method described by Efron (1982). The original version of the ICPIN program produced 95% confidence intervals that contained the true value less than 95% of the time for small datasets. Consequently, a second version of the program (i.e., the version used here) was created to calculate original and expanded 95% confidence intervals for all datasets containing six or less replicates (Norberg-King 1993). In the present study, all tests consisted of less than six replicates and therefore expanded confidence intervals are reported.

4.6 Incorporation of site-specific data into current Manitoba Surface Water Quality Objectives (MSWQO)

To determine how site-specific data influence acute criteria, the acute-value (AV) in Equation 6 (i.e., an intermediate equation for Equations 10, 3* and 6*) was replaced with the LC50 generated for each locally tested fish species. When more than one test was conducted for a single fish species, the geometric mean of all appropriate LC50s replaced the AV in Equation 6. This approach assumes that each of the five locally-tested fish species was more sensitive to acute NH₃

exposure than the ranked dataset pooled by the US EPA (refer to Section 3.3.1). Second, it is consistent with the US EPA approach that the AV be replaced by the SMAV (in this case, the LC50) of a commercially or recreationally important species should the SMAV be lower than the calculated AV (refer to Section 3.3.1). Site-Specific Water Quality Objectives (SSWQO) for each of the five locally tested fish species acutely exposed to NH_3 in Red River water were compared with current Manitoba Surface Water Quality Objectives (MSWQO) for pHs between 6.5 and 9.0 (i.e., pH values between which Manitoba Conservation considers MSWQO to be applicable).

Similarly, the lower of an LC20 or EC20 generated for locally tested fish species replaced the factor 'a' in Equations 1* (i.e., Equation 11) and 2* and replaced the factor 'b' in Equations 4* (i.e., Equation 12) and 5*. This approach assumes that each of the five locally-tested fish species was more sensitive to chronic NH_3 exposure than the ranked dataset pooled by the US EPA (refer to Section 3.3.2). Consequently, the LC20 or EC20 for each resident fish species replaced the GMCV for bluegill and the temperature-adjusted GMCV for *Hyaella* in the chronic criteria-developing equations (refer to Section 3.3.2). This approach assumes that all other data factored into the chronic criteria-developing equations remained equal. For example, using regression analysis of toxicity data from ten aquatic genera, the US EPA determined the maximum ammonia concentration below which 95% of genera should be protected from the effects of ammonia toxicity. This value occurred at a level 15.6% lower than the lowest

GMCV. In this thesis, chronic SSWQO were also determined using a value that is 15.6% lower than the GMCV, except that the GMCV was replaced with site-specific results (described above). Equations 4* (i.e., Equation 12) and 5* apply when ELS fish are absent or when water temperatures are $\leq 5^{\circ}\text{C}$. Since two of the five resident fish species (i.e., northern pike and white sucker) were tested at a larval age class only, and since they hatch from eggs at water temperatures greater than 5°C (Scott and Crossman 1973), only channel catfish, fathead minnow and walleye data could be incorporated into these equations. Data generated with all five locally tested fish species were incorporated into Equations 1* (i.e., Equation 11) and 2*, which apply when ELS fish are present or when water temperatures are $>5^{\circ}\text{C}$. SSWQO for each locally tested species were compared with the current MSWQO for temperatures between 0°C and 30°C and pHs between 6.5 and 9.0 (i.e., temperature and pH values between which Manitoba Conservation considers MSWQO to be applicable).

5.0 RESULTS AND DISCUSSION

The degree to which acute and chronic-exposure toxicity-tests conducted with fish satisfied either ASTM (1996) or US EPA (1991a, 1993) protocols is shown in Appendix I, Tables I-1 and I-2. Explanations are provided when test-conditions deviated from recommended test-acceptability criteria.

5.1 Results of acute-exposure testing

Ten acute-exposure tests were completed under static, semi-static or flow-through conditions using young (i.e., larval or juvenile) walleye, white sucker, fathead minnow, channel catfish and northern pike. All acute-exposure test results are summarized in Table 5-1 together with related environmental data. Dose-response curves for each test are illustrated in Figures 5-1a to 5-10a, and are paired with time-to-response curves illustrated in Figures 5-1b to 5-10b. The data used to calculate all LC50 values are tabulated in Appendix J, Tables J-1 to J-10. ICPIN printouts are provided in Appendix K.

NH₃ was acutely toxic to fish in seven of the ten test-populations (c.f., Table 5-1). Mortalities of two groups of juvenile fathead minnows and one batch of juvenile walleye were insufficient to calculate LC50 values because less than 50% of the fish died at the highest concentrations tested. Presumably, the LC50 values for these three tests are greater than the most concentrated ammonia-solution (i.e., 0.76 mg NH₃/L for Test 7 with fathead minnows; 0.36 mg NH₃/L for Test 8 with

**Table 5-1. Acute-exposure toxicity of ammonia-fortified river water to five fish species:
Test conditions and 96-hour LC50 values.**

Species (Test No. – Age Class)	Test Type	DO ^a mean (range) (mg/L)	DO ^a mean (range) (% saturation)	pH mean (range)	Temperature mean (range) (°C)	96-h LC50 (95% CI ^b) (mg NH ₃ /L)
Channel catfish (6 – juvenile)	Flow-through	11.1 (9.1-12.4)	98.6 (82.5-111.1)	8.4 (7.9-8.7)	10.2 (9.5-11.7)	0.69 (--) ^c
Fathead minnow (5A – larval)	Semi-static	8.2 (7.7-8.8)	91.3 (86.4-96.8)	8.6 (8.4-8.8)	20.7 (20.1-21.0)	0.36 (0.03-0.58)
Fathead minnow (7 – juvenile)	Flow-through	11.5 (10.3-12.4)	101.4 (91.2-109.9)	8.4 (8.2-8.6)	9.8 (9.3-10.3)	>0.76 ^d
Fathead minnow (8 – juvenile)	Flow-through	12.0 (10.0-13.7)	104.4 (87.5-117.1)	8.5 (8.4-8.6)	9.4 (8.1-12.3)	>0.36 ^d
Northern pike (9 – larval)	Flow-through	10.1 (9.1-10.8)	103.5 (94.2-110.6)	8.5 (8.4-8.6)	16.7 (16.2-17.2)	0.52 (0.48-0.57)
Walleye (1 – larval)	Static	7.7 (2.8-10.5)	77.3 (28.4-104.1)	8.3 (8.0-8.5)	15.6 (14.0-18.5)	0.33 (0.30-0.40)
Walleye (10 – juvenile)	Flow-through	9.2 (8.6-10.0)	96.7 (89.0-107.8)	8.4 (8.3-8.5)	17.9 (16.6-19.3)	>0.48 ^d
White sucker (2 – larval)	Semi-static	7.5 (3.9-9.6)	75.0 (39.5-96.2)	8.3 (8.0-8.5)	15.5 (14.5-18.5)	0.49 (0.42-0.79)
White sucker (3A – larval)	Semi-static	6.1 (5.4-7.6)	61.2 (53.4-77.8)	8.2 (8.0-8.4)	15.5 (14.0-17.0)	0.22 (0.14-0.25)
White sucker (4A – larval)	Semi-static	7.3 (6.4-8.3)	76.0 (66.2-87.7)	8.2 (7.9-8.3)	17.5 (16.0-20.0)	0.52 (0.37-0.76)

Notes:

- a) DO = dissolved oxygen
- b) CI = confidence interval
- c) A confidence interval could not be calculated because the number of re-samples that were generated using the Bootstrap Method is not a multiple of 40.
- d) A point estimate of the lethal NH₃ concentration could not be computed because the exposure concentrations did not bracket the expected response-mean. The highest concentration of NH₃ tested is reported instead, and it is expected that the actual LC50 value would be greater than this value.

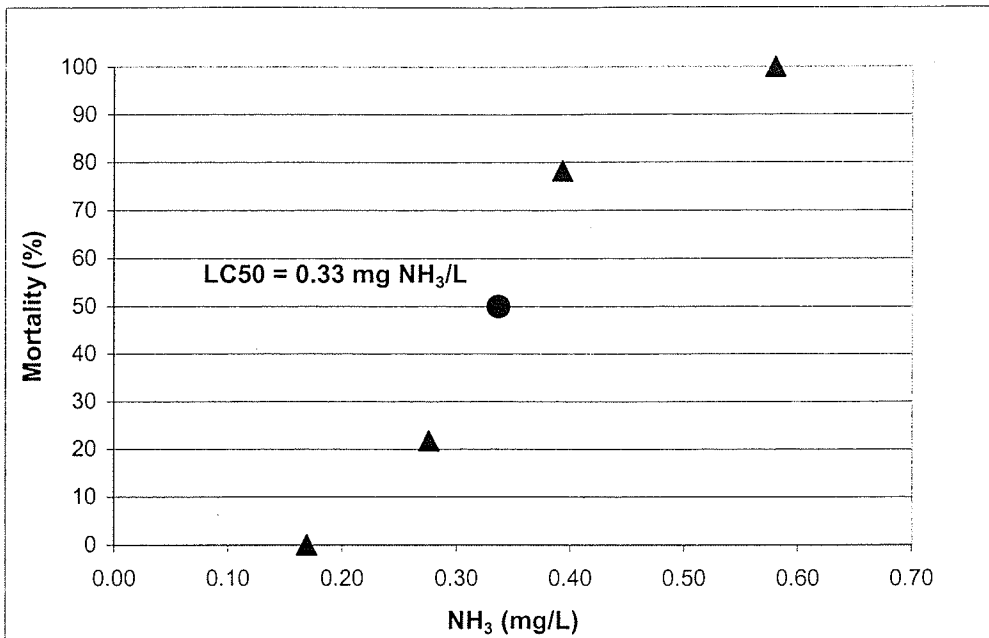


Figure 5-1a. 96-hr LC50 and mortality curve for larval walleye exposed to Red River water fortified with NH₃ (T1).

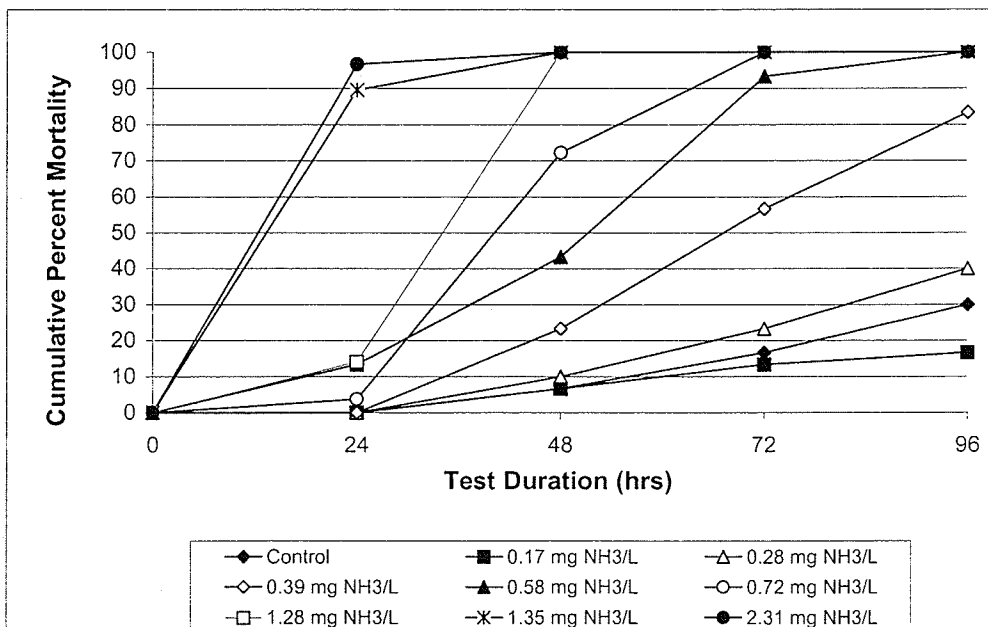


Figure 5-1b. Time to mortality curve for larval walleye exposed to NH₃ in Red River water for 96 hours (T1).

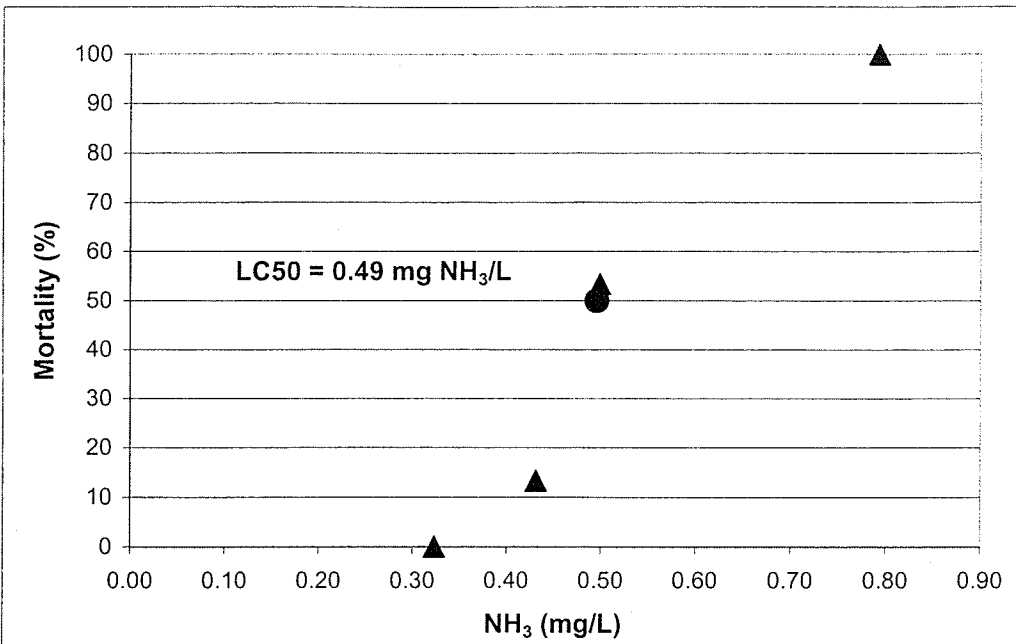


Figure 5-2a. 96-hr LC50 and mortality curve for larval white sucker exposed to Red River water fortified with NH₃ (T2).

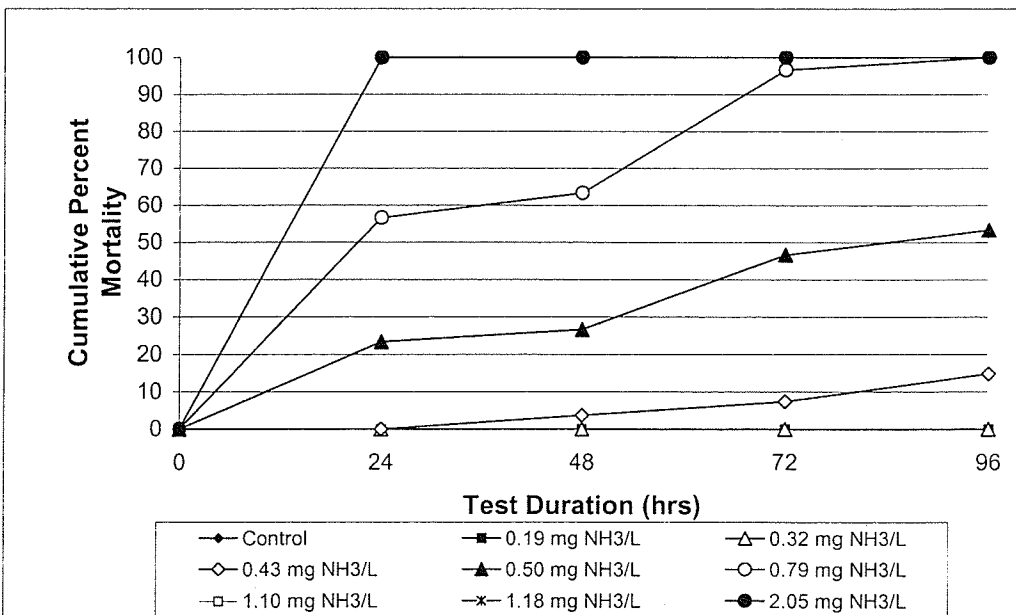


Figure 5-2b. Time to mortality curve for larval white sucker exposed to NH₃ in Red River Water for 96 hours (T2).

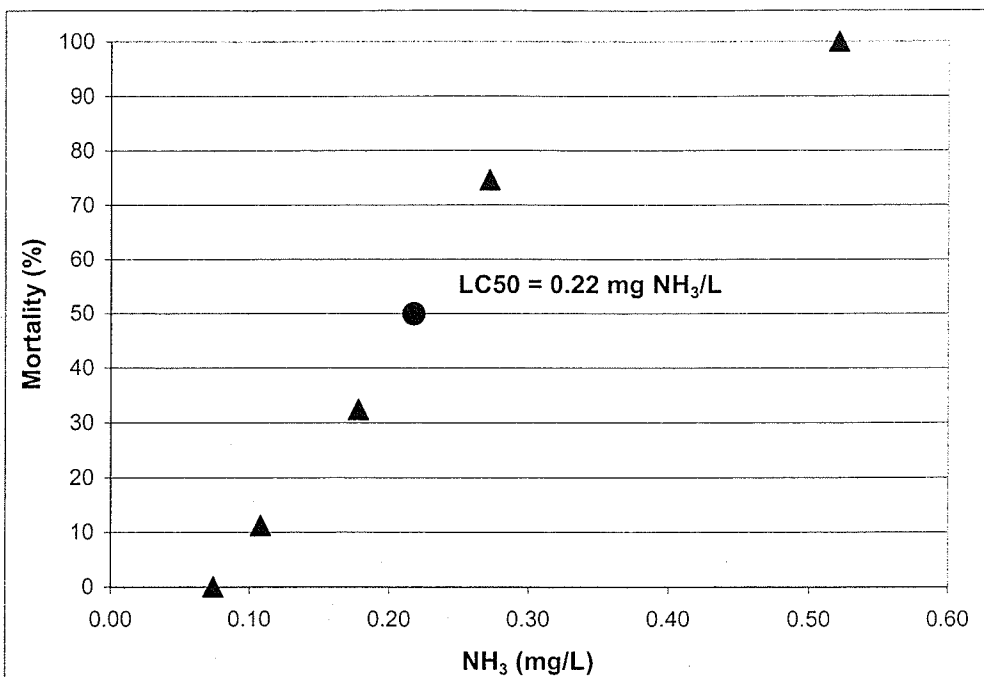


Figure 5-3a. 96-hr LC50 and mortality curve for larval white sucker exposed to Red River water fortified with NH₃ (T3A).

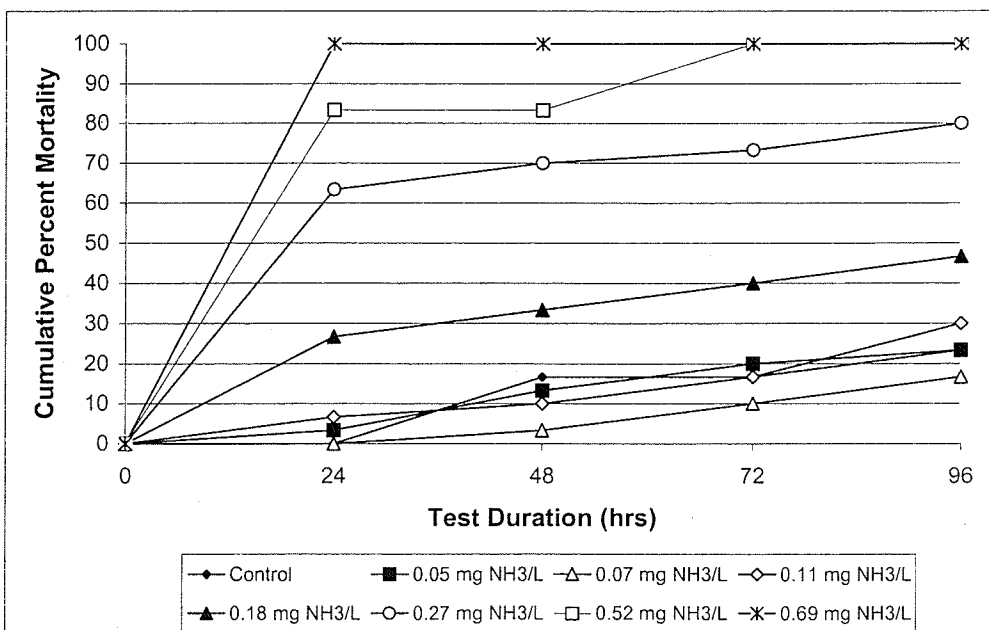
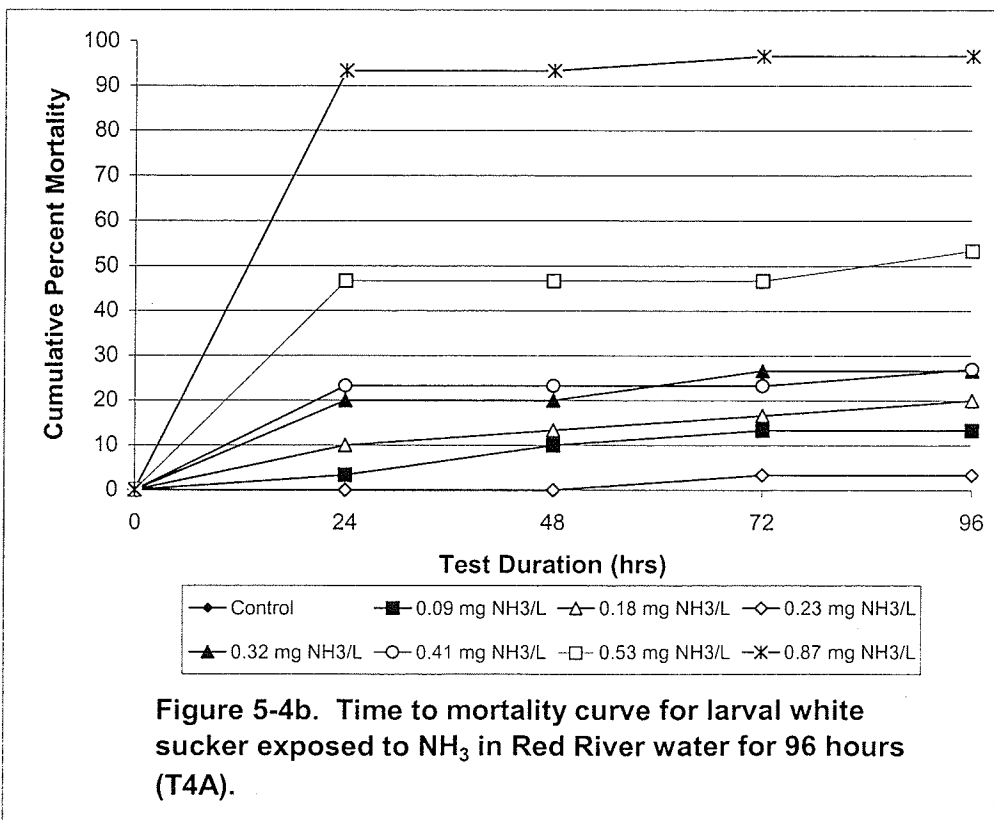
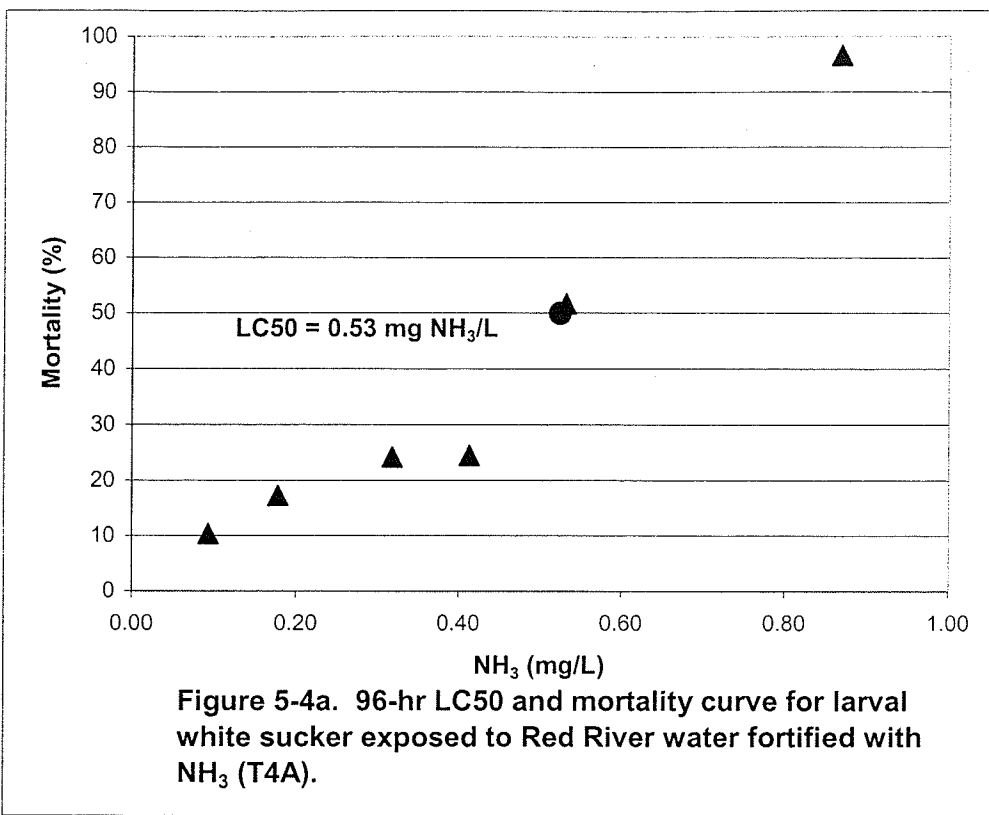


Figure 5-3b. Time to mortality curve for larval white sucker exposed to NH₃ in Red River Water for 96 hours (T3A).



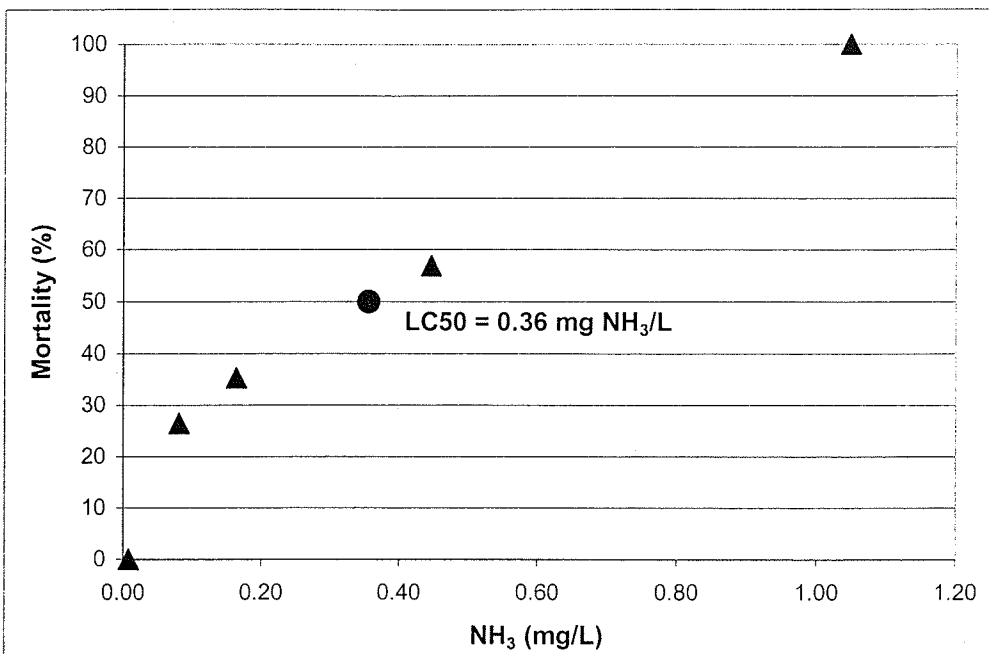


Figure 5-5a. 96-hr LC50 and mortality curve for larval fathead minnow exposed to Red River water fortified with NH₃ (T5A).

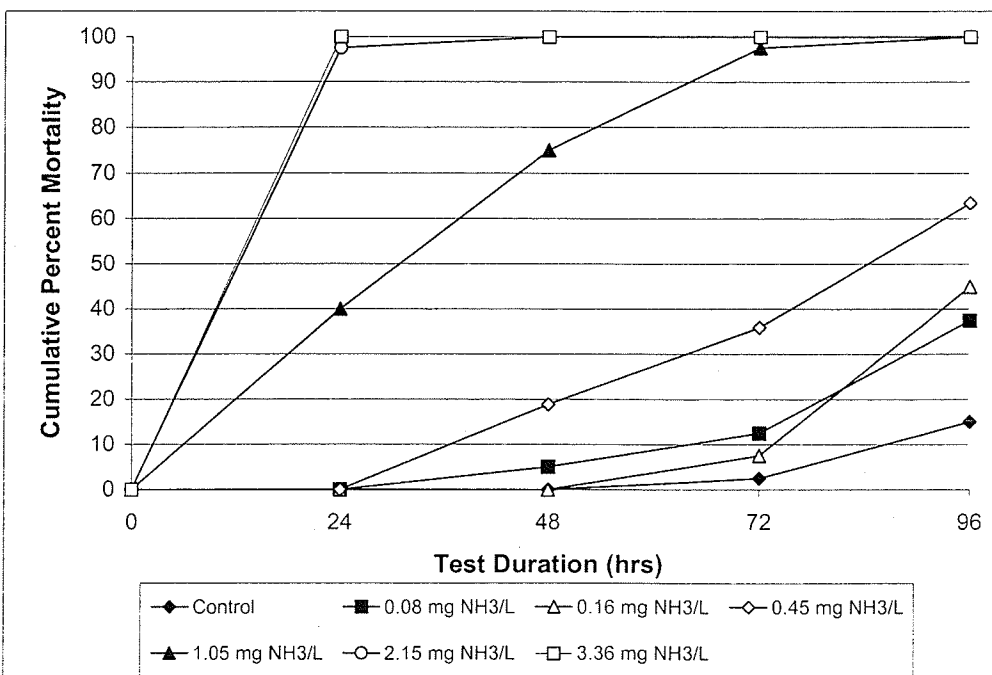
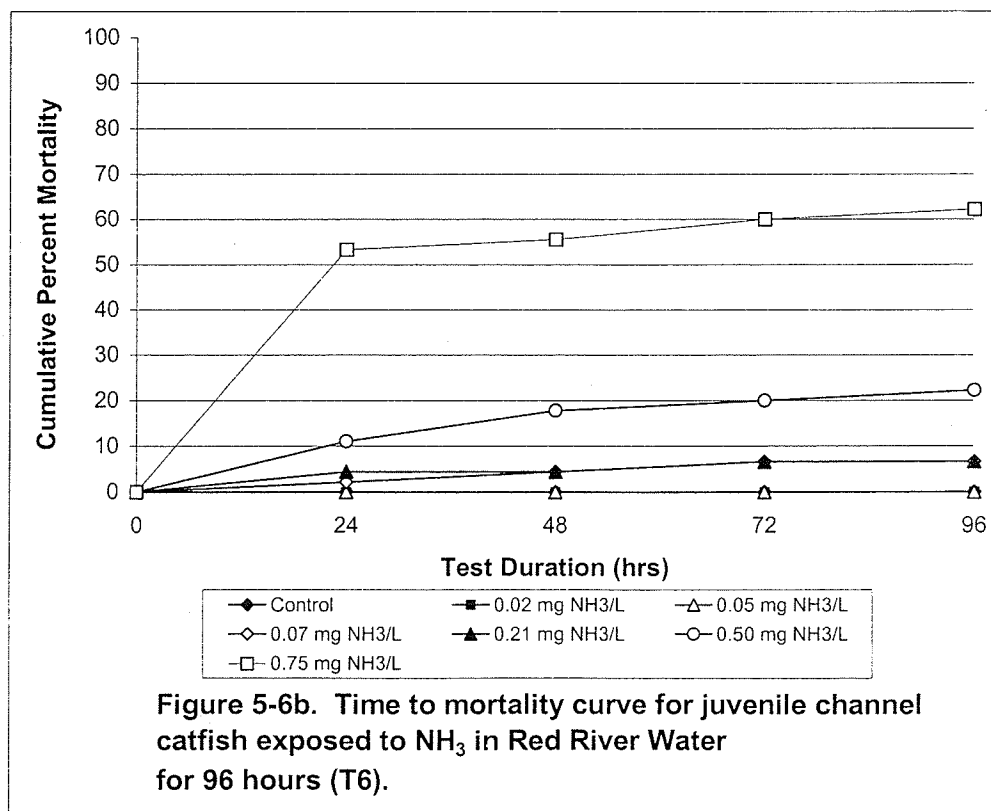
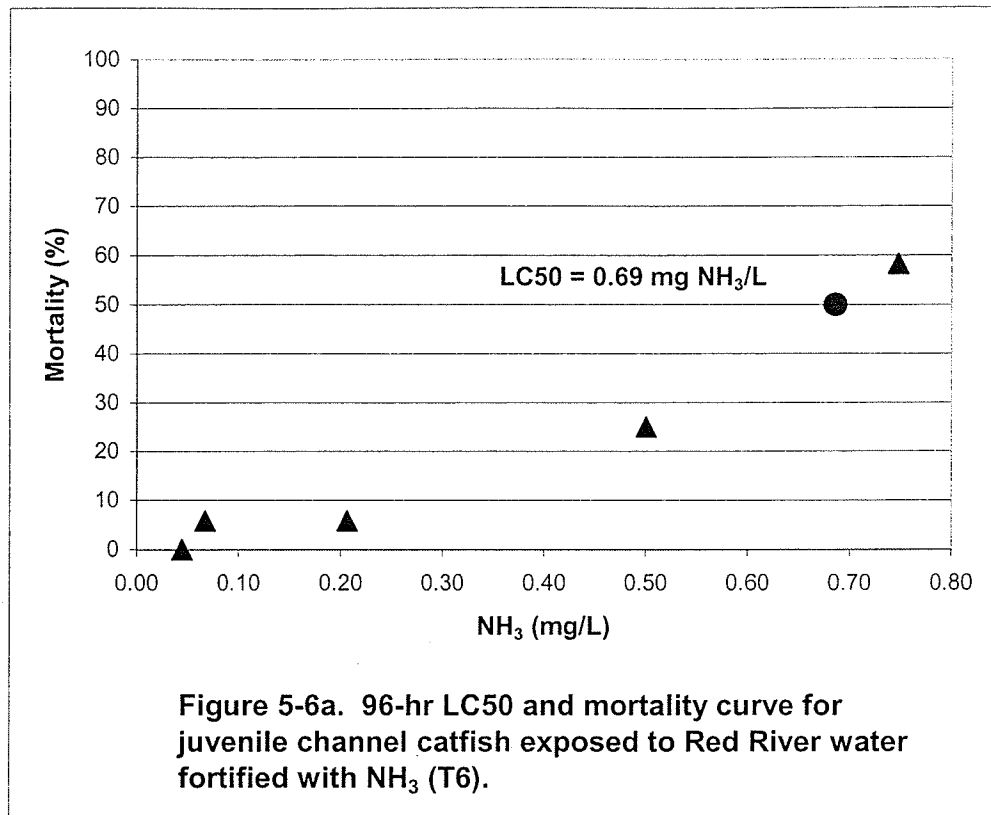


Figure 5-5b. Time to mortality curve for larval fathead minnow exposed to NH₃ in Red River Water for 96 hours (T5A).



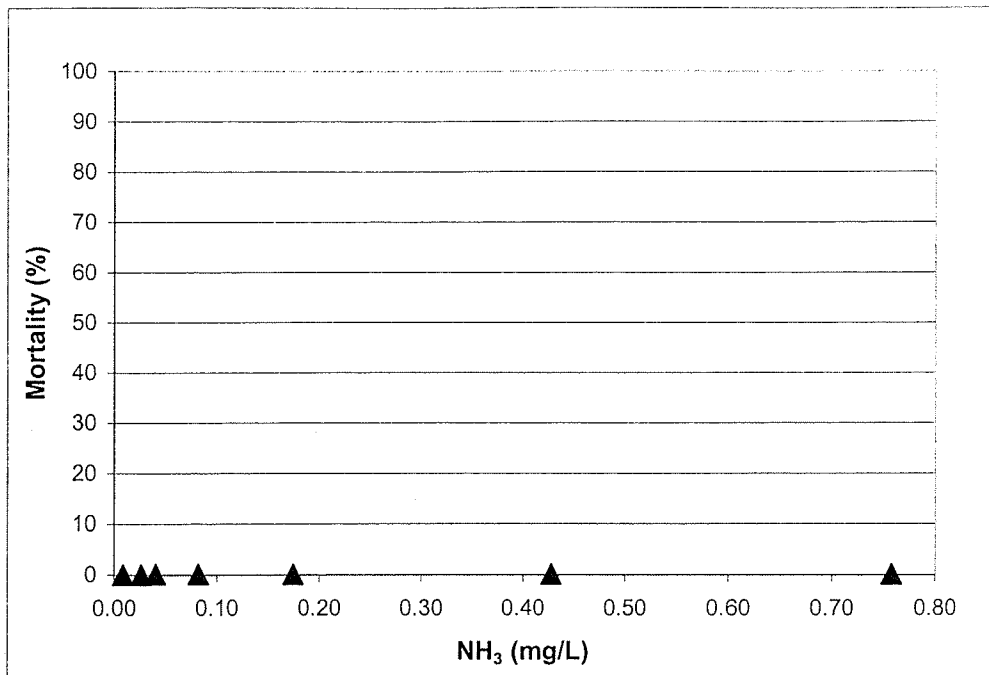


Figure 5-7a. 96-hr LC50 and mortality curve for juvenile fathead minnows exposed to Red River water fortified with NH₃ (T7).

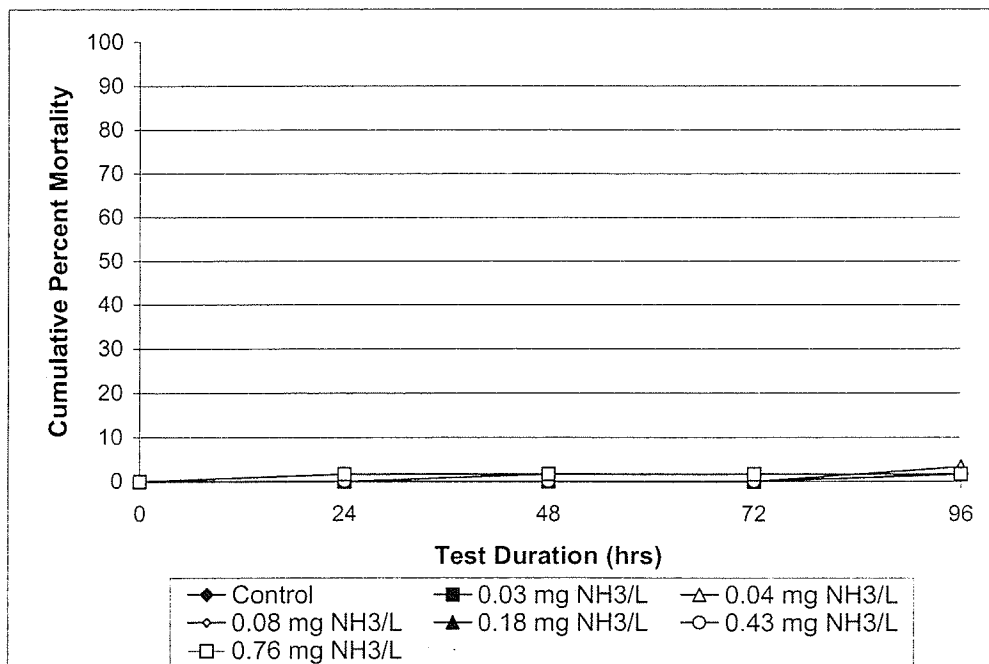
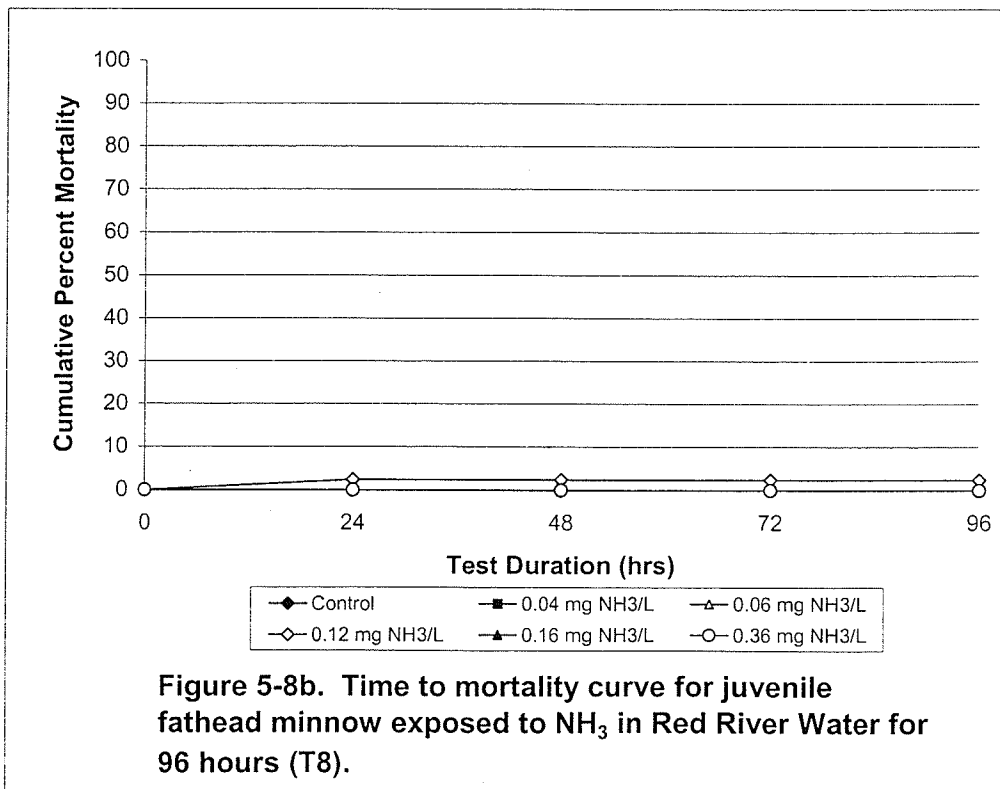
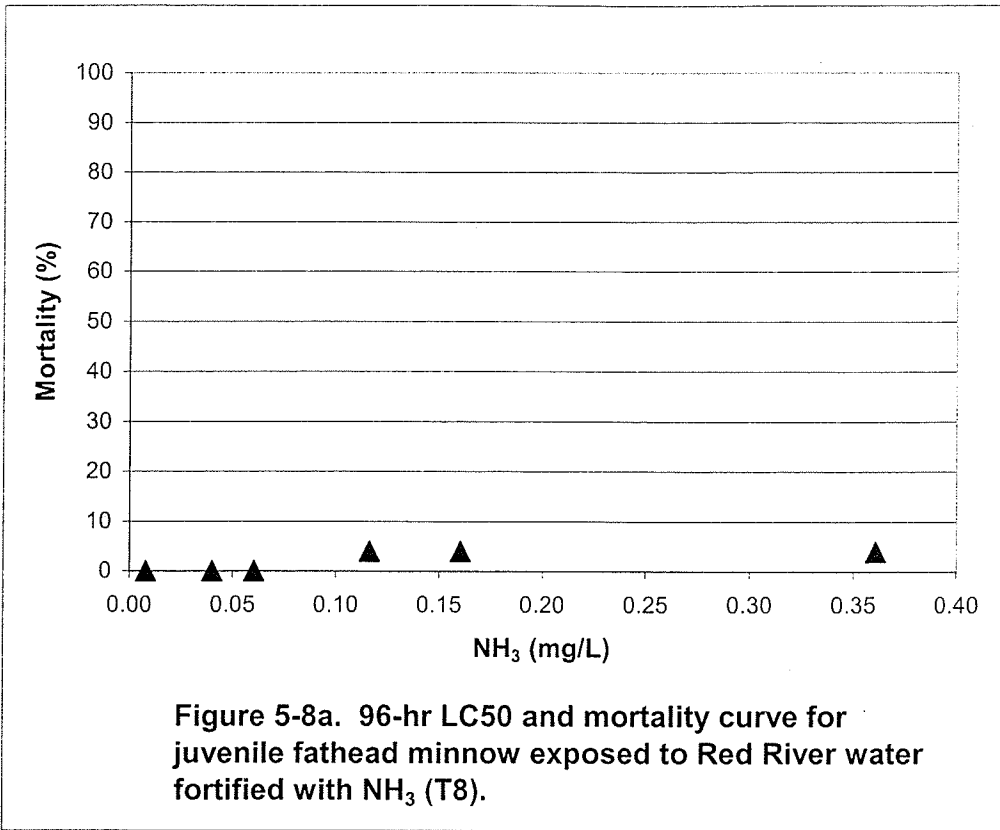


Figure 5-7b. Time to mortality curve for juvenile fathead minnow exposed to NH₃ in Red River water for 96 hours (T7).



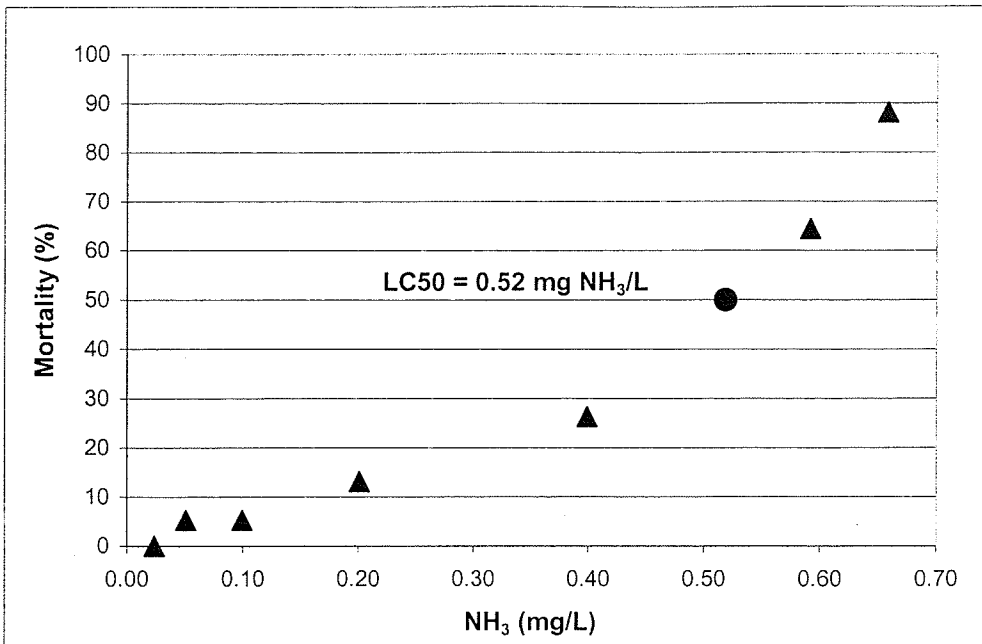


Figure 5-9a. 96-hr LC50 and mortality curve for larval northern pike exposed to Red River water fortified with NH₃ (T9).

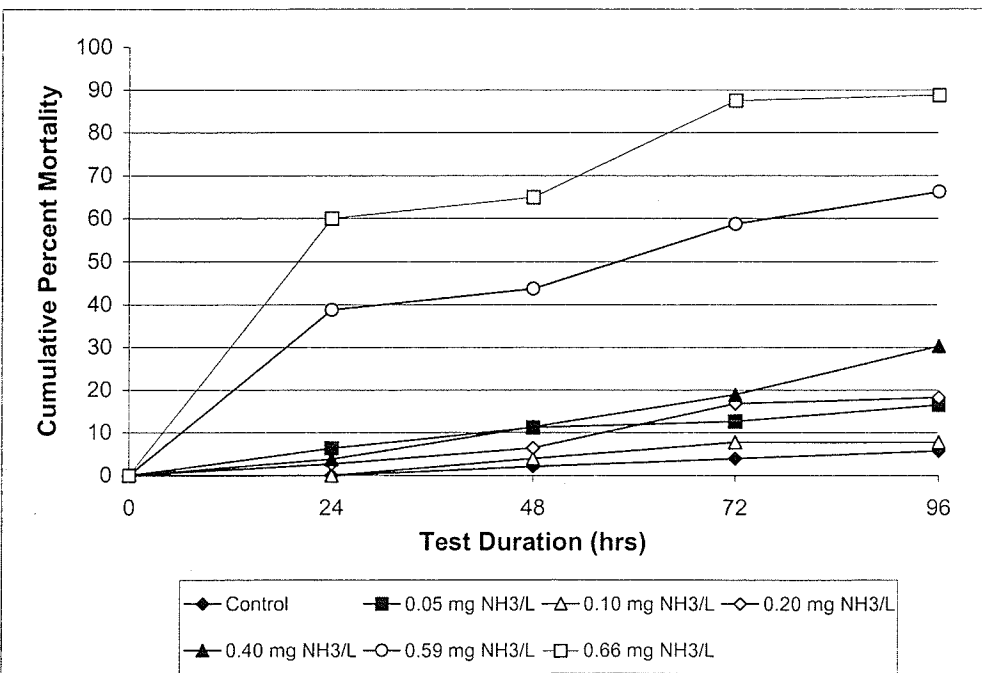
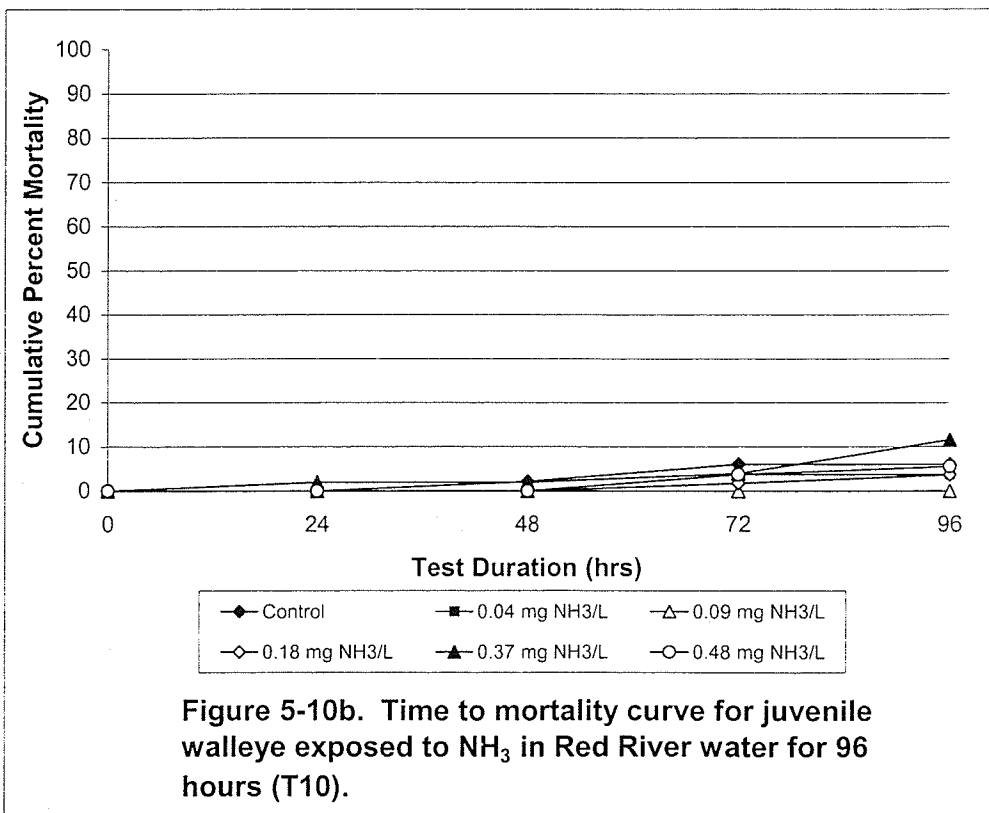
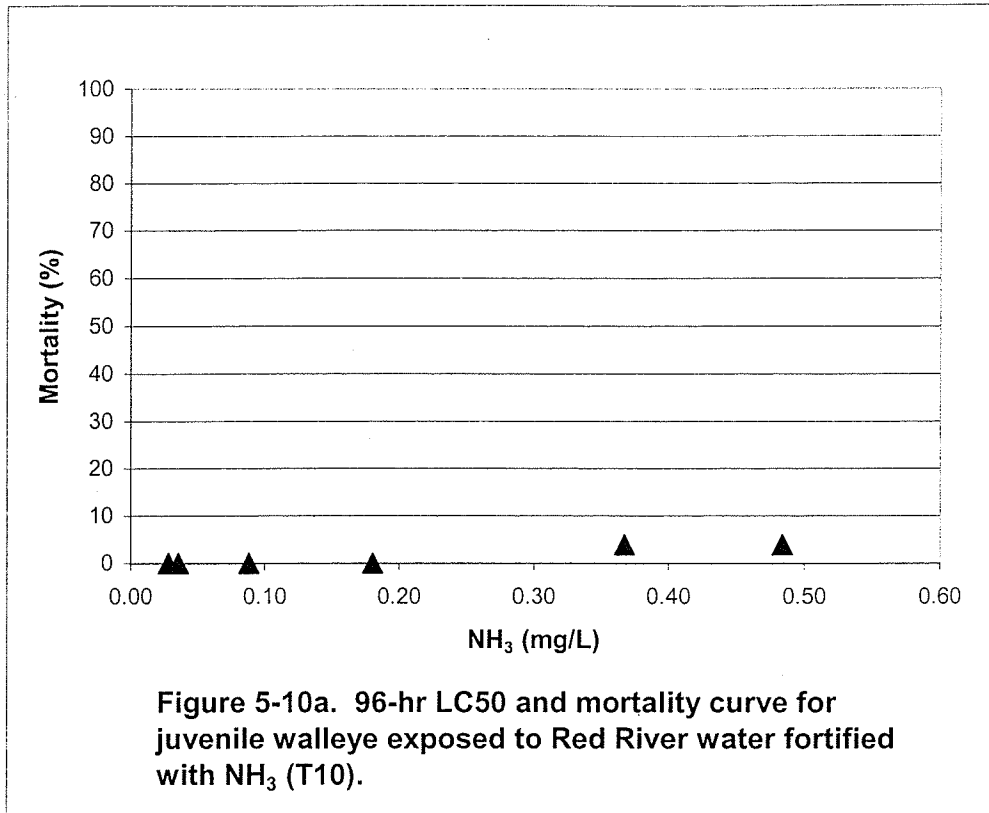


Figure 5-9b. Time to mortality curve for larval northern pike exposed to NH₃ in Red River Water for 96 hours (T9).



fathead minnows; and 0.48 mg NH₃/L for Test 10 with walleye) (c.f., Table 5-1). Juvenile channel catfish also experienced low mortality rates of less than 10% in four of six exposure concentrations (Figures 5-6a and 5-6b). All tests conducted with larval fish rather than older fish produced greater mortalities at lower toxicant concentrations as shown in Figures 5-1b-5-6b, and 5-9b. Therefore, the age class of tested fish may have been the most influential factor governing their sensitivities to NH₃.

5.2 Discussion of acute-exposure test results

Channel catfish

Juvenile channel catfish were the most tolerant fish tested in the present study yielding an LC50 of 0.69 mg NH₃/L [95% confidence intervals (C.I.) could not be generated using the Bootstrap method, Table 5-1]. Similar studies reported 96-hour LC50 values ranging from 0.5 to 3.8 mg NH₃/L (Colt and Tchobanoglous 1976, 1978, Swigert and Spacie 1983, Arthur et al. 1987, DeGraeve et al. 1987). Although other reported LC50s bracket the LC50 generated with channel catfish exposed to NH₃-fortified Red River water, the public literature generally suggests that channel catfish can tolerate higher levels of ammonia (i.e., up to 5.5 times greater) than results of the present study indicate (Table 5-2). There are several possible explanations for this difference, each of which are described below.

Table 5-2. Comparisons between public domain results and results of the present study for acute-exposure NH₃ toxicity tests.

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp. (°C)	Avg. test-pH	96-hr LC50 ^b (mg/L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Channel catfish	Juvenile	5.0	Red River, Winnipeg, MB	Flow-through	10.2	8.4	0.69	Present study	
	Juvenile	5.8	Mississippi River, Monticello, MN	Flow-through	3.5	8.0	0.50	Arthur et al. (1987)	Lower
		6.4			14.6	8.1	0.98		Higher
		3.5			19.6	7.8	1.29		Higher
	Juvenile	3-4	Not reported	Static	22	8.7	2.4	Colt and Tchobanoglous (1976)	Higher
					26	8.7	2.9		Higher
					30	8.7	3.8		Higher
	Juvenile	1-1.4	Well-water	Flow-through	28	8.36	1.95 ^b	Colt and Tchobanoglous (1978)	Higher
	Juvenile	Not reported	Well water	Flow-through	6	7.40	0.44	DeGraeve et al. (1987)	Lower
					10	7.44	0.81		Higher
					15	7.40	0.96		Higher
					20	7.42	1.10		Higher
					20	7.44	0.97		Higher
					25	7.38	1.50		Higher
					25	7.35	1.53		Higher
30					7.36	1.72	Higher		
Juvenile	0.5	Well water	Flow-through	25.7	7.8	1.45 ^b	Swigert and Spacie (1983)	Higher	

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp. (°C)	Avg. test-pH	96-hr LC50 ^b (mg/L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Fathead minnow	Larval	Not measured	Red River, Winnipeg, MB	Semi-static	20.7	8.6	0.36	Present study	
	Juvenile	1.3		Flow-through	9.8	8.5	>0.76		
		0.47			9.4	8.5	>0.36		
	Adults	1.8	Mississippi River, Monticello, MN	Flow-through	12.1	8.1	1.83	Arthur et al. (1987)	Higher, indeterminable, indeterminable
		1.6			17.1	8.0	1.97		Higher, indeterminable, indeterminable
		1.9			3.4	7.9	2.41		Higher, indeterminable, indeterminable
		1.7			26.1	8.1	2.55		Higher, indeterminable, indeterminable
	Not reported	Not reported	Well water	Flow-through	14	8.1	1.59	DeGraeve et al. (1980)	Higher, indeterminable, indeterminable
	Juvenile	Not reported	Well water	Flow-through	6	7.40	0.46	DeGraeve et al. (1987)	Higher, lower, indeterminable
					10	7.44	0.66		Higher, lower, indeterminable
					15	7.40	0.65		Higher, lower, indeterminable
					20	7.42	0.96		Higher, indeterminable, indeterminable
					20	7.44	0.90		Higher, indeterminable, indeterminable
25					7.38	1.60	Higher, indeterminable, indeterminable		

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp. (°C)	Avg. test-pH	96-hr LC50 ^b (mg/L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Fathead minnows	Juvenile	Not reported	Well water	Flow-through	25	7.35	1.40	DeGraeve et al. (1987)	Higher, indeterminable, indeterminable
					30	7.36	1.69		Higher, indeterminable, indeterminable
	Juvenile	0.28	Tittabawasee River, MI	Flow-through	22.0	8.07 ^e	1.82 ^d	Mayes et al. (1986)	Higher, indeterminable, indeterminable
	Larval	Not reported	St. Vrain River, CO	Flow-through	19.8	7.8 ^e	0.94	Nimmo et al. (1989)	Higher, indeterminable, indeterminable
					20.6	7.9 ^e	1.12		Higher, indeterminable, indeterminable
					6.2	8.2 ^e	0.19		Lower, lower, lower
					19.6	8.1 ^e	1.40		Higher, indeterminable, indeterminable
	Juvenile		St. Vrain River, CO	Flow-through	5.8	8.1 ^e	0.40		Higher, lower, indeterminable
					6.2	8.2 ^e	0.30		Lower, lower, lower
	Juvenile	0.2	Well water	Flow-through	25.9	7.78	1.75 ^d	Swigert and Spacie (1983)	Higher, indeterminable, indeterminable
		0.5			25.6	7.80	1.87 ^d		Higher, indeterminable, indeterminable
	Adults	1.9	Ground-water spring	Flow-through	13.0	6.51	0.240	Thurston et al. (1981b)	Lower, lower, lower
13.8					7.01	0.452	Higher, lower, indeterminable		
12.0					7.82	1.078	Higher, indeterminable, indeterminable		

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp. (°C)	Avg. test-pH	96-hr LC50 ^b (mg/L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Fathead minnow	Adults	1.9	Ground-water spring	Flow-through	11.8	7.83	0.793	Thurston et al. (1981b)	Higher, indeterminable, indeterminable
					13.5	8.51	1.676		Higher, indeterminable, indeterminable
					13.2	9.03	1.469		Higher, indeterminable, indeterminable
	Larval/Juvenile	0.09	Ground-water spring	Flow-through	16.3	7.91	1.50	Thurston et al. (1983)	Higher, indeterminable, indeterminable
					13.1	7.89	1.10		Higher, indeterminable, indeterminable
					13.6	7.64	0.754		Lower and higher
					13.5	7.68	0.908		Higher, indeterminable, indeterminable
		0.13	0.19	0.22	22.1	8.03	2.73		Higher, indeterminable, indeterminable
					22.0	8.06	2.59		Higher, indeterminable, indeterminable
					13.9	7.67	0.832		Higher, indeterminable, indeterminable
					13.0	8.05	2.33		Higher, indeterminable, indeterminable
	0.31	0.35	0.42	13.6	8.05	2.17	Higher, indeterminable, indeterminable		
				19.1	7.94	1.61	Higher, indeterminable, indeterminable		
				19.0	7.76	1.27	Higher, indeterminable, indeterminable		

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp. (°C)	Avg. test-pH	96-hr LC50 ^b (mg/L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Fathead minnow	Juvenile	0.47	Ground-water spring	Flow-through	13.4	7.66	0.775	Thurston et al. (1983)	Higher, indeterminable, indeterminable
		0.47			15.8	7.87	1.51		Higher, indeterminable, indeterminable
		0.5			22.0	7.83	1.85		Higher, indeterminable, indeterminable
		0.8			18.9	7.91	1.73		Higher, indeterminable, indeterminable
		1.0			14.3	7.77	1.22		Higher, indeterminable, indeterminable
	Adults	1.4			14.1	7.77	1.31		Higher, indeterminable, indeterminable
		1.4			22.4	8.04	2.16		Higher, indeterminable, indeterminable
		1.4			21.4	8.08	2.73		Higher, indeterminable, indeterminable
		1.4			21.4	8.16	3.44		Higher, indeterminable, indeterminable
		1.4			21.7	7.88	2.04		Higher, indeterminable, indeterminable
		1.4			12.9	7.68	1.23		Higher, indeterminable, indeterminable
		1.4			12.3	7.74	1.10		Higher, indeterminable, indeterminable
		1.4			13.2	7.63	1.10		Higher, indeterminable, indeterminable
		1.4							Higher, indeterminable, indeterminable

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp. (°C)	Avg. test-pH	96-hr LC50 ^b (mg/L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Fathead minnow	Adults	1.4	Ground-water spring	Flow-through	11.7	7.62	0.983	Thurston et al. (1983)	Higher, indeterminable, indeterminable
		1.5			13.6	7.93	1.37		Higher, indeterminable, indeterminable
		1.5			12.6	7.77	1.45		Higher, indeterminable, indeterminable
		1.5			12.5	7.83	1.12		Higher, indeterminable, indeterminable
		1.5			12.9	7.76	1.73		Higher, indeterminable, indeterminable
		1.7			21.7	7.84	2.03		Higher, indeterminable, indeterminable
		2.0			16.0	7.90	0.952		Higher, indeterminable, indeterminable
		2.0			15.5	7.92	1.18		Higher, indeterminable, indeterminable
		2.1			13.1	7.76	1.09		Higher, indeterminable, indeterminable
		2.2			12.8	7.74	0.796		Higher, indeterminable, indeterminable
		2.3			15.9	7.91	1.34		Higher, indeterminable, indeterminable
Northern Pike	Larval	Not measured	Red River, Winnipeg, MB	Flow-through	16.7	8.5	0.52	Present study	
Walleye	Larval	Not measured	Red River, Winnipeg, MB	Static	15.6	8.3	0.33	Present study	

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp. (°C)	Avg. test-pH	96-hr LC50 ^b (mg/L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Walleye	Juvenile	0.30-0.50	Not measured	Flow-through	17.9	8.4	>0.48	Present study	
	Juvenile	13.4	Mississippi River, Monticello, MN	Flow-through	19.0	8.3	0.51	Arthur et al. (1987)	Higher, indeterminable
		22.6			3.7	7.9	0.52		Higher, indeterminable
		19.4			11.1	7.7	1.10		Higher, indeterminable
Juvenile	3.0	Tittabawasee River, MI	Flow-through	21.5	8.03	1.26 ^d	Mayes et al. (1986)	Higher, indeterminable	
White Sucker	Larval	Not measured	Red River, Winnipeg, MB	Static	15.5	8.3	0.49	Present study	
				Semi-static	15.5	8.2	0.22		
					17.5	8.2	0.52		
	Juvenile	5.6	Mississippi River, Monticello, MN	Flow-through	3.6	7.8	0.76	Arthur et al. (1987)	Higher, higher, higher
		12.6			12.6	8.2	1.73		Higher, higher, higher
		5.2			11.3	8.1	1.87		Higher, higher, higher
		9.6			15.3	8.2	2.22		Higher, higher, higher
Adult	Not reported	St. Vrain River, CO	Flow-through	20.2	7.8	>0.94	Nimmo et al. (1989)	Higher, higher, higher	
Juvenile	11.4	Well water	Flow-through	22.5	7.8	0.79 ^b	Swigert and Spacie (1983)	Higher, higher, higher	

Notes:

- a As much as possible, age classes reported in the public literature were recorded in Table 5-2. When age classes were not reported, they were determined using length or weight data measured at the beginning of each test in combination with length, weight and age of sexual maturation information provided in Scott and Crossman (1973).
- b LC50 = The concentration of NH₃ that was lethal to 50% of the exposed-fish.

- c In cases where more than one test was conducted per species, each test-result was compared to the public domain values in the order that they appear in Table 5-2. For example, three white sucker tests were conducted under site-specific conditions and have been compared with results reported by Arthur et al. (1987). For all three tests, the white sucker were more sensitive to NH_3 than those tested by Arthur et al. (1987) so “higher, higher, higher” is recorded in the final column of the Table 5-2.
- d LC50 values were reported as $\text{NH}_3\text{-N}$ in the literature; they have been converted to NH_3 in this thesis.
- e Median pH recorded instead of average pH.

Colt and Tchobanoglous (1978) found that the sensitivity of juvenile channel catfish to NH_3 increased by a factor of 1.5 to 2.4 compared to a similar study conducted two years earlier (i.e., Colt and Tchobanoglous 1976). The fish used during the 1976 study weighed between 3 g and 4 g whereas those used in 1978 weighed only 1 g. Although Colt and Tchobanoglous (1978) stated that it was unknown whether size and age are factors that can influence ammonia toxicity, reviews by the US EPA (1998, 1999) have reported that younger fish may be more sensitive to NH_3 than older ones. Larger (and presumably older) fish were used in the present study compared with those tested by Colt and Tchobanoglous in both 1976 and 1978 (c.f., Table 5-2), and so their heightened sensitivity is not a matter of size differences.

Colt and Tchobanoglous (1978) suggested that fish tested under flow-through conditions might be more susceptible to the toxic effects of NH_3 than those tested under static conditions. Lloyd and Orr (1969) reported that handling stress induces a diuretic response in fish that may help them to acclimate to subsequent ammonia exposures by increasing NH_3 outputs to ambient water via urine excretion. Since Colt and Tchobanoglous (1978) believed that handling stress is greater for fish tested under static conditions than flow-through conditions, they claimed that fish might be more tolerant to elevated ambient ammonia concentrations when held under static conditions. Whether or not fish undergo more handling stress under static conditions than flow-through conditions is debatable, but there appears to be a correlation between the tolerance of

channel catfish to NH_3 and the type of test used to measure NH_3 toxicity. Table 5-2 shows that juvenile channel catfish tested under static conditions by Colt and Tchobanoglous (1976) yielded higher LC50s than fish tested by other researchers under flow-through conditions. However, not only did Colt and Tchobanoglous (1976) use static conditions, but they also tested the fish at high pHs (i.e., pH = 8.7). NH_3 toxicity decreases with increasing pH (reviewed in Section 2.5) and may have been an influential factor in enabling fish used by Colt and Tchobanoglous (1976) to withstand higher NH_3 concentrations.

The relatively low LC50 value obtained in the present study compared with the public literature is likely a result of differences in test temperatures. The optimal temperature for channel catfish growth is 28°C (reviewed by Colt and Tchobanoglous 1978). In this study, channel catfish were tested at 10.2°C whereas Colt and Tchobanoglous (1976 and 1978) maintained test temperatures closer to the optimum for that species (i.e., 22°C , 26°C , 28°C and 30°C). Arthur et al. (1987) exposed juvenile channel catfish to several NH_3 concentrations at 3.5°C , 14.6°C and 19.6°C and obtained LC50 values of 0.5 mg/L, 0.98 mg/L and 1.29 mg/L, respectively (c.f., Table 5-2). These results are in good agreement with those obtained in the present study since an LC50 of 0.69 mg/L generated at a test temperature of 10.2°C is bracketed by the LC50s generated by Arthur et al. (1987) at lower and higher temperatures. Similarly, DeGraeve et al. (1987) conducted tests at cold water temperatures of 6°C and 10°C and generated LC50s marginally different from that produced in the present study (i.e., 0.44

mg/L at 6°C and 0.81 mg/L at 10°C versus 0.69 mg/L at 10.2°C). Since NH₃ toxicity increases with decreasing pH, and since DeGraeve et al. (1987) tested their fish at relatively low pHs (i.e., 7.46), one would have expected DeGraeve's LC50 at 10°C to have been lower than 0.69 mg/L, the value obtained in the present study at 10.2°C.

Fathead minnow

Three tests were completed in the present study with fathead minnows of larval and juvenile age classes and, as mentioned above, only one test (i.e., Test 5 with larval fish) produced a point estimate of the LC50 at 0.36 mg NH₃/L (95% C.I., 0.03-0.58). The two juvenile fathead minnow tests (i.e., Tests 7 and 8) produced indeterminate LC50 values greater than 0.76 and 0.36 mg NH₃/L, respectively (c.f., Table 5-1). LC50 values for 33 acute toxicity tests conducted on larval and juvenile fathead minnows and reported in the public domain range between 0.19 and 2.73 mg NH₃/L (DeGraeve et al. 1980, Thurston et al. 1981b, Swigert and Spacie 1983, Thurston et al. 1983, Mayes et al. 1986, Arthur et al. 1987, DeGraeve et al. 1987, and Nimmo et al. 1989) (c.f., Table 5-2). Of this dataset, one group of juvenile and one group of larval fathead minnows tested under low temperatures (i.e., 6.2°C, Nimmo et al. 1989) (c.f., Table 5-2) were more sensitive to NH₃ exposure than locally tested fish. At temperatures similar to those used in the present study, (i.e., approximately 20°C), larval fish tested by Nimmo et al. (1989) were at least 2.5 times more tolerant to NH₃-exposure than those tested locally. This higher degree of tolerance was observed in spite of the

fact that Nimmo et al. (1989) tested larval fathead minnows at pHs that were up to 0.8 pH units lower than the pHs maintained in the present study (i.e., 7.8 versus 8.6). Since the locally tested juveniles did not have a mortality response great enough to generate LC50 values, it is impossible to compare their susceptibility to NH_3 with that reported in the public domain.

Northern pike

A single test with larval northern pike exposed to NH_3 -fortified Red River water generated an LC50 of 0.52 mg NH_3 /L (95% C.I., 0.48-0.57). No comparisons can be made with other studies for this species since it has not been studied elsewhere.

Walleye

50% of the locally tested larval walleye (i.e., Test 1) died at a concentration of 0.33 mg NH_3 /L (95% C.I., 0.30-0.40), but the juvenile walleye were not acutely affected by NH_3 up to a concentration of 0.48 mg/L, the highest concentration tested (c.f., Table 5-1). Similar studies conducted on larger, juvenile walleye generated LC50 values ranging from 0.50 mg NH_3 /L to 1.26 mg NH_3 /L (Mayes et al. 1986, Arthur et al. 1987) (c.f., Table 5-2).

White sucker

Three tests conducted with larval white sucker (i.e., Tests 2, 3A, and 4A) produced LC50 values of 0.49 mg NH_3 /L (95% C.I., 0.42-0.79), 0.22 mg NH_3 /L

(95% C.I., 0.14-0.25) and 0.52 mg NH₃/L (95% C.I., 0.37-0.76), respectively (c.f., Table 5-1). The geometric mean of these three tests is 0.38 mg/L. All three tests were conducted under semi-static conditions with average temperatures of 15.5°C to 17.5°C and pHs of 8.2 to 8.3. Test-fish were obtained from the same stock and were of similar ages (c.f., Table 4-2).

Arthur et al. (1987) conducted four acute toxicity tests on juvenile white sucker exposed to NH₃ at various temperatures to determine the relative sensitivities of white sucker to the toxicant at ambient seasonal temperatures. Results of this test produced 96-hour LC50 ranging between 0.76 and 2.22 mg NH₃/L (c.f., Table 5-2). NH₃ toxicity generally increased with decreasing temperature. At a test temperature and pH similar to those used in this study (i.e., temp. = 15.3°C, pH = 8.2), juvenile white sucker were at least four times more tolerant to NH₃ than larval white sucker (i.e., LC50 for juvenile fish = 2.22 mg/L, LC50 for larval fish = 0.52 mg/L) (c.f., Table 5-2).

Adult white sucker exposed to NH₃ in river water from a Colorado stream did not have a mortality response great enough to define the LC50, which is reported by Nimmo et al. (1989) as >0.94 mg NH₃/L, the highest exposure concentration tested (c.f., Table 5-2).

5.3 Results of chronic-exposure testing

Six semi-static or flow-through chronic-exposure tests ranging in length between 10 and 30 days were completed using juvenile or larval channel catfish, fathead minnow, northern pike, walleye and white sucker (c.f., Appendix D). Survival and growth effects were measured for each test and EOT LC20 and EC20 values are summarized in Table 5-3 together with related environmental data. Survival responses over the range of NH_3 concentrations tested plus the ICPIN-generated LC20s are illustrated in Figures 5-11a to 5-16a. Time-to-mortality curves are illustrated in Figures 5-11b to 5-16b. Growth responses for each test are illustrated in Figures 5-17 to 5-22. The data used to calculate all LC20 and EC20 values are tabulated in Appendix J, Tables J-11 to J-16 and J-17 to J-22. ICPIN printouts are provided in Appendix K.

NH_3 was lethal to 20% of the juvenile channel catfish, larval northern pike, juvenile walleye, and larval white sucker test-populations at concentrations of 0.16 mg/L (95% C.I., 0.00-0.28), 0.13 mg/L (95% C.I., 0.01-0.27), 0.20 mg/L (95% C.I., indeterminable), and 0.36 mg/L (95% C.I., 0.00-0.77), respectively (c.f., Table 5-3). Mortalities of two groups of juvenile fathead minnows were too low to calculate LC20 values because less than 20% of the fish died at the highest concentrations tested. Presumably, the LC20 values for these two tests are greater than the most concentrated ammonia-test-solution (i.e., 0.58 mg NH_3 /L for Test 7 and 0.30 mg NH_3 /L for Test 8) (c.f., Table 5-3). These data

Table 5-3. Chronic-exposure toxicity of ammonia-fortified river water to five fish species: Test conditions and End-of-Test (EOT) LC20 and EC20^a values.

Species (Test No. – Age Class)	DO ^b mean (range) (mg/L)	DO ^b mean (range) (% saturation)	pH mean (range)	Temperature mean (range) (°C)	EOT LC20 (95% CI ^c) (mg NH ₃ /L)	EOT EC20 (95% CI ^c) (mg NH ₃ /L)
Channel catfish (6 – juvenile)	12.0 (7.70-14.4)	102.7 (69.0-115.7)	8.4 (7.9-8.7)	8.8 (5.5-13.2)	0.16 (0.00-0.28)	**
Fathead minnow (7 – juvenile)	12.3 (9.1-14.2)	104.4 (83.5-114.3)	8.4 (7.9-8.6)	8.3 (5.0-11.3)	>0.58 ^d	0.52 (--) ^e
Fathead minnow (8 – juvenile)	13.1 (10.0-14.8)	108.1 (87.5-117.1)	8.4 (8.3-8.6)	7.1 (4.0-12.3)	>0.30 ^d	**
Northern pike (9 – larval)	10.0 (5.2-10.9)	102.6 (55.5-112.9)	8.5 (7.8-8.6)	16.7 (16.2-18.3)	0.13 (0.01-0.27)	**
Walleye (10 – juvenile)	8.3 (6.5-10.0)	87.1 (71.5-106.7)	8.0 (7.7-8.5)	17.8 (15.4-20.2)	0.20 (--) ^e	**
White sucker (4A – larval)	6.6 (2.3-8.3)	69.5 (23.8-87.7)	8.2 (7.7-8.4)	17.4 (16.0-20.0)	0.36 (0.00-0.77)	**

Notes:

- a) EC20 values are based on growth measurements where dry weights at test-termination were used as growth metrics.
- b) DO = dissolved oxygen
- c) CI = confidence interval
- d) A point estimate of the lethal or effective NH₃ concentration could not be computed because the exposure concentrations did not bracket the expected response-mean. When survival is the endpoint, the highest concentration of NH₃ tested is reported instead, and it is expected that the actual LC20 value would be the greater value.
- e) A confidence interval could not be calculated because the number of re-samples that were generated using the Bootstrap Method is not a multiple of 40.

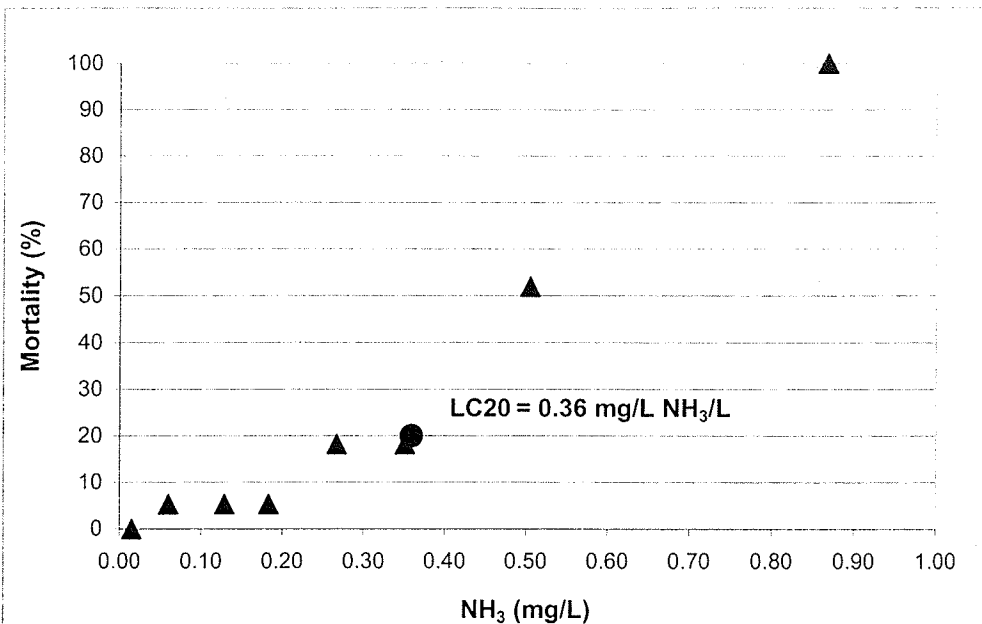


Figure 5-11a. 10-d LC20 and mortality curve for larval white sucker exposed to Red River water fortified with NH₃ (T4A).

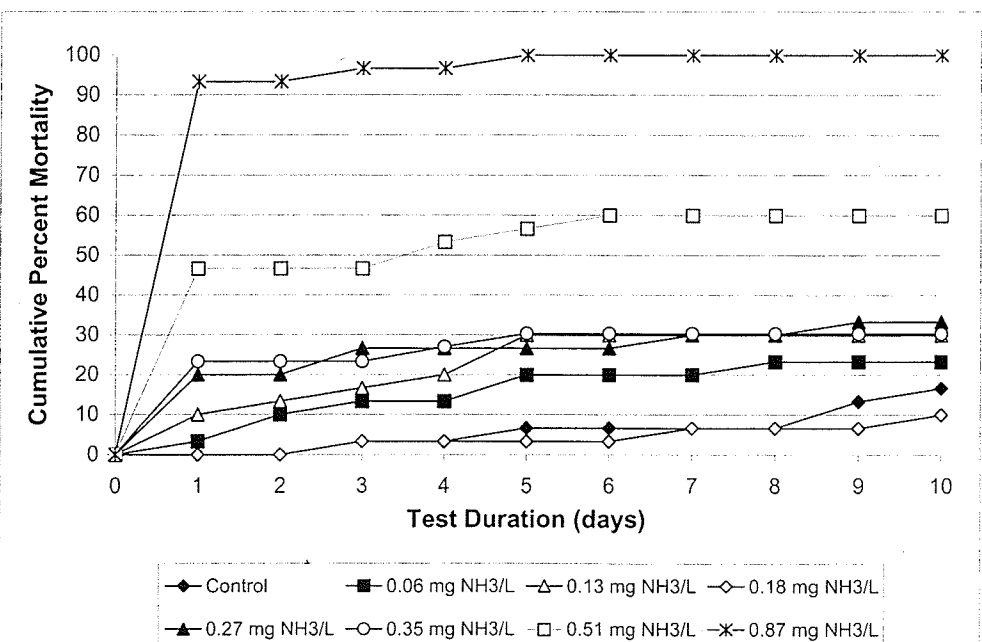


Figure 5-11b. Time to mortality curve for larval white sucker exposed to NH₃ in Red River Water for 10 days (T4A).

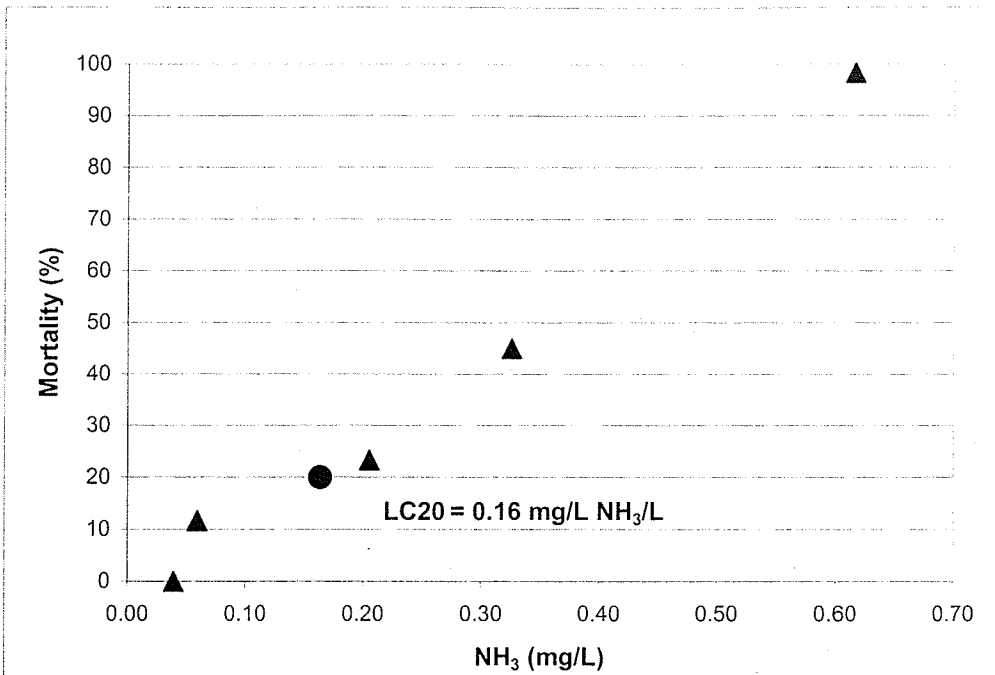


Figure 5-12a. 30-d LC20 and mortality curve for juvenile channel catfish exposed to Red River water fortified with NH₃ (T6).

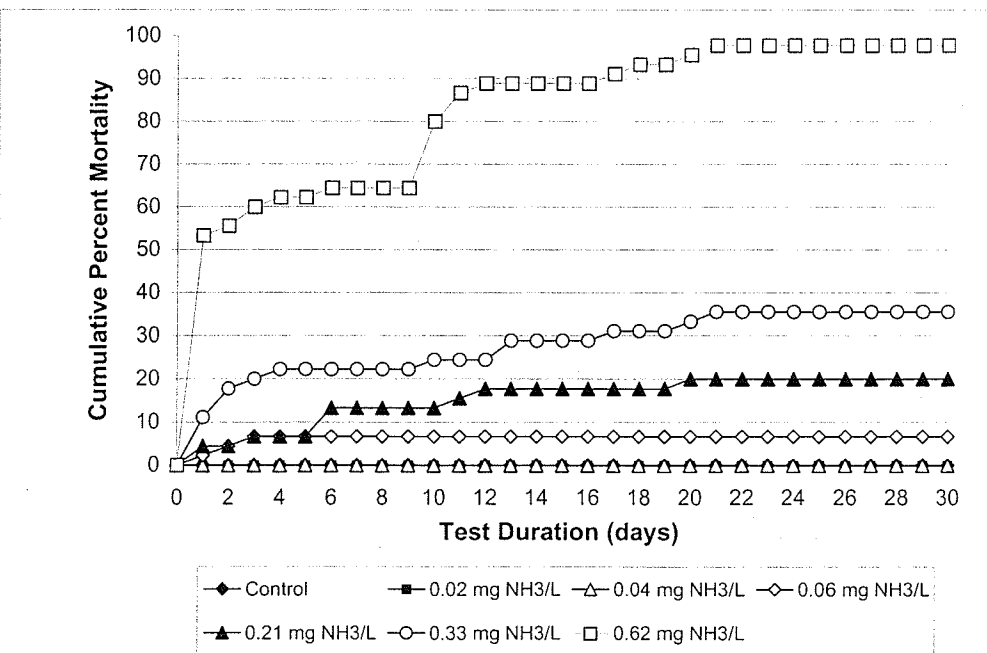


Figure 5-12b. Time to mortality curve for juvenile channel catfish exposed to NH₃ in Red River water for 30 days (T6).

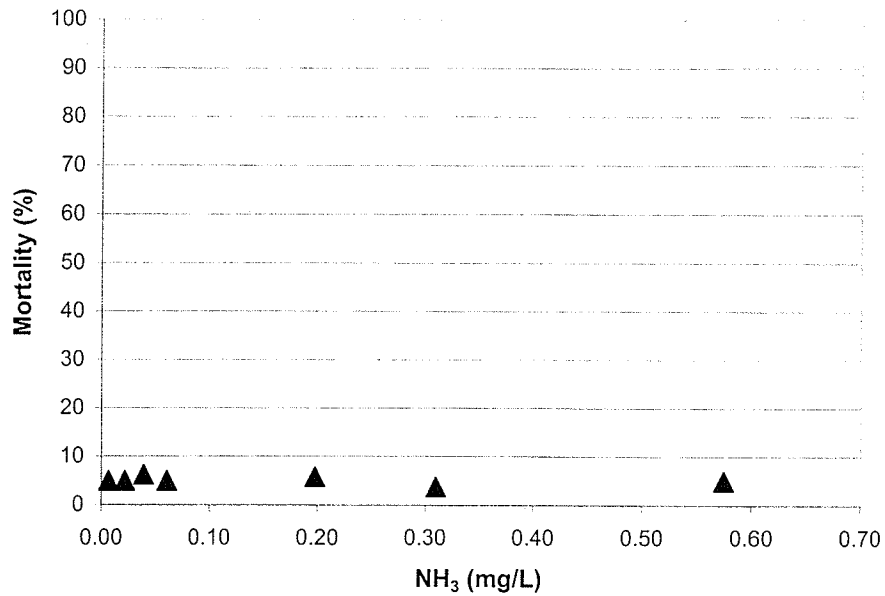


Figure 5-13a. Mortality curve for juvenile fathead minow exposed to Red River water fortified with NH₃ (T7).

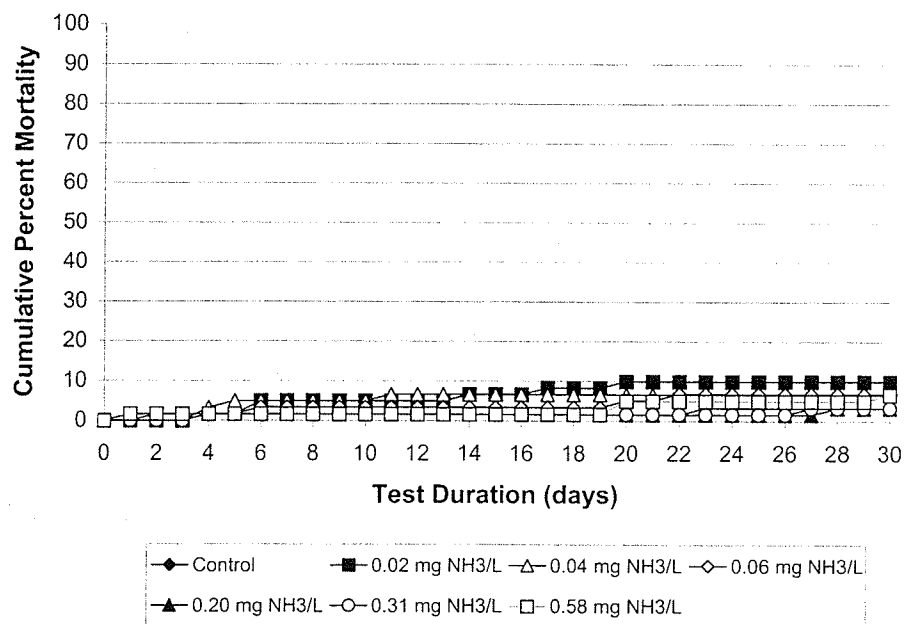


Figure 5-13b. Time to mortality curve for juvenile fathead minnow exposed to NH₃ in Red River Water for 30 days (T7).

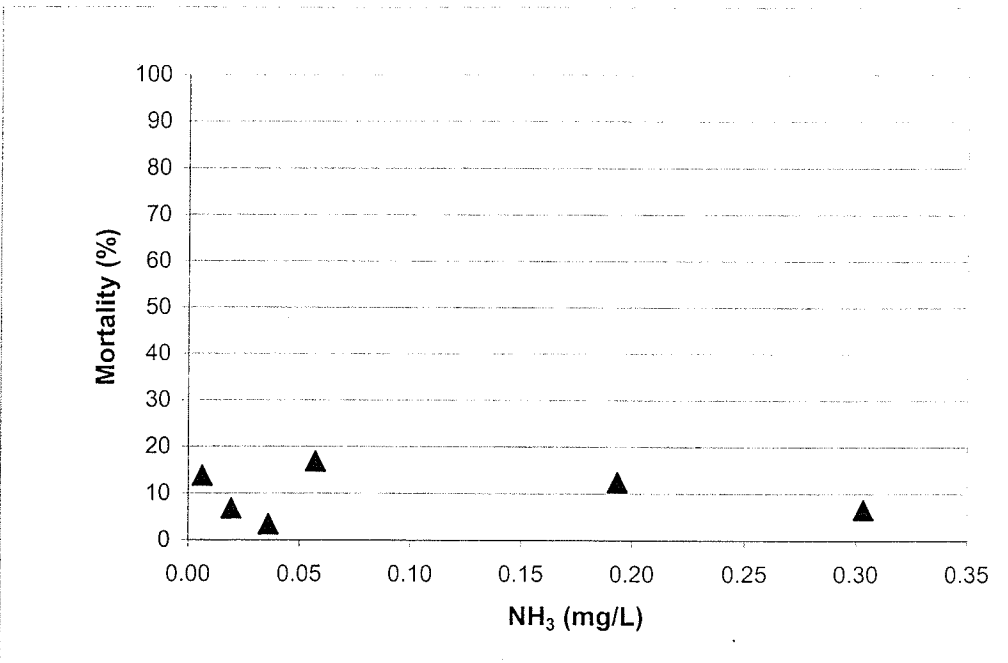


Figure 5-14a. 29-d LC20 and mortality curve for juvenile fathead minnow exposed to Red River water fortified with NH₃ (T8).

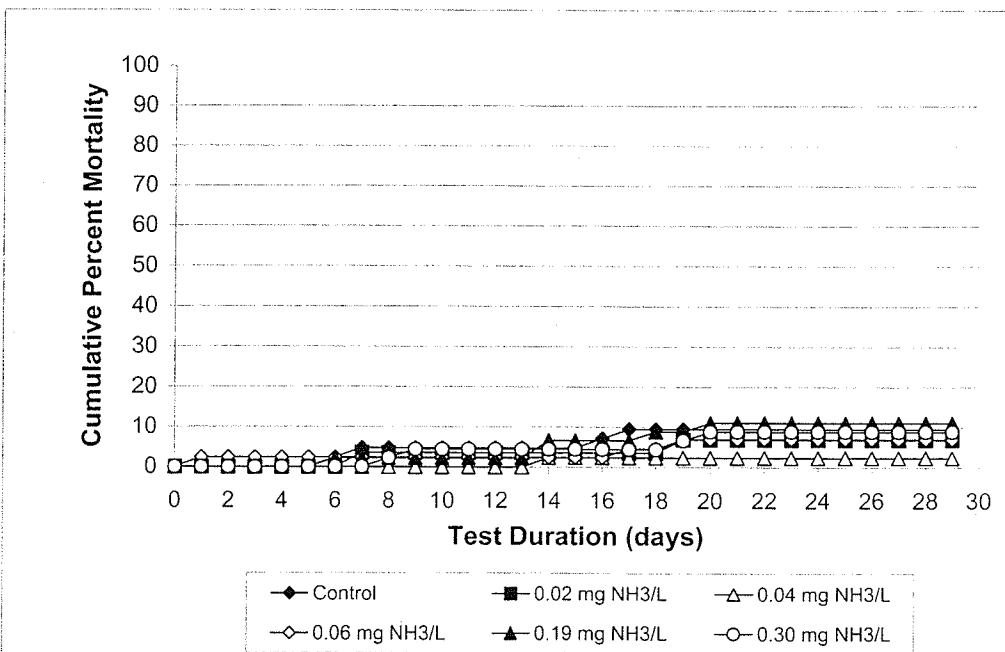


Figure 5-14b. Time to mortality curve for juvenile fathead minnow exposed to NH₃ in Red River water for 29 days (T8).

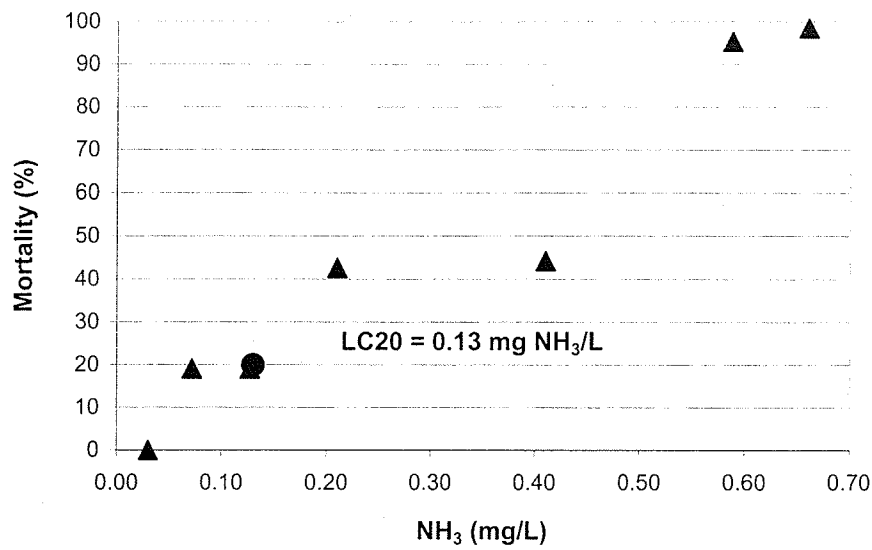


Figure 5-15a. 12-d LC_{20} and mortality curve for larval northern pike exposed to Red River water fortified with NH_3 (T9).

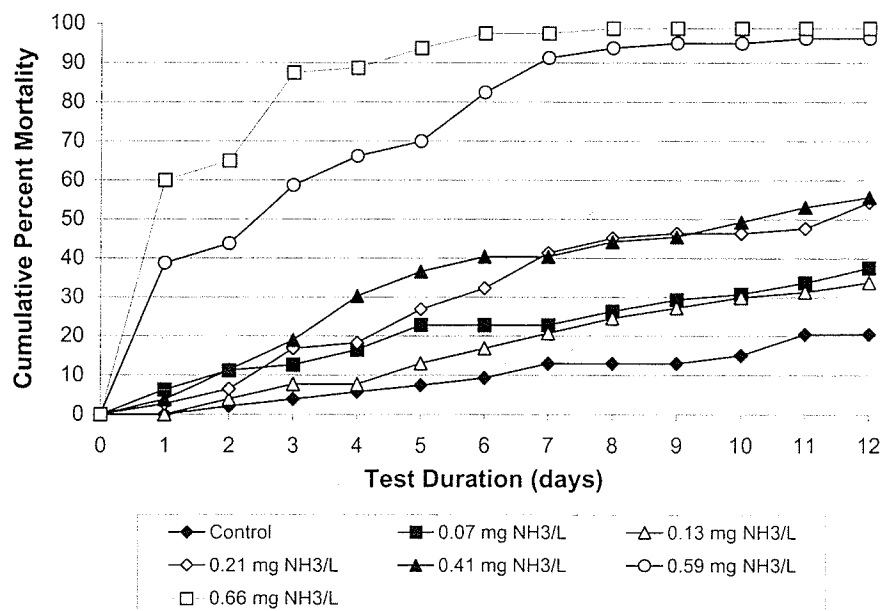


Figure 5-15b. Time to mortality curve for larval northern pike exposed to NH_3 in Red River Water for 12 days (T9).

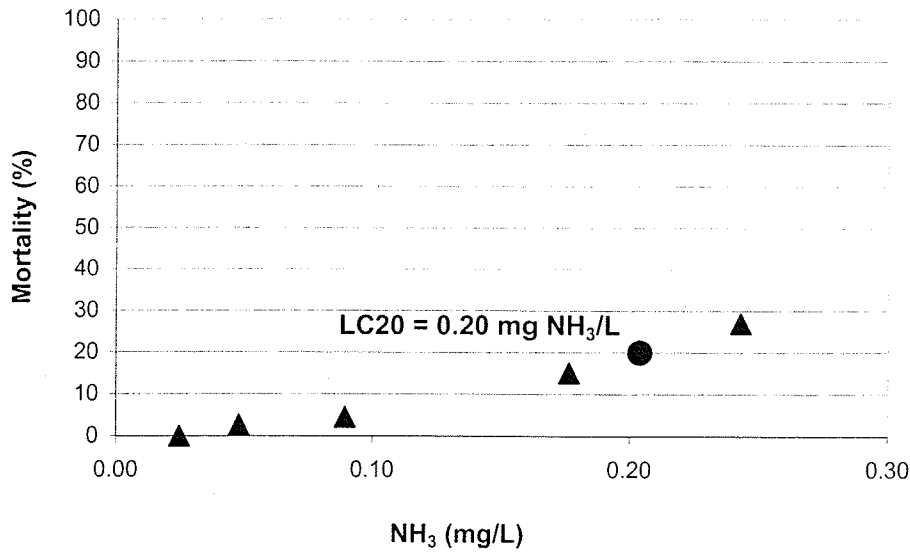


Figure 5-16a. 30-d LC20 and mortality curve for juvenile walleye exposed to Red River water fortified with NH₃ (T10).

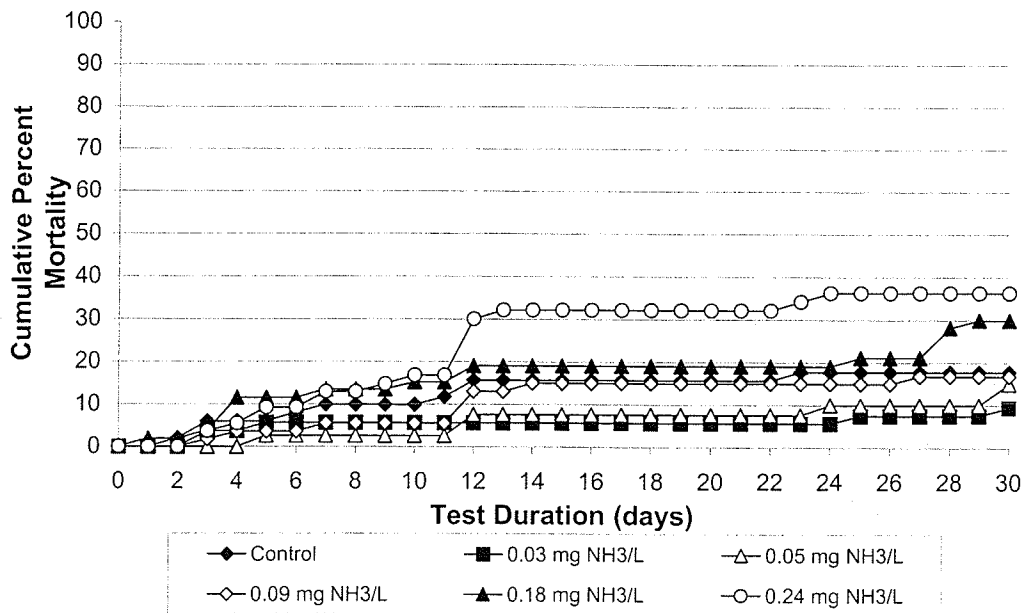


Figure 5-16a. Time to mortality curve for juvenile walleye exposed to NH₃ in Red River Water for 30 days (T10).

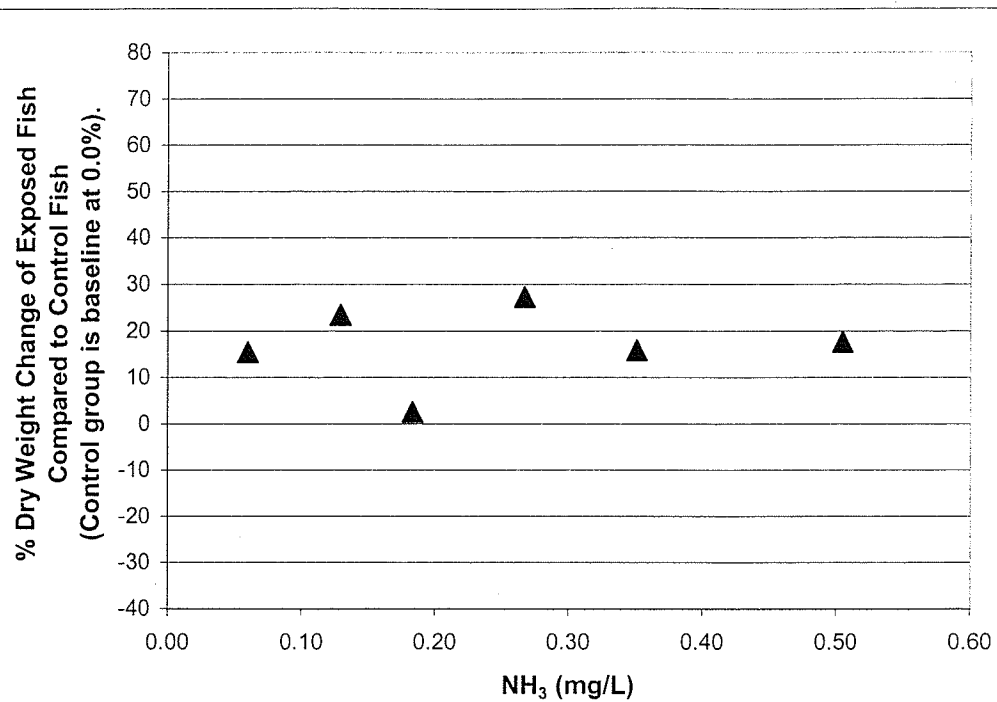


Figure 5-17. Growth of larval white sucker exposed to Red River water fortified with NH₃ (T4A).

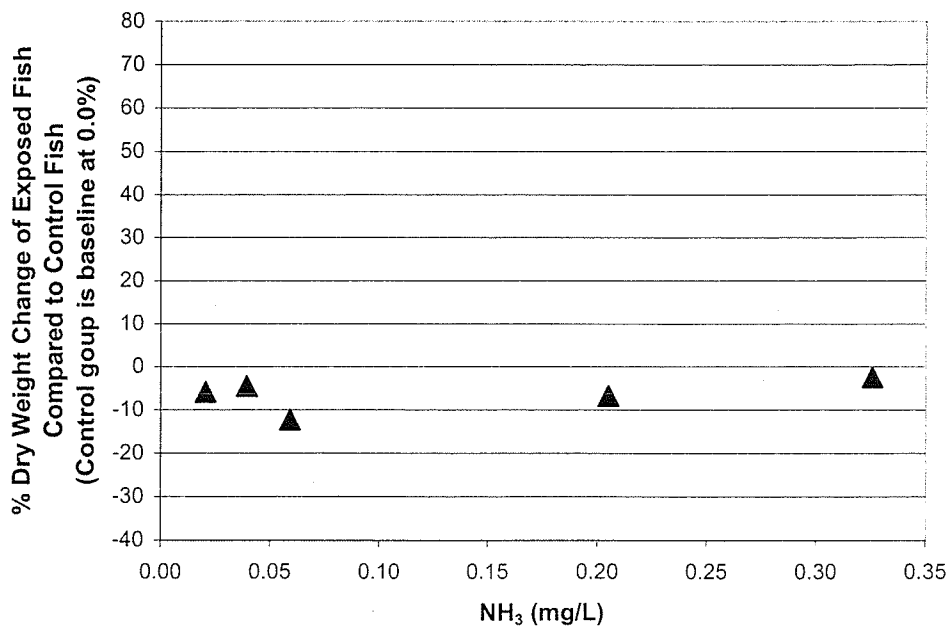
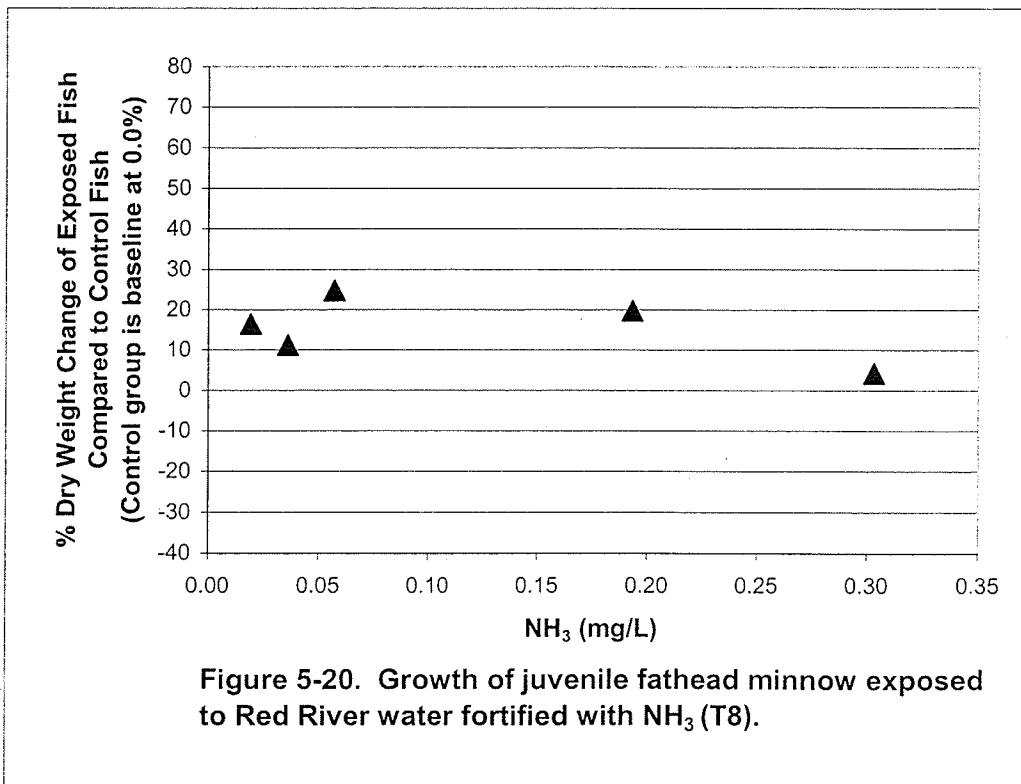
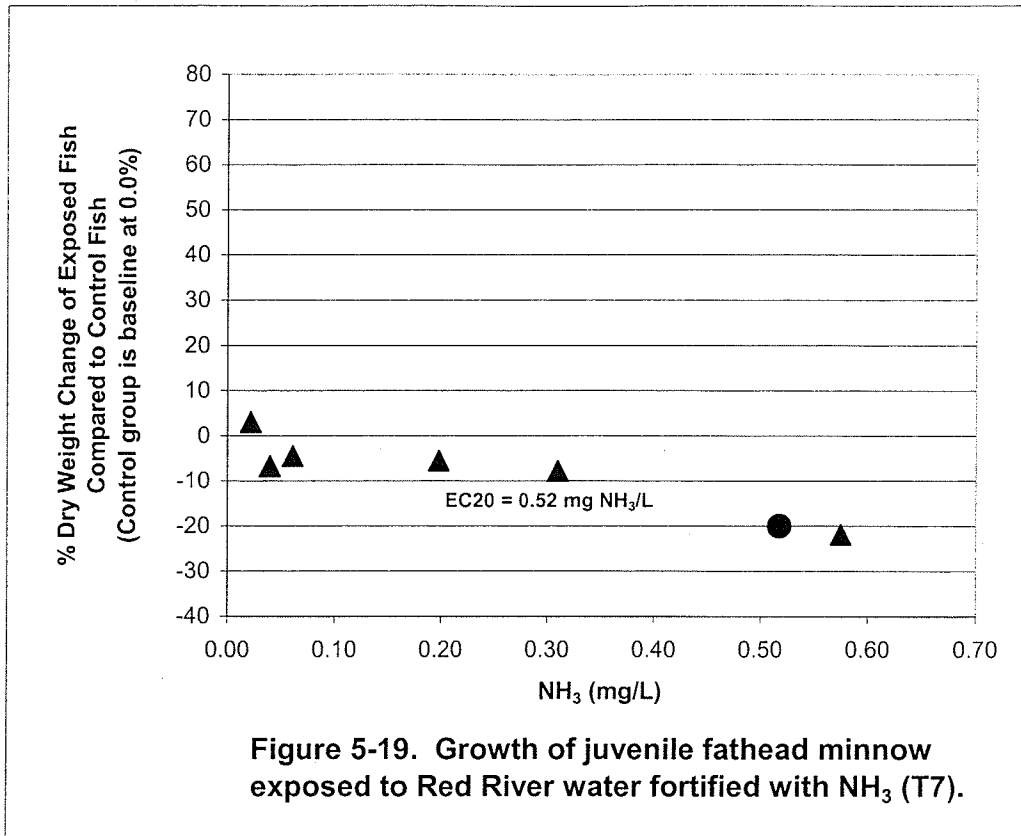


Figure 5-18. Growth of juvenile channel catfish exposed to Red River water fortified with NH₃ (T6).



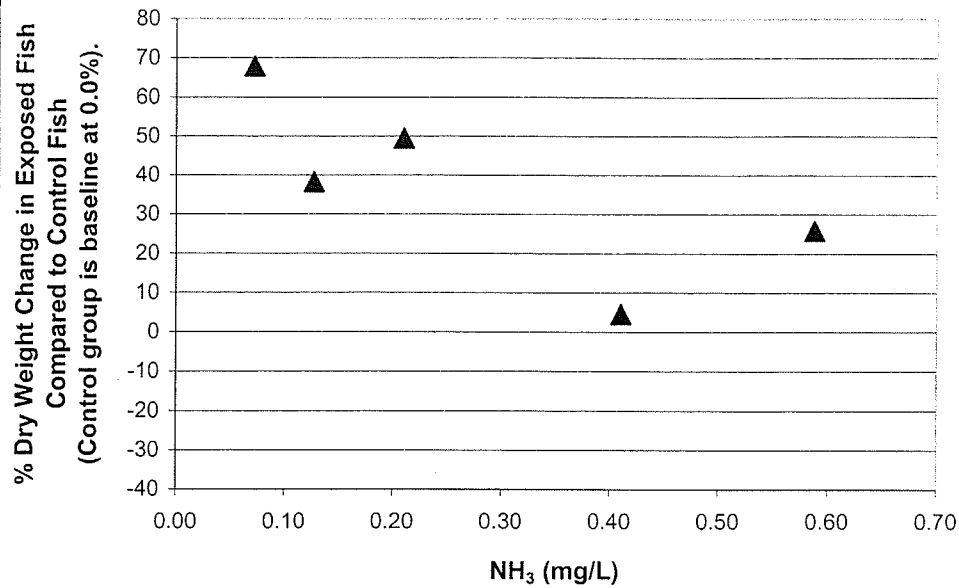


Figure 5-21. Growth of larval northern pike exposed to Red River water fortified with NH₃ (T9).

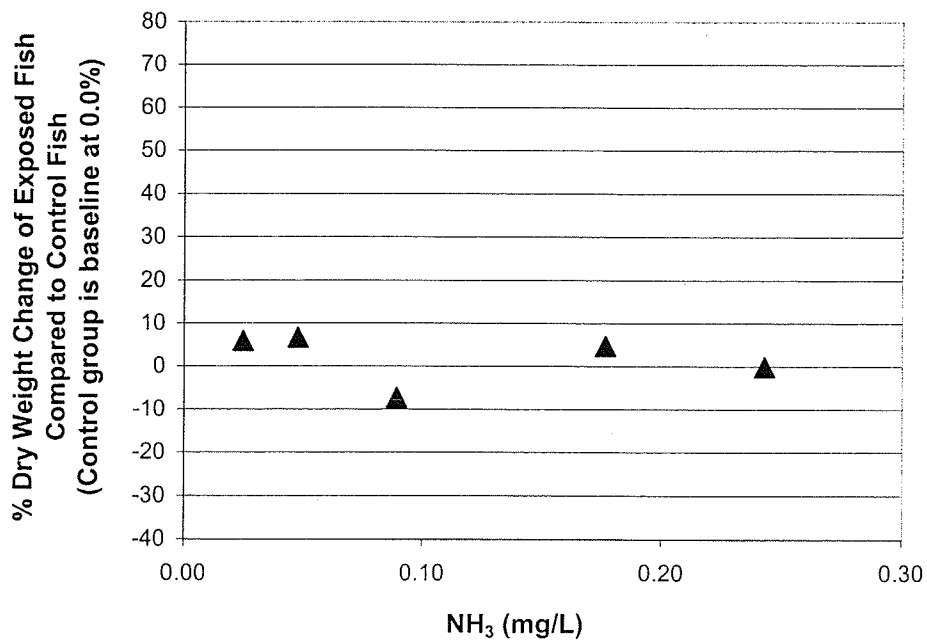


Figure 5-22. Growth of juvenile walleye exposed to Red River water fortified with NH₃ (T10).

suggest the following relative species sensitivity (statistical significance not determined because several variables differed among tests): larval northern pike > juvenile channel catfish > juvenile walleye > larval white sucker > juvenile fathead minnow.

Inhibitory growth effects (based on dry weight measurements) were found in one test (i.e., Test 7), during which locally collected juvenile fathead minnows were exposed to NH_3 for 30 days under flow-through conditions. Results of this test yielded an EC20 of 0.52 mg NH_3 /L (c.f., Table 5-3).

5.4 Discussion of chronic-exposure test results

Historically, results of chronic tests have been reported in the literature as either No Observable Effects Concentrations (i.e., NOEC) or Lowest Observable Effects Concentrations (i.e., LOEC) (US EPA 1999). The NOEC is the highest concentration at which toxicological responses of test-organisms are not statistically different from that of the control group. The LOEC is the lowest concentration at which a toxicological response of test-organisms is statistically different from that of the control group. To facilitate comparisons between datasets, tests conducted by other researchers whose chronic values were not already reported as LC20 or EC20 values were converted in this thesis using the raw data provided by the authors and the ICPIN program. Standardized test results are summarized in Table 5-4.

Table 5-4. Comparisons between public domain results and results of the present study for chronic-exposure NH₃ toxicity tests.

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp (°C)	Avg. test-pH	Test-duration (days)	Endpoint	96-hr EC20 or LC20 ^b (mg NH ₃ /L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Channel catfish	Juvenile	5.0	Red River, Winnipeg, MB	Flow-through	8.8	8.4	30	Survival	0.16	Present study	
	Juvenile	1-1.4	Well water	Flow-through	27.9	8.36	31	Survival	Between 0.95 and 1.20 ^d	Colt and Tchobanoglous (1978)	Higher
	Juvenile	Not reported	Well water	Flow-through	6	7.73	30	Survival	0.04 ^e	DeGraeve et al. (1987)	Lower
					10	7.61		Survival	0.02 ^e		Lower
					15	7.62		Survival	0.01 ^e		Lower
					15	7.48		Survival	>0.13 ^e		Indeterminable
					20	7.46		Survival	0.01 ^e		Lower
					20	7.27		Survival	>0.12 ^e		Indeterminable
					25	7.23		Survival	0.01 ^e		Lower
	30	7.14	Survival	>0.42 ^e	Higher						
Juvenile	6.4	Mississippi River	Experimental stream	18.2	7.5-8.1 ^f	117	Survival	>0.08-0.16 ^g	Hermanutz et al. (1987)	Indeterminable	
	8.0			16.8	7.6-8.1 ^f		36	Survival		>0.11-0.15 ^g	Indeterminable

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp (°C)	Avg. test-pH	Test-duration (days)	Endpoint	96-hr EC20 or LC20 ^b (mg NH ₃ /L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Channel catfish	Juvenile	18.8	Mississippi River	Experimental stream	21.1	8.1-8.2 ^f	89	Survival	Between 0.04-0.07 and 0.09-0.21 ^g	Hermanutz et al, (1987)	Indeterminable
	Larval	Not reported	Well water	Flow-through	26.0	7.82	30	Survival	>0.48	Reinbold and Pescitelli (1982)	Higher
	Larval	Not reported	Well water	Flow-through	26.9	7.78	30	Survival	0.55 ^e	Swigert and Spacie (1983)	Higher
	Juvenile	5.0	Red River, Winnipeg, MB	Flow-through	8.8	8.4	30	Growth	--^h	Present study	
	Juvenile	1-1.4	Well water	Flow-through	27.9	8.36	31	Growth	0.26 ^e (based on dry weights)	Colt and Tchobanoglous (1978)	Indeterminable
									0.30 ^e (based on wet weights)		Indeterminable
	Juvenile	Not reported	Well water	Flow-through	6	7.73	30	Growth	0.04 ^e	DeGraeve et al. (1987)	Indeterminable
10					7.61	0.09 ^e			Indeterminable		
20					7.46	0.01 ^e			Indeterminable		
25					7.23	0.01 ^e			Indeterminable		

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp (°C)	Avg. test-pH	Test-duration (days)	Endpoint	96-hr EC20 or LC20 ^b (mg NH ₃ /L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Channel catfish	Juvenile	Not reported	Well water	Flow-through	30	7.14	30	Growth	0.03 ^e	DeGraeve et al. (1987)	Indeterminable
	Juvenile	6.4	Mississippi River	Experimental stream	18.2	7.5-8.1 ^f	117	Growth	Between 0.01-0.03 and 0.02-0.05 ^g	Hermanutz et al. (1987)	Indeterminable
		18.8			21.2	8.1-8.2 ^f	89		Between 0.09-0.21 and 0.43-0.77 ^g		Indeterminable
	Larval	Not reported	Well water	Flow-through	26.9	7.78	30	Growth	0.21 ^e	Swigert and Spacie (1983)	Indeterminable
Fathead minnow	Juvenile	1.3	Red River, Winnipeg, MB	Flow-through	8.3	8.4	30	Survival	>0.58	Present study	
		0.47			7.1	8.4	29	Survival	>0.30		
	Juvenile	Not reported	Well water	Flow-through	6	7.75	30	Survival	0.12 ^e	DeGraeve et al. (1987)	Lower, lower
					10	7.73	30	Survival	0.12 ^e		Lower, lower
					25	7.44	30	Survival	0.55 ^e		Lower, indeterminable
					30	7.27	30	Survival	0.52 ^e		Lower, indeterminable
	Larval	Not reported	Tittawabasee River, MI	Flow-through	24.8	7.97	28	Survival	0.28 ^e	Mayes et al. (1986)	Lower, lower

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp (°C)	Avg. test-pH	Test-duration (days)	Endpoint	96-hr EC20 or LC20 ^b (mg NH ₃ /L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Fathead minnow	Larval	Not reported	Well water	Flow-through	25.1	7.87	28	Survival	0.62 ^e	Swigert and Spacie (1983)	Indeterminable, indeterminable
	Larval	Not reported	Ground-water	Flow-through	24.2	8.00	Approx. 1-year – Parental fish survival measured at (a) 0-30 days, (b) 30-60 days, (c) 60 days to start of spawning and (d) during spawning	Survival ^f	(a) 0.55 ^{e,j}	Thurston et al. (1986)	Lower, indeterminable
									(b) 0.80 ^{e,j}		Indeterminable, indeterminable
									(c) 0.54 ^{e,j}		Lower, indeterminable
									(d) 0.83 ^{e,j}		Indeterminable, indeterminable
	Juvenile	1.3	Red River, Winnipeg, MB	Flow-through	8.3	8.4	30	Growth	0.52	Present study	
Larval	Not reported	Well water	Flow-through	25.1	7.87	28	Growth	0.26 ^e	Swigert and Spacie (1983)	Lower	

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp (°C)	Avg. test-pH	Test-duration (days)	Endpoint	96-hr EC20 or LC20 ^b (mg NH ₃ /L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
Fathead minnow	Larval	Not reported	Ground-water	Flow-through	24.2	8.00	0-30 days	Growth of parental fish (based on length) ⁱ	0.74 ^{e,j}	Thurston et al. (1986)	Higher
								Hatching success of eggs ⁱ	0.12 ^{e,j}		Lower
Northern pike	Larval	Not measured	Red River, Winnipeg MB	Flow-through	16.7	8.5	12	Survival	0.13	Present study	
	Larval	Not measured	Red River, Winnipeg MB	Flow-through	16.7	8.5	12	Growth	-- ^h	Present study	
Walleye	Juvenile	0.3-0.5	Red River, Winnipeg, MB	Flow-through	17.8	8.1	30	Survival	0.20	Present study	
	Juvenile	97.7 18.7	Mississippi River	Experimental stream	24.1	8.1	46	Survival	0.23 ^e	Hermanutz et al. (1987)	Higher
					16.7	8.4	43		0.28 ^e		Higher
Juvenile	0.3-0.5	Red River, Winnipeg, MB	Flow-through	17.8	8.1	30	Growth	-- ^h	Present study		

Species	Age class at test-start ^a	Wet weight at test-start (g)	Source of test-water	Test-type	Avg. test-temp (°C)	Avg. test-pH	Test-duration (days)	Endpoint	96-hr EC20 or LC20 ^b (mg NH ₃ /L)	Source	Relative tolerance of fish reported in public data set compared to present data set ^c
White sucker	Larval	Not measured	Red River, Winnipeg, MB	Semi-static	17.4	8.2	10	Survival	0.36	Present study	
	Larval	Not reported	Well water	Flow-through	18.6	8.24	31	Survival	>0.23	Reinbold and Pescitelli (1982)	Indeterminable
	Larval	Not measured	Red River, Winnipeg, MB	Semi-static	17.4	8.2	10	Growth	-- ⁿ	Present study	
	Larval	Not reported	Well water	Flow-through	18.6	8.24	31	Growth % larvae swimming up @ 72-hours post hatch	>0.23 0.08 ^e	Reinbold and Pescitelli (1982)	Indeterminable Indeterminable

Notes:

- a As much as possible, age classes reported in the public literature were recorded in Table 5-4. When age classes were not reported, they were determined using length or weight data measured at the beginning of each test in combination with length, weight and age of sexual maturation information provided in Scott and Crossman (1973).
- b EC20 or LC20 = Effective concentration where a sublethal response was different than the response of control organisms by 20% or lethal concentration affecting 20% of the test-population.
- c Lethal endpoints were compared with one another; sublethal endpoints were compared with one another. In cases where more than one test was conducted per species for the present study, each test-result is compared with the public domain

values in the order that they appear in Table 5-4. For example, two fathead minnow tests were conducted and compared with results reported by DeGraeve et al. (1987). At temperatures $\leq 10^{\circ}\text{C}$, the fish used by DeGraeve et al. (1987) were more sensitive to NH_3 -exposure than the fish used in the present study. Consequently, "lower, lower" is recorded in the final column of Table 5-4.

- d An LC20 was not determined by the authors and could not be calculated from the information provided in the research paper. Instead, the range of values within which the LC20 would fall is reported in Table 5-4.
- e LC20 or EC20 values were calculated using the Linear Interpolation Method and the ICPIN based on raw data provided by the authors.
- f The authors reported average pH concentrations for each treatment (i.e., NH_3 exposure-concentration). The range of average pH values is reported in Table 5-4 due to the relatively large pH differences among treatments.
- g Each treatment consisted of exposing fish to NH_3 in four experimental streams. Concentration gradients with a significant degree of variance were established in three of four experimental streams; results are reported as ranges representing the minimum and maximum average NH_3 concentration in any of the three treatments.
- h The NH_3 concentration did not produce a response that was different from the control response by at least 20%.
- i A life-cycle test was conducted and the following endpoints were tested: (a) parental fish survival, (b) parental fish growth, (c) egg production, (d) egg viability, (e) egg hatchability, (f) F_1 larvae survival, (g) F_1 larvae growth, (h) occurrence of brain lesions, and (i) occurrence of other histological changes. Parental fish survival and growth are recorded because they are most directly comparable to the present study. Egg hatchability is recorded because it was the most sensitive endpoint of those that are commonly used to measure the chronic effects of NH_3 toxicity (i.e., survival, growth, and reproductive success).
- j Two tests were analyzed together because their results were so similar. During EC20 derivation via the linear interpolation method, both sets of results and their average NH_3 concentrations were entered into the ICPIN program.

Channel catfish

Juvenile channel catfish were among the more sensitive locally tested fish yielding an LC20 of 0.16 mg NH₃/L. Although channel catfish are typically more tolerant to NH₃ than other freshwater fish (reviewed by US EPA 1999, Colt and Tchobanoglous 1978), a wide range of sensitivities is reported for this species and literature values bracket the responses observed in the present study.

Several tests conducted on larval and juvenile channel catfish generated LC20 values ranging from 0.01 mg NH₃/L to between 0.95 and 1.20 mg NH₃/L (Colt and Tchobanoglous 1978, DeGraeve et al. 1987, Hermanutz et al. 1987, Reinbold and Pescitelli 1982, Swigert and Spacie 1983) (c.f., Table 5-4). The upper tolerance limit reported for this species (i.e., 0.95 - 1.20 mg NH₃/L) was determined by Colt and Tchobanoglous (1978) and is approximately six to seven times greater than the NH₃ level required to reduce survival of locally tested channel catfish by 20%. The test-design used by Colt and Tchobanoglous (1978) was similar to that used in the present study with the primary distinction being a three-fold difference in test temperatures (c.f., Table 5-4). Channel catfish exposed to ammonia-fortified Red River water were tested under cold water conditions with a mean temperature of 8.8°C; Colt and Tchobanoglous maintained a mean test temperature of 27.9°C. 28°C is optimal for maximum growth of channel catfish (reviewed by Colt and Tchobanoglous 1978) and likely increased survival rates of the fish compared with those tested under cold water

conditions. Additionally, higher temperatures (and pHs) reduce the toxicity of NH_3 to catfish and rainbow trout (reviewed by Hermanutz et al. 1987).

Reinbold and Pescitelli (1982) and Swigert and Spacie (1983) measured survival rates of larval channel catfish exposed to NH_3 for 30 days at warm water temperatures (i.e., 26-27°C). Results of both tests indicate that, despite testing a more sensitive life stage (i.e., larval versus juvenile), channel catfish are at least three times more tolerant to chronic NH_3 -exposure than results of the present study suggest (c.f., Table 5-4). Temperature differences between research efforts and related effects on NH_3 toxicity may have caused the variation between test results.

DeGraeve et al. (1987) conducted a series of NH_3 toxicity tests on juvenile channel catfish at temperatures ranging from 6°C to 30°C and pHs of 7.16 to 7.74 (c.f., Table 5-4). With the exception of data generated at a temperature of 30°C, survival of the test-fish was compromised at NH_3 concentrations up to 15 times lower than those required to induce similar effects in locally tested channel catfish (c.f., Table 5-4). However, results reported by DeGraeve et al. (1987) might have been confounded because Acriflavine, a prophylactic treatment, was added to the holding water daily up to two days prior to the start of the test. Secondly, DO levels in some treatments were below recommended limits (US EPA 1999). Both variables may have increased the sensitivity of channel catfish to NH_3 exposure.

Hermanutz et al. (1987) conducted a field study, which exposed channel catfish and several other fish and invertebrate species to NH_3 , to assess the reliability of laboratory data in predicting environmental impacts on aquatic life and the applicability of laboratory data as a tool for deriving protective criteria. The study utilized an outdoor experimental stream continuously supplied with Mississippi River water that facilitated long-term testing. Results of the field study showed reasonable agreement with laboratory data, a relationship that is supported with results of the present study.

Hermanutz et al. (1987) exposed 6.4 g and 8.0 g channel catfish to NH_3 levels that were insufficient to reduce survival by 20% compared with the control group. Consequently, the LC20s for these two tests are higher than the highest exposure concentrations tested obtaining values $>0.08 - 0.16 \text{ mg NH}_3/\text{L}$ and $>0.11 - 0.15 \text{ mg NH}_3/\text{L}$, respectively (c.f., Table 5-4). (The LC20s are reported as ranges because concentration gradients formed in the experimental streams and the true exposure concentrations to the test-populations were indeterminable). Results obtained with locally tested channel catfish (weighing 5.0 g each) yielded a higher value of $0.16 \text{ mg NH}_3/\text{L}$ (c.f., Table 5-4). Exposure periods varied substantially between research projects; locally tested fish were exposed to NH_3 for 30 days whereas Hermanutz et al. (1987) held the channel catfish in NH_3 -fortified Mississippi water for up to 177 days. Since the mortality rate of channel catfish used in the present study stabilized over the 30-day exposure period (c.f.,

Figure 5-12b), it is assumed that the LC20 would not have changed significantly had the test continued for a longer period of time.

Growth rates were compromised in juvenile channel catfish exposed to NH_3 -fortified Red River water for 30 days (c.f., Figure 5-18). Mean tissue mass (based on EOT dry weights) of exposed fish weighed between 87.7% and 98.6% of the mean weight measured for control fish (c.f., Appendix J). However, a 20% difference in dry weight of exposed fish versus control fish was required to generate an EC20.

Using data reported by Colt and Tchobanoglous (1978), the ICPIN-generated EC20 for juvenile channel catfish ranged between 0.26 mg NH_3/L (based on dry weight data) and 0.30 mg NH_3/L (based on wet weight data) (c.f., Table 5-4). These results are consistent with an EC20 of 0.21 mg NH_3/L for larval channel catfish generated using data provided by Swigert and Spacie (1983).

Conversely, growth data generated by DeGraeve et al. (1987) and Hermanutz et al. (1987) indicate that growth effects in juvenile channel catfish may be observed at significantly lower levels of NH_3 exposure (e.g., 0.03 mg NH_3/L , c.f., Table 5-4). The heightened sensitivity of fish tested by DeGraeve et al. (1987) may have been due to confounding factors described above. Hermanutz et al. (1987) may have documented growth effects at lower NH_3 exposures due to the

prolonged exposure period (i.e., 177 days), thereby enabling the magnitude of sub-lethal effects to be fully realized.

Fathead minnow

Of the five fish species tested under site-specific conditions, juvenile fathead minnows were the most tolerant to chronic NH_3 exposure. Two fathead minnow tests generated LC_{20} s >0.58 mg NH_3/L and >0.30 mg NH_3/L (i.e., concentrations that were higher than those required to reduce survival by 20% in channel catfish, northern pike, walleye and white sucker test populations).

DeGraeve et al. (1987) evaluated 30-day mortality responses of juvenile fathead minnows exposed to NH_3 at several temperatures. The concentrations of NH_3 used in two of the tests (i.e., those conducted at 15°C and 20°C) were not great enough to elicit a mortality response that was significantly different from the control group. However, exposure concentrations were sufficiently high to significantly decrease survival rates by 20% in the tests run at 6, 10, 25 and 30°C . Results of these four tests produced ICPIN-based LC_{20} values of 0.12, 0.12, 0.55, and 0.52 mg NH_3/L , respectively (c.f., Table 5-4). At test temperatures most similar to those used in the present study (i.e., 6 and 10°C), the NH_3 concentrations were at least 2.5 times lower than those required to kill 20% of the fish in NH_3 -fortified Red River water. NH_3 toxicity increases with decreasing pH (Thurston et al. 1981b, several studies reviewed by Erickson 1985, Sheehan and Lewis 1986, US EPA 1999). Since DeGraeve et al. (1987)

used test pHs that were lower than those used in the present study (i.e., 7.3-7.8 versus 8.4, respectively) the differences in test results may have been due to a pH effect (c.f., Table 5-4).

Thurston et al. (1986) conducted a one-year, life-cycle study on fathead minnows beginning with a population of larval fish. As the larval fish matured, their survival rate was monitored throughout four consecutive development stages: (a) from Day 0 to Day 30 (b) from Day 30 to Day 60 (c) from Day 60 to the start of the spawning period and (d) during spawning. Growth rates of parental fish and the hatching success of their eggs were also measured throughout the study. ICPIN-based LC20 and EC20 values calculated with the data gathered by Thurston et al. (1986) are tabulated in Table 5-4. Of the commonly measured endpoints, (i.e., survival, growth and reproduction), the hatching success of eggs was the most sensitive yielding an EC20 of 0.12 mg NH₃/L. Based on growth data, the fish tested by Thurston et al. (1986) were more tolerant to chronic NH₃ exposure than locally tested fish. It is unclear if this observation would hold if survival data were compared because the highest exposure concentrations used during the present study were insufficient to generate a 20% response in the fathead minnows.

Mayes et al. (1986) and Swigert and Spacie (1983) conducted 28-day chronic NH₃ exposure tests on larval fathead minnows. Although the test temperatures were three times higher than the temperatures used in the present study (c.f.,

Table 5-4), the locally tested fish were more tolerant to elevated NH_3 concentrations. Using data reported by Mayes et al. (1986), an LC20 of 0.28 mg NH_3/L was generated compared with an LC20 >0.30 mg NH_3/L for the present study. Swigert and Spacie (1983) observed growth effects at NH_3 levels that were half as concentrated as those required to induce a similar response in locally tested fish (c.f., Table 5-4).

Growth (based on dry weights of surviving fish at test-termination) of one of the two groups of locally tested fathead minnows was compromised by 20% at a concentration of 0.52 mg NH_3/L compared to control fish (c.f., Table 5-4, Figure 5-19). A review by Colt and Tchobanoglous (1978) identified several mechanisms by which sublethal levels of ammonia may reduce fish growth. They include: (a) decreased ability to assimilate oxygen into the circulatory system due to gill damage, (b) increased expenditure of energy towards nitrogen excretion via alternate detoxification pathways, (c) elevated loss of ions due to increased urine flow, (d) inhibition of sodium uptake and (e) damage to various tissues. Additionally, the growth response may have been due to a reduction in food consumption or hormonal changes in stressed organisms.

Growth of the second group of locally tested fathead minnows appeared to have been stimulated by NH_3 exposure (c.f., Figure 5-20). The stimulation of growth by low levels of potentially toxic agents is known as hormesis, an effect that has been observed in a wide range of taxa (including fish) following exposure to a

variety of agents (including ammonia) (Stebbing, 1982). Stebbing (1982) proposed that growth hormesis "may be the consequence of regulatory overcorrections by biosynthetic control mechanisms to low levels of inhibitory challenge, resulting in growth that is greater than normal".

Northern pike

Larval northern pike exposed to ammonia-fortified Red River water for 12 days produced an LC20 of 0.13 mg NH₃/L (95% C.I. = 0.01-0.27 mg NH₃/L) and was the most sensitive fish species tested under chronic conditions. Adverse growth effects were not observed in northern pike fry; conversely, the presence of NH₃ in the test water appeared to stimulate growth. Figure 5-21 shows that at NH₃ concentrations between approximately 0.07 mg/L – 0.21 mg/L, northern pike fry had individual dry weights that were 38% to 68% greater than the dry weights of control fish. At higher exposure concentrations (i.e., >0.40 mg NH₃/L), this effect was significantly reduced, but continued to exceed growth levels obtained by the controls (i.e., individual tissue weights of exposed fish were greater than 0%, the baseline for the controls).

One possible explanation for the increase in tissue mass in exposed fish compared to control fish is hormesis (i.e., a stimulatory effect caused by low levels of potentially toxic agents, described above). Stimulatory levels of a toxicant are typically an order of magnitude lower than inhibitory levels (Stebbing, 1982). Since the northern pike were exhibiting a mortality response in addition to

gaining weight at low levels of NH_3 , it is likely that an effect other than hormesis was causing exposed fish to gain tissue mass more quickly than control organisms. Another possible explanation is that low levels of NH_3 exposure (i.e., 0.07 mg/L) increased mortality rates compared with control exposures thus reducing competition for available food resources and lowering stress levels associated with being in the presence of other predators, two factors favouring growth. At higher NH_3 concentrations (i.e., >0.40 mg/L), survival rates continued to decline, but only the strongest (and presumably the largest) fish survived thereby outweighing unstressed, control organisms on an individual basis.

Walleye

Results of one chronic-exposure toxicity test conducted on juvenile walleye held in NH_3 -fortified Red River water for 30 days yielded an LC20 of 0.20 mg NH_3 /L. No growth effects were found (c.f., Figure 5-22). Other chronic data obtained during laboratory testing are not available for this species. Reinbold and Pescitelli (1982) attempted to conduct a chronic exposure test on ELS walleye but failed because 80% of the newly hatched fish died prior to testing.

Hermanutz et al. (1987) conducted 46 day and 43 day field studies on two groups of walleye (i.e., average weight = 97.7 g and 18.7 g, respectively) held in control, low, medium, and high ammonia-exposure experimental streams supplied with Mississippi River water. ICPIN-generated LC20 values determined using data reported by Hermanutz et al. (1987) were 0.23 mg NH_3 /L and 0.28 mg

NH₃/L compared with the LC20 of 0.20 mg NH₃/L for locally tested fish (c.f., Table 5-4). This difference is marginal considering fish tested in the field were at least 62 times bigger (based on wet weights) than fish tested locally in a laboratory.

White sucker

Larval white sucker exposed to NH₃-fortified Red River water for 10 days had a higher LC20 (i.e., 0.36 mg NH₃/L) than other locally tested fish with the exception of juvenile fathead minnows. Although neither fathead minnow test yielded a point estimate LC20, the EC20 of 0.52 mg NH₃/L obtained with one of the two groups is 1.4 times greater than the LC20 of 0.36 mg NH₃/L for white sucker.

Based on individual dry weights of surviving fish at test-termination, larval white sucker growth was stimulated in enclosures containing NH₃ compared with the controls (c.f., Figure 5-17). This effect was similar to those observed in larval northern pike and one group of fathead minnows and may have been due to the explanations provided above.

Few studies have examined white sucker sensitivity to NH₃. Among them are a laboratory study by Reinbold and Pescitelli (1982) and a field study by Hermanutz et al. (1987). Reinbold and Pescitelli (1982) exposed larval white sucker to ammonia concentrations that were insufficient to elicit a 20% mortality or growth response. Consequently, the LC20 and EC20 (based on growth) for

this test were greater than the highest exposure-concentration tested of 0.226 mg NH₃/L. Delayed responses in swim-up of larvae were observed at 0.08 mg NH₃/L (c.f., Table 5-4).

Hermanutz et al. (1987) exposed two groups of juvenile white sucker to three NH₃ treatments (i.e., high, medium and low) and a control stream for 183 days and 88-days, respectively. Survival in the control stream for the first test was less than 25% and cannot be used for comparison with results of the present study. For the second test, control survival was approximately 70% but did not differ significantly from survival in the treatments. Growth data between control and treatment exposures were also very similar. These results are not included in Table 5-4.

5.5 Results and discussion of parallel tests conducted with ammonia-fortified Red River water with and without treated effluent

Three pairs of semi-static tests (i.e., T3A&B, T4A&B and T5A&B) were conducted on larval fathead minnow and larval white sucker species using treatments of either (a) Red River water containing NH₄Cl (i.e., A series) or (b) Red River water containing NH₄Cl and NEWPCC treated effluent (i.e., B series). Results of the effluent plus NH₄Cl toxicity tests (i.e., T3B, T4B and T5B) are shown in Table 5-5 together with related environmental data. All LC₅₀ values for

**Table 5-5. Toxicity of ammonia- and effluent-fortified river water to five fish species:
Test conditions and LC50 values.**

Species (Test No. - Age class)	Test-duration	DO ^a mean (range) (mg/L)	DO ^a mean (range) (% saturation)	pH mean (range)	Temperature mean (range) (°C)	LC50 ^b (95% CI ^c) (mg NH ₃ /L)
Fathead minnow (5B - larval)	72 hours	8.1 (7.2-8.7)	90.2 (80.8-95.7)	7.9 (7.2-8.8)	20.9 (20.8-21.0)	0.19 (0.19-0.20)
White sucker (3B - larval)	96 hours	6.2 (5.4-7.2)	62.0 (53.4-74.5)	7.8 (7.1-8.3)	15.5 (14.0-17.0)	0.18 (0.17-0.19)
White sucker (4B - larval)	96 hours	7.2 (6.1-8.2)	73.9 (63.1-85.6)	7.9 (7.2-8.3)	16.8 (15.5-20.0)	0.321 (--) ^d
	10 days	6.4 (2.7-8.2)	66.7 (27.9-85.6)	7.9 (6.9-8.4)	16.9 (15.5-20.0)	0.28 (0.26-0.29)

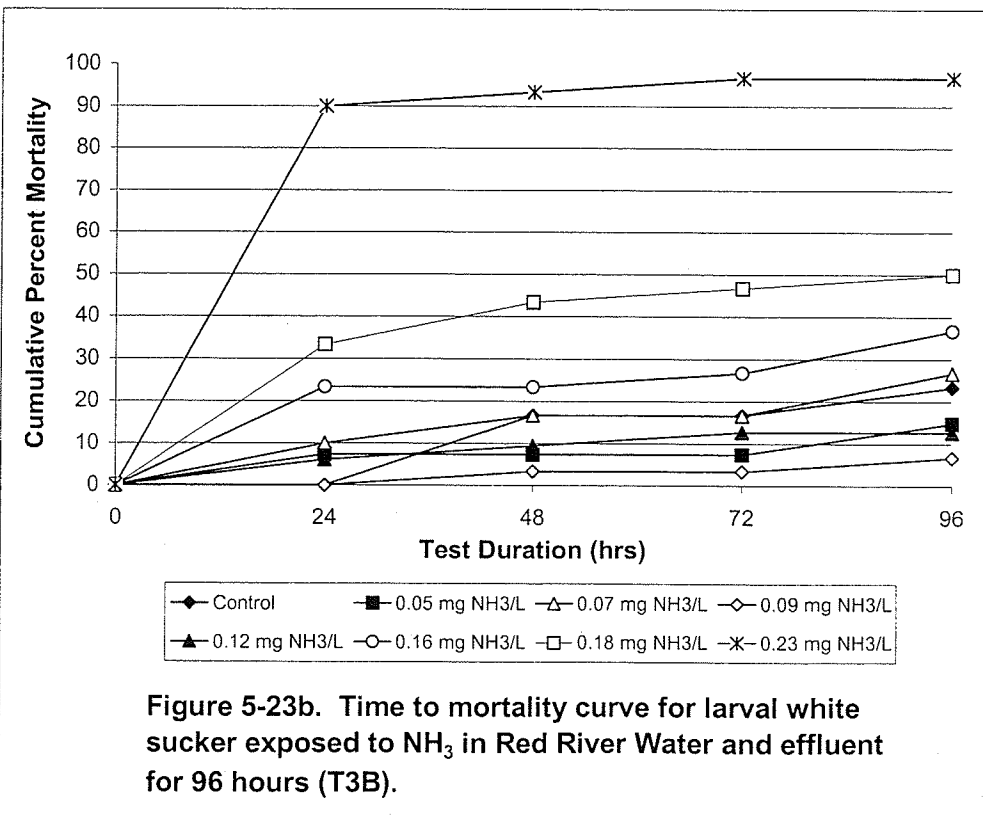
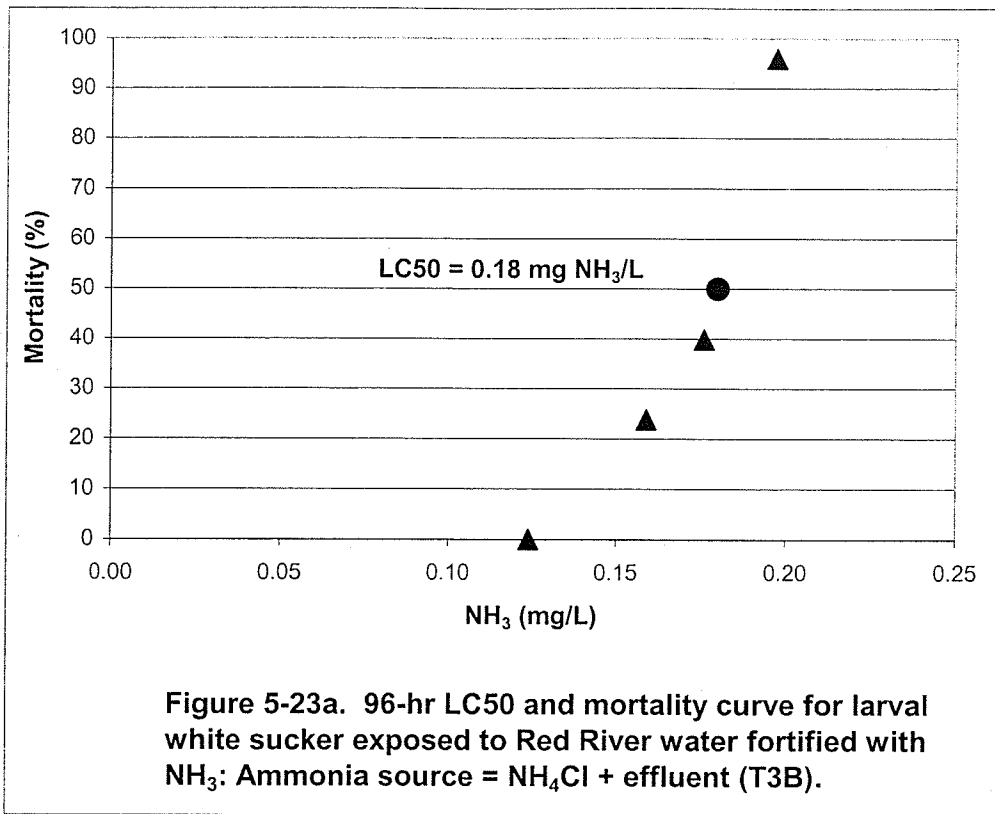
Notes:

- (a) DO = dissolved oxygen
- (b) For T3B and T4B (after 96 hours and 10 days), the LC50 was obtained when the test-solution consisted of 50% to 100% effluent. For T5B, the LC50 was obtained when the test-solution consisted of 25% to 50% effluent.
- (c) CI = confidence interval
- (d) The Bootstrap Method was unable to compute the confidence interval.

each test plus the associated mortality curves are shown in Figures 5-23a and 5-23b to 5-26a and 5-26b. The raw data used to calculate all LC50 values are tabulated in Appendix J, Tables J-23 to J-26. ICPIN printouts are provided in Appendix K.

96-hour LC50s for T3B and T4B with white sucker were 0.18 mg NH₃/L (95% C.I. = 0.17 to 0.19 mg/L) and 0.32 mg NH₃/L (95% C.I. indeterminable), respectively. The higher of the two values was calculated using slightly older fish obtained from the same genetic stock (c.f., Table 4-2). The 10-day LC50 for T4B was 0.28 mg NH₃/L (95% C.I. = 0.26 - 0.29 mg/L). T5B conducted with fathead minnows yielded a 72-hour LC50 of 0.19 mg NH₃/L (95% CI = 0.19 - 0.20 mg/L). This test was terminated after 72 hours rather than 96 hours because the number of mortalities in the control group was approaching 10%, the upper limit recommended by the US EPA (1993) (c.f., Table 5-5).

Average pHs for all three effluent plus NH₄Cl tests ranged between 7.8 and 7.9; average temperatures ranged between 15.5°C and 20.9°C (c.f., Table 5-5). Average DO levels of at least 6.2 mg/L (i.e., 62% saturation) were maintained throughout all but one test and met minimum standards recommended by the US EPA (1993, 1999) and ASTM (1996). During the final days of the chronic-exposure test (i.e., T4B), DO concentrations as low as 2.7 mg/L were measured in some test-chambers. These low levels were observed infrequently (i.e., less than 5% of the time) and occurred only in the most concentrated test-solutions



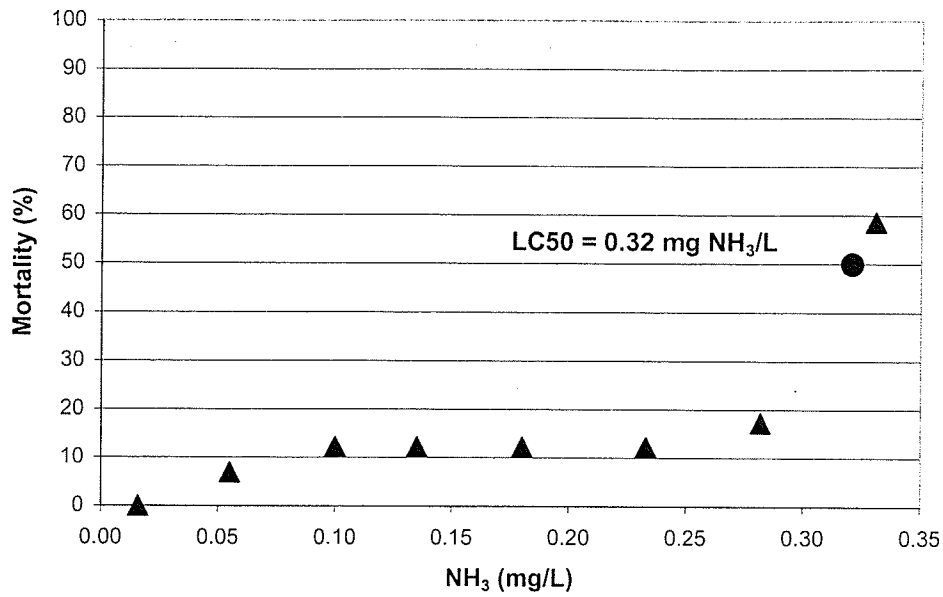


Figure 5-24a. 96-hr LC50 and mortality curve for larval white sucker exposed to Red River water fortified with NH₃: Ammonia source = NH₄Cl + effluent (T4B).

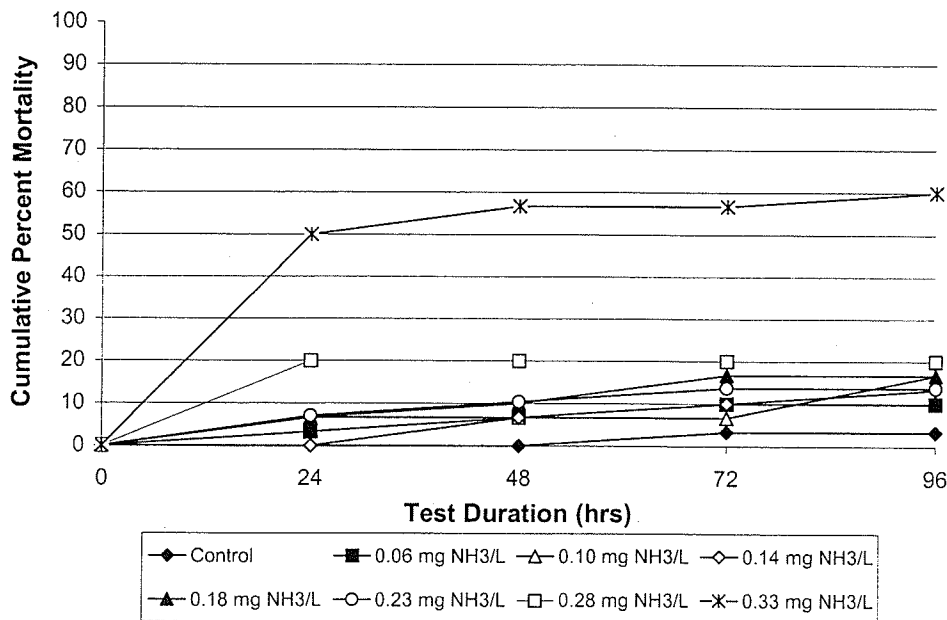


Figure 5-24b. Time to mortality curve for larval white sucker exposed to NH₃ in Red River Water and effluent for 96 hours (T4B).

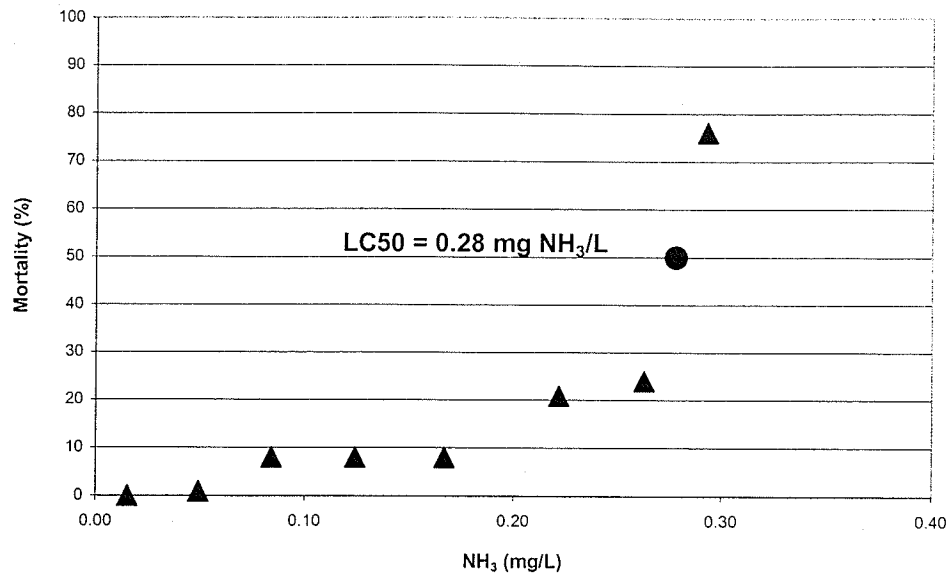


Figure 5-25a. 10-d LC50 and mortality curve for larval white sucker exposed to Red River water fortified with NH₃: Ammonia source = NH₄Cl + effluent (T4B).

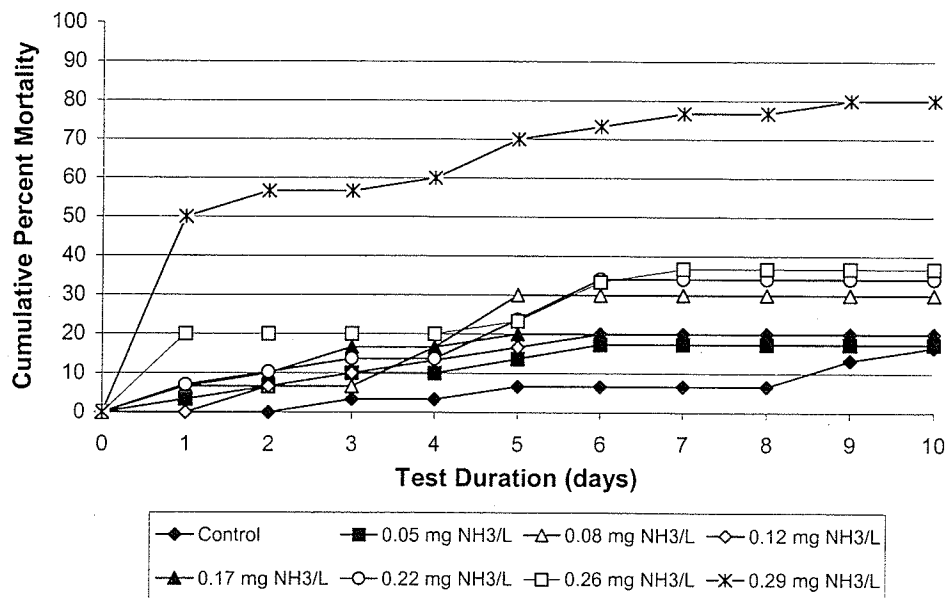
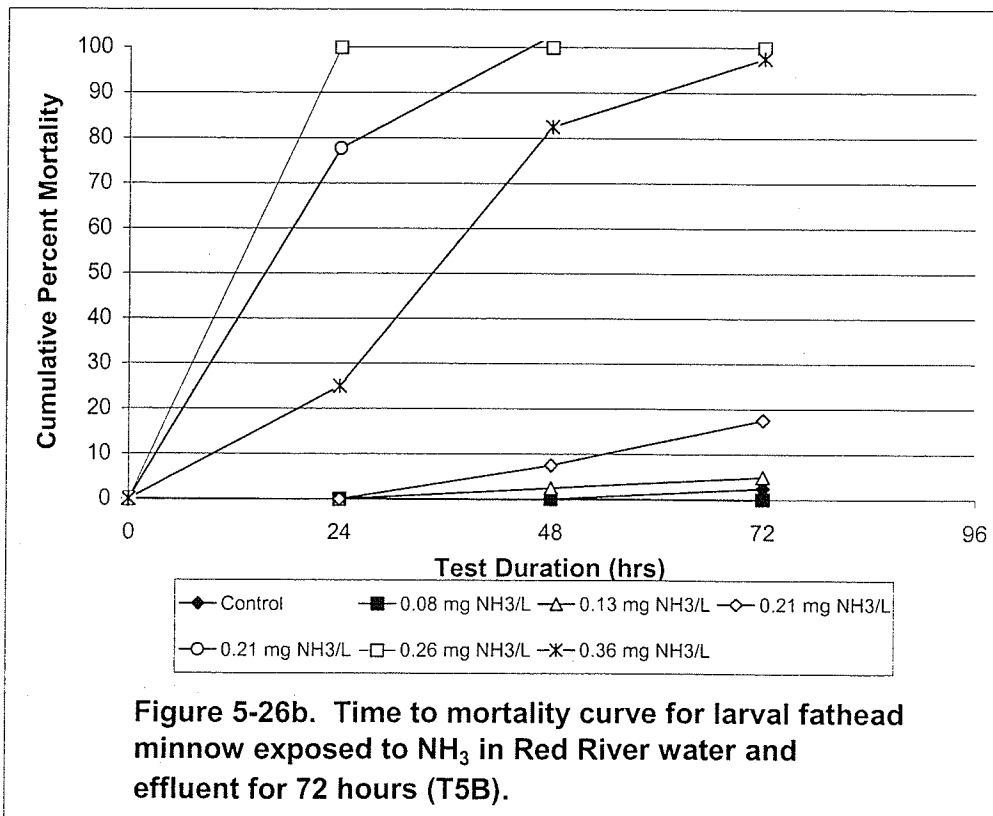
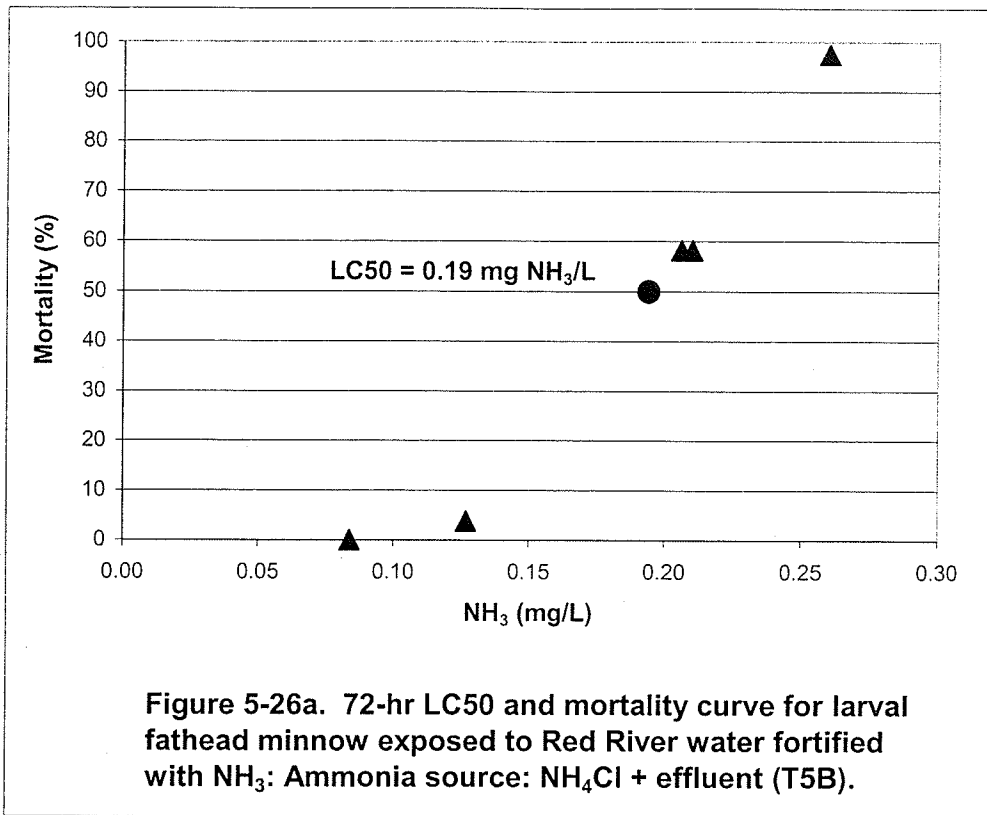


Figure 5-25b. Time to mortality curve for larval white sucker exposed to NH₃ in Red River water and effluent for 10 days (T4B).



containing dead fish. Since the bodies of dead fish were observed to decompose almost entirely within the 24-hour period between mortality counts, it is assumed that decay processes acting on the dead fish and any uneaten food consumed much of the available DO. It is less likely that the presence of highly concentrated NH_3 reduced oxygen levels causing the fish to die because low DO levels were not measured consistently in upper level treatments.

Two anomalies occurred during T5B with fathead minnows that did not occur during any other tests conducted throughout the test program. First, the highest nominal total ammonia-N exposure-concentration used in T5B did not contain the highest concentration of NH_3 . A possible explanation for this effect may be due to differences in pHs among exposure concentrations. The presence of effluent in the test-water significantly reduced pHs in upper level treatments, as noted by the large pH ranges shown in Table 5-5. Since the fraction of total ammonia that exists in its un-ionized form decreases with decreasing pH, test-chambers containing the highest concentrations of effluent contained lower NH_3 levels than more dilute test solutions.

Secondly, the greatest mortality response did not occur at the highest NH_3 concentration (nor at the highest effluent concentrations), but rather in test chambers containing the most total ammonia (c.f., Figure 5-26b). This suggests that ammonia toxicity was not solely attributed to NH_3 , but likely resulted from the

toxic action of both the un-ionized and ionized forms. This theory is supported in the literature (reviewed by Colt and Tchobanoglous 1978, US EPA 1999).

Results of all paired tests (i.e., T3A&B, T4A&B and T5A&B) conducted with and without treated effluent are summarized in Table 5-6. All LC50 values for tests that exposed fish to NH_4Cl and treated effluent from the City of Winnipeg's NEWPCC were lower than the LC50 values generated for duplicate tests using NH_4Cl as the sole source of ammonia. This difference was significant in two of the three tests (i.e., T4 after 10 days of exposure and T5; $\alpha \leq 0.10$) as indicated in Table 5-6. These results suggest that the fish were more sensitive to NH_3 in the presence of NEWPCC effluent possibly due to a constituent of the effluent that was toxic to fish in addition to the NH_3 or because the constituent was acting synergistically with ammonia to enhance ammonia toxicity. Alexander et al. (1977) evaluated the toxicity of the City of Winnipeg's NEWPCC effluent and reported that copper, zinc and methylene blue-active substances may be present in quantities great enough to contribute to effluent toxicity in addition to NH_3 .

Nimmo et al. (1989) compared the effects of NH_3 in St. Vrain River water to that in Longmont, Colorado wastewater and also found that the toxicity of ammonia was enhanced by a constituent of or characteristic of the effluent. Under warm water conditions (i.e., test temperature = approx. 20°C) the LC50 for larval fathead minnow in wastewater was $0.56 \text{ mg NH}_3/\text{L}$ (95% C.I. = $0.52\text{-}0.61$) versus $0.94 \text{ mg NH}_3/\text{L}$ (95% C.I. = $0.87\text{-}1.02$) in river water. The LC50 for adult white

Table 5-6. Comparison between duplicate tests conducted with and without treated effluent.

Species (Test No. - Age class)	Test-duration	LC50 (95% CI ^a) (mg NH ₃ /L) Ammonia source: NH₄Cl only ('A' series)	LC50 (95% CI ^a) (mg NH ₃ /L) Ammonia source: NH₄Cl + effluent ('B' series)	Statistically significant? (Y=yes; N=no; I=indeterminate)
Fathead minnow (5 - larval)	72 hours	0.57 ^b (0.39-0.71)	0.19 (0.19-0.20)	Y
White sucker (3 - larval)	96 hours	0.22 (0.14-0.25)	0.18 (0.17-0.19)	N
White sucker (4 - larval)	96 hours	0.53 (0.37-0.76)	0.32 (--) ^c	I
	10 days	0.50 ^d (0.29-0.84)	0.28 (0.26-0.29)	Y

Notes:

(a) CI = confidence interval

(b) T5A was conducted for 96 hours. A 72 hour LC50 has been calculated, here, to facilitate comparisons between its duplicate test, T5B.

(c) The Bootstrap Method was unable to compute the confidence interval.

(d) An LC20 was originally derived for Test 4A. An LC50 is reported here to facilitate comparisons between its duplicate test, T4B.

sucker was 0.57 mg NH₃/L (95% C.I. = 0.45-0.74) in wastewater versus >0.94 mg NH₃/L in river water. Nimmo et al. (1989) suggested that aluminum, copper, and possibly nickel and zinc present in Longmont wastewater were increasing the overall toxicity of effluent in addition to NH₃.

5.6 Comparison of site-specific test results with current Manitoba Surface Water Quality Objectives (MSWQO)

The development of site-specific criteria has been promoted by various regulatory agencies (e.g., US EPA, CCME, Manitoba Conservation) in an effort to regulate water quality without being overprotective or underprotective for a particular substance in a given area. National or Provincial objectives are designed to protect a diverse range of ecosystems, whereas Site-Specific Water Quality Objectives (SSWQO) are tailored to a single receiving system and consider the physical, chemical, and biological factors of that system, which affect the toxicity of a substance to aquatic organisms. In this study, existing MSWQO were modified to include site-specific data generated with five fish species resident to the Winnipeg reaches of the Red and Assiniboine Rivers. Site-specific criteria for each of the five fish species evaluated were compared with the current MSWQO to estimate the effectiveness of Provincial criteria in protecting each species of fish. No attempts were made to re-define Provincial criteria or to derive site-specific criteria using the entire site-specific dataset simultaneously.

To compare site specific data with current MSWQO, acute LC50s and chronic LC20s or EC20s computed in the present study were incorporated into Equations 1* to 6* as described in Section 4.6. Acute criteria (i.e., the CMC) and chronic criteria (i.e., the CCC) change with changing temperature and pH. Therefore, all site-specific data were incorporated into Equations 1* to 6* for temperatures ranging from 0°C to 25°C and for pHs ranging from 7.8 to 8.4; conditions that represent average monthly minima and maxima in the study area for these two parameters (c.f., Table 4-1). Figure 5-27 compares the acute and chronic MSWQO with site-specific criteria developed for each of the five species tested in the present study.

Chronic criteria are highest (i.e., least conservative) at low temperatures and pHs and they are lowest (i.e., most conservative) at high temperatures and pHs. Therefore, Figure 5-27 displays chronic criteria derived under extreme conditions (i.e., temp. = 0°C, pH = 7.8 and temp. = 25°C, pH = 8.4). Acute criteria are highest (i.e., least conservative) at low pHs and lowest (i.e., most conservative) at high pHs, independent of temperature. Acute MSWQO and related site-specific objectives are therefore illustrated on Figure 5-27 for pHs of 7.8 and 8.4. Since Equations 5* and 2* are simply magnified versions of Equations 4* and 1* respectively (i.e., magnified by a factor of 2.5 as discussed in Section 4.6), only criteria developed with the former are plotted on Figure 5-27. The data plotted on this figure are tabulated in Appendix L, Tables L-1 and L-2.

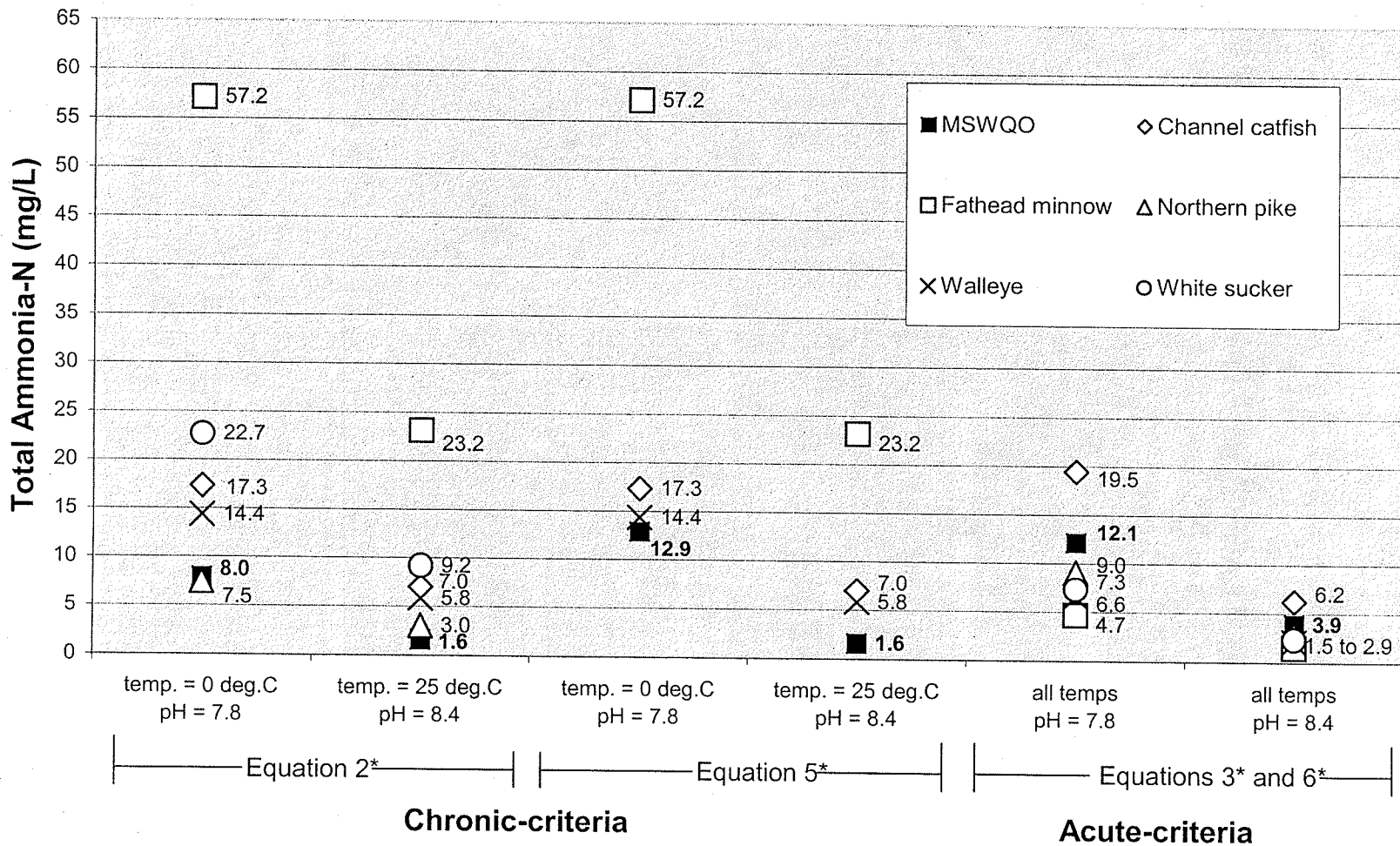


Figure 5-27. Relationship between current Manitoba Surface Water Quality Objectives (MSWQO) and Site-Specific Water Quality Objectives (SSWQO)

Figure 5-27 shows that, with the exception of larval northern pike, all other resident fish species tested in the present study are adequately protected by current MSWQO developed for chronic ammonia-exposure when ELS fish are present or water temperatures are greater than 5°C (i.e., when Equation 2* applies). At low temperatures (e.g. 0°C) and pHs (e.g., 7.8), the chronic site-specific criterion for northern pike falls just below the MSWQO by a factor of 0.06 (i.e., SSWQO = 7.5 mg total ammonia-N/L versus MSWQO = 8.0 mg total ammonia-N/L). At a temperature of 15°C, the SSWQO for northern pike is equal to the MSWQO, regardless of pH. At 25°C, the current MSWQO is almost twice as conservative as the SSWQO recommended for this species (c.f., Figure 5-27).

Northern pike spawn in the spring immediately after the ice melts (i.e., April to early May) when water temperatures are between 4.4°C and 11.1°C (Scott and Crossman 1973). The length of time to hatch is dependent on ambient water temperatures; as waters warm, less time is required for the developing embryo to hatch (Piper et al. 1982). For example, at water temperatures of 10°C northern pike eggs typically hatch within 12 to 14 days post-fertilization. As water temperatures warm above 15°C, the risks of ammonia toxicity to northern pike lessen. Therefore, the most critical period for northern pike may be the period immediately post-hatch when water temperatures are less than 15°C. Since northern pike is a valued sport species in the Red and Assiniboine Rivers (CEC 1992), the development of less restrictive criteria for surface waters of Manitoba may threaten young northern pike. This effect may be nullified by the fact that

river water flows are greatest and ammonia concentrations are lowest (i.e., most dilute) in the spring when larval northern pike are present. Since only one test was conducted on northern pike during this study and no other results are available from the literature, further testing of this species is recommended to determine with higher confidence, safe chronic ammonia levels for larval northern pike.

Site-specific walleye, channel catfish and fathead minnow data derived with juvenile fish were substituted into Equation 5* to evaluate MSWQO when water temperatures are less than or equal to 5°C or when ELS-fish are absent. Up to a temperature of 10°C, SSWQO calculated for juvenile walleye were very similar to the MSWQO, regardless of pH; SSWQO for juvenile walleye exceed MSWQO at higher temperatures (c.f., Figure 5-27). Fathead minnow data and channel catfish data generated SSWQO that were up to 14 times greater than current MSWQO suggesting that Provincial criteria are adequately protective or overprotective for these species. White sucker and northern pike data were not substituted into Equation 5* because the data were generated under conditions that do not apply to Equation 5*. Specifically, the data were generated using larval fish rather than older fish and white sucker and northern pike larvae are not present at water temperatures less than 5°C.

Provincial and site-specific criteria for acute ammonia exposure are contrary to the trend observed for chronic ammonia exposure; four of the five resident

species tested yielded acute SSWQO that are lower than current MSWQO (c.f., Figure 5-27). Current MSWQO are up to four times higher (i.e., less conservative) than the level required to protect the most acutely sensitive fish species tested under site-specific conditions (i.e., the fathead minnow). Any modifications to the Provincial criteria should project the maximum acceptable ammonia limits down to a level that would meet site-specific objectives. Based on the results of this study, Provincial objectives for acute ammonia exposure are only protective to channel catfish.

Several researchers have used site-specific data to determine the most appropriate level of regulation for an area receiving ammonia inputs (Alexander et al. 1986, Nimmo et al. 1989, and Diamond et al. 1993). Alexander et al. (1986) measured the acute and chronic effects of several fish species and one invertebrate species using Tittawabasee River water to develop site-specific criteria for Midland, Michigan. This project was initiated by the Dow Chemical Company, the Consumers Power Company, and the City of Midland Waste Water Treatment Plant (WWTP) in response to proposed water quality criteria revisions by the State of Michigan Department of Natural Resources (MDNR). Results of the study succeeded in shifting upwards the maximum acceptable water quality limits for NH_3 at critical times of the year (i.e., June through September when temperatures and pHs are highest and river flows are lowest) and minimized discharge restrictions for the three proponents.

Nimmo et al. (1989) conducted several site-specific toxicity tests on johnny darters, white suckers, and fathead minnows, using St. Vrain River water, Colorado. The tests were conducted to facilitate the development of site-specific ammonia criteria for the St. Vrain River in the vicinity of Longmont, Colorado. A secondary objective was to determine whether additional wastewater treatment facilities for Longmont would affect the long-term survival of johnny darters, a State-listed threatened species. Site-specific data generated for the johnny darter were incorporated in the data pools used to generate both acute and chronic criteria. The study produced a site-specific chronic criterion of 0.05 mg NH₃-N/L, which was strongly supported by the state-wide standard of 0.06 mg NH₃-N/L generally applied to Class I warm-water streams in Colorado. Additionally, a site-specific acute criterion of 0.56 mg NH₃-N/L was determined. The authors reported that the characteristics of the St. Vrain River water were not unique and did not significantly influence ammonia toxicity compared with duplicate tests performed using laboratory (well) water. In other words, adjustments were not made to the acute and chronic criteria for water effects.

A study by Diamond et al. (1993) designed to derive site-specific ammonia criteria for an effluent-dominated headwater stream also reported that the site-water had no effect on NH₃ toxicity. The study area consisted of an intermittent freshwater, wooded stream located on the Delmarva Peninsula on the eastern shore of the United States. For at least four months of the year, the stream is made up, primarily, of effluent from a food-processing plant. Acute and chronic

toxicity tests were conducted on nine resident species (four of which were fish species) using plant well water. Well water was considered appropriate for site-specific testing because it was used as the process water for the plant and consequently made up most of the stream volume. Tests were conducted at both cold water (i.e., 12°C) and warm water (i.e., 20°C) temperatures. Results of the chronic tests yielded proposed site-specific criteria that were three times higher than the national criteria (i.e., site-specific criteria = 0.05 mg NH₃/L) for cold water temperatures and two times greater for warm water temperatures (i.e., site-specific criteria = 0.09 mg NH₃/L). Site-specific criteria for acute-exposure to NH₃ were not significantly different from the national criteria (i.e., site-specific criteria = 0.30 to 0.62 mg/L for temperatures between 12°C and 20°C). Diamond et al. (1993) concluded that aquatic life residing in small or intermittent headwater streams on the eastern shore of the United States may be able to tolerate higher concentrations of NH₃ than lake or large stream biota.

The work of Alexander et al. (1986), Nimmo et al. (1989) and Diamond et al. (1993) demonstrate the value of using site-specific data to approximate appropriate regulations for a given site. Two of the three studies (i.e., Alexander et al. 1986 and Diamond et al. 1993) successfully generated site-specific criteria that are different from (and greater than) national criteria thereby facilitating Publicly Owned Treatment Works (POTW) and industrial effluent dischargers to meet compliance schedules. Nimmo et al. (1989) established appropriate site-specific criteria for the St. Vrain River in the Longmont, Colorado region, a

necessity for the promotion of additional wastewater treatment facilities in a state that previously had no recommendations for acute NH_3 exposure. In addition, the work of these three research groups has shown that site-specific criteria are more likely to differ from national criteria due to the relative sensitivities of resident species rather than an effect of the site water.

In the present study, site-water effects were not measured, but resident species generally showed heightened sensitivities to NH_3 than results reported in the public literature. Northern pike, a previously untested species, was the most sensitive resident species tested under chronic conditions. Current MSWQO for chronic NH_3 exposure are at a level that is appropriate for the protection of key fish species to the Red and Assiniboine Rivers, except possibly larval northern pike at cold water temperatures (i.e., $<15^\circ\text{C}$). Alternately, current MSWQO for acute NH_3 exposure are underprotective for the majority of locally tested fish species and may be imposing risks to resident fish populations in the study area.

6.0 SUMMARY

Site-specific data can be used as a tool for developing criteria that reflect the unique biological, chemical, and physical conditions of a particular area. This study was designed to develop site-specific ammonia toxicity data for fish species resident to the Red and Assiniboine Rivers and to determine whether current Manitoba ammonia objectives are appropriate for the protection of a cool water fishery. The study area included the Winnipeg reaches of the Red and Assiniboine Rivers and their tributaries.

For this study, site specificity was established by testing resident fish species in ammonia-fortified Red River water. The river water was used as control- and dilution water for all tests and was altered minimally from its natural state. Ten acute-exposure tests and six chronic-exposure tests were completed using several fish species: channel catfish, fathead minnow, northern pike, walleye and white sucker. Most groups of test-fish were obtained from local sources or sites within the Lake Winnipeg watershed to use genetic strains most similar to those found within the study area. Only young (i.e., larval or juvenile) fish were tested to generate conservative estimates of species sensitivity to ammonia.

LC50s for species tested in ammonia-fortified Red River water were generally lower than those reported in the public domain suggesting that resident fish species were more sensitive to acute ammonia exposure under site-specific

conditions. Consequently, Provincial WQO, which are based on a large, public dataset, are underprotective for the study area. Only site-specific data for channel catfish generated SSWQOs that were higher than (i.e., less conservative) than current MSWQOs.

This study was the first to have successfully generated ammonia toxicity data on northern pike, a key sport species and top predator of the Red and Assiniboine Rivers. Northern pike were more sensitive to chronic ammonia-exposure than locally tested channel catfish, fathead minnow, walleye and white sucker yielding a 12-day LC20 of 0.13 mg NH₃/L. SSWQO derived with northern pike data were lower (i.e., more conservative) than current chronic Manitoba ammonia objectives at water temperatures below 15°C whereas SSWQO for channel catfish, fathead minnow, walleye and white sucker were higher (i.e., less conservative) than existing MSWQO. Since few data exist for northern pike, further testing of this species would assist regulators in determining an appropriate control for protecting northern pike populations.

Based on the results of paired tests conducted with and without treated effluent from the City of Winnipeg's NEWPCC, evidence exists that a constituent of the effluent increases the overall toxicity of ammonia or is itself toxic. Further study of NEWPCC effluent toxicity would ensure that ammonia objectives are not understated should a constituent of the effluent be increasing either the potency of ammonia or the sensitivity of fish to ammonia exposure.

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APPENDIX A

EQUATIONS USED BY MANITOBA CONSERVATION TO DETERMINE AMMONIA WATER QUALITY OBJECTIVES (WQO) FOR THE PROTECTION OF COOL WATER AQUATIC LIFE AND WILDLIFE

(Source: Manitoba Conservation 2001)

<u>Water Quality Variable</u>	<u>Units and Form</u>	<u>Water Use</u>	<u>Tier II - Water Quality Objectives^(a)</u>	<u>Applicable Period</u>	<u>Averaging Duration</u>	<u>Allowable Exceedance Frequency</u>	<u>Design Flow^(b)</u>	<u>References</u>
Ammonia	mg/L Total Ammonia as N	Surface Water: Cool Water Aquatic Life and Wildlife (continued)	$= \left[\left(\left[\frac{0.0577}{1 + 10^{7.688 - \text{pH}}} \right] + \left[\frac{2.487}{1 + 10^{\text{pH} - 7.688}} \right] \right) \times b \right] \text{ (Eq. 4)}$ <p>where $b = 1.45 \times 10^{0.028 \times (25 - c)}$</p> <p>and</p> <p>$c = \text{Maximum Temperature or } 7^\circ\text{C}$</p> <p>whichever is greater</p> <p>and $\text{pH} \geq 6.5$ and ≤ 9.0;</p> <p>and</p>	Water Temperature $\leq 5^\circ\text{C}$ or Early Life Stages are Absent	30 Days ^(c)	Not More Than Once Each 3 Years, On Average	30-Day, 3-Year or 30Q10	
			$= 2.5 \times \left[\left(\left[\frac{0.0577}{1 + 10^{7.688 - \text{pH}}} \right] + \left[\frac{2.487}{1 + 10^{\text{pH} - 7.688}} \right] \right) \times b \right] \text{ (Eq. 5)}$ <p>where $b = 1.45 \times 10^{0.028 \times (25 - c)}$</p> <p>and</p> <p>$c = \text{Maximum Temperature or } 7^\circ\text{C}$</p> <p>whichever is greater</p> <p>and $\text{pH} \geq 6.5$ and ≤ 9.0;</p> <p>and</p>	Water Temperature $\leq 5^\circ\text{C}$ or Early Life Stages are Absent	4 Days ^(c)	Not More Than Once Each 3 Years, On Average	4-Day, 3-Year or 7Q10	
			$= \left[\frac{0.411}{1 + 10^{7.204 - \text{pH}}} \right] + \left[\frac{58.4}{1 + 10^{\text{pH} - 7.204}} \right] \text{ (Eq. 6)}$	All Periods	1 Hour ^(d)	Not More Than Once Each 3 Years, On Average	1-Day, 3-Year or 1Q10	

APPENDIX B

LIVE FISH COLLECTION AND HANDLING PERMITS

MANITOBA DEPARTMENT OF NATURAL RESOURCES
FISH HABITAT MANAGEMENT SECTION

LIVE FISH HANDLING PERMIT

Issued under the authority of the Fisheries Act (Federal and Provincial) and the Manitoba Fishery Regulations, the Fishing Licence Fee Regulation, and the Fishing Licence Fee Regulations made thereunder.

Issued to: Mr. Don Harron

of: TetRES Consultants, 603-386 Broadway Wpg. MB

is hereby authorized to handle live fish subject to the following conditions:

1. Purpose of Permit: See Attached
2. Location: See Attached
3. Effective Date: 3-May-99 to 30-Jun-99 inclusive.
4. Special Conditions: Destroy all fish on completion of studies.

Shelley Matkowski

Issued By

Fish Enhancement Biologist

Title

4-May-99

Date of Issue

Signature of Permittee

Permit Number: 99-08

Attachment to Live Fish Handling Permit # 99-08

To transport and hold walleye, pike and white sucker fry for toxicity tests in Winnipeg. The walleye and pike fry will be acquired from Qu'Appelle Hatchery, SK; the white sucker will be hatched in a temporary facility on the La Salle River in Starbuck, MB from eggs collected from the Saskatchewan River spawners at Grand Rapids.

MANITOBA DEPARTMENT OF NATURAL RESOURCES
FISH HABITAT MANAGEMENT SECTION

SCIENTIFIC COLLECTION PERMIT

Issued under the authority of the Fisheries Act (Manitoba) and the Fishing Licence Regulation and Fishing Licence Fee Regulation made thereunder.

Issued to: Marlene Gifford, TetRes Consultants

of: 603-386 Broadway, Winnipeg MB 942-2505

is hereby authorized to collect, transport and possess fish within the Province of Manitoba subject to the following conditions:

1. Release live fish only in the water from which they were taken.
2. Fish may not be sold, traded or bartered.
3. The use of chemicals and explosives as aids in collecting fish is prohibited.
4. This permit expires on 31-Jul-99 following date of issue.
5. A report must be submitted to Fisheries Branch, Box 40, 200 Saulteaux Crescent, Winnipeg, MB R3J 3W3 upon expiration of this permit indicating: location, species, number and disposition of the collected specimens.
6. Special Conditions: See Attached.

Shelley Matkowski

Issued By

Fish Enhancement Biologist

Title

11-May-99

Date of Issue

Signature of Permittee

Permit Number: 99-06

Attachment to Scientific Collection Permit #99-06

May 11, 1999

To collect live specimens of indigenous fish species from the Red River and its tributaries using seine nets, dip nets, and minnow traps. Up to 10 specimens of each species may be used for educational display in aquariums in the city of Winnipeg. Please contact local Natural Resource Officer prior to each collection (945-7258).

MANITOBA DEPARTMENT OF NATURAL RESOURCES
FISH HABITAT MANAGEMENT SECTION

LIVE FISH HANDLING PERMIT

Issued under the authority of the Fisheries Act (Federal and Provincial) and the Manitoba Fishery Regulations, the Fishing Licence Fee Regulation, and the Fishing Licence Fee Regulations made thereunder.

Issued to: Marlene Gifford, TetRES Consultants

of: 603-386 Broadway, Winnipeg MB R3C 3R6

is hereby authorized to handle live fish subject to the following conditions:

1. Purpose of Permit: To import fish into Manitoba for toxicity testing purposes.
2. Location: City of Winnipeg, North End Pollution Centre
3. Effective Date: 22-Jun-99 to 30-Nov-99 inclusive.
4. Special Conditions: Fish fry will be tested at the North End Pollution Control Centre. All fish must be euthanized at the termination of the study. Water in which the fish are shipped and tested must be disposed of via the sewer system at the treatment plant.

Lorimer Thompson

Issued By

Chief, Fish Habitat

Title

22-Jun-99

Date of Issue

Signature of Permittee

Permit Number: 99-16

MANITOBA DEPARTMENT OF NATURAL RESOURCES
FISH HABITAT MANAGEMENT SECTION

SCIENTIFIC COLLECTION PERMIT

Issued under the authority of the Fisheries Act (Manitoba) and the Fishing Licence Regulation and Fishing Licence Fee Regulation made thereunder.

Issued to: Karen Mathers, Donald Harron

of: TetrES Consultants Inc. 603-386 Broadway, Winnipeg, MB R3C 3R6

is hereby authorized to collect, transport and possess fish within the Province of Manitoba subject to the following conditions:

1. Release live fish only in the water from which they were taken.
2. Fish may not be sold, traded or bartered.
3. The use of chemicals and explosives as aids in collecting fish is prohibited.
4. This permit expires on 30-Sep-99 following date of issue.
5. A report must be submitted to Fisheries Branch, Box 40, 200 Saulteaux Crescent, Winnipeg, MB R3J 3W3 upon expiration of this permit indicating; location, species, number and disposition of the collected specimens.
6. Special Conditions: As on attached Schedule 1.

Shelley Matkowski

Issued By

Stock Enhancement Biologist

Title

5-Jul-99

Date of Issue

Signature of Permittee

Permit Number: 99-33

SCHEDULE 1. SCIENTIFIC COLLECTION PERMIT NO. 99-33

Authorization to collect 3,000 *Pyganodon grandis* mussels (northern floater) and 2,500 *Sphaerium simile* (grooved fingernail clam) of the ages 1-3 years from the LaSalle River near Starbuck, MB, by means of shovels and screens. All other fauna captured incidentally are to be released immediately on site.

Those clams that are retained will be held in the LaSalle River at the residence of TetraE's biologist Don Harron of Starbuck, or the City of Winnipeg North End Water Pollution Control Centre (NEWPCC), until needed for *in-situ* toxicity tests in the Red River. The clams will then be deployed in cages within 200 m of the NEWPCC until late September when they will be removed and evaluated. All clams are to be destroyed at the conclusion of the study. Shells may be retained for scientific purposes.

Ministry of
Natural Resources

Ministère des
Richesses naturelles



Ontario

Scientific Collection and Live Fish Transfer Licence

Under the Fish and Wildlife Conservation Act and the Regulations, and subject to the limitations thereof and the limitations of the Fisheries Act (Canada) and the Ontario Fishery Regulations, 1989 as amended, this licence is granted to :

NAME: Amy Partridge and Assistants
of Tetres Consultants Inc.
603- 386 Broadway
Winnipeg, Manitoba
R3C 3R6

To collect sucker spawn and milt from the waters of Kenora Ministry of Natural Resources District excluding any waters that are classified as fish sanctuaries or have closed seasons for that species for the purposes of water toxicity studies to be conducted at the home site or auxiliary building of Tetres Consultants Inc. . Harvesting of the fish for milking is to be done by legal angling means normally available to an angler licenced under an Ontario sport fishing licence .

The authorized collection period will cover the time April 28th through to and including May 10th .

Live spawn and milt may be transported from Ontario to the Tetres Consultant facilities in Manitoba . No adult fish may be transported under this licence and no live spawn may be released into any waterbody in Ontario or Manitoba other than the host waterbody and the Tetres holding tanks.

Any Manitoba authorization required for importation will be the responsibility of the applicant licensee .

Issued at the Kenora District Ministry of Natural Resources office this 28th day of April 2000 .

Licencee

Licence Issuer / Fisheries Officer

MANITOBA CONSERVATION
FISH HABITAT MANAGEMENT SECTION

LIVE FISH HANDLING PERMIT

Issued under the authority of the Fisheries Act (Federal and Provincial) and the Manitoba Fishery Regulations, the Fishing Licence Fee Regulation, and the Fishing Licence Fee Regulations made thereunder.

Issued to: Mr. Don Harron

of: TetrES Consultants, Winnipeg, MB 942-2505

is hereby authorized to handle live fish subject to the following conditions:

1. Purpose of Permit: To import or collect and hold fish and fish eggs as listed on permit attachment #00-03
2. Location: Fish & Eggs will be held at the City of Winnipeg Northend Water Pollution Control Centre or residence of Mr. Don Harron.
3. Effective Date: 4-Apr-00 to 31-Jul-00 inclusive.
4. Special Conditions: All fish to be destroyed upon completion of research.

Shelley Matkowski

Issued By

Fish Enhancement Biologist

Title

4-Apr-00

Date of Issue

Signature of Permittee

Permit Number: 00-03

Attachment to Live Fish Handling Permit #00-03

The following fish and fish eggs may be imported/collected and held

- 1000 northern pike eggs/fry from Qu'Appelle Hatchery, Saskatchewan
- 1000 walleye eggs/fry from Qu'Appelle Hatchery, Saskatchewan
- 1000 lake whitefish eggs/fry from Grand Rapids Hatchery &
- 1000 lake trout fingerlings from Grand Rapids Hatchery
- 800 fathead minnows from Aquatic Biosystems, Colorado
- 5000 white sucker eggs/fry from spawning runs in southern Manitoba streams
- 1000 walleye fingerlings from a hatchery in Southern Ontario

MANITOBA CONSERVATION
FISH HABITAT MANAGEMENT SECTION

SCIENTIFIC COLLECTION PERMIT

Issued under the authority of the Fisheries Act (Manitoba) and the Fishing Licence Regulation and Fishing Licence Fee Regulation made thereunder.

Issued to: Mr. Don Harron

of: Tetres Consultants - Winnipeg MB 942-2505

is hereby authorized to collect, transport and possess fish within the Province of Manitoba subject to the following conditions:

1. Release live fish only in the water from which they were taken.
2. Fish may not be sold, traded or bartered.
3. The use of chemicals and explosives as aids in collecting fish is prohibited.
4. This permit expires on 31-Jul-00 following date of issue.
5. A report must be submitted to Fisheries Branch, Box 40, 200 Saulteaux Crescent, Winnipeg, MB R3J 3W3 upon expiration of this permit indicating location, species, number and disposition of the collected specimens.
6. Special Conditions: To collect and hold 1,500 fingernail clams from the LaSalle River near Starbuck, Manitoba. All clams to be destroyed upon completion of research. Notify local Natural Resource Officer prior to collection.

Shelley Matkowski

Issued By

Fish Enhancement Biologist

Title

Signature of Permittee

4-Apr-00

Date of Issue

Permit Number: 00-11

APPENDIX C

PROCEDURE FOR OBTAINING FERTILIZED FISH EGGS

Procedure for obtaining fertilized fish eggs

- Gently press the lower abdomen of “ripe” female and male fish in an anterior to posterior motion to obtain eggs and sperm. Perform this procedure over a metal mixing bowl as soon as fish are retrieved from the net, or retain live fish in a holding tub with water until it is convenient to do so. Sperm does not have to be added immediately to the eggs but eggs should be fertilized within ten minutes.
- Add one to two cups of lake/river water and gently mix the eggs and sperm together with a feather. The eggs and sperm of several fish can be mixed in one batch.
- Sprinkle approximately two tablespoons of bentonite clay (“grout”) over each liter of mixture and continue to stir until reasonably well mixed. Some clumping of clay may occur.
- Pour eggs/sperm/clay mixture into a large zip-lock bag and enclose in a second zip-lock bag to minimize accidental leakage. Do not press excess air from bags before sealing.
- Mark each bag with:
 - species
 - date and time
 - approximate location
- Float the bags in a cooler with water and ice. Avoid direct contact of eggs with ice.

- At the rearing site, place the fertilized eggs in a pool (diameter: 1.5-m) receiving a fresh supply of river or lake water. Adjust flows so that eggs circulate slightly. Gentle agitation of eggs will keep them adequately aerated and assist in culling unfertilized eggs.
- Unfertilized eggs will float and can be removed with a net, siphon, or can be allowed to spill over the lip of the pool. Healthy eggs will hatch within seven to ten days.

APPENDIX D
TEST CONDITIONS

Table D-1. Test conditions for static and semi-static testing.

TEST NUMBER	1	2	3A&B	4A&B	5A&B
SPECIES – Common name - <i>Scientific name</i>	Walleye <i>Stizostedion vitreum</i>	White sucker <i>Catostomus commersoni</i>	White sucker <i>Catostomus commersoni</i>	White sucker <i>Catostomus commersoni</i>	Fathead minnow <i>Pimephales promelas</i>
AGE AT TEST-START	8 days	18 days	24 days	29 days	72 hours
LIFE-CYCLE STAGE ^a	Larval	Larval	Larval	Larval	Larval
WET WEIGHT (g)	Not measured	Not	Not measured	Not measured	Not measured
TEST TYPE (Static, Semi-static, flow-through)	Static	Static	Semi-static	Semi-static	Semi-static
DURATION	96 hrs	96 hrs	96 hrs	10 days	T5A – 96 hrs T5B - 72 hrs
AMMONIA SOURCE	NH ₄ Cl	NH ₄ Cl	T3A – NH ₄ Cl T3B – NH ₄ Cl + effluent	T4A – NH ₄ Cl T4B – NH ₄ Cl + effluent	T5A – NH ₄ Cl T5B – NH ₄ Cl + effluent
NOMINAL NH ₃ -N CONCENTRATION (mg/L)	1.0-125.0	1.0-125.0	0.5-32.0	1.0-64.0	1.0-32.0
DILUTION FACTOR	0.5	0.5	0.5	0.5	0.5
NUMBER OF EXPOSURE GROUPS	8	8	7	7	6
EXPOSURE VOLUME	600 mL	600 mL	500 mL	500 mL	750 mL
REPLACEMENT RATE	None	None	500 mL/d	500 mL/d	750 mL/d
NUMBER OF ORGANISMS/VESSEL	10	10	10	10	10
NUMBER OF REPLICATES/CON.	3	3	3	3	4
AERATION RATE (mL/Min.)	None	None	None	none	~45 mL/min
FOOD TYPE	Liquid Baby Fish Food	Liquid Baby Fish Food	Not fed	Live Shrimp Brine	Live Shrimp Brine
FEEDING RATE	1 drop on day 3	1 drop on day 3	n/a	2X daily ~ 4.4 mg dry wt.	2X daily ~ 4.4 mg dry wt.

Notes:

a Larval = very young or newly hatched fish.

Table D-2. Test conditions for flow-through testing.

TEST NUMBER	6	7	8	9	10
SPECIES – Common name - Scientific name	Channel catfish <i>Ictalurus punctatus</i>	Fathead minnow <i>Pimephales promelas</i>	Fathead minnow <i>Pimephales promelas</i>	Northern pike <i>Esox lucius</i>	Walleye <i>Stizostedion vitreum</i>
AGE AT TEST-START	117 days	~ 90 days ^a	35 – 45 days	1½ weeks	39 days
LIFE-CYCLE STAGE ^b	Juvenile	Juvenile	Juvenile	Larval	Juvenile
WET WEIGHT (g)	~ 5.0	~ 1.3	~ 0.50	Not measured	0.3-0.5
TEST TYPE (Static, Semi-static, flow-through)	Flow-through	Flow-through	Flow-through	Flow-through	Flow-through
DURATION	30 days	30 days	29 days	12 days	30 days
AMMONIA SOURCE	NH ₄ Cl	NH ₄ Cl	NH ₄ Cl	NH ₄ Cl	NH ₄ Cl
NOMINAL NH ₃ -N CONCENTRATION (mg/L)	0.5-16.0	0.5-16.0	0.5-8.0	0.5-8.0	0.5-6.0
DILUTION FACTOR	0.5	0.5	0.5	0.25 between three most concentrated solutions; 0.5 between three most dilute solutions	0.25 between two most concentrated solutions; 0.5 between three most dilute solutions
NUMBER OF EXPOSURE GROUPS	6	6	5	6	5
EXPOSURE VOLUME	10 L	10 L	1 L	700 mL	8 L
REPLACEMENT RATE	200 mL/min	200 mL/min	200 mL/min	400 mL/min	400 mL/min
NUMBER OF ORGANISMS/VESSEL	15	20	15	20	20
NUMBER OF REPLICATES/CON.	4	4	4	4	3
AERATION RATE (mL/Min.)	none	none	None	none	~310 mL/min
FOOD TYPE	Trout Chow	Trout Chow	Live Shrimp Brine	live daphnia	(a) live daphnia or (b) frozen daphnia
FEEDING RATE	2X daily Day 1 - 23: 1.0g Day 24 - 30: 1.5g	2X daily Day 1 - 23: 1.0g Day 24 - 30: 1.5g	2X daily 0.2 g wet weight	3X daily 2.6 g wet weight	3X daily (a) 2.6 g or (b) 2.7g wet weight

Notes:

- a Fish were captured locally, therefore, exact age approximated.
b Larval = very young or newly hatched fish;
Juvenile = young fish that have not reached sexual maturity.

APPENDIX E

PROTOCOL FOLLOWED WHILE PREPARING TEST- SOLUTIONS FOR STATIC AND SEMI-STATIC TESTS

Ammonia stock preparation:

Time prepared: beginning of test

Ammonia source: ammonium chloride (NH₄Cl)

Desired stock concentration: 1 mg NH₃-N/ml solution

Q: To prepare 1000 mL of ammonia stock with an NH₃-N concentration of 1.0 mg/ml, 1000 mg NH₃-N is needed. If NH₄Cl is the NH₃-N source, how many grams of NH₄Cl must be added to each 1.0 L of de-ionized water to produce the desired stock concentration?

Molecular weight of N = 14.007 u
Molecular weight of H = 1.008 u
Molecular weight of Cl = 35.453
Molecular weight of NH₄Cl = 53.492 u

$$\text{So, } \frac{1.0 \text{ g NH}_3\text{-N}}{x \text{ g NH}_4\text{Cl}} = \frac{14.007 \mu}{53.492 \mu} \quad x = 3.82 \text{ g NH}_4\text{Cl}$$

A: 3.82 g NH₄Cl must be added to each 1.0 L of de-ionized water to prepare a 1.0 mg NH₃-N/ml ammonia-stock solution.

Procedure for preparing river water (RW) stock:

Time Prepared: (a) prior to initiation of static-tests

(b) prior to initiation of semi-static tests and once daily in 24-hour

intervals following test-start

Assume: (a) Test-solution volume per replicate test-chamber = 500 ml

(b) Highest nominal $\text{NH}_3\text{-N}$ exposure-concentration = 32 mg/L

Procedure:

- (1) Pour 32 ml of ammonia-stock (@1.0 mg $\text{NH}_3\text{-N}/\text{ml}$) into a 1.0 L graduated cylinder and fill to 1.0 L with river water (i.e., concentration = 32 mg/L) (Figure E-1).
- (2) Pour 500 ml of (1) into one replicate test-chamber marked for the highest exposure-concentration. (Note: 500 ml of RW-stock will remain in graduated cylinder; do not discard) (c.f., Figure E-1).
- (3) Refill graduated cylinder to 1.0 L with river water (i.e., add 500 ml of RW thus diluting the solution by a factor of 0.5).
- (4) Repeat steps (2) and (3) until $\text{NH}_3\text{-N}$ concentration is equal to the lowest exposure-concentration in the dilution-series.
- (5) Repeat steps (1) to (4) for each set of replicates for each test.

Procedure for preparing effluent (E) stock:

Time Prepared: (a) prior to initiation of static-tests

(b) prior to initiation of semi-static tests and once daily in 24-hour

intervals following test-start

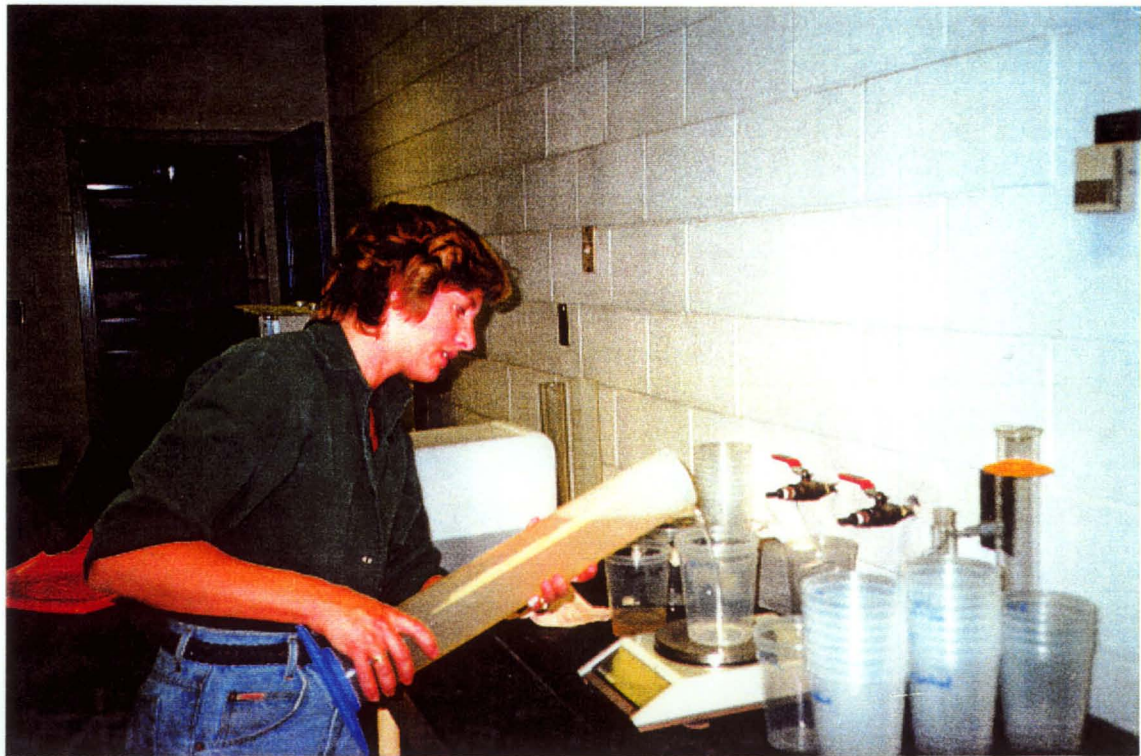
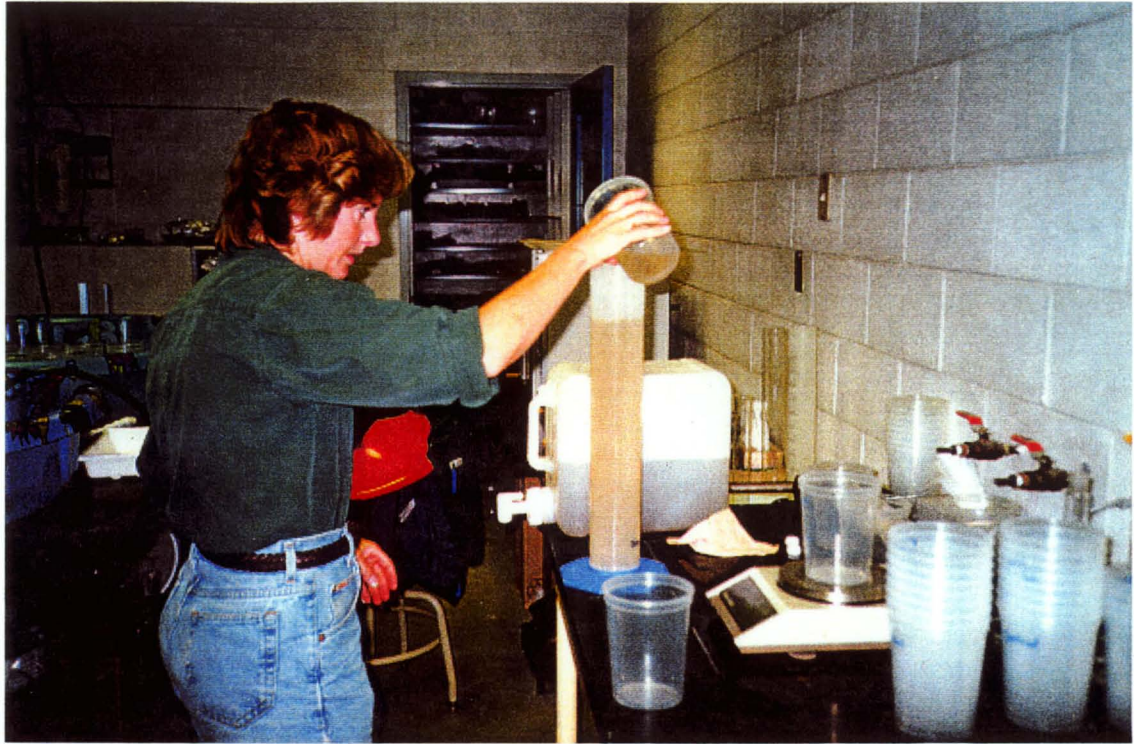


Figure E-1. Preparing test solutions for static and semi-static tests.

Assume: (a) Test-solution volume = 500 mL

(b) Highest nominal $\text{NH}_3\text{-N}$ concentration = 32 mg/L

Procedure:

(1) Obtain a grab sample of effluent and measure the $\text{NH}_3\text{-N}$ concentration using a CHEMet® Ammonia Kit.

If the measured $\text{NH}_3\text{-N}$ concentration is less than the highest nominal concentration:

(2) Perform the following calculation: subtract the measured concentration from the desired concentration. (E.g., If effluent is at 20 mg/L and 32 mg/L is desired, then add $32-20=12$ mg $\text{NH}_3\text{-N/L}$ E-stock).

(3) Multiply the result of (2) by the volume of E-stock that needs to be prepared. In this case, 1.0 L of E-stock is needed for each replicate exposure (i.e., 500 mL for the highest exposure-concentration and 500 mL for the dilution series). If there are 4 replicate test-chambers for each $\text{NH}_3\text{-N}$ exposure-concentration, 4.0 L of E-stock is required. So: $12 \text{ mg } \text{NH}_3\text{-N/L E-stock} * 4 \text{ L E-stock} = 48 \text{ mg}$.

(4) Since the ammonia-stock concentration = 1.0 mg $\text{NH}_3\text{-N/mL}$, the number of milligrams of $\text{NH}_3\text{-N}$ determined in (3) equals the number of milliliters of ammonia-stock that must be added to the effluent to obtain the highest nominal exposure-concentration. Drain an equal volume of effluent that will be replaced with ammonia-stock and add the ammonia-stock.

If the measured NH₃-N concentration is greater than the highest nominal concentration:

(2) Determine the concentration of NH₃-N in the volume of effluent required for the dilution-series. (E.g., If effluent contains 34 mg NH₃-N/L, 4.0 L contains 136 mg/L).

(3) Perform the following calculation and solve for x where x is the total volume of E-stock required:

$$\begin{aligned}x &= \text{mass NH}_3\text{-N determined in (2) / highest desired nominal conc.} \\ &= 136 \text{ mg} / 32 \text{ mg /L} \\ &= 4.25 \text{ L}\end{aligned}$$

(4) Determine the volume of RW that must be added to the E-stock in order to dilute it the desired NH₃-N concentration. That is, subtract the volume of effluent from the total volume of E-stock required. (E.g., 4.25 L – 4.0 L = 0.25 L).

(5) Perform appropriate serial-dilutions as described for the RW-stock (i.e., steps (2)-(5)).

Notes:

(1) Variations to the above procedures include mixing and diluting larger volumes of stock to (a) accommodate increasing test-chamber volumes and (b) reduce the number of times steps (2) –(5) described for preparing RW-stock have to be repeated.

- (2) The volume of solution added to each test-chamber was determined in one of two ways. (a) Place each test-chamber on a tared scale and fill with stock until the weight equals the desired volume (assumption: stock density = 1.0 g/ml) (c.f., Figure E-1); or (b) fill each test-chamber to a pre-measured depth indicative of the desired volume.

APPENDIX F

NEWPCC LABORATORY RESULTS FOR DAILY TOTAL
AMMONIA-N CONCENTRATIONS

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY	D. De Luca	REPORT TO	Marlene
DATE SUBMITTED	June 04, 1999	ADDRESS	Tetres
DATE SAMPLED	June 04, 1999	POSTAL CODE	
SAMPLED BY	Marlene	PHONE #	942-2505
SAMPLE TYPE	River Samples		
LOCATION	North End Plant		
PROJECT	Ammonia Study		

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample	Date	NH3-N	Concentration - mg/L			
Description						
SB-Cont1	08-Jun-99	<1.0				
SB-Cont2	08-Jun-99	<1.0				
SB-1	08-Jun-99	2.2				
SB-2	08-Jun-99	4.5				
SB-4	08-Jun-99	6.7				
SA-8	08-Jun-99	8.7				
SB1-8	08-Jun-99	11.6				
SB2-8	08-Jun-99	11.7				
SB3-8	08-Jun-99	11.6				
SC-8	08-Jun-99	4.1				
SB-15	08-Jun-99	19.8				
SB-30	08-Jun-99	23.6				
SB-62	08-Jun-99	29.1				
SB-125	08-Jun-99	63.2				
WB-Cont1	08-Jun-99	<1.0				
WB-Cont2	08-Jun-99	<1.0				
WB-1	08-Jun-99	1.8				
WB-2	08-Jun-99	4.0				
WB-4	08-Jun-99	6.8				
WA-8	08-Jun-99	8.3				
WB1-8	08-Jun-99	12.1				
WB2-8	08-Jun-99	12.2				
WB3-8	08-Jun-99	12.2				
WC-8	08-Jun-99	8.6				
WB-15	08-Jun-99	14.4				
WB-30	08-Jun-99	26.0				
WB-62	08-Jun-99	33.2				
WB-125	08-Jun-99	61.1				
La Salle 1	08-Jun-99	<1.0				
La Salle 2	08-Jun-99	<1.0				
La Salle 3	08-Jun-99	<1.0				
OC data (verification stds)						
OC data (standard additions)						

Remarks: The 0-50 ppm NH3-N range (w/ 1/4 dia n loop) was used for this analysis

Date Analyzed: 4 Jun 99

Verified by:

Analyst: Dawn De Luca

Date: JUN 16 1999

File:

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: <u>Mariene</u>	REPORT TO: <u>Mariene</u>
DATE SUBMITTED: <u>8-Jun-99</u>	ADDRESS: <u>Tetres</u>
DATE SAMPLED: <u>8-Jun-99</u>	POSTAL CODE: _____
SAMPLED BY: <u>Mariene</u>	PHONE #: <u>942-2505</u>

SAMPLE TYPE: <u>River Samples</u>
LOCATION: <u>North End Plant</u>
PROJECT: <u>Ammonia Study</u>

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample	Description	Date	Concentration - mg/L			
			NH3-N			
1	SB-Cont1	08-Jun-99	1.3			
2	SB-Cont2	08-Jun-99	1.0			
3	SB-1	08-Jun-99	3.6			
4	SB-2	08-Jun-99	5.8			
5	SB-4	08-Jun-99	8.1			
6	SA-8	08-Jun-99	10.0			
7	SB1-8	08-Jun-99	11.0			
8	SB2-8	08-Jun-99	11.1			
9	SB3-8	08-Jun-99	11.1			
10	SC-8	08-Jun-99	4.8			
11	SB-15	08-Jun-99	13.4			
12	WB-Cont1	08-Jun-99	1.6			
13	WB-Cont2	08-Jun-99	1.0			
14	WB-1	08-Jun-99	3.9			
15	WB-2	08-Jun-99	5.6			
16	<u>WA-4</u> WB-2	08-Jun-99	5.7			
17	WB1-4	08-Jun-99	7.4			
18	WB2-4	08-Jun-99	7.7			
19	WB3-4	08-Jun-99	7.7			
20	WC-4	08-Jun-99	5.0			
21	WA-8	08-Jun-99	9.1			
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Remarks: The 0-50 ppm range (w/ 1/4 di/n loop) was used for this NH3 analysis.

Date Analyzed: 8-Jun-99 Verified by: _____
 Analyst: Dom De Luca Date: JUN 16 1999

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: <u>Marlene</u>	REPORT TO: <u>Marlene</u>
DATE SUBMITTED: <u>June 10, 1999</u>	ADDRESS: <u>Tetres</u>
DATE SAMPLED: <u>June 10, 1999</u>	POSTAL CODE: _____
SAMPLED BY: <u>Marlene</u>	PHONE #: <u>942-2505</u>
SAMPLE TYPE: <u>River water samples</u>	
LOCATION: _____	
PROJECT: <u>Ammonia Study</u>	

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample	Description	Date	NH3-N	Concentration - mg/L			
1	SA-E-CONTROL	10-Jun-99	22.3				
2	SA-E -.5	10-Jun-99	1.3				
3	SA-E -1	10-Jun-99	2.2				
4	SA-E -2	10-Jun-99	2.8				
5	SA-E -4	10-Jun-99	4.9				
6	SA-E -8	10-Jun-99	7.2				
7	SA-E -16	10-Jun-99	9.5				
8	SA-E -32	10-Jun-99	27.1				
9	SB-E-CONTROL	10-Jun-99	22.5				
10	SB-E -.5	10-Jun-99	1.4				
11	SB-E -1	10-Jun-99	1.8				
12	SB-E -2	10-Jun-99	2.4				
13	SB-E -4	10-Jun-99	4.2				
14	SB1-E-8	10-Jun-99	7.0				
15	SB2-E-8	10-Jun-99	7.0				
	SB-E -16	10-Jun-99	12.3				
	SB-E -32	10-Jun-99	27.1				
16	SC-RW-CONTROL	10-Jun-99	<1.0				
17	SC-RW -.5	10-Jun-99	1.2				
18	SC-RW -1	10-Jun-99	2.2				
19	SC-RW -2	10-Jun-99	3.2				
20	SC-RW -4	10-Jun-99	5.2				
21	SC-RW -8	10-Jun-99	6.7				
22	SC-RW -16	10-Jun-99	12.8				
23	SC-RW -32	10-Jun-99	18.0				
24	SD-RW-CONTROL	10-Jun-99	<1.0				
25	SD-RW -.5	10-Jun-99	1.8				
26	SD-RW -1	10-Jun-99	2.2				
27	SD-RW -2	10-Jun-99	3.2				
28	SD-RW -4	10-Jun-99	5.0				
29	SD1-RW-8	10-Jun-99	6.3				
30	SD2-RW-8	10-Jun-99	6.2				
31	SD-RW -16	10-Jun-99	8.7				
32	SD-RW -32	10-Jun-99	19.7				
33	SE-E-CONTROL	10-Jun-99	22.2				
34	SE-E -.5	10-Jun-99	<1.0				
35	SE-E -1	10-Jun-99	1.4				
36	SE-E -2	10-Jun-99	2.3				
37	SE-E -4	10-Jun-99	4.4				
38	SE-E -8	10-Jun-99	6.8				
39	OC data: (verification stats)						
40							
41							
42							
43	OC data: (standard additions)						
44							
45							
46							

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dil'n loop) was used for this analysis

CONTINUED ON NEXT PAGE

Date Analyzed: 11-Jun-99

Verified by: _____

Analyst: Dom De luca

Date: JUN 16 1999

File: _____

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: Marlene	REPORT TO: Marlene
DATE SUBMITTED: June 11, 1999	ADDRESS: Tetres
DATE SAMPLED: June 11, 1999	POSTAL CODE: _____
SAMPLED BY: Marlene	PHONE # 942-2505
SAMPLE TYPE: River Samples	
LOCATION: North End Plant	
PROJECT: Ammonia Study	

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample		Concentration - mg/L			
Description	Date	NH3-N			
SA-E-CONTROL	11-Jun-99	over range			
SA-E -0.5	11-Jun-99	1.1			
SA-E -1	11-Jun-99	1.6			
SA-E -2	11-Jun-99	2.9			
SA-E -4	11-Jun-99	4.5			
SA-E -8	11-Jun-99	7.4			
SA-E -16	11-Jun-99	15.4			
SA-E -32	11-Jun-99	36.5			
SB-E-CONTROL	11-Jun-99	over range			
SB-E -0.5	11-Jun-99	1.0			
SB-E -1	11-Jun-99	1.7			
SB-E -2	11-Jun-99	2.6			
SB-E -4	11-Jun-99	5.5			
SB1-E-8 -8	11-Jun-99	9.3			
SB2-E-8 -8	11-Jun-99	9.2			
SB-E -16	11-Jun-99	11.8			
EFFLUENT	11-Jun-99	28.4			
SC-RW-CONTROL	11-Jun-99	<0.5			
SC-RW -0.5	11-Jun-99	0.9			
SC-RW -1	11-Jun-99	1.3			
SC-RW -2	11-Jun-99	2.6			
SC-RW -4	11-Jun-99	3.7			
SC-RW -8	11-Jun-99	7.4			
EFFLUENT	11-Jun-99	28.9			
RIVER WATER	11-Jun-99	<0.5			
SD-RW-CONTROL	11-Jun-99	<0.5			
SD-RW -0.5	11-Jun-99	0.7			
SD-RW -1	11-Jun-99	1.4			
SD-RW -2	11-Jun-99	2.6			
SD-RW -4	11-Jun-99	4.6			
SD1-RW -8	11-Jun-99	8.4			
SD2-RW -8	11-Jun-99	8.4			
SD-RW -16	11-Jun-99	11.7			
RIVER WATER	11-Jun-99	<0.5			
SE-E-CONTROL	11-Jun-99	28.6			
SE-E -0.5	11-Jun-99	0.8			
SE-E -1	11-Jun-99	1.7			
SE-E -2	11-Jun-99	2.6			
SE-E -4	11-Jun-99	4.5			
SE-E -8	11-Jun-99	8.0			
QC data: (verification stds)					
QC data: (standard additions)					

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dil'n loop) was used for this analysis

CONTINUED ON NEXT PAGE

Date Analyzed: 11-Jun-99

Verified by:

Analyst: Dom De Luca

Date: JUN 16 1999

File

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: <u>Marlene</u>	REPORT TO: <u>Marlene</u>
DATE SUBMITTED: <u>June 12, 1999</u>	ADDRESS: <u>Tetres</u>
DATE SAMPLED: <u>June 12, 1999</u>	POSTAL CODE: _____
SAMPLED BY: <u>Marlene</u>	PHONE # <u>942-2505</u>
SAMPLE TYPE: <u>River Samples</u>	
LOCATION: <u>North End Plant</u>	
PROJECT: <u>Ammonia Study</u>	

PARAMETERS REQUESTED: Total Ammonia mg/L

RESULTS

Sample	Description	Date	Concentration - mg/L			
			NH3-N			
1	SA-E-CONTROL	12-Jun-99	25.8			
2	SA-E -0.5	12-Jun-99	1.0			
3	SA-E -1	12-Jun-99	1.3			
4	SA-E -2	12-Jun-99	2.7			
5	SA-E -4	12-Jun-99	3.6			
6	SA-E -8	12-Jun-99	6.2			
7	SA-E -16	12-Jun-99	13.0			
8	SA-E -32	12-Jun-99	33.7			
9	SB-E-CONTROL	12-Jun-99	25.9			
10	SB-E -0.5	12-Jun-99	1.0			
11	SB-E -1	12-Jun-99	1.7			
12	SB-E -2	12-Jun-99	2.6			
13	SB-E -4	12-Jun-99	3.2			
14	SB-E- SB1-E-8	12-Jun-99	6.3			
15	SB-E- SB2-E-8	12-Jun-99	6.3			
16	SC-RW-CONTROL	12-Jun-99	0.7			
17	SC-RW -0.5	12-Jun-99	0.9			
18	SC-RW -1	12-Jun-99	1.2			
19	SC-RW -2	12-Jun-99	1.7			
20	SC-RW -4	12-Jun-99	2.8			
21	SC-RW -8	12-Jun-99	4.6			
22	SD-RW-CONTROL	12-Jun-99	0.7			
23	SD-RW -0.5	12-Jun-99	1.0			
24	SD-RW -1	12-Jun-99	1.2			
25	SD-RW -2	12-Jun-99	1.7			
26	SD-RW -4	12-Jun-99	2.2			
27	SD-RW- SD1-RW-8	12-Jun-99	4.3			
28	SD-RW- SD2-RW-8	12-Jun-99	4.4			
29	SE-E-CONTROL	12-Jun-99	26.6			
30	SE-E -0.5	12-Jun-99	1.2			
31	SE-E -1	12-Jun-99	1.6			
32	SE-E -2	12-Jun-99	2.5			
33	SE-E -4	12-Jun-99	4.2			
34	SE-E -8	12-Jun-99	7.9			
35	SE-E -16	12-Jun-99	7.6			
36	SF-RW-CONTROL	12-Jun-99	0.7			
37	SF-RW -0.5	12-Jun-99	0.7			
38	SF-RW -1	12-Jun-99	0.9			
39	SF-RW -2	12-Jun-99	1.5			
40	SF-RW -4	12-Jun-99	2.8			
41	SF-RW -8	12-Jun-99	5.2			
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100						
OC data (standard additions)						
• 5.0 ppm NH3-N						
• 10.0 ppm NH3-N						
Hach verification standard (10.0 mg/L NH3-N)	June 12, 1999	9.7				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dil'n loop) was used for this analysis

Date Analyzed: 14-Jun-99

Verified by: _____

Analyst: Dom De Luca

Date: JUN 16 1999

File

City of Winnipeg
Laboratory Services Division

Analytical Report

13 samples were preserved and analyzed on the 14th

SAMPLES SUBMITTED BY: Marlene	REPORT TO: Marlene
DATE SUBMITTED: June 14, 1999	ADDRESS: Tetes
DATE SAMPLED: June 14, 1999	POSTAL CODE: _____
SAMPLED BY: Marlene	PHONE #: 942-2505
SAMPLE TYPE: River Samples	
LOCATION: North End Plant	
PROJECT: Ammonia Study	

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample	Date	NH3-N	Concentration - mg/L			
SA-E-CONTROL	14-Jun-09	22.2				
SA-E -0.5	14-Jun-09	1.4				
SA-E -1	14-Jun-09	1.8				
SA-E -2	14-Jun-09	2.4				
SA-E -4	14-Jun-09	4.7				
SA-E -8	14-Jun-09	5.8				
SA-E -16	14-Jun-09	11.2				
SA-E -32	14-Jun-09	32.6				
SB-E-CONTROL	14-Jun-09	21.9				
SB-E -0.5	14-Jun-09	1.2				
SB-E -1	14-Jun-09	1.3				
SB-E -2	14-Jun-09	1.9				
SB-E -4	14-Jun-09	3.3				
SB1-E-8	14-Jun-09	6.2				
SB2-E-8	14-Jun-09	6.0				
SB-E -16	14-Jun-09	13.1				
SC-RW-CONTROL	14-Jun-09	<1.0				
SC-RW -0.5	14-Jun-09	1.0				
SC-RW -1	14-Jun-09	1.4				
SC-RW -2	14-Jun-09	1.8				
SC-RW -4	14-Jun-09	2.4				
SC-RW -8	14-Jun-09	4.8				
SD-RW-CONTROL	14-Jun-09	<1.0				
SD-RW -0.5	14-Jun-09	<1.0				
SD-RW -1	14-Jun-09	1.2				
SD-RW -2	14-Jun-09	1.8				
SD-RW -4	14-Jun-09	2.6				
SD1-RW -8	14-Jun-09	4.7				
SD2-RW -8	14-Jun-09	4.7				
SE-E-CONTROL	14-Jun-09	22.0				
SE-E -0.5	14-Jun-09	1.2				
SE-E -1	14-Jun-09	1.6				
SE-E -2	14-Jun-09	2.4				
SE-E -4	14-Jun-09	4.1				
SE-E -8	14-Jun-09	6.7				
SE-E -16	14-Jun-09	8.5				
SF-RW-CONTROL	14-Jun-09	<1.0				
SF-RW -0.5	14-Jun-09	<1.0				
SF-RW -1	14-Jun-09	<1.0				
SF-RW -2	14-Jun-09	1.2				
SF-RW -4	14-Jun-09	1.6				
SF-RW -8	14-Jun-09	2.6				
OC data (verification stds)						
Hach verification standard (10.0 mg/L NH3-N)		9.7				
2.0 mg/L std		1.9				
OC data (standard additions)						
Riverwater Control + 5.0 ppm NH3-N		81				
Riverwater Control + 10.0 ppm NH3-N		73				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dil'n loop) was used for this analysis

Date Analyzed: 14-Jun-99

Analyst: Dom Daluca, Ted Poniatowski

Verified by: _____

Date: JUN 16 1999

File: _____

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY	Marlene	REPORT TO	Marlene
DATE SUBMITTED	June 15, 1999	ADDRESS	Terres
DATE SAMPLED	June 15, 1999	POSTAL CODE	
SAMPLED BY	Marlene	PHONE #	942-2505

SAMPLE TYPE	River Samples
LOCATION	North End Plant
PROJECT	Ammonia Study

PARAMETERS REQUESTED Total Ammonia

RESULTS

Sample	Date	NH3-N	Concentration - mg/L				
SA-RW-CONTROL	15-Jun-99	<1.0					
SA-RW -2	15-Jun-99	2.7					
SA-RW -4	15-Jun-99	4.5					
SA-RW -8	15-Jun-99	4.1					
SA-RW -16	15-Jun-99	6.3					
SA-RW -32	15-Jun-99	18.5					
SB-RW-CONTROL	15-Jun-99	<1.0					
SB-RW -2	15-Jun-99	3.7					
SB-RW -4	15-Jun-99	6.3					
SB-RW -8	15-Jun-99	7.0					
SB-RW -16	15-Jun-99	10.0					
SB-RW -32	15-Jun-99	20.8					
SC-RW-CONTROL	15-Jun-99	<1.0					
SC-RW -2	15-Jun-99	3.0					
SC-RW -4	15-Jun-99	4.3					
SC-RW -8	15-Jun-99	7.3					
SC-RW -16	15-Jun-99	10.1					
SC-RW -32	15-Jun-99	12.4					
SD-RW-CONTROL	15-Jun-99	<1.0					
SD-RW -2	15-Jun-99	3.6					
SD-RW -4	15-Jun-99	5.8					
SD-RW -8	15-Jun-99	9.0					
SD-RW -16	15-Jun-99	13.8					
SD-RW -32	15-Jun-99	14.6					
SA-E-CONTROL	15-Jun-99	26.6					
SA-E -2	15-Jun-99	2.6					
SA-E -4	15-Jun-99	4.1					
SA-E -8	15-Jun-99	5.7					
SA-E -16	15-Jun-99	8.8					
SA-E -32	15-Jun-99	36.9					
SB-E-CONTROL	15-Jun-99	26.5					
SB-E -2	15-Jun-99	3.3					
SB-E -4	15-Jun-99	5.1					
SB-E -8	15-Jun-99	6.8					
SB-E -16	15-Jun-99	12.6					
SB-E -32	15-Jun-99	32.6					
SC-E-CONTROL	15-Jun-99	26.8					
SC-E -2	15-Jun-99	2.4					
SC-E -4	15-Jun-99	3.3					
SC-E -8	15-Jun-99	5.6					
SC-E -16	15-Jun-99	12.6					
SC-E -32	15-Jun-99	31.3					
SA-RW-0.5	15-Jun-99	1.7					
SB-RW-0.5	15-Jun-99	1.5					
SC-RW-0.5	15-Jun-99	1.5					
SA-RW-1	15-Jun-99	2.0					
SB-RW-1	15-Jun-99	2.9					
SC-RW-1	15-Jun-99	2.0					
SA-E-0.5	15-Jun-99	1.2					
SB-E-0.5	15-Jun-99	1.2					
SC-E-0.5	15-Jun-99	1.5					
SA-E-1	15-Jun-99	1.2					
SB-E-1	15-Jun-99	1.9					
SC-E-1	15-Jun-99	2.8					
OC data (verification stds)							
Hach verification standard (10.0 mg/L NH3-N)		9.5					
OC data (standard additions)			% recovery	% recovery	% recovery	% recovery	% recovery
NEWPCC Final Eff. - 10.0 ppm NH3-N			103				
NEWPCC Final Eff. - 20.0 ppm NH3-N			98				

Remarks: The 0.25 ppm NH3-N range (w/ 1/4 pin loop) was used for this analysis

Date Analyzed: 15 Jun 99
 Analyst: Dawn Debra Ted Powis
 Verified by: [Signature]
 Date: [Signature]

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY	Amy Partridge	REPORT TO	Amy Partridge
DATE SUBMITTED	June 16 1999	ADDRESS	Tetes
DATE SAMPLED	June 16 1999	POSTAL CODE	
SAMPLED BY	Amy Partridge	PHONE #	942-2505

SAMPLE TYPE	River Samples
LOCATION	North End Plant
PROJECT	Ammonia Study

PARAMETERS REQUESTED Total Ammonia

RESULTS

Sample	Description	Date	NH3-N	Concentration - mg/L			
1	SA-RW-CONTROL	June 16, 1999	<0.4				
2	SA-RW-5	June 16, 1999	1.3				
3	SA-RW-1.0	June 16, 1999	1.8				
4	SA-RW-2	June 16, 1999	2.6				
5	SA-RW-4	June 16, 1999	4.6				
6	SA-RW-8	June 16, 1999	6.3				
7	SA-RW-16	June 16, 1999	8.2				
8	SB-RW-CONTROL	June 16, 1999	<0.4				
9	SB-RW-5	June 16, 1999	1.9				
10	SB-RW-1.0	June 16, 1999	2.8				
11	SB-RW-2	June 16, 1999	3.4				
12	SB-RW-4	June 16, 1999	5.1				
13	SB-RW-8	June 16, 1999	7.4				
14	SB-RW-16	June 16, 1999	8.8				
15	SB-RW-32	June 16, 1999	9.6				
16	SC-RW-CONTROL	June 16, 1999	<0.4				
17	SC-RW-5	June 16, 1999	2.2				
18	SC-RW-1.0	June 16, 1999	4.1				
19	SC-RW-2	June 16, 1999	6.4				
20	SC-RW-4	June 16, 1999	7.9				
21	SC-RW-8	June 16, 1999	10.6				
22	SC-RW-16	June 16, 1999	15.5				
23	SC-RW-32	June 16, 1999	17.0				
24	SA-E-CONTROL	June 16, 1999	34.9				
25	SA-E-0.5	June 16, 1999	1.4				
26	SA-E-1.0	June 16, 1999	2.2				
27	SA-E-2	June 16, 1999	3.1				
28	SA-E-4	June 16, 1999	4.4				
29	SA-E-8	June 16, 1999	7.5				
30	SA-E-16	June 16, 1999	15.6				
31	SA-E-32	June 16, 1999	18.1				
32	SB-E-CONTROL	June 16, 1999	44.0				
33	SB-E-5	June 16, 1999	1.9				
34	SB-E-1.0	June 16, 1999	2.9				
35	SB-E-2	June 16, 1999	3.9				
36	SB-E-4	June 16, 1999	6.7				
37	SB-E-8	June 16, 1999	8.4				
38	SB-E-16	June 16, 1999	17.4				
39	SB-E-32	June 16, 1999	24.2				
40	SC-E-CONTROL	June 16, 1999	44.2				
41	SC-E-5	June 16, 1999	1.5				
42	SC-E-1.0	June 16, 1999	3.2				
43	SC-E-2	June 16, 1999	3.0				
44	SC-E-4	June 16, 1999	6.0				
45	SC-E-8	June 16, 1999	8.5				
46	SC-E-16	June 16, 1999	12.9				
47	SC-E-32	June 16, 1999	21.5				
QC data:							
Hach Verification Standard (10.0 mg/L NH3-N)							
	0.0 ppm std. check		10.3				
	2.0 ppm std. check		0.2				
	7.0 ppm std. check		1.9				
	5.0 ppm std. check		4.9				
	10.0 ppm std. check		10.0				
	20.0 ppm std. check		19.8				
Standard additions							
	River Water (17 June 09) filtered	+ 10.0 ppm NH3-N	101				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 d/n loop) was used for this analysis. MOL <0.4 mg/L NH3-N
N.B. This report supersedes any previously submitted copies

Date Analyzed June 16, 1999

Verified by: G. Gay

Analyst: Dawn Deluca, Test Preparation

Date: 25 Jun 99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge	REPORT TO: Amy Partridge
DATE SUBMITTED: June 17, 1999	ADDRESS: Tetras
DATE SAMPLED: June 17, 1999	POSTAL CODE: _____
SAMPLED BY: Amy Partridge	PHONE #: 942-2505
SAMPLE TYPE: River Samples	
LOCATION: North End Plant	
PROJECT: Ammonia Study	

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample Description	Date	NH3-N	Concentration - mg/L			
SA-RW-CONTROL	June 17, 1999	<0.4				
SA-RW-0.5	June 17, 1999	2.4				
SA-RW-1.0	June 17, 1999	3.2				
SA-RW-2	June 17, 1999	4.1				
SA-RW-4	June 17, 1999	5.5				
SA-RW-8	June 17, 1999	7.2				
SA-RW-16	June 17, 1999	7.0				
SB-RW-CONTROL	June 17, 1999	<0.4				
SB-RW-0.5	June 17, 1999	1.1				
SB-RW-1.0	June 17, 1999	2.3				
SB-RW-2	June 17, 1999	3.4				
SB-RW-4	June 17, 1999	3.6				
SB-RW-8	June 17, 1999	5.4				
SB-RW-16	June 17, 1999	8.8				
SB-RW-32	June 17, 1999	19.8				
SC-RW-CONTROL	June 17, 1999	<0.4				
SC-RW-0.5	June 17, 1999	1.3				
SC-RW-1.0	June 17, 1999	2.2				
SC-RW-2	June 17, 1999	3.5				
SC-RW-4	June 17, 1999	4.6				
SC-RW-8	June 17, 1999	7.1				
SC-RW-16	June 17, 1999	11.6				
SC-RW-32	June 17, 1999	24.7				
SA-E-CONTROL	June 17, 1999	35.4				
SA-E-0.5	June 17, 1999	1.1				
SA-E-1.0	June 17, 1999	1.6				
SA-E-2	June 17, 1999	2.6				
SA-E-4	June 17, 1999	3.8				
SA-E-8	June 17, 1999	5.8				
SA-E-16	June 17, 1999	9.8				
SA-E-32	June 17, 1999	33.4				
SB-E-CONTROL	June 17, 1999	35.5				
SB-E-0.5	June 17, 1999	1.9				
SB-E-1.0	June 17, 1999	2.2				
SB-E-2	June 17, 1999	3.3				
SB-E-4	June 17, 1999	5.0				
SB-E-8	June 17, 1999	7.2				
SB-E-16	June 17, 1999	11.4				
SB-E-32	June 17, 1999	32.7				
SC-E-CONTROL	June 17, 1999	34.9				
SC-E-0.5	June 17, 1999	1.2				
SC-E-1.0	June 17, 1999	2.1				
SC-E-2	June 17, 1999	3.5				
SC-E-4	June 17, 1999	4.8				
SC-E-8	June 17, 1999	7.2				
SC-E-16	June 17, 1999	11.4				
SC-E-32	June 17, 1999	32.9				
OC data						
Hach Verification Standard	10.0 mg/L NH3-N	9.9				
	0.0 ppm nit check	0.2				
	2.0 ppm nit check	1.8				
	5.0 ppm nit check	4.9				
	10.0 ppm nit check	10.0				
	20.0 ppm nit check	19.8				
Standard additions						
River Water (17 June 99) filtered	+ 10.0 ppm NH3-N	99				
River Water (17 June 99) unfiltered	+ 10.0 ppm NH3-N	104				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dfn loop) was used for this analysis. MDL <0.4 mg/L NH3-N
N.B. This report supersedes any previously submitted copies

Date Analyzed 17 Jun 99

Analyst Dawn DeLuca

Verified by G. Gay

Date 25 Jun 99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY	Amy Partridge	REPORT TO	Amy Partridge
DATE SUBMITTED	June 18, 1999	ADDRESS	Tetres
DATE SAMPLED	June 18, 1999	POSTAL CODE	
SAMPLED BY	Amy Partridge	PHONE #	942-2505
SAMPLE TYPE	River Samples		
LOCATION	North End Plant		
PROJECT	Ammonia Study		

PARAMETERS REQUESTED Total Ammonia

RESULTS

Sample Description	Date	NH3-N	Concentration - mg/L				
SA-RW-CONTROL	June 18, 1999	<0.4					
SA-RW-5	June 18, 1999	0.6					
SA-RW-10	June 18, 1999	1.0					
SA-RW-2	June 18, 1999	2.4					
SA-RW-4	June 18, 1999	3.4					
SA-RW-8	June 18, 1999	4.8					
SA-RW-16	June 18, 1999	12.2					
SB-RW-CONTROL	June 18, 1999	<0.4					
SB-RW-5	June 18, 1999	1.8					
SB-RW-10	June 18, 1999	4.6					
SB-RW-2	June 18, 1999	4.9					
SB-RW-4	June 18, 1999	5.6					
SB-RW-8	June 18, 1999	9.3					
SB-RW-16	June 18, 1999	10.9					
SB-RW-32	June 18, 1999	16.4					
SC-RW-CONTROL	June 18, 1999	<0.4					
SC-RW-5	June 18, 1999	0.9					
SC-RW-10	June 18, 1999	1.5					
SC-RW-2	June 18, 1999	3.3					
SC-RW-4	June 18, 1999	4.5					
SC-RW-8	June 18, 1999	6.7					
SC-RW-16	June 18, 1999	13.6					
SA-E-CONTROL	June 18, 1999	35.6					
SA-E-0.5	June 18, 1999	0.8					
SA-E-1.0	June 18, 1999	1.5					
SA-E-2	June 18, 1999	3.0					
SA-E-4	June 18, 1999	4.4					
SA-E-8	June 18, 1999	6.8					
SA-E-16	June 18, 1999	14.4					
SA-E-32	June 18, 1999	33.7					
SB-E-CONTROL	June 18, 1999	<0.4					
SB-E-0.5	June 18, 1999	0.9					
SB-E-1.0	June 18, 1999	1.3					
SB-E-2	June 18, 1999	2.5					
SB-E-4	June 18, 1999	3.4					
SB-E-8	June 18, 1999	7.5					
SB-E-16	June 18, 1999	10.8					
SB-E-32	June 18, 1999	33.3					
SC-E-CONTROL	June 18, 1999	35.5					
SC-E-0.5	June 18, 1999	1.2					
SC-E-1.0	June 18, 1999	2.1					
SC-E-2	June 18, 1999	3.0					
SC-E-4	June 18, 1999	5.4					
SC-E-8	June 18, 1999	9.2					
SC-E-16	June 18, 1999	15.1					
SC-E-32	June 18, 1999	33.2					
High Verification Standard (10.0 mg/L NH3-N)		10.0					
0.0 ppm and check		0.2					
2.0 ppm and check		1.9					
5.0 ppm and check		4.7					
10.0 ppm and check		10.1					
20.0 ppm and check		19.9					
Standard additions							
River Water (18 June 99)	10.0 ppm NH3-N	108					
Standard Blank	10.0 ppm NH3-N	108					

Remarks: The 0.25 ppm NH3-N range (w/ 1/4 d'n loop) was used for this analysis. MDL = 0.4 mg/L NH3-N
N.B. This report supersedes any previously submitted copies

Date Analyzed: 18 July 99
Analyst: Amy Partridge
Verified by: G. G. G.
Date: 20 June 99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY	Amy Partridge	REPORT TO:	Amy Partridge
DATE SUBMITTED	June 19, 1999	ADDRESS:	Tetras
DATE SAMPLED	June 19, 1999	POSTAL CODE:	
SAMPLED BY	Amy Partridge	PHONE #	942-2505
SAMPLE TYPE	River Samples		
LOCATION	North End Plant		
PROJECT	Ammonia Study		

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample Description	Date	NH3-N	Concentration - mg/L			
SA-RW-CONTROL	June 19, 1999	<0.4				
SA-RW-0.5	June 19, 1999	1.1				
SA-RW-1.0	June 19, 1999	0.9				
SA-RW-2	June 19, 1999	1.7				
SA-RW-4	June 19, 1999	2.1				
SA-RW-8	June 19, 1999	5.3				
SA-RW-16	June 19, 1999	3.7				
SB-RW-CONTROL	June 19, 1999	<0.4				
SB-RW-0.5	June 19, 1999	1.0				
SB-RW-1.0	June 19, 1999	4.9				
SB-RW-2	June 19, 1999	5.3				
SB-RW-4	June 19, 1999	8.6				
SB-RW-8	June 19, 1999	8.3				
SB-RW-16	June 19, 1999	6.7				
SB-RW-32	June 19, 1999	20.2				
SC-RW-CONTROL	June 19, 1999	<0.4				
SC-RW-0.5	June 19, 1999	1.1				
SC-RW-1.0	June 19, 1999	3.1				
SC-RW-2	June 19, 1999	4.3				
SC-RW-4	June 19, 1999	6.4				
SC-RW-8	June 19, 1999	6.9				
SC-RW-16	June 19, 1999	<0.4				
SA-E-CONTROL	June 19, 1999	40.2				
SA-E-0.5	June 19, 1999	0.8				
SA-E-1.0	June 19, 1999	1.0				
SA-E-2	June 19, 1999	2.1				
SA-E-4	June 19, 1999	3.7				
SA-E-8	June 19, 1999	6.4				
SA-E-16	June 19, 1999	9.3				
SA-E-32	June 19, 1999	33.8				
SB-E-CONTROL	June 19, 1999	40.2				
SB-E-0.5	June 19, 1999	1.0				
SB-E-1.0	June 19, 1999	1.4				
SB-E-2	June 19, 1999	2.9				
SB-E-4	June 19, 1999	4.6				
SB-E-8	June 19, 1999	8.3				
SB-E-16	June 19, 1999	14.5				
SB-E-32	June 19, 1999	33.6				
SC-E-CONTROL	June 19, 1999	39.9				
SC-E-0.5	June 19, 1999	0.9				
SC-E-1.0	June 19, 1999	1.9				
SC-E-2	June 19, 1999	2.3				
SC-E-4	June 19, 1999	3.9				
SC-E-8	June 19, 1999	7.0				
SC-E-16	June 19, 1999	9.4				
SC-E-32	June 19, 1999	33.7				
QC data						
Each Verification Standard (10.0 ppm NH3-N)		9.9				
0.0 ppm std. check		0.1				
2.0 ppm std. check		1.8				
5.0 ppm std. check		4.8				
10.0 ppm std. check		9.9				
20.0 ppm std. check		19.6				
Standard additions						
NEWPCG Final Eff (2/16/99) + 10.0 ppm NH3-N		100				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dfl loop) was used for this analysis. MDL <0.4 mg/L NH3-N
N.B. This report supersedes any previously submitted copies

Date Analyzed: 21 Jun 99
Analyst: Dean DeLuca, Ted Prinsley
Verified by: G. Gay
Date: 25 Jun 99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge	REPORT TO: Amy Partridge
DATE SUBMITTED: June 20, 1999	ADDRESS: Tetes
DATE SAMPLED: June 20, 1999	POSTAL CODE: _____
SAMPLED BY: Amy Partridge	PHONE #: 942-2505
SAMPLE TYPE: River Samples	
LOCATION: North End Plant	
PROJECT: Ammonia Study	

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample Description	Date	NH3-N	Concentration - mg/L			
SA-RW-CONTROL	June 20, 1999	<0.4				
SA-RW-0.5	June 20, 1999	0.3				
SA-RW-1.0	June 20, 1999	1.7				
SA-RW-2	June 20, 1999	1.8				
SA-RW-4	June 20, 1999	3.4				
SA-RW-8	June 20, 1999	3.8				
SA-RW-16	June 20, 1999	3.5				
SB-RW-CONTROL	June 20, 1999	<0.4				
SB-RW-0.5	June 20, 1999	0.7				
SB-RW-1.0	June 20, 1999	0.8				
SB-RW-2	June 20, 1999	1.6				
SB-RW-4	June 20, 1999	1.8				
SB-RW-8	June 20, 1999	3.0				
SB-RW-16	June 20, 1999	10.8				
SC-RW-CONTROL	June 20, 1999	1.0				
SC-RW-0.5	June 20, 1999	<0.4				
SC-RW-1.0	June 20, 1999	1.8				
SC-RW-2	June 20, 1999	3.0				
SC-RW-4	June 20, 1999	4.7				
SC-RW-8	June 20, 1999	7.2				
SC-RW-16	June 20, 1999	5.3				
SA-E-CONTROL	June 20, 1999	34.6				
SA-E-0.5	June 20, 1999	<0.4				
SA-E-1.0	June 20, 1999	0.6				
SA-E-2	June 20, 1999	1.2				
SA-E-4	June 20, 1999	3.0				
SA-E-8	June 20, 1999	8.2				
SA-E-16	June 20, 1999	9.2				
SA-E-32	June 20, 1999	35.3				
SB-E-CONTROL	June 20, 1999	34.7				
SB-E-0.5	June 20, 1999	0.8				
SB-E-1.0	June 20, 1999	1.7				
SA-E-2	June 20, 1999	2.1				
SA-E-4	June 20, 1999	4.6				
SA-E-8	June 20, 1999	8.9				
SA-E-16	June 20, 1999	13.4				
SA-E-32	June 20, 1999	33.9				
SC-E-CONTROL	June 20, 1999	33.8				
SC-E-0.5	June 20, 1999	1.0				
SC-E-1.0	June 20, 1999	1.8				
SC-E-2	June 20, 1999	1.6				
SC-E-4	June 20, 1999	2.7				
SC-E-8	June 20, 1999	6.6				
SC-E-16	June 20, 1999	10.0				
SC-E-32	June 20, 1999	34.2				
OC data:						
Hach Verification Standard (10.0 mg/L NH3-N)	June 20, 1999	9.9				
0.0 ppm std. check		0.1				
2.0 ppm std. check		1.8				
5.0 ppm std. check		4.8				
10.0 ppm std. check		9.9				
20.0 ppm std. check		19.6				
Standard additions:						
NEWPCC Final Eff (2/16/99) + 10.0 ppm NH3-N		100				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dia loop) was used for this analysis. MDL <0.4 mg/L NH3-N
N.B. This report supersedes any previously submitted copies

Date Analyzed: 21 Jun 99
Analyst: Dawn Delella, Ted Ptasnikowski
Verified by: G. Gray
Date: 29 Jun 99

City of Winnipeg
Laboratory Services Division
Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge	REPORT TO: Amy Partridge
DATE SUBMITTED: June 21, 1999	ADDRESS: Tetres
DATE SAMPLED: June 21, 1999	POSTAL CODE: _____
SAMPLED BY: Amy Partridge	PHONE #: 942-2505
SAMPLE TYPE: River Samples	
LOCATION: North End Plant	
PROJECT: Ammonia Study	

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample Description	Date	Concentration - mg/L				
		NH3-N				
SA-RW-CONTROL	June 21, 1999	<0.4				
SA-RW-0.5	June 21, 1999	<0.4				
SA-RW-1.0	June 21, 1999	0.8				
SA-RW-2	June 21, 1999	1.5				
SA-RW-4	June 21, 1999	2.6				
SA-RW-8	June 21, 1999	3.2				
SA-RW-16	June 21, 1999	7.3				
SB-RW-CONTROL	June 21, 1999	<0.4				
SB-RW-0.5	June 21, 1999	0.6				
SB-RW-1.0	June 21, 1999	1.8				
SB-RW-2	June 21, 1999	2.4				
SB-RW-4	June 21, 1999	4.2				
SB-RW-8	June 21, 1999	5.6				
SB-RW-16	June 21, 1999	10.2				
SC-RW-CONTROL	June 21, 1999	0.0				
SC-RW-0.5	June 21, 1999	<0.4				
SC-RW-1.0	June 21, 1999	1.2				
SC-RW-2	June 21, 1999	2.6				
SC-RW-4	June 21, 1999	2.7				
SC-RW-8	June 21, 1999	3.8				
SC-RW-16	June 21, 1999	8.1				
SA-E-CONTROL	June 21, 1999	30.3				
SA-E-0.5	June 21, 1999	0.6				
SA-E-1.0	June 21, 1999	0.8				
SA-E-2	June 21, 1999	1.7				
SA-E-4	June 21, 1999	1.3				
SA-E-8	June 21, 1999	5.4				
SA-E-16	June 21, 1999	10.1				
SA-E-32	June 21, 1999	30.8				
SB-E-CONTROL	June 21, 1999	29.3				
SB-E-0.5	June 21, 1999	0.5				
SB-E-1.0	June 21, 1999	0.8				
SA-E-2	June 21, 1999	1.9				
SA-E-4	June 21, 1999	2.8				
SA-E-8	June 21, 1999	6.6				
SA-E-16	June 21, 1999	13.5				
SA-E-32	June 21, 1999	30.8				
SC-E-CONTROL	June 21, 1999	29.7				
SC-E-0.5	June 21, 1999	0.6				
SC-E-1.0	June 21, 1999	0.9				
SC-E-2	June 21, 1999	1.2				
SC-E-4	June 21, 1999	2.1				
SC-E-8	June 21, 1999	4.8				
SC-E-16	June 21, 1999	11.0				
SC-E-32	June 21, 1999	30.2				
OC data						
Hach Verification Standard (10.0 mg/L NH3-N)		9.9				
0.0 ppm std. check		0.2				
2.0 ppm std. check		1.7				
5.0 ppm std. check		4.7				
10.0 ppm std. check		9.8				
20.0 ppm std. check		19.7				
Standard additions						
NEWPCC Final ER (22/5/99) - 10.0 ppm NH3-N		9.1				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dia/n loop) was used for this analysis. MDL <0.4 mg/L NH3-N
N.B. This report supersedes any previously submitted copies

Date Analyzed: 25 Jun 99
Analyst: Dean Dubata, Ted Poniatowski
Verified by: G. Gray
046 25 Jun 99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY	Amy Partridge	REPORT TO	Amy Partridge
DATE SUBMITTED	June 22, 1999	ADDRESS	Tetes
DATE SAMPLED	June 22, 1999	POSTAL CODE	
SAMPLED BY	Amy Partridge	PHONE #	942-2505

SAMPLE TYPE	River Samples
LOCATION	North End Plant
PROJECT	Ammonia Study

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample Description	Date	NH3-N	Concentration - mg/L			
SA-RW-CONTROL	22-Jun-99	<0.4				
SA-RW-0.5	22-Jun-99	0.3				
SA-RW-1.0	22-Jun-99	0.8				
SA-RW-2	22-Jun-99	1.7				
SA-RW-4	22-Jun-99	2.8				
SA-RW-8	22-Jun-99	5.8				
SA-RW-16	22-Jun-99	10.7				
SB-RW-CONTROL	22-Jun-99	<0.4				
SB-RW-0.5	22-Jun-99	0.7				
SB-RW-1.0	22-Jun-99	1.2				
SB-RW-2	22-Jun-99	2.3				
SB-RW-4	22-Jun-99	3.5				
SB-RW-8	22-Jun-99	7.6				
SB-RW-16	22-Jun-99	13.3				
SC-RW-CONTROL	22-Jun-99	0.0				
SC-RW-0.5	22-Jun-99	<0.4				
SC-RW-1.0	22-Jun-99	1.1				
SC-RW-2	22-Jun-99	2.3				
SC-RW-4	22-Jun-99	5.0				
SC-RW-8	22-Jun-99	5.4				
SC-RW-16	22-Jun-99	8.2				
SA-E-CONTROL	22-Jun-99	36.3				
SA-E-0.5	22-Jun-99	0.8				
SA-E-1.0	22-Jun-99	1.0				
SA-E-2	22-Jun-99	2.7				
SA-E-4	22-Jun-99	3.6				
SA-E-8	22-Jun-99	7.0				
SA-E-16	22-Jun-99	11.7				
SA-E-32	22-Jun-99	32.6				
SB-E-CONTROL	22-Jun-99	36.2				
SB-E-0.5	22-Jun-99	1.1				
SB-E-1.0	22-Jun-99	1.9				
SA-E-2	22-Jun-99	3.0				
SA-E-4	22-Jun-99	5.0				
SA-E-8	22-Jun-99	8.2				
SA-E-16	22-Jun-99	14.5				
SA-E-32	22-Jun-99	32.4				
SC-E-CONTROL	22-Jun-99	36.1				
SC-E-0.5	22-Jun-99	1.2				
SC-E-1.0	22-Jun-99	1.9				
SC-E-2	22-Jun-99	3.7				
SC-E-4	22-Jun-99	4.2				
SC-E-8	22-Jun-99	5.3				
SC-E-16	22-Jun-99	10.8				
SC-E-32	22-Jun-99	31.9				
OC data						
Hach Verification Standard (10.0 mg/L NH3-N)		9.9				
0.0 ppm std. check		0.2				
2.0 ppm std. check		1.7				
5.0 ppm std. check		4.7				
10.0 ppm std. check		9.8				
20.0 ppm std. check		19.7				
Standard additions						
NEWPCC Final EH (22-5-99)	10.0 ppm NH3-N	9.1				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 d/d loop) was used for this analysis. MDL <0.4 mg/L NH3-N
N.B. This report supersedes any previously submitted copies

Date Analyzed: 22 Jun 99

Verified by: G. Hay

Analyst: Leah Debra, Ted Pouchowski

Date: 25 Jun 99

City of Winnipeg
Laboratory Services Division
Analytical Report

SAMPLES SUBMITTED BY	Amy Partridge	REPORT TO	Amy Partridge
DATE SUBMITTED	June 23, 1999	ADDRESS	Tetres
DATE SAMPLED	June 23, 1999	POSTAL CODE	
SAMPLED BY	Amy Partridge	PHONE #	942-2505
SAMPLE TYPE(S)	River Water & NEWPCC Final Effluent		
LOCATION	North End Plant		
PROJECT	Ammonia Study		

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample	Description	Date	Concentration - mg/L				
			NH3-N				
1	SA-RW-CONTROL	23-Jun-99	< 0.4				
2	SA-RW-0.5	23-Jun-99	< 0.4				
3	SA-RW-1.0	23-Jun-99	0.4				
4	SA-RW-2	23-Jun-99	1.2				
5	SA-RW-4	23-Jun-99	2.9				
6	SA-RW-8	23-Jun-99	3.2				
7	SA-RW-16	23-Jun-99	9.0				
8	SB-RW-CONTROL	23-Jun-99	< 0.4				
9	SB-RW-0.5	23-Jun-99	< 0.4				
10	SB-RW-1.0	23-Jun-99	0.6				
11	SB-RW-2	23-Jun-99	1.6				
12	SB-RW-4	23-Jun-99	1.6				
13	SB-RW-8	23-Jun-99	3.7				
14	SB-RW-16	23-Jun-99	6.2				
15	SC-RW-CONTROL	23-Jun-99	10.4				
16	SC-RW-0.5	23-Jun-99	< 0.4				
17	SC-RW-1.0	23-Jun-99	0.6				
18	SC-RW-2	23-Jun-99	1.5				
19	SC-RW-4	23-Jun-99	1.9				
20	SC-RW-8	23-Jun-99	3.1				
21	SC-RW-16	23-Jun-99	4.5				
22	SC-RW-16	23-Jun-99	7.3				
23	SA-E-CONTROL	23-Jun-99	15.9				
24	SA-E-0.5	23-Jun-99	0.5				
25	SA-E-1.0	23-Jun-99	0.5				
26	SA-E-2	23-Jun-99	1.8				
27	SA-E-4	23-Jun-99	3.4				
28	SA-E-8	23-Jun-99	5.3				
29	SA-E-16	23-Jun-99	9.1				
30	SA-E-32	23-Jun-99	30.4				
31	SB-E-CONTROL	23-Jun-99	15.3				
32	SB-E-0.5	23-Jun-99	0.6				
33	SB-E-1.0	23-Jun-99	1.0				
34	SB-E-2	23-Jun-99	2.2				
35	SB-E-4	23-Jun-99	4.0				
36	SB-E-8	23-Jun-99	7.3				
37	SB-E-16	23-Jun-99	12.8				
38	SB-E-32	23-Jun-99	29.9				
39	SC-E-CONTROL	23-Jun-99	15.0				
40	SC-E-0.5	23-Jun-99	0.7				
41	SC-E-1.0	23-Jun-99	1.2				
42	SC-E-2	23-Jun-99	2.0				
43	SC-E-4	23-Jun-99	3.4				
44	SC-E-8	23-Jun-99	6.8				
45	SC-E-16	23-Jun-99	10.3				
46	SC-E-32	23-Jun-99	30.0				
47	OC data						
48	High Verification Standard (10.0 mg/L NH3-N)		10.1				
49	0.0 ppm std. check		0.2				
50	2.0 ppm std. check		1.9				
51	5.0 ppm std. check		5.1				
52	10.0 ppm std. check		10.3				
53	20.0 ppm std. check		20.2				
54	Standard additions		% recovery	% recovery	% recovery	% recovery	% recovery
55	NEWPCC Final Eff (24/6/99) - 10.0 ppm NH3-N		96				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 di/n loop) was used for this analysis. MDL <0.4 mg/L NH3-N

Date Analyzed: June 24, 1999
Analyst: Don DeLuca
Verified by: G. Goy
Date: 30 June 99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY	Amy Partridge	REPORT TO	Amy Partridge
DATE SUBMITTED	June 24, 1999	ADDRESS	Tetes
DATE SAMPLED	June 24, 1999	POSTAL CODE	
SAMPLED BY	Amy Partridge	PHONE #	942-2505

SAMPLE TYPE	River Water & NEWPCC Final Effluent
LOCATION	North End Plant
PROJECT	Ammonia Study

PARAMETERS REQUESTED: Total Ammonia

RESULTS

Sample Description	Date	NH3-N	Concentration - mg/L			
SA-RW-CONTROL	24-Jun-99	<0.4				
SA-RW-0.5	24-Jun-99	0.7				
SA-RW-1.0	24-Jun-99	1.1				
SA-RW-2	24-Jun-99	2.0				
SA-RW-4	24-Jun-99	3.9				
SA-RW-8	24-Jun-99	5.0				
SA-RW-16	24-Jun-99	8.0				
SB-RW-CONTROL	24-Jun-99	<0.4				
SB-RW-0.5	24-Jun-99	1.0				
SB-RW-1.0	24-Jun-99	1.9				
SB-RW-2	24-Jun-99	3.1				
SB-RW-4	24-Jun-99	5.0				
SB-RW-8	24-Jun-99	7.0				
SB-RW-16	24-Jun-99	9.7				
SC-RW-CONTROL	24-Jun-99	<0.4				
SC-RW-0.5	24-Jun-99	1.0				
SC-RW-1.0	24-Jun-99	1.7				
SC-RW-2	24-Jun-99	2.5				
SC-RW-4	24-Jun-99	5.0				
SC-RW-8	24-Jun-99	5.4				
SC-RW-16	24-Jun-99	7.7				
SA-E-CONTROL	24-Jun-99	22.3				
SA-E-0.5	24-Jun-99	0.9				
SA-E-1.0	24-Jun-99	1.6				
SA-E-2	24-Jun-99	2.7				
SA-E-4	24-Jun-99	4.3				
SA-E-8	24-Jun-99	5.1				
SA-E-16	24-Jun-99	9.4				
SA-E-32	24-Jun-99	30.2				
SB-E-CONTROL	24-Jun-99	22.1				
SB-E-0.5	24-Jun-99	1.5				
SB-E-1.0	24-Jun-99	2.1				
SB-E-2	24-Jun-99	3.7				
SB-E-4	24-Jun-99	5.5				
SB-E-8	24-Jun-99	8.2				
SB-E-16	24-Jun-99	12.9				
SB-E-32	24-Jun-99	30.0				
SC-E-CONTROL	24-Jun-99	21.3				
SC-E-0.5	24-Jun-99	1.2				
SC-E-1.0	24-Jun-99	1.8				
SC-E-2	24-Jun-99	3.6				
SC-E-4	24-Jun-99	3.5				
SC-E-8	24-Jun-99	5.6				
SC-E-16	24-Jun-99	11.3				
SC-E-32	24-Jun-99	30.1				
Hach Verification Standard (10.0 mg/L NH3-N)		10.1				
0.0 ppm std. check		0.2				
2.0 ppm std. check		1.9				
5.0 ppm std. check		5.1				
10.0 ppm std. check		10.3				
20.0 ppm std. check		20.2				
Standard additions		% recovery	% recovery	% recovery	% recovery	% recovery
NEWPCC Final Eff (24/6/99) - 10.0 ppm NH3-N		96				

Remarks: The 0-25 ppm NH3-N range (w/ 1/4 dil'n loop) was used for this analysis. MDL = 0.4 mg/L NH3-N

Date Analyzed: June 24, 1999
 Analyst: Amy Partridge
 Verified by: G. Gay
 Date: 30 June 99

City of Winnipeg
Laboratory Services Division

Fish test #6

Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge DATE SUBMITTED: 16-Aug-99 DATE SAMPLED: Aug 13 to 16 1999 SAMPLED BY: Amy Partridge		REPORT TO: Amy Partridge ADDRESS: Tetras POSTAL CODE: _____ PHONE: 942-2595	
SAMPLE TYPE: River Samples LOCATION: North End Park PROJECT: Ammonia Study		Sampler/Submitter Remarks:	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ -N) <input type="checkbox"/> Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>		Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> Total Phosphorus (P) <input type="checkbox"/>	

RESULTS

Sample	Description / Identification	Date	Concentration (mg/L)			
			NH ₃ -N	NO ₂ /NO ₃ -N	Analysis Date	Analysis Date
8/13/99	8ch7 RW	13-Aug-99	0.18		16-Aug-99	
8/13/99	Control	13-Aug-99	30.9		24-Aug-99	
8/13/99	E1	13-Aug-99	0.7		24-Aug-99	
8/13/99	E2	13-Aug-99	1.4		24-Aug-99	
8/13/99	E4	13-Aug-99	3.2		24-Aug-99	
8/13/99	E8	13-Aug-99	7.3		24-Aug-99	
8/13/99	E16	13-Aug-99	16.8		24-Aug-99	
8/13/99	E32	13-Aug-99	32.3		24-Aug-99	
8/13/99	RW 1mg/L	13-Aug-99	0.4		24-Aug-99	
8/13/99	RW 2mg/L	13-Aug-99	0.8		24-Aug-99	
8/13/99	RW 4mg/L	13-Aug-99	2.3		24-Aug-99	
8/13/99	RW 8mg/L	13-Aug-99	5.7		24-Aug-99	
8/13/99	RW 16mg/L	13-Aug-99	14.6		24-Aug-99	
8/13/99	RW 32 mg/L	13-Aug-99	28.9		24-Aug-99	
8/14/99	E1	14-Aug-99	0.5		24-Aug-99	
8/14/99	E2	14-Aug-99	1.0		24-Aug-99	
8/14/99	E4	14-Aug-99	3.0		24-Aug-99	
8/14/99	E8	14-Aug-99	7.3		24-Aug-99	
8/14/99	E16	14-Aug-99	18.1		24-Aug-99	
8/14/99	RW 1mg/L	14-Aug-99	0.3		24-Aug-99	
8/14/99	RW 2mg/L	14-Aug-99	0.6		24-Aug-99	
8/14/99	RW 4mg/L	14-Aug-99	2.2		24-Aug-99	
8/14/99	RW 8mg/L	14-Aug-99	6.0		24-Aug-99	
8/14/99	RW 16mg/L	14-Aug-99	15.5		24-Aug-99	
8/15/99	E1	15-Aug-99	0.5		24-Aug-99	
8/15/99	E2	15-Aug-99	0.8		24-Aug-99	
8/15/99	E4	15-Aug-99	2.2		24-Aug-99	
8/15/99	E8	15-Aug-99	6.1		24-Aug-99	
8/15/99	RW 1mg/L	15-Aug-99	0.4		24-Aug-99	
8/15/99	RW 2mg/L	15-Aug-99	0.7		24-Aug-99	
8/15/99	RW 4mg/L	15-Aug-99	2.3		24-Aug-99	
8/15/99	RW 8mg/L	15-Aug-99	6.3		24-Aug-99	
8/16/99	E1	16-Aug-99	0.3		24-Aug-99	
8/16/99	E2	16-Aug-99	0.9		24-Aug-99	
8/16/99	E4	16-Aug-99	2.7		24-Aug-99	
8/16/99	E8	16-Aug-99	6.6		24-Aug-99	
8/16/99	RW 1mg/L	16-Aug-99	0.5		24-Aug-99	
8/16/99	RW 2mg/L	16-Aug-99	1.3		24-Aug-99	
8/16/99	RW 4mg/L	16-Aug-99	3.2		24-Aug-99	
8/16/99	RW 8mg/L	16-Aug-99	7.2		24-Aug-99	
QC data Low level Standard Check 0.5 mg/L 0.41 % recovery NH ₃ -N std check 1.0 0.8 NH ₃ -N std check 2.0 2.0 NH ₃ -N std check 4.0 4.0 NH ₃ -N std check 8.0 8.0 Verification Standard (5.0 mg/L NH ₃ -N) 5.2 Standard solutions 8/13/99 E1 + 2.0 mg/L #6 8/13/99 E2 + 2.0 mg/L #7 8/13/99 E2 + 2.0 mg/L #5						

Analyst Remarks:

Date Reported: 24-Aug-99
 Analyst: J. Ponikvarski, D. DeLucca
 Verified by: _____
 Date: 8 Sept. 99

NOTE: only one anal value per [NH₃] (i.e. samples not taken for each replicates b/c large stream as no d.l.n were made and 2 replicates taken for each

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLE SUBMITTED BY: Amy Partridge DATE SUBMITTED: Oct 23 25, 1999 DATE SAMPLED: Oct 23 25, 1999 SAMPLED BY: Amy Partridge		REPORT TO: Amy Partridge ADDRESS: Tetres POSTAL CODE: _____ PHONE: _____	
SAMPLE TYPE: River water LOCATION: 2230 Main St PROJECT: Toxicity Testing		Sampler/Submitter Remarks:	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ N) <input type="checkbox"/> Total Ammonia (NH ₃ N) <input checked="" type="checkbox"/> Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>	Ortho phosphate (PO ₄ P) <input type="checkbox"/> Total Phosphorus (P) <input type="checkbox"/>	Total Nitrate (NO ₃ /NO ₂ N) <input type="checkbox"/> Total Ammonia (NH ₃ N) <input checked="" type="checkbox"/> Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>	Ortho phosphate (PO ₄ P) <input type="checkbox"/> Total Phosphorus (P) <input type="checkbox"/>

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
10/23/99 RW CONTROL	23-Oct-99	<0.3	26-Oct-99								
10/23/99 0.5	23-Oct-99	0.3	26-Oct-99								
10/23/99 1.0	23-Oct-99	0.5	26-Oct-99								
10/23/99 2.0	23-Oct-99	1.0	26-Oct-99								
10/23/99 4.0	23-Oct-99	7.5	26-Oct-99								
10/23/99 8.0	23-Oct-99	12.1	26-Oct-99								
10/23/99 16 CC	23-Oct-99	23.8	26-Oct-99								
10/23/99 16.0 FFM	23-Oct-99	23.5	26-Oct-99								
10/23/99 16.0 LFM	23-Oct-99	23.6	26-Oct-99								
10/23/99 58	23-Oct-99	80	26-Oct-99								
10/24/99 RW CONTROL	24-Oct-99	<0.3	26-Oct-99								
10/24/99 0.5	24-Oct-99	0.8	26-Oct-99								
10/24/99 1.0	24-Oct-99	0.7	26-Oct-99								
10/24/99 2.0	24-Oct-99	0.5	26-Oct-99								
10/24/99 4.0	24-Oct-99	2.7	26-Oct-99								
10/24/99 8.0 CC	24-Oct-99	9.1	26-Oct-99								
10/24/99 8.0 FFM	24-Oct-99	5.0	26-Oct-99								
10/24/99 8.0 LFM	24-Oct-99	9.5	26-Oct-99								
10/24/99 16.0	24-Oct-99	8.9	26-Oct-99								
10/24/99 58	24-Oct-99	75	26-Oct-99								
10/25/99 RW CONTROL	25-Oct-99	0.1	26-Oct-99								
10/25/99 0.5	25-Oct-99	0.2	26-Oct-99								
10/25/99 1.0	25-Oct-99	0.4	26-Oct-99								
10/25/99 2.0	25-Oct-99	0.6	26-Oct-99								
10/25/99 4.0	25-Oct-99	2.0	26-Oct-99								
10/25/99 8.0 CC-A	25-Oct-99	5.9	26-Oct-99								
10/25/99 8.0 FFM-A	25-Oct-99	5.8	26-Oct-99								
10/25/99 8.0 LFM-A	25-Oct-99	6.3	26-Oct-99								
10/25/99 16.0	25-Oct-99	15.6	26-Oct-99								
10/25/99 58	25-Oct-99	61	26-Oct-99								
QC data:			% recovery		% recovery		% recovery		% recovery		% recovery
end of run std check:	0.0 mg/L NH ₃ -N	0.1									
end of run std check:	1.0 mg/L NH ₃ -N	1.0									
end of run std check:	2.0 mg/L NH ₃ -N	2.0									
end of run std check:	4.0 mg/L NH ₃ -N	4.0									
end of run std check:	8.0 mg/L NH ₃ -N	8.1									
Verification Std (HACH):	5.0 PPM NH ₃ -N	5.0	101%								
Verification Std (HACH):	0.5 PPM NH ₃ -N	0.5	104%								
Standard additions:			% recovery		% recovery		% recovery		% recovery		% recovery
10/23/99 2.0 repeat		1.0	100%								
10/23/99 2.0 + 0.4 ppm NH ₃ -N			106%								
10/24/99 2.0 repeat		0.4	80%								
10/24/99 2.0 + 0.4 ppm NH ₃ -N			81%								

Analyst Remarks

Date Reported: 10/25/99
 Analyst: E. Poirier, J. D. DeLong
 Verified by: J. Long
 Date: 10/25/99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge DATE SUBMITTED: Oct 23 25, 1999 DATE SAMPLED: Oct 23 25, 1999 SAMPLED BY: Amy Partridge		REPORT TO: Amy Partridge ADDRESS: Tetras POSTAL CODE: _____ PHONE: _____	
SAMPLE TYPE: River Samples LOCATION: N Main PROJECT: Toxicity		Sampler/Submitter Remarks:	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ -NO ₂ -N) <input type="checkbox"/> Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>		Ortho phosphate (PO ₄ -P) <input type="checkbox"/> Total Phosphorus (P) <input type="checkbox"/>	

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)								
		NH ₃ -N		NO ₃ -N		TKN		P		Ortho PO ₄ -P
		Analysis Date	Analysis Date	Analysis Date	Analysis Date	Analysis Date	Analysis Date	Analysis Date	Analysis Date	Analysis Date
10/23/99 RW CONTROL	23-Oct-99	<0.2	26-Oct-99							
10/23/99 0.5	23-Oct-99	0.3	26-Oct-99							
10/23/99 1.0	23-Oct-99	0.5	26-Oct-99							
10/23/99 2.0	23-Oct-99	1.0	26-Oct-99							
10/23/99 4.0	23-Oct-99	7.5	26-Oct-99							
10/23/99 8.0	23-Oct-99	12.1	26-Oct-99							
10/23/99 16 CC	23-Oct-99	23.8	26-Oct-99							
10/23/99 16.0 FFM	23-Oct-99	23.5	26-Oct-99							
10/23/99 16.0 LFM	23-Oct-99	23.6	26-Oct-99							
10/23/99 58.0	23-Oct-99	79.7	26-Oct-99							
10/24/99 RW CONTROL	24-Oct-99	0.2	26-Oct-99							
10/24/99 0.5	24-Oct-99	0.8	26-Oct-99							
10/24/99 1.0	24-Oct-99	0.7	26-Oct-99							
10/24/99 2.0	24-Oct-99	0.5	26-Oct-99							
10/24/99 4.0	24-Oct-99	2.7	26-Oct-99							
10/24/99 8.0 CC	24-Oct-99	9.1	26-Oct-99							
10/24/99 8.0 FFM	24-Oct-99	5.0	26-Oct-99							
10/24/99 8.0 LFM	24-Oct-99	9.5	26-Oct-99							
10/24/99 16.0	24-Oct-99	8.9	26-Oct-99							
10/24/99 58	24-Oct-99	75.3	26-Oct-99							
10/25/99 RW CONTROL	25-Oct-99	<0.2	26-Oct-99							
10/25/99 0.5	25-Oct-99	0.2	26-Oct-99							
10/25/99 1.0	25-Oct-99	0.4	26-Oct-99							
10/25/99 2.0	25-Oct-99	0.6	26-Oct-99							
10/25/99 4.0	25-Oct-99	2.0	26-Oct-99							
10/25/99 8.0 CC-A	25-Oct-99	5.9	26-Oct-99							
10/25/99 8.0 FFM-A	25-Oct-99	5.8	26-Oct-99							
10/25/99 8.0 LFM-A	25-Oct-99	6.3	26-Oct-99							
10/25/99 16.0	25-Oct-99	15.6	26-Oct-99							
10/25/99 58.0	25-Oct-99	60.8	26-Oct-99							
QC data:			% recovery		% recovery		% recovery		% recovery	
end of run std. check	0.0 mg/L NH ₃	0.1								
end of run std. check	1.0 mg/L NH ₃	1.0								
end of run std. check	2.0 mg/L NH ₃	2.0								
end of run std. check	4.0 mg/L NH ₃	4.0								
end of run std. check	8.0 mg/L NH ₃	8.1								
Verification Std (HACH)	5.0 PPM NH ₃	5.0	101%							
Verification Std (HACH)	0.5 PPM NH ₃	0.5	104%							
Standard additions			% recovery		% recovery		% recovery		% recovery	
10/23/99 2.0 repeat		1.0								
10/23/99 2.0 +0.4 ppm NH ₃										
10/24/99 2.0 repeat		0.4								
10/24/99 2.0 +0.4 ppm NH ₃										

Analyst Remarks

Date Reported: 10/23/99

Verified by: [Signature]

Analyst: [Signature]

Date: 10/23/99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED: Oct 26 TO NOV 1, 1999 DATE SAMPLED: Oct 26 TO NOV 1, 1999 SAMPLED BY: Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetres POSTAL CODE: _____ PHONE: _____	
SAMPLE TYPE: River water LOCATION: 2230 Main St. PROJECT: Toxicity Testing		Sampler/Submitter Remarks: _____	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) <input type="checkbox"/> check Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>	Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> check Total Phosphorus (P) <input type="checkbox"/>	check	

RESULTS

Sample	Description / Identification	Date	Concentration (mg/L)											
			NH ₃ -N		NO ₃ -N		TKN		Total P		Ortho PO ₄ -P			
				Analysis Date		Analysis Date		Analysis Date		Analysis Date		Analysis Date		
3	10/26/99 RW CONTROL	10/26/99	<0.3	2-Nov-99										
	10/26/99 0.5	10/26/99	28	2-Nov-99										
	10/26/99 1.0	10/26/99	0.5	2-Nov-99										
	10/26/99 2.0	10/26/99	2.0	2-Nov-99										
	10/26/99 4.0	10/26/99	1.7	2-Nov-99										
	10/26/99 8-CC	10/26/99	6.5	2-Nov-99										
	10/26/99 8-FFM	10/26/99	6.8	2-Nov-99										
	10/26/99 8 LFM	10/26/99	6.5	2-Nov-99										
	10/26/99 16.0	10/26/99	12.1	2-Nov-99										
	10/26/99 58	10/26/99	63	2-Nov-99										
	4	10/27/99 RW CONTROL	10/27/99	<0.3	2-Nov-99									
10/27/99 0.5		10/27/99	0.3	2-Nov-99										
10/27/99 1.0		10/27/99	0.3	2-Nov-99										
10/27/99 2.0		10/27/99	1.6	2-Nov-99										
10/27/99 4.0		10/27/99	0.3	2-Nov-99										
10/27/99 8-CC		10/27/99	2.7	2-Nov-99										
10/27/99 8-FFM		10/27/99	2.7	2-Nov-99										
10/27/99 8 LFM		10/27/99	2.4	2-Nov-99										
10/27/99 16.0		10/27/99	5.3	2-Nov-99										
10/27/99 58		10/27/99	62	2-Nov-99										
5		10/28/99 RW CONTROL	10/28/99	<0.3	2-Nov-99									
	10/28/99 0.5	10/28/99	<0.3	2-Nov-99										
	10/28/99 1.0	10/28/99	0.3	2-Nov-99										
	10/28/99 2.0	10/28/99	3.7	2-Nov-99										
	10/28/99 4.0	10/28/99	1.0	2-Nov-99										
	10/28/99 8-CC	10/28/99	3.0	2-Nov-99										
	10/28/99 8-FFM	10/28/99	2.6	2-Nov-99										
	10/28/99 8 LFM	10/28/99	3.5	2-Nov-99										
	10/28/99 16.0	10/28/99	13.3	2-Nov-99										
	10/28/99 58	10/28/99	53	2-Nov-99										
	6	10/29/99 RW CONTROL	10/29/99	<0.3	2-Nov-99									
10/29/99 0.5		10/29/99	0.3	2-Nov-99										
10/29/99 1.0		10/29/99	0.4	2-Nov-99										
10/29/99 2.0		10/29/99	0.4	2-Nov-99										
10/29/99 4.0		10/29/99	3.0	2-Nov-99										
10/29/99 8-CC		10/29/99	5.2	2-Nov-99										
10/29/99 8-FFM		10/29/99	5.9	2-Nov-99										
10/29/99 8 LFM		10/29/99	5.3	2-Nov-99										
10/29/99 16.0		10/29/99	12.8	2-Nov-99										
10/29/99 58		10/29/99	63	2-Nov-99										
7		10/30/99 RW CONTROL	10/30/99	<0.3	2-Nov-99									
	10/30/99 0.5	10/30/99	0.3	2-Nov-99										
	10/30/99 1.0	10/30/99	0.4	2-Nov-99										
	10/30/99 2.0	10/30/99	0.3	2-Nov-99										
	10/30/99 4.0	10/30/99	2.7	2-Nov-99										
	10/30/99 8-CC	10/30/99	5.9	2-Nov-99										
	10/30/99 8-FFM	10/30/99	5.7	2-Nov-99										
	10/30/99 8 LFM	10/30/99	5.9	2-Nov-99										
	10/30/99 16.0	10/30/99	13.2	2-Nov-99										
	10/30/99 58	10/30/99	57	2-Nov-99										

outlier removed fr. dataset of stressed fish

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge DATE SUBMITTED: Oct 26 TO NOV 1, 1999 DATE SAMPLED: Oct 26 TO NOV 1, 1999 SAMPLED BY: Amy Partridge	REPORT TO: Amy Partridge ADDRESS: Tetres POSTAL CODE: _____ PHONE: _____
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SAMPLE TYPE: River water LOCATION: 2230 Main St. PROJECT: Toxicity Testing	Sampler/Submitter Remarks _____ _____
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) <input type="checkbox"/> check Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>	Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> check Total Phosphorus (P) <input type="checkbox"/> check

RESULTS

Sample			Concentration (mg/L)									
Description / Identification	Date	NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	Total P	Analysis Date	Ortho PO ₄ -P	Analysis Date	
10/31/99 RW CONTROL	10/31/99	<0.3	2-Nov-99									
10/31/99 0.5	10/31/99	2.7	2-Nov-99									
10/31/99 1.0	10/31/99	1.3	2-Nov-99									
8 10/31/99 2.0	10/31/99	3.2	2-Nov-99									
10/31/99 4.0	10/31/99	1.7	2-Nov-99									
10/31/99 8-CC	10/31/99	8.1	2-Nov-99									
10/31/99 8-FFM	10/31/99	4.7	2-Nov-99									
10/31/99 8 LFM	10/31/99	8.3	2-Nov-99									
10/31/99 16.0	10/31/99	23.3	2-Nov-99									
10/31/99 58	10/31/99	90	2-Nov-99									
11/01/99 RW CONTROL	11/01/99	<0.3	2-Nov-99									
11/01/99 0.5	11/01/99	<0.3	2-Nov-99									
11/01/99 1.0	11/01/99	0.4	2-Nov-99									
9 11/01/99 2.0	11/01/99	0.1	2-Nov-99									
11/01/99 4.0	11/01/99	2.4	2-Nov-99									
11/01/99 8-CC	11/01/99	1.3	2-Nov-99									
11/01/99 8-FFM	11/01/99	1.0	2-Nov-99									
11/01/99 8 LFM	11/01/99	1.1	2-Nov-99									
11/01/99 16.0	11/01/99	13.8	2-Nov-99									
11/01/99 58	11/01/99	45	2-Nov-99									
QC data:		% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	
end of run std. check: 0.0 mg/L NH ₃ -N		0.1										
end of run std. check: 1.0 mg/L NH ₃ -N		0.9										
end of run std. check: 2.0 mg/L NH ₃ -N		1.9										
end of run std. check: 4.0 mg/L NH ₃ -N		4.0										
end of run std. check: 8.0 mg/L NH ₃ -N		8.0										
Verification Std. (HAQ1): 5.0 PPM NH ₃ -N		5.1	102%									
Verification Std. (HAQ1): 0.5 PPM NH ₃ -N		0.6	120%									
Standard additions:		% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	% recovery	
10/26/99 2.0 repeat		1.9	96%									
10/26/99 2.0 + 4.0 ppm NH ₃ -N			101%									
10/26/99 4.0 repeat		1.7	100%									
10/26/99 4.0 + 4.0 ppm NH ₃ -N			100%									

Analyst Remarks:

Date Reported: 02-Nov-99

Verified by:

Analyst: T. Poniatowski, D. Deluca

Date:

NOV 03 1999

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED NOV. 2-7/99 DATE SAMPLED NOV. 2-7/99 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetres POSTAL CODE _____ PHONE _____	
SAMPLE TYPE River Samples LOCATION N. Main PROJECT Toxicity		Sampler/Submitter Remarks	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) <input type="checkbox"/> check Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>		Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> check Total Phosphorus (P) <input type="checkbox"/>	

RESULTS

Sample			Concentration (mg/L)									
Description / Identification	Date		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
11/02/99 RW CONTROL	11/02/99		<0.3	8-Nov-99								
11/02/99 0.5	11/02/99		<0.3	8-Nov-99								
11/02/99 1	11/02/99		<0.3	8-Nov-99								
11/02/99 2	11/02/99		0.5	8-Nov-99								
11/02/99 4	11/02/99		3.5	8-Nov-99								
11/02/99 8-CC	11/02/99		5.7	8-Nov-99								
11/02/99 8-FFM	11/02/99		5.3	8-Nov-99								
11/02/99 8 LFM	11/02/99		5.7	8-Nov-99								
11/02/99 16	11/02/99		8.3	8-Nov-99								
11/02/99 58	11/02/99		20.3	8-Nov-99								
11/03/99 RW CONTROL	11/03/99		<0.3	8-Nov-99								
11/03/99 0.5	11/03/99		0.3	8-Nov-99								
11/03/99 1	11/03/99		0.3	8-Nov-99								
11/03/99 2	11/03/99		<0.3	8-Nov-99								
11/03/99 4	11/03/99		2.2	8-Nov-99								
11/03/99 8-CC	11/03/99		3.4	8-Nov-99								
11/03/99 8-FFM-A	11/03/99		4.5	8-Nov-99								
11/03/99 8 LFM-A	11/03/99		3.2	8-Nov-99								
11/03/99 16	11/03/99		9.3	8-Nov-99								
11/03/99 58	11/03/99		51.7	8-Nov-99								
11/04/99 1	11/04/99		0.3	8-Nov-99								
11/04/99 8	11/04/99		5.5	8-Nov-99								
11/04/99 58	11/04/99		59.9	8-Nov-99								
used checkeds lit readings see pg. 64 lab book 12												
11/05/99 RW CONTROL	11/05/99		<0.3	8-Nov-99								
11/05/99 0.5	11/05/99		<0.3	8-Nov-99								
11/05/99 1	11/05/99		0.3	8-Nov-99								
11/05/99 2	11/05/99		0.7	8-Nov-99								
11/05/99 4	11/05/99		4.1	8-Nov-99								
11/05/99 8-CC	11/05/99		4.8	8-Nov-99								
11/05/99 8-FFM	11/05/99		5.2	8-Nov-99								
11/05/99 8 LFM	11/05/99		4.8	8-Nov-99								
11/05/99 16	11/05/99		13.6	8-Nov-99								
11/05/99 58	11/05/99		56.3	8-Nov-99								
11/06/99 RW CONTROL	11/06/99		<0.3	8-Nov-99								
11/06/99 0.5	11/06/99		<0.3	8-Nov-99								
11/06/99 1	11/06/99		0.4	8-Nov-99								
11/06/99 2	11/06/99		0.4	8-Nov-99								
11/06/99 4	11/06/99		2.7	8-Nov-99								
11/06/99 8-CC	11/06/99		9.5	8-Nov-99								
11/06/99 8-FFM	11/06/99		6.8	8-Nov-99								
11/06/99 8 LFM	11/06/99		6.8	8-Nov-99								
11/06/99 16	11/06/99		10.4	8-Nov-99								
11/06/99 58	11/06/99		59.1	8-Nov-99								

RESULTS

Sample			Concentration (mg/L)								
Description / Identification	Date	NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
11/07/99 RW CONTROL	11/07/99	<0.3	8 Nov 99								
11/07/99 0.5	11/07/99	<0.3	8 Nov 99								
11/07/99 1	11/07/99	0.4	8 Nov 99								
11/07/99 2	11/07/99	0.5	8 Nov 99								
11/07/99 4	11/07/99	0.6	8 Nov 99								
11/07/99 8-CC	11/07/99	3.3	8 Nov 99								
11/07/99 8-FFM	11/07/99	10.8	8 Nov 99								
11/07/99 8-IFM	11/07/99	6.9	8 Nov 99								
11/07/99 16	11/07/99	37.0	8 Nov 99								
11/07/99 58	11/07/99	58.5	8 Nov 99								
QC data:			% recovery		% recovery		% recovery		% recovery		% recovery
end of run std. check	0.0 mg/L NH ₃	0.1									
end of run std. check	1.0 mg/L NH ₃	0.9									
end of run std. check	2.0 mg/L NH ₃	1.9									
end of run std. check	4.0 mg/L NH ₃	4.0									
end of run std. check	8.0 mg/L NH ₃	8.1									
Verification Std (HACH)	5.0 PPM NH ₃	5.1	102%								
Verification Std (HACH)	0.5 PPM NH ₃	0.5	106%								
Standard additions:			% recovery		% recovery		% recovery		% recovery		% recovery
11/02/99	2.0 repeat	0.7	134%								
11/02/99	2.0 +4.0 ppm NH ₃		88%								
11/03/99	2.0 repeat	0.3									
11/03/99	2.0 +4.0 ppm NH ₃		97%								

Analyst Remarks:

Date Reported: 08-Nov-99

Verified by: G. Gay

Analyst: T. Poniatowski, D. Deluca

Date: 10-Nov-99

RESULTS

Sample			Concentration (mg/L)									
Description / Identification	Date		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
11/13/99 RW CONTROL	11/13/99		<0.3	15-Nov-99								
11/13/99 0.5	11/13/99		<0.3	15-Nov-99								
11/13/99 1	11/13/99		<0.3	15-Nov-99								
2 11/13/99 2	11/13/99		0.6	15-Nov-99								
11/13/99 4	11/13/99		6.6	15-Nov-99								
11/13/99 8-CC	11/13/99		5.4	15-Nov-99								
11/13/99 8-FFM	11/13/99		5.4	15-Nov-99								
11/13/99 8 LFM	11/13/99		6.8	15-Nov-99								
11/13/99 16	11/13/99		11.3	15-Nov-99								
11/13/99 58	11/13/99		57	15-Nov-99								
11/14/99 RW CONTROL	11/14/99		<0.3	15-Nov-99								
11/14/99 0.5	11/14/99		<0.3	15-Nov-99								
11/14/99 1	11/14/99		0.3	15-Nov-99								
11/14/99 2	11/14/99		0.5	15-Nov-99								
D 11/14/99 4	11/14/99		4.5	15-Nov-99								
11/14/99 8-CC	11/14/99		5.9	15-Nov-99								
11/14/99 8-FFM	11/14/99		6.2	15-Nov-99								
11/14/99 8 LFM	11/14/99		6.0	15-Nov-99								
11/14/99 16	11/14/99		10.1	15-Nov-99								
11/14/99 58	11/14/99		31	15-Nov-99								
QC data:				% recovery		% recovery		% recovery		% recovery		% recovery
end of run std. check:	0.0 mg/L NH ₃		0.0									
end of run std. check:	1.0 mg/L NH ₃		0.8									
end of run std. check:	2.0 mg/L NH ₃		1.8									
end of run std. check:	4.0 mg/L NH ₃		3.8									
end of run std. check:	8.0 mg/L NH ₃		7.6									
Verification Std. (HACH):	5.0 PPM NH ₃		5.0	101%								
Verification Std. (HACH):	0.5 PPM NH ₃		0.6	112%								
Standard additions:				% recovery		% recovery		% recovery		% recovery		% recovery
11/08/99	2.0 repeat		0.6	100%								
11/08/99	2.0 +4.0 ppm NH ₃			97%								
11/09/99	2.0 repeat		1.1	113%								
11/09/99	2.0 +4.0 ppm NH ₃			96%								

Analyst Remarks:

Date Reported: 15-Nov-99

Verified by:

Analyst: T. Poniatowski, D. Deluca

Date:

NOV 16 1999

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge DATE SUBMITTED: NOV.15-21/99 DATE SAMPLED: NOV.15-21/99 SAMPLED BY: Amy Partridge		REPORT TO: Amy Partridge ADDRESS: Tetres POSTAL CODE: _____ PHONE: _____	
SAMPLE TYPE: River Samples LOCATION: N. Main PROJECT: Toxicity		Sampler/Submitter Remarks: _____	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) <input type="checkbox"/> check Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>		Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> check Total Phosphorus (P) <input type="checkbox"/>	

RESULTS

Sample		Concentration (mg/L)									
Description / Identification	Date	NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
11/15/99 RW CONTROL	11/15/99	<0.3	22-Nov-99								
11/15/99 0.5	11/15/99	<0.3	22-Nov-99								
11/15/99 1	11/15/99	<0.3	22-Nov-99								
23 11/15/99 2	11/15/99	0.6	22-Nov-99								
11/15/99 4	11/15/99	2.9	22-Nov-99								
11/15/99 8-CC	11/15/99	5.8	22-Nov-99								
11/15/99 8-FFM	11/15/99	5.2	22-Nov-99								
11/15/99 8 LFM	11/15/99	5.8	22-Nov-99								
11/15/99 16	11/15/99	9.0	22-Nov-99								
11/15/99 58	11/15/99	63	22-Nov-99								
11/16/99 RW CONTROL	11/16/99	<0.3	22-Nov-99								
11/16/99 0.5	11/16/99	<0.3	22-Nov-99								
11/16/99 1	11/16/99	0.4	22-Nov-99								
24 11/16/99 2	11/16/99	0.4	22-Nov-99								
11/16/99 4	11/16/99	2.0	22-Nov-99								
11/16/99 8-CC	11/16/99	5.2	22-Nov-99								
11/16/99 8-FFM	11/16/99	5.2	22-Nov-99								
11/16/99 8 LFM	11/16/99	5.2	22-Nov-99								
11/16/99 16	11/16/99	12.0	22-Nov-99								
11/16/99 58	11/16/99	50	22-Nov-99								
11/17/99 RW CONTROL	11/17/99	17.0	22-Nov-99								
11/17/99 0.5	11/17/99	0.4	22-Nov-99								
11/17/99 1	11/17/99	0.4	22-Nov-99								
11/17/99 2	11/17/99	0.5	22-Nov-99								
25 11/17/99 4	11/17/99	2.7	22-Nov-99								
11/17/99 8-CC	11/17/99	3.6	22-Nov-99								
11/17/99 8-FFM	11/17/99	6.4	22-Nov-99								
11/17/99 8 LFM	11/17/99	5.0	22-Nov-99								
11/17/99 16	11/17/99	9.5	22-Nov-99								
11/17/99 58	11/17/99	41	22-Nov-99								
11/18/99 RW CONTROL	11/18/99	<0.3	22-Nov-99								
11/18/99 0.5	11/18/99	<0.3	22-Nov-99								
11/18/99 1	11/18/99	<0.3	22-Nov-99								
26 11/18/99 2	11/18/99	1.0	22-Nov-99								
11/18/99 4	11/18/99	1.6	22-Nov-99								
11/18/99 8-CC	11/18/99	3.3	22-Nov-99								
11/18/99 8-FFM	11/18/99	3.4	22-Nov-99								
11/18/99 8 LFM	11/18/99	3.6	22-Nov-99								
11/18/99 16	11/18/99	11.3	22-Nov-99								
11/18/99 58	11/18/99	54	22-Nov-99								

→ outlier
omit fr. data set

RESULTS

Sample			Concentration (mg/L)								
Description / Identification	Date	NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
11/19/99 RW CONTROL	11/19/99	<0.3	22-Nov-99								
11/19/99 0.5	11/19/99	<0.3	22-Nov-99								
11/19/99 1	11/19/99	0.4	22-Nov-99								
11/19/99 2	11/19/99	<0.3	22-Nov-99								
27 11/19/99 4	11/19/99	1.4	22-Nov-99								
11/19/99 8-CC	11/19/99	4.7	22-Nov-99								
11/19/99 8-FFM	11/19/99	5.0	22-Nov-99								
11/19/99 8 LFM	11/19/99	4.7	22-Nov-99								
11/19/99 16	11/19/99	12.0	22-Nov-99								
11/19/99 58	11/19/99	57	22-Nov-99								
11/20/99 RW CONTROL	11/20/99	<0.3	22-Nov-99								
11/20/99 0.5	11/20/99	0.3	22-Nov-99								
11/20/99 1	11/20/99	0.4	22-Nov-99								
11/20/99 2	11/20/99	0.7	22-Nov-99								
28 11/20/99 4	11/20/99	2.3	22-Nov-99								
11/20/99 8-CC	11/20/99	5.3	22-Nov-99								
11/20/99 8-FFM	11/20/99	5.1	22-Nov-99								
11/20/99 8 LFM	11/20/99	4.8	22-Nov-99								
11/20/99 16	11/20/99	9.6	22-Nov-99								
11/20/99 58	11/20/99	56	22-Nov-99								
11/21/99 RW CONTROL	11/21/99	<0.3	22-Nov-99								
11/21/99 0.5	11/21/99	<0.3	22-Nov-99								
11/21/99 1	11/21/99	<0.3	22-Nov-99								
11/21/99 2	11/21/99	0.6	22-Nov-99								
29 11/21/99 4	11/21/99	0.8	22-Nov-99								
11/21/99 8-CC	11/21/99	2.7	22-Nov-99								
11/21/99 8-FFM	11/21/99	2.2	22-Nov-99								
11/21/99 8 LFM	11/21/99	1.7	22-Nov-99								
11/21/99 16	11/21/99	9.4	22-Nov-99								
11/21/99 58	11/21/99	31	22-Nov-99								
QC data:			% recovery		% recovery		% recovery		% recovery		% recovery
end of run std. check: 0.0 mg/L NH3		0.0		0.9							
end of run std. check: 1.0 mg/L NH3		2.0		4.2							
end of run std. check: 2.0 mg/L NH3		7.9									
end of run std. check: 4.0 mg/L NH3		5.1	102%								
end of run std. check: 8.0 mg/L NH3		0.5	100%								
Verification Std. (HACH): 5.0 PPM NH3											
Verification Std. (HACH): 0.5 PPM NH3											
Standard additions:											
11/15/99 2.0 repeat		0.7	-								
11/15/99 2.0 +4.0 ppm NH3		-	105%								
11/15/99 4.0 repeat		3.1	-								
11/15/99 4.0 +4.0 ppm NH3		-	101%								

Analyst Remarks:

Date Reported: 22-Nov-99

Verified by: G. Gay

Analyst: T. Poniatowski, D. Deluca

Date: 23-Nov-99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge DATE SUBMITTED: 22-25 Nov 99 DATE SAMPLED: 22-25 Nov 99 SAMPLED BY: Amy Partridge		REPORT TO: Amy Partridge ADDRESS: Tetres INSTAL CODE: _____ PHONE: _____	
SAMPLE TYPE: River Samples LOCATION: 2230 Main PROJECT: Toxcity		Sampler/Submitter Remarks:	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ , N) <input type="checkbox"/> Total Ammonia (NH ₄ , N) <input checked="" type="checkbox"/> Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>	Ortho phosphate (PO ₄ , P) <input type="checkbox"/> Total Phosphorus (P) <input type="checkbox"/>		

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
11/22/99 RW CONTROL	11/22/99	<0.3	26-Nov-99								
11/22/99 0.5	11/22/99	<0.3	26-Nov-99								
11/22/99 1	11/22/99	<0.3	26-Nov-99								
(17) 11/22/99 2	11/22/99	<0.3	26-Nov-99								
11/22/99 4	11/22/99	0.5	26-Nov-99								
11/22/99 8	11/22/99	1.3	26-Nov-99								
11/22/99 16	11/22/99	3.5	26-Nov-99								
11/22/99 58	11/22/99	11.3	26-Nov-99								
		72	26-Nov-99								
11/23/99 RW CONTROL	11/23/99	<0.3	26-Nov-99								
11/23/99 0.5	11/23/99	<0.3	26-Nov-99								
(18) 11/23/99 1	11/23/99	<0.3	26-Nov-99								
11/23/99 2	11/23/99	0.4	26-Nov-99								
11/23/99 4	11/23/99	0.6	26-Nov-99								
11/23/99 8	11/23/99	2.4	26-Nov-99								
11/23/99 58	11/23/99	5.4	26-Nov-99								
		65	26-Nov-99								
11/24/99 RW CONTROL	11/24/99	<0.3	26-Nov-99								
11/24/99 0.5	11/24/99	<0.3	26-Nov-99								
(19) 11/24/99 1	11/24/99	<0.3	26-Nov-99								
11/24/99 2	11/24/99	0.3	26-Nov-99								
11/24/99 4	11/24/99	0.8	26-Nov-99								
11/24/99 8	11/24/99	2.3	26-Nov-99								
11/24/99 58	11/24/99	5.3	26-Nov-99								
		47	26-Nov-99								
11/25/99 RW CONTROL	11/25/99	<0.3	26-Nov-99								
11/25/99 0.5	11/25/99	<0.3	26-Nov-99								
(20) 11/25/99 1	11/25/99	<0.3	26-Nov-99								
11/25/99 2	11/25/99	0.3	26-Nov-99								
11/25/99 4	11/25/99	0.7	26-Nov-99								
11/25/99 8	11/25/99	2.4	26-Nov-99								
11/25/99 58	11/25/99	3.9	26-Nov-99								
		66	26-Nov-99								
QC data:											
end of run std check	0.0 mg/L NH ₃	0.0									
end of run std check	1.0 mg/L NH ₃	0.9									
end of run std check	2.0 mg/L NH ₃	1.9									
end of run std check	4.0 mg/L NH ₃	4.1									
end of run std check	8.0 mg/L NH ₃	8.0									
Verification Std (NH ₃)	5.0 ppm NH ₃	5.0	101%								
Verification Std (NH ₃)	0.5 ppm NH ₃	0.5	96%								
Standard additions:											
11/22/99 2.0 repeat		0.6	119%								
11/22/99 2.0 +4.0 ppm NH ₃			89%								
11/22/99 4.0 repeat		1.6	116%								
11/22/99 4.0 +4.0 ppm NH ₃			92%								

Analyst Remarks:

Date Reported: 26-Nov-99

Verified by: G. Gay

Analyst: J. Popatowski, D. Doherty

Date: 26-Nov-99

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 5/23/2000 - 5/28/2000 DATE SAMPLED 5/23/2000 - 5/28/2000 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetras POSTAL CODE PHONE	
SAMPLE TYPE (they water) LOCATION PROJECT NH3 - Toxicity		Sample/Submitter Remarks	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) <input type="checkbox"/> check Total Ammonia (NH ₄ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>		Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> check Total Phosphate (P) <input type="checkbox"/>	

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)											
		NH ₄ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date		
05/23/00 CONT. FH-A	23-May-00	<0.3	20-May-00										
05/23/00 0.5 FH-C	23-May-00	0.4	20-May-00										
05/23/00 1 FH-B	23-May-00	0.8	20-May-00										
05/23/00 2 FH-A	23-May-00	2.0	20-May-00										
05/23/00 4 FH-A	23-May-00	3.4	20-May-00										
05/23/00 4-WFT-C	23-May-00	3.4	20-May-00										
05/23/00 6 FH-C	23-May-00	6.0	20-May-00										
05/23/00 8 FH-A	23-May-00	7.8	20-May-00										
05/23/00 29.0 ppm	23-May-00	27.7	20-May-00										
05/24/00 CONT. FH-C	24-May-00	<0.3	20-May-00										
05/24/00 0.5 FH-D	24-May-00	6.6	20-May-00										
05/24/00 1.0 FH-B	24-May-00	6.8	20-May-00										
05/24/00 2 FH-D	24-May-00	1.8	20-May-00										
05/24/00 4 WE-C	24-May-00	3.3	20-May-00										
05/24/00 4 NP-C	24-May-00	3.4	20-May-00										
05/24/00 4-WFT-B	24-May-00	3.3	20-May-00										
05/24/00 4 FH-D	24-May-00	3.4	20-May-00										
05/24/00 6 FH-C	24-May-00	5.7	20-May-00										
05/24/00 8 FH-B	24-May-00	7.5	20-May-00										
05/24/00 29ppm	24-May-00	27.1	20-May-00										
05/25/00 CONT. A Y2K-4-FH	25-May-00	<0.3	20-May-00										
05/25/00 0.5 B Y2K-4-FH	25-May-00	0.4	20-May-00										
05/25/00 1.0 B Y2K-4-FH	25-May-00	0.8	20-May-00										
05/25/00 2.0 B Y2K-4-FH	25-May-00	1.8	20-May-00										
05/25/00 4.0 C Y2K-6NP	25-May-00	4.4	20-May-00										
05/25/00 4.0 C Y2K-SWE	25-May-00	3.4	20-May-00										
05/25/00 4.0 A Y2K-4-FH	25-May-00	3.1	20-May-00										
05/25/00 4.0A Y2K-3WF	25-May-00	3.1	20-May-00										
05/25/00 6.0 B Y2K-4-FH	25-May-00	5.5	20-May-00										
05/25/00 8.0 A Y2K-4-FH	25-May-00	8.2	20-May-00										
05/25/00 8.0 A Y2K-4-FH	25-May-00	27.8	20-May-00										
05/26/00 -CONT. FH-A	26-May-00	<0.3	20-May-00										
05/26/00 -0.5 FH-A	26-May-00	0.4	20-May-00										
05/26/00 -1 FH-D	26-May-00	0.8	20-May-00										
05/26/00 -2 NP-D	26-May-00	2.0	20-May-00										
05/26/00 -2 FH-C	26-May-00	1.8	20-May-00										
05/26/00 -4 FH-C	26-May-00	3.1	20-May-00										
05/26/00 -6 FH-D	26-May-00	5.6	20-May-00										
05/26/00 -8 FH-A	26-May-00	7.4	20-May-00										
05/26/00 -29 ppm	26-May-00	25.8	20-May-00										
05/27/00 -CONT. Y2K-4	27-May-00	<0.3	20-May-00										
05/27/00 -0.5D Y2K-3	27-May-00	0.4	20-May-00										
05/27/00 -0.5 D Y2K-4	27-May-00	0.4	20-May-00										
05/27/00 -0.5 B Y2K-6	27-May-00	6.4	20-May-00										
05/27/00 -1.0 C Y2K-4	27-May-00	6.8	20-May-00										
05/27/00 -2.0 C Y2K-4	27-May-00	1.8	20-May-00										
05/27/00 -6.0 B Y2K-4	27-May-00	3.8	20-May-00										
05/27/00 -6.0 Y2K-4	27-May-00	4.8	20-May-00										
05/27/00 -8.0 Y2K-4	27-May-00	5.5	20-May-00										
05/27/00 29.0 ppm	27-May-00	25.8	20-May-00										
05/28/00 CONT. -N-P-C	28-May-00	<0.3	20-May-00										
05/28/00 0.5 NP-A	28-May-00	0.7	20-May-00										
05/28/00 1 WF-B	28-May-00	1.0	20-May-00										
05/28/00 1 NP-A	28-May-00	1.0	20-May-00										
05/28/00 2 NP-D	28-May-00	1.8	20-May-00										
05/28/00 4 NP-C	28-May-00	3.0	20-May-00										
05/28/00 6 NP-C	28-May-00	6.2	20-May-00										
05/28/00 8 NP-D	28-May-00	7.6	20-May-00										
05/28/00 29 ppm	28-May-00	26.7	20-May-00										
QC data:													
end of run std check:	0.0 mg/L NH3	0.00											
end of run std check:	1.0 mg/L NH3	0.99											
end of run std check:	2.0 mg/L NH3	1.97											
end of run std check:	4.0 mg/L NH3	3.95											
end of run std check:	8.0 mg/L NH3	8.05											
Recovery %:													
0.0 ppm NH3		5.07											
1.0 ppm NH3		102%											
2.0 ppm NH3		98%											
4.0 ppm NH3		95%											
8.0 ppm NH3		101%											
Recovery %:													
05/23/00 1-FH-B		0.59											
05/23/00 1-FH-B +0.3 mg/L NH3													
05/23/00 2-FH-A		1.70											
05/23/00 2-FH-A +0.3 mg/L NH3													

Analyst Remarks

Date Reported: 29-May-00

Verified by: G. GAY

Analyst: I. Ponomarev, D. Dutka

Date: 29-May-00

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 5/29/2000 - 06/04/00 DATE SAMPLED 5/29/2000 - 06/04/00 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetras POSTAL CODE PHONE	
SAMPLE TYPE river water LOCATION PROJECT NH3 - Toxicity		Sampler/Submitter Remarks	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) <input type="checkbox"/> Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>		Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> Total Phosphorus (P) <input type="checkbox"/>	

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
5/29/00 CONT. NP-A	29-May-00	0.3	5-Jun-00								
5/29/00 -0.5 NP-D	29-May-00	0.8	5-Jun-00								
5/29/00 -1 NP-C	29-May-00	1.0	5-Jun-00								
5/29/00 -2 NP-B	29-May-00	2.2	5-Jun-00								
5/29/00 -4 NP-A	29-May-00	4.3	5-Jun-00								
5/29/00 -6 NP-D	29-May-00	6.7	5-Jun-00								
5/29/00 -8 NP-D	29-May-00	8.3	5-Jun-00								
5/29/00 29 ppm	29-May-00	28.9	5-Jun-00								
5/30/00 -CONT. NP-C	30-May-00	<0.3	5-Jun-00								
5/30/00 -0.5 NP-D	30-May-00	1.0	5-Jun-00								
5/30/00 -1 NP-D	30-May-00	1.6	5-Jun-00								
5/30/00 -2 NP-C	30-May-00	2.1	5-Jun-00								
5/30/00 -4 WE-D	30-May-00	3.9	5-Jun-00								
5/30/00 -4 NP-D	30-May-00	4.4	5-Jun-00								
5/30/00 -6 NP-A	30-May-00	6.2	5-Jun-00								
5/30/00 -8 NP-C	30-May-00	8.1	5-Jun-00								
5/30/00 -29 ppm	30-May-00	27.6	5-Jun-00								
5/31/00 -CONT. NP-B	31-May-00	<0.3	5-Jun-00								
5/31/00 -0.5 NP-A	31-May-00	0.3	5-Jun-00								
5/31/00 -1.0 NP-C	31-May-00	0.9	5-Jun-00								
5/31/00 -2.0 NP-A	31-May-00	1.9	5-Jun-00								
5/31/00 -4.0 NP-B	31-May-00	3.8	5-Jun-00								
5/31/00 -6 NP-D	31-May-00	5.8	5-Jun-00								
5/31/00 -8 NP-B	31-May-00	7.6	5-Jun-00								
05/31/00 29 ppm	31-May-00	26.0	5-Jun-00								
06/01/00 -CONT. NP-D	01-Jun-00	0.4	5-Jun-00								
06/01/00 -0.5 NP-A	01-Jun-00	0.5	5-Jun-00								
06/01/00 -1 NP-C	01-Jun-00	1.2	5-Jun-00								
06/01/00 -2 NP-B	01-Jun-00	2.0	5-Jun-00								
06/01/00 -4 NP-B	01-Jun-00	5.0	5-Jun-00								
06/01/00 -6 NP-A	01-Jun-00	5.8	5-Jun-00								
06/01/00 -8 NP-B	01-Jun-00	8.5	5-Jun-00								
06/01/00 -29 ppm	01-Jun-00	26.5	5-Jun-00								
06/02/00 -CONT. NP-D	02-Jun-00	0.5	5-Jun-00								
06/02/00 -0.5 NP-A	02-Jun-00	1.1	5-Jun-00								
06/02/00 -1 NP-B	02-Jun-00	1.5	5-Jun-00								
06/02/00 -2 NP-A	02-Jun-00	2.0	5-Jun-00								
06/02/00 -4 NP-B	02-Jun-00	3.9	5-Jun-00								
06/02/00 -6 NP-C	02-Jun-00	6.0	5-Jun-00								
06/02/00 -8 NP-D	02-Jun-00	8.0	5-Jun-00								
06/02/00 -29 ppm	02-Jun-00	25.8	5-Jun-00								
05/03/00 -CONT. NP-A	03-Jun-00	0.3	5-Jun-00								
05/03/00 -0.5 NP-D	03-Jun-00	0.6	5-Jun-00								
05/03/00 -1.0 NP-C	03-Jun-00	1.3	5-Jun-00								
05/03/00 -2.0 NP-C	03-Jun-00	2.1	5-Jun-00								
05/03/00 -4 NP-B	03-Jun-00	4.2	5-Jun-00								
05/03/00 -6 NP-C	03-Jun-00	5.4	5-Jun-00								
05/03/00 -8 NP-A	03-Jun-00	7.0	5-Jun-00								
05/03/00 -29 ppm	03-Jun-00	24.0	5-Jun-00								
06/04/00 -CONT. NP-A	04-Jun-00	0.3	5-Jun-00								
06/04/00 -0.5 NP-D	04-Jun-00	0.5	5-Jun-00								
06/04/00 -1.0 NP-B	04-Jun-00	1.3	5-Jun-00								
06/04/00 -2 NP-A	04-Jun-00	2.0	5-Jun-00								
06/04/00 -4 LTA	04-Jun-00	3.6	5-Jun-00								
06/04/00 NP-A	04-Jun-00	4.1	5-Jun-00								
06/04/00 -6 NP-C	04-Jun-00	6.3	5-Jun-00								
06/04/00 -8 LT-C	04-Jun-00	7.9	5-Jun-00								
06/04/00 -29 ppm	04-Jun-00	25.7	5-Jun-00								

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY <u>Amy Partridge</u> DATE SUBMITTED <u>5/29/2000 - 06/04/00</u> DATE SAMPLED <u>5/29/2000 - 06/04/00</u> SAMPLED BY <u>Amy Partridge</u>		REPORT TO <u>Amy Partridge</u> ADDRESS <u>Tetres</u> POSTAL CODE _____ PHONE _____	
SAMPLE TYPE <u>river water</u> LOCATION _____ PROJECT <u>NH3 - Toxicity</u>		Sampler/Submitter Remarks _____	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) <input type="checkbox"/> check Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>	Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> check Total Phosphorus (P) <input type="checkbox"/>		

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
QC data:											
end of run std. check: 0.0 mg/L NH3		0.1									
end of run std. check: 1.0 mg/L NH3		0.9	95%								
end of run std. check: 2.0 mg/L NH3		2.0	102%								
end of run std. check: 4.0 mg/L NH3		4.1	101%								
end of run std. check: 8.0 mg/L NH3		8.0	100%								
Verification Std. (HACI): 5.0 PPM NH3											
Standard additions:											
05/29/00 2-NP-B		2.2	100%								
05/29/00 2-NP-B+0.3 mg/L NH3			100%								
05/30/00 2-NP-C		2.2	103%								
05/30/00 2-NP-C+0.3 mg/L NH3			101%								

Analyst Remarks:

Date Reported: 05-Jun-00
 Analyst: T. Poniatowski, D. Deluca

Verified by: G. GAY
 Date: 5-Jun-00

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 06/05/00 - 06/11/00 DATE SAMPLED 06/05/00 - 06/11/00 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetres POSTAL CODE PHONE	
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SAMPLE TYPE river water LOCATION PROJECT NH3 - Toxicity	Sampler/Submitter Remarks		
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ /N) <input type="checkbox"/> check Total Ammonia (NH ₃ /N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>	Ortho phosphate (PO ₄ /P) <input type="checkbox"/> check	Total Phosphorus (P) <input type="checkbox"/> check	<input type="checkbox"/> check

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
06/05/00 CONTROL	05-Jun-00	<0.2	12-Jun-00								
06/05/00 0.5-LTC	05-Jun-00	0.4	12-Jun-00								
06/05/00 1.0-LTD	05-Jun-00	0.8	12-Jun-00								
06/05/00 2.0-LTA	05-Jun-00	1.7	12-Jun-00								
06/05/00 4.0-LTB	05-Jun-00	3.3	12-Jun-00								
06/05/00 6-LTB	05-Jun-00	5.6	12-Jun-00								
06/05/00 8.0-LTA	05-Jun-00	7.5	12-Jun-00								
29ppm		23.7	12-Jun-00								
06/06/00 CONT-LTC	06-Jun-00	0.2	12-Jun-00								
06/06/00 0.5 LTC	06-Jun-00	0.3	12-Jun-00								
06/06/00 1.0 LTA	06-Jun-00	0.9	12-Jun-00								
06/06/00 2.0 LTC	06-Jun-00	1.8	12-Jun-00								
06/06/00 4.0 LTB	06-Jun-00	3.7	12-Jun-00								
06/06/00 6.0 LTA	06-Jun-00	5.9	12-Jun-00								
06/06/00 8.0 LTA	06-Jun-00	7.8	12-Jun-00								
29 ppm	06-Jun-00	22.9	12-Jun-00								
06/07/00 CONT ALT	07-Jun-00	0.2	12-Jun-00								
06/07/00 0.5 DLT	07-Jun-00	0.3	12-Jun-00								
06/07/00 1.0 CLT	07-Jun-00	0.8	12-Jun-00								
06/07/00 2.0 ALT	07-Jun-00	2.0	12-Jun-00								
06/07/00 4.0 BLT	07-Jun-00	3.8	12-Jun-00								
06/07/00 6.0 BLT	07-Jun-00	6.2	12-Jun-00								
06/07/00 8.0 DLT	07-Jun-00	7.6	12-Jun-00								
29 ppm	07-Jun-00	23.0	12-Jun-00								
06/08/00 CONT LTB	08-Jun-00	0.2	12-Jun-00								
06/08/00 0.5-LTA	08-Jun-00	0.2	12-Jun-00								
06/08/00 1.0-LTA	08-Jun-00	0.7	12-Jun-00								
06/08/00 2.0-LTA	08-Jun-00	1.8	12-Jun-00								
06/08/00 4.0-LTB	08-Jun-00	3.8	12-Jun-00								
06/08/00 6.0-LTC	08-Jun-00	5.4	12-Jun-00								
06/08/00 6.0 WEA	08-Jun-00	5.5	12-Jun-00								
06/08/00 8.0 LTB	08-Jun-00	7.4	12-Jun-00								
29 ppm	08-Jun-00	25.7	12-Jun-00								
06/09/00 CONT-WE-C	09-Jun-00	0.2	12-Jun-00								
06/09/00 0.5 WE-C	09-Jun-00	0.2	12-Jun-00								
06/09/00 1.0 WE-B	09-Jun-00	0.7	12-Jun-00								
06/09/00 2.0 WE-A	09-Jun-00	1.6	12-Jun-00								
06/09/00 4.0 LT-C	09-Jun-00	3.5	12-Jun-00								
06/09/00 4.0 WE-A	09-Jun-00	3.6	12-Jun-00								
06/09/00 6.0 WE-C	09-Jun-00	5.4	12-Jun-00								
06/09/00 8.0 LT-B	09-Jun-00	7.5	12-Jun-00								
29 ppm	09-Jun-00	30.2	12-Jun-00								
06/10/00 CONTROL B WE	10-Jun-00	0.3	12-Jun-00								
06/10/00 0.5-C-WE	10-Jun-00	0.5	12-Jun-00								
06/10/00 1.0 A-WE	10-Jun-00	1.0	12-Jun-00								
06/10/00 2.0 B-WE	10-Jun-00	2.2	12-Jun-00								
06/10/00 4.0 B-LT	10-Jun-00	3.8	12-Jun-00								
06/10/00 4.0 C-WE	10-Jun-00	3.8	12-Jun-00								
06/10/00 6.0 B-WE	10-Jun-00	5.7	12-Jun-00								
06/10/00 8.0 C-LT	10-Jun-00	7.2	12-Jun-00								
06/10/00 29.0 p	10-Jun-00	32.4	12-Jun-00								
06/11/00 CONTROL B WE	11-Jun-00	0.3	12-Jun-00								
06/11/00 0.5 B WE	11-Jun-00	0.5	12-Jun-00								
06/11/00 1.0 C WE	11-Jun-00	0.9	12-Jun-00								
06/11/00 1.0 C LT	11-Jun-00	0.8	12-Jun-00								
06/11/00 2.0 C WE	11-Jun-00	1.8	12-Jun-00								
06/11/00 4.0 B WE	11-Jun-00	3.7	12-Jun-00								
06/11/00 A WE	11-Jun-00	5.2	12-Jun-00								
06/11/00 8.0 B LT	11-Jun-00	7.1	12-Jun-00								
06/11/00 29.0 p	11-Jun-00	28.3	12-Jun-00								

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 06/05/00 - 06/11/00 DATE SAMPLED 06/05/00 - 06/11/00 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetras POSTAL CODE _____ PHONE _____	
SAMPLE TYPE river water LOCATION _____ PROJECT NH3 - Toxicity		Sampler/Submitter Remarks _____	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ -N) <input type="checkbox"/> check Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>	Ortho phosphate (PO ₄ -P) <input type="checkbox"/> check	Total Phosphorus (P) <input type="checkbox"/> check	_____

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
QC data:											
end of run std. check: 0.0 mg/L NH ₃		0.0	% recovery		% recovery				% recovery		% recovery
end of run std. check: 1.0 mg/L NH ₃		1.3	130%								
end of run std. check: 2.0 mg/L NH ₃		1.9	95%								
end of run std. check: 4.0 mg/L NH ₃		4.0	100%								
end of run std. check: 8.0 mg/L NH ₃		8.0	100%								
Verification Std (HACH): 5.0 PPM NH ₃											
Standard additions:											
06/05/00 2.0 LTA					% recovery		% recovery		% recovery		% recovery
06/05/00 2.0 LTA+0.3 mg/L NH ₃		1.7	98%								
06/06/00 2.0-LTC											
06/06/00 2.0-LTC+0.3 mg/L NH ₃		1.8	99%								

Analyst Remarks:

Date Reported: 12-Jun-00
Analyst: T. Ponatowski, D. Deluca

Verified by: G. GAY
Date: 5-Jun-00

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 06/12/00 - 06/19/00 DATE SAMPLED 06/12/00 - 06/19/00 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tees POSTAL CODE PHONE	
SAMPLE TYPE river water LOCATION PROJECT NH3 - Toxicity		Samples/Submitter Remarks	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) Total Ammonia (NH ₃ -N) Total Kjeldahl Nitrogen (TKN)	check X	Ortho-phosphate (PO ₄ -P) Total Phosphorus (P)	check check

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
06/12/00-CONT-WE-B	12-Jun-00	<0.3	20-Jun-00								
06/12/00-0.5 WE-C	12-Jun-00	0.4	20-Jun-00								
06/12/00-1.0 WE-C	12-Jun-00	0.9	20-Jun-00								
06/12/00-2.0 WE-A	12-Jun-00	1.8	20-Jun-00								
06/12/00-2.0 LT-A	12-Jun-00	1.8	20-Jun-00								
06/12/00-4 WE-C	12-Jun-00	3.6	20-Jun-00								
06/12/00-6.0 WE-B	12-Jun-00	5.5	20-Jun-00								
06/12/00-8.0 LT-A	12-Jun-00	7.7	20-Jun-00								
06/12/00-29 PPM	12-Jun-00	25.4	20-Jun-00								
06/13/00 CONT-WE-C	13-Jun-00	<0.3	20-Jun-00								
06/13/00 0.5 WE-B	13-Jun-00	0.4	20-Jun-00								
06/13/00 1.0 WE-A	13-Jun-00	0.9	20-Jun-00								
06/13/00 2.0 WE-B	13-Jun-00	1.7	20-Jun-00								
06/13/00 4.0 LT-B	13-Jun-00	3.1	20-Jun-00								
06/13/00 4.0 WE-B	13-Jun-00	3.2	20-Jun-00								
06/13/00 6.0 WE-B	13-Jun-00	5.8	20-Jun-00								
06/13/00 8.0 LT-A	13-Jun-00	6.8	20-Jun-00								
06/13/00 29 PPM	13-Jun-00	25.6	20-Jun-00								
06/14/00 CONT-WE-B	14-Jun-00	<0.3	20-Jun-00								
06/14/00 0.5 WE-A	14-Jun-00	0.4	20-Jun-00								
06/14/00 1.0 WE-C	14-Jun-00	0.8	20-Jun-00								
06/14/00 2.0 WE-B	14-Jun-00	1.8	20-Jun-00								
06/14/00 4.0 WE-A	14-Jun-00	3.5	20-Jun-00								
06/14/00 6.0 LT-A	14-Jun-00	5.8	20-Jun-00								
06/14/00 6 WE-C	14-Jun-00	5.9	20-Jun-00								
06/14/00 8.0 LT-B	14-Jun-00	7.6	20-Jun-00								
06/14/00 29 PPM	14-Jun-00	31.2	20-Jun-00								
06/15/00 CONT B-LT	15-Jun-00	<0.3	20-Jun-00								
06/15/00 CONT C-WE	15-Jun-00	<0.3	20-Jun-00								
06/15/00 0.5 C-WE	15-Jun-00	0.5	20-Jun-00								
06/15/00 1.0 B-WE	15-Jun-00	1.0	20-Jun-00								
06/15/00 2.0 A-WE	15-Jun-00	2.2	20-Jun-00								
06/15/00 4.0 A-WE	15-Jun-00	3.4	20-Jun-00								
06/15/00 6.0 C-WE	15-Jun-00	6.1	20-Jun-00								
06/15/00 8.0 B-WE	15-Jun-00	8.0	20-Jun-00								
06/15/00 29 PPM	15-Jun-00	26.7	20-Jun-00								
06/16/00 CONT-WE-B	16-Jun-00	<0.3	20-Jun-00								
06/16/00 0.5 LT-B	16-Jun-00	<0.3	20-Jun-00								
06/16/00 0.5 WE-B	16-Jun-00	<0.3	20-Jun-00								
06/16/00 1-WE-A	16-Jun-00	<0.3	20-Jun-00								
06/16/00 2-WE-B	16-Jun-00	<0.3	20-Jun-00								
06/16/00 4-WE-C	16-Jun-00	<0.3	20-Jun-00								
06/16/00 6-WE-A	16-Jun-00	0.6	20-Jun-00								
06/16/00 8-LT-B	16-Jun-00	0.3	20-Jun-00								
06/16/00 22 PPM	16-Jun-00	20.9	20-Jun-00								
06/17/00 CONT-WE-A	17-Jun-00	<0.3	20-Jun-00								
06/17/00 0.5 WE-A	17-Jun-00	0.8	20-Jun-00								
06/17/00 1.0 WE-C	17-Jun-00	1.1	20-Jun-00								
06/17/00 2.0 WE-A	17-Jun-00	2.1	20-Jun-00								
06/17/00 4.0 WE-C	17-Jun-00	4.6	20-Jun-00								
06/17/00 6.0 WE-C	17-Jun-00	5.7	20-Jun-00								
06/17/00 6.0 LT-B	17-Jun-00	5.8	20-Jun-00								
06/18/00 CONT B-WE	18-Jun-00	<0.3	20-Jun-00								
06/18/00 0.5 C-WE	18-Jun-00	0.6	20-Jun-00								
06/18/00 1.0 A-WE	18-Jun-00	1.2	20-Jun-00								
06/18/00 2.0C-LT	18-Jun-00	2.2	20-Jun-00								
06/18/00 2.0 B-WE	18-Jun-00	2.4	20-Jun-00								
06/18/00 4.0 C-WE	18-Jun-00	6.0	20-Jun-00								
06/18/00 6.0 A-WE	18-Jun-00	6.0	20-Jun-00								
06/18/00 22 PPM	18-Jun-00	28.2	20-Jun-00								
06/19/00 CONT-LTA	19-Jun-00	0.4	20-Jun-00								
06/19/00 CONT-WE-C	19-Jun-00	0.3	20-Jun-00								
06/19/00 0.5 WE-B	19-Jun-00	0.7	20-Jun-00								
06/19/00 1.0 WE-A	19-Jun-00	1.4	20-Jun-00								
06/19/00 2.0 WE-B	19-Jun-00	2.2	20-Jun-00								
06/19/00 4.0 WE-A	19-Jun-00	4.7	20-Jun-00								
06/19/00 6.0 WE-B	19-Jun-00	6.2	20-Jun-00								
06/19/00 22 PPM	19-Jun-00	22.3	20-Jun-00								

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City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 06/12/00 - 06/19/00 DATE SAMPLED 06/12/00 - 06/19/00 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetres POSTAL CODE PHONE	
SAMPLE TYPE river water LOCATION PROJECT NH3 - Toxicity		Sampler/Submitter Remarks	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) Total Ammonia (NH ₃ -N) Total Kjeldahl Nitrogen (TKN)	check X	Ortho-phosphate (PO ₄ -P) check	check

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)				
		NH ₃ -N	NO ₃ -N	TKN	P	Ortho PO ₄ -P
QC data:						
end of run std. check: 0.0 mg/L NH ₃		0.14				
end of run std. check: 1.0 mg/L NH ₃		0.98	98%			
end of run std. check: 2.0 mg/L NH ₃		1.98	99%			
end of run std. check: 4.0 mg/L NH ₃		4.05	101%			
end of run std. check: 8.0 mg/L NH ₃		8.02	100%			
Verification Std. (NH ₃) 5.0 mg/L NH ₃		5.00	100%			
Standard addition:						
05/12/00 2.0 WE-A		1.7				
05/12/00 2.0 WE-A+0.3 mg/L NH ₃			102%			
05/13/00 2.0 WE-B		1.8				
05/13/00 2.0 WE-B+0.3 mg/L NH ₃			98%			

Analyst Remarks: Shaded values for 2.0 B-WE and 2.0 C-WE are suspect due to possibly switched samples.

Date Reported: 20-Jun-00
Analyst: T. Poniatowski, D. Deluca

Verified by: G. GAY
Date: 21-Jun-00

City of Winnipeg
Laboratory Services Division

13001

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 06/20/00 - 06/26/00 DATE SAMPLED 06/20/00 - 06/26/00 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetres POSTAL CODE PHONE	
SAMPLE TYPE river water LOCATION PROJECT NH3 - Toxicity		Sampler/Submitter Remarks	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) <input type="checkbox"/> check Total Ammonia (NH ₃ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>		Ortho-phosphate (PO ₄ -P) <input type="checkbox"/> check Total Phosphorus (P) <input type="checkbox"/>	

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
06/20/00-CONT WE-C	20-Jun-00	0.2	27-Jun-00								
06/20/00-0.5 WE-A	20-Jun-00	0.7	27-Jun-00								
06/20/00-1.0 WE-A	20-Jun-00	0.9	27-Jun-00								
06/20/00-2.0 WE-B	20-Jun-00	1.6	27-Jun-00								
06/20/00- 2.0 LT-B	20-Jun-00	1.6	27-Jun-00								
06/20/00-4 WE-A	20-Jun-00	3.5	27-Jun-00								
06/20/00-6.0 WE-B	20-Jun-00	4.7	27-Jun-00								
06/20/00-22 PPM	20-Jun-00	21.7	27-Jun-00								
06/21/00 CONT WE-B	21-Jun-00	0.1	27-Jun-00								
06/21/00 0.5 WE-A	21-Jun-00	0.4	27-Jun-00								
06/21/00 1.0 WE-B	21-Jun-00	1.2	27-Jun-00								
06/21/00 2.0 WE-A	21-Jun-00	1.7	27-Jun-00								
06/21/00 4.0 WE-A	21-Jun-00	3.9	27-Jun-00								
06/21/00 4.0 LT-C	21-Jun-00	3.9	27-Jun-00								
06/21/00 6.0 WE-A	21-Jun-00	5.7	27-Jun-00								
06/21/00 22 PPM	21-Jun-00	21.5	27-Jun-00								
06/22/00 CONT WE-A	22-Jun-00	0.2	27-Jun-00								
06/22/00 0.5 WE-C	22-Jun-00	0.5	27-Jun-00								
06/22/00 1.0 WE-A	22-Jun-00	1.0	27-Jun-00								
06/22/00 2.0 WE-B	22-Jun-00	1.8	27-Jun-00								
06/22/00 2.0 LT-B	22-Jun-00	1.8	27-Jun-00								
06/22/00 4 WE-A	22-Jun-00	3.8	27-Jun-00								
06/22/00 6 WE-A	22-Jun-00	5.1	27-Jun-00								
06/22/00 22 PPM	22-Jun-00	31.7	27-Jun-00								
06/23/00 CONT WE-B	23-Jun-00	0.1	27-Jun-00								
06/23/00 0.5 WE-B	23-Jun-00	0.4	27-Jun-00								
06/23/00 1.0 WE-C	23-Jun-00	0.9	27-Jun-00								
06/23/00 2.0 WE-B	23-Jun-00	1.7	27-Jun-00								
06/23/00 4.0 WE-C	23-Jun-00	3.8	27-Jun-00								
06/23/00 6.0 WE-B	23-Jun-00	5.3	27-Jun-00								
06/23/00 6 LT-A	23-Jun-00	5.1	27-Jun-00								
06/23/00 22 PPM	23-Jun-00	24.0	27-Jun-00								
06/24/00 CONT WE-A	24-Jun-00	0.2	27-Jun-00								
06/24/00 0.5 WE-B	24-Jun-00	0.4	27-Jun-00								
06/24/00 1 LT-A	24-Jun-00	0.9	27-Jun-00								
06/24/00 1 WE-A	24-Jun-00	1.0	27-Jun-00								
06/24/00 2 WE-B	24-Jun-00	1.5	27-Jun-00								
06/24/00 4 WE-A	24-Jun-00	3.5	27-Jun-00								
06/24/00 6 WE-A	24-Jun-00	5.1	27-Jun-00								
06/24/00 22 PPM	24-Jun-00	23.0	27-Jun-00								
06/25/00 CONT WE-B	25-Jun-00	0.2	27-Jun-00								
06/25/00 0.5 WE-C	25-Jun-00	0.5	27-Jun-00								
06/25/00 1.0 WE-A	25-Jun-00	0.9	27-Jun-00								
06/25/00 2.0 WE-C	25-Jun-00	1.6	27-Jun-00								
06/25/00 2.0 LT-A	25-Jun-00	1.6	27-Jun-00								
06/25/00 4.0 WE-C	25-Jun-00	3.5	27-Jun-00								
06/25/00 6.0 WE-B	25-Jun-00	5.1	27-Jun-00								
06/25/00 22 PPM	25-Jun-00	26.7	27-Jun-00								

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 06/20/00 - 06/26/00 DATE SAMPLED 06/20/00 - 06/26/00 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetres POSTAL CODE _____ PHONE: _____	
SAMPLE TYPE river water LOCATION _____ PROJECT NH3 - Toxicity		Sampler/Submitter Remarks	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) Total Ammonia (NH ₃ -N) Total Kjeldahl Nitrogen (TKN)	check X	Ortho-phosphate (PO ₄ -P) Total Phosphorus (P)	check check

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
06/26/00 CONT. WE-A	26-Jun-00	0.4	27-Jun-00								
06/26/00 0.5WE-B	26-Jun-00	0.6	27-Jun-00								
06/26/00 1.0 WE-A	26-Jun-00	1.0	27-Jun-00								
06/26/00 2.0 WE-B	26-Jun-00	1.6	27-Jun-00								
06/26/00 4.0 WE-A	26-Jun-00	3.6	27-Jun-00								
06/26/00 6.0 WE-C	26-Jun-00	5.1	27-Jun-00								
06/26/00 6.0 C-LT	26-Jun-00	5.3	27-Jun-00								
06/26/00 22 PPM	26-Jun-00	26.0	27-Jun-00								
QC data:											
end of run std. check:	0.0 mg/L NH ₃	0.03		% recovery		% recovery		% recovery		% recovery	
end of run std. check:	1.0 mg/L NH ₃	1.06	106%								
end of run std. check:	2.0 mg/L NH ₃	4.02	100%								
end of run std. check:	4.0 mg/L NH ₃	8.18	102%								
end of run std. check:	8.0 mg/L NH ₃	4.97	99%								
Verification Std. (HACH)	5.0 PPM NH ₃										
Standard additions:											
	06/20/00 2.0 WE-B	1.6	101%			% recovery		% recovery		% recovery	
	06/20/00 2.0 WE-B+ 5.0 mg/L NH ₃										
	06/20/00 2.0-LT-B	1.7	102%								
	06/20/00 2.0-LT-B+ 5.0 mg/L NH ₃										

Analyst Remarks:

Date Reported: 27-Jun-00

Verified by: G. GAY / G. LEVESQUE

Analyst: T. Poniatowski, D. Deluca

Date: 28-Jun-00

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY Amy Partridge DATE SUBMITTED 06/27/00 07/03/00 DATE SAMPLED 06/27/00 07/03/00 SAMPLED BY Amy Partridge		REPORT TO Amy Partridge ADDRESS Tetres POSTAL CODE PHONE	
SAMPLE TYPE river water LOCATION PROJECT NH3 - Toxicity		Sampler/Submitter Remarks	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ -N) <input type="checkbox"/> check Total Ammonia (NH ₄ -N) <input checked="" type="checkbox"/> X Total Kjeldahl Nitrogen (TKN) <input type="checkbox"/>		Ortho phosphate (PO ₄ -P) <input type="checkbox"/> check Total Phosphorus (P) <input type="checkbox"/>	

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
06/27/00-CONT WE-A	27-Jun-00	<0.3	4-Jul-00								
06/27/00-0.5 WE-C	27-Jun-00	0.5	4-Jul-00								
06/27/00-1.0 WE-C	27-Jun-00	0.7	4-Jul-00								
06/27/00-2.0 WE-A	27-Jun-00	1.5	4-Jul-00								
06/27/00-2.0 LT-A	27-Jun-00	3.5	4-Jul-00								
06/27/00-4 WE-A	27-Jun-00	3.4	4-Jul-00								
06/27/00-6.0 WE-A	27-Jun-00	5.0	4-Jul-00								
06/27/00-22 PPM	27-Jun-00	35.7	4-Jul-00								
06/28/00 CONT WE-C	28-Jun-00	<0.3	4-Jul-00								
06/28/00 0.5 WE-B	28-Jun-00	0.4	4-Jul-00								
06/28/00 1.0 WE-A	28-Jun-00	0.7	4-Jul-00								
06/28/00 2.0 LT-C	28-Jun-00	1.5	4-Jul-00								
06/28/00 2.0 WE-B	28-Jun-00	1.5	4-Jul-00								
06/28/00 4.0 WE-B	28-Jun-00	3.5	4-Jul-00								
06/28/00 6.0 WE-A	28-Jun-00	4.6	4-Jul-00								
06/28/00 22 PPM	28-Jun-00	24.7	4-Jul-00								
06/29/00 CONT WE-A	29-Jun-00	<0.3	4-Jul-00								
06/29/00 0.5 WE-C	29-Jun-00	0.4	4-Jul-00								
06/29/00 1.0 WE-B	29-Jun-00	0.8	4-Jul-00								
06/29/00 2.0 WE-C	29-Jun-00	1.1	4-Jul-00								
06/29/00 4 WE-C	29-Jun-00	3.3	4-Jul-00								
06/29/00 6 LT-C	29-Jun-00	4.7	4-Jul-00								
06/29/00 6 WE-A	29-Jun-00	4.3	4-Jul-00								
06/29/00 22 PPM	29-Jun-00	21.5	4-Jul-00								
06/30/00 CONT WE-C	30-Jun-00	<0.3	4-Jul-00								
06/30/00 0.5 WE-A	30-Jun-00	0.4	4-Jul-00								
06/30/00 1.0 WE-B	30-Jun-00	0.9	4-Jul-00								
06/30/00 2.0 WE-B	30-Jun-00	1.6	4-Jul-00								
06/30/00 4.0 WE-C	30-Jun-00	3.5	4-Jul-00								
06/30/00 4 LT-B	30-Jun-00	3.6	4-Jul-00								
06/30/00 6 WE-B	30-Jun-00	5.1	4-Jul-00								
06/30/00 22 PPM	30-Jun-00	20.6	4-Jul-00								
07/01/00 CONT WE-A	01-Jul-00	<0.3	4-Jul-00								
07/01/00 0.5 WE-B	01-Jul-00	0.4	4-Jul-00								
07/01/00 1 WE-A	01-Jul-00	0.9	4-Jul-00								
07/01/00 1-LT-B	01-Jul-00	0.9	4-Jul-00								
07/01/00 2 WE-B	01-Jul-00	1.5	4-Jul-00								
07/01/00 4 WE-B	01-Jul-00	3.3	4-Jul-00								
07/01/00 6 WE-C	01-Jul-00	4.6	4-Jul-00								
07/01/00 22 PPM	01-Jul-00	24.1	4-Jul-00								
07/02/00 CONT WE-A	02-Jul-00	<0.3	4-Jul-00								
07/02/00 0.5 WE-C	02-Jul-00	0.8	4-Jul-00								
07/02/00 1.0 WE-C	02-Jul-00	1.1	4-Jul-00								
07/02/00 2.0 WE-A	02-Jul-00	1.8	4-Jul-00								
07/02/00 4.0 WE-A	02-Jul-00	3.7	4-Jul-00								
07/02/00 4.0 LT-B	02-Jul-00	3.7	4-Jul-00								
07/02/00 6 WE-B	02-Jul-00	5.4	4-Jul-00								
07/02/00 22 PPM	02-Jul-00	21.0	4-Jul-00								

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLE SUBMITTED BY Amy Partridge	REPORT TO Amy Partridge
DATE SUBMITTED 06/27/00 - 07/03/00	ADDRESS Totes
DATE SAMPLED 06/27/00 - 07/03/00	POSTAL CODE
SAMPLED BY Amy Partridge	PHONE

SAMPLE TYPE river water	Sampler/Submitter Remarks		
LOCATION			
PROJECT NH3 - Toxicity			
REQUESTED PARAMETER(S)	Total Nitrate (NO ₃ /NO ₂ /N)	check	
	Total Ammonia (NH ₃ , N)	X	
	Total Kjeldahl Nitrogen (TKN)		
	Ortho-phosphate (PO ₄ -P)	check	
	Total Phosphorus (P)		

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)									
		NH ₃ -N	Analysis Date	NO ₃ -N	Analysis Date	TKN	Analysis Date	P	Analysis Date	Ortho PO ₄ -P	Analysis Date
07/03/00 CONT WE-C	03-Jul-00	0.4	4-Jul-00								
07/03/00 0.5 WE-C	03-Jul-00	0.6	4-Jul-00								
07/03/00 1.0 WE-C	03-Jul-00	1.0	4-Jul-00								
07/03/00 2.0 WE-A	03-Jul-00	1.9	4-Jul-00								
07/03/00 2.0 LT-C	03-Jul-00	2.1	4-Jul-00								
07/03/00 4.0 WE-B	03-Jul-00	3.9	4-Jul-00								
07/03/00 6.0 WE-C	03-Jul-00	5.2	4-Jul-00								
07/03/00 22 PPM	03-Jul-00	22.0	4-Jul-00								
QC data:			% recovery		% recovery		% recovery		% recovery		% recovery
end of run std check	0.0 mg/L NH3	0.07									
end of run std check	1.0 mg/L NH3	0.89	89%								
end of run std check	2.0 mg/L NH3	1.98	99%								
end of run std check	4.0 mg/L NH3	4.08	102%								
end of run std check	8.0 mg/L NH3	7.91	99%								
Verification Std (HACH)	5.0 PPM NH3	5.09	102%								
Standard additions			% recovery		% recovery		% recovery		% recovery		% recovery
	06/27/00 2.0 WE-B	1.6	107%								
	06/27/00 2.0 WE-B + 5.0 mg/L NH3		109%								
	06/20/00 2.0 LT-B	1.7	117%								
	06/20/00 2.0 LT-B + 5.0 mg/L NH3		105%								

Analyst Remarks

Date Reported: 05-Jul-00

Verified by: Gerry Levesque

Analyst: T. Poniatowski, D. Deluca

Date: July 5 / 00

City of Winnipeg
Laboratory Services Division

Analytical Report

SAMPLES SUBMITTED BY: Amy Partridge DATE SUBMITTED: 07/04 - 07/07/00 DATE SAMPLED: 07/04 - 07/07/00 SAMPLED BY: Amy Partridge		REPORT TO: Amy Partridge ADDRESS: Tetras POSTAL CODE: _____ PHONE: _____	
SAMPLE TYPE: river water LOCATION: _____ PROJECT: NH3 - Toxicity		Sampler/Submitter Remarks: _____	
REQUESTED PARAMETER(S): Total Nitrate (NO ₃ /NO ₂ -N) Total Ammonia (NH ₃ -N) Total Kjeldahl Nitrogen (TKN)	CHECK: X	Ortho phosphate (PO ₄ -P) Total Phosphorus (P)	CHECK: _____

RESULTS

Sample Description / Identification	Date	Concentration (mg/L)											
		NH ₃ -N		NO ₃ -N		TKN		P		Ortho PO ₄ -P			
			Analysis Date		Analysis Date		Analysis Date		Analysis Date		Analysis Date		Analysis Date
0704/00 CONT WE-C	04-Jul-00	0.2	7-Jul-00										
0704/00 0.5 WE-C	04-Jul-00	0.5	7-Jul-00										
0704/00 1.0 WE-A	04-Jul-00	1.0	7-Jul-00										
0704/00 2.0 WE-B	04-Jul-00	1.8	7-Jul-00										
0704/00 4 WE-A	04-Jul-00	3.5	7-Jul-00										
0704/00 6.0 WE-C	04-Jul-00	5.0	7-Jul-00										
0704/00 22 PPM	04-Jul-00	24.5	7-Jul-00										
0705/00 CONT WE-B	05-Jul-00	0.2	7-Jul-00										
0705/00 0.5 WE-B	05-Jul-00	0.5	7-Jul-00										
0705/00 1.0 WE-A	05-Jul-00	1.1	7-Jul-00										
0705/00 2.0 WE-A	05-Jul-00	1.9	7-Jul-00										
0705/00 4.0 WE-B	05-Jul-00	4.3	7-Jul-00										
0705/00 6.0 WE-C	05-Jul-00	5.4	7-Jul-00										
0705/00 22 PPM	05-Jul-00	22.4	7-Jul-00										
0706/00 CONT WE-A	06-Jul-00	0.2	7-Jul-00										
0706/00 0.5 WE-B	06-Jul-00	0.5	7-Jul-00										
0706/00 1.0 WE-B	06-Jul-00	1.1	7-Jul-00										
0706/00 2.0 WE-C	06-Jul-00	2.2	7-Jul-00										
0706/00 4 WE-C	06-Jul-00	4.0	7-Jul-00										
0706/00 6 WE-B	06-Jul-00	5.3	7-Jul-00										
0706/00 22 PPM	06-Jul-00	22.5	7-Jul-00										
0707/00 CONT WE-B	07-Jul-00	0.4	7-Jul-00										
0707/00 0.5 WE-B	07-Jul-00	0.6	7-Jul-00										
0707/00 1.0 WE-B	07-Jul-00	1.1	7-Jul-00										
0707/00 2.0 WE-B	07-Jul-00	2.1	7-Jul-00										
0707/00 4.0 WE-B	07-Jul-00	4.2	7-Jul-00										
0707/00 6 WE-A	07-Jul-00	5.2	7-Jul-00										
0707/00 22 PPM	07-Jul-00	20.2	7-Jul-00										
QC data:			% recovery		% recovery		% recovery		% recovery		% recovery		% recovery
end of run std. check	0.0 mg/L NH ₃	0.01											
end of run std. check	1.0 mg/L NH ₃	0.90	90%										
end of run std. check	2.0 mg/L NH ₃	1.93	97%										
end of run std. check	4.0 mg/L NH ₃	4.06	101%										
end of run std. check	8.0 mg/L NH ₃	8.20	100%										
Verification Std (NH ₃)	5.0 PPM NH ₃												
Standard additions			% recovery		% recovery		% recovery		% recovery		% recovery		% recovery
07/04/00 2.0 WE-B		1.7	103%										
07/04/00 2.0 WE-B + 5.0 mg/L NH ₃			106%										
07/05/00 2.0 WE-A		1.9	100%										
07/05/00 2.0 WE-A + 5.0 mg/L NH ₃			106%										

Analyst Remarks

Date Reported: 07 Jul 00
Analyst: B. Debra

Verified by: Gerry Lozupolo
Date: July 7/00

APPENDIX G

DETERMINING FLOW RATES AND THE NH_4Cl STOCK CONCENTRATION FOR THE FLOW-THROUGH EXPOSURE SYSTEM

Calculating flow rates to diluters at each test-table used during 1999

testing:

ASTM (1996) protocol E 1192-88 states that a minimum daily replacement rate of five times per day should be used in flow-through exposure-systems and a ten-fold replacement rate is recommended. Each test-table in the toxicology-laboratory held twelve-10 L test-chambers (4 of which contained 1 L 'insert' containers for the one-month-old fathead minnows used in Test 8). Therefore, the minimum flow-rate required to pass through the diluters at each testing-table to replace the test-solutions ten times per day was 0.83 L/min.

Sample calculation:

$$12 \text{ test chambers} * \frac{10 \text{ L}}{\text{test-chamber}} * \frac{10 \text{ replacements}}{\text{day}} * \frac{1 \text{ day}}{1400 \text{ min}} = 0.83 \text{ L/min}$$

In order to maintain a sufficient flow-rate through the distribution manifold to supply all test chambers with an equal volume of test-solution, a flow-rate of 3.6 L/min was used (i.e., a rate four times greater than the minimum replacement rate).

Each diluter was supplied with a constant source of river water and NH₄Cl-stock. The flow rates of NH₄Cl-stock were chosen empirically and are tabulated in Table G-1. The river water flow rates were calculated by subtracting the corresponding

Table G-1. Flow rates of river water and NH₄Cl-stock entering each diluter.

Nominal [NH ₃ -N] (mg/L)	NH ₄ Cl-stock flow rate (ml/min)	River water flow rate (ml/min)	Total flow rate to diluter (ml/min)
Control	--	3600	3600
0.5	31	3569	3600
1	63	3537	3600
2	125	3475	3600
4	250	3350	3600
8	500	3100	3600
16	1000	2600	3600

NH₄Cl-stock flow rate at each exposure-concentration from the ideal measured flow rate to the diluter system (i.e., 3.6 L/min).

Determining NH₄Cl-stock concentration in 1999:

An LMI™ dosing-pump withdrew 10 ml of NH₄Cl-stock per minute or 14.4 L/day (i.e. 0.01 L/min * 60 min/hr * 24 hr/day) from a NH₄Cl-stock storage-container and deposited it into the ammonia-mixing chamber. River water from the river-water-distribution-chamber was also added to the ammonia-mixing chamber at a rate of 4650 ml/min (i.e., a good working-flow-rate). Therefore, 4660 ml of solution (i.e., 4650 ml +10 ml) was added to the ammonia-mixing chamber every minute for the duration of each test.

The maximum total ammonia-N concentration needed for each test was 16 mg/L. In one minute, the total volume of test-solution entering each diluter was 3.6 L, 1.0 L (c.f., Table G-1) of which contained 57.6 mg of total ammonia-N at the highest exposure-concentration.

$$\text{Sample Calculation: } \frac{16 \text{ mg}}{\text{L}} = \frac{x \text{ mg}}{3.6 \text{ L}} \quad x = 57.6 \text{ mg}$$

So, the minimum total ammonia-N concentration in the ammonia-mixing chamber was 57.6 mg/L. In one minute, the ammonia-mixing chamber received 4.66 L of test-solution, therefore 268.4 mg of total ammonia-N had to be supplied by the 10 ml input of NH₄Cl-stock.

Sample Calculation: $57.6 \text{ mg/L} * 4.66 \text{ L} = 268.4 \text{ mg}$

$268.4 \text{ mg NH}_3\text{-N}/10 \text{ ml NH}_4\text{Cl stock} = \mathbf{26.84 \text{ g/L}}$

Using molecular weights, the concentration of $\text{NH}_3\text{-N}$ required by the exposure-system (i.e., 26.84 g/L) was converted to the concentration of NH_4Cl needed to prepare the NH_4Cl -stock.

Sample Calculation:

Molecular weight of N = 14.01μ

Molecular weight of NH_4Cl = 53.49μ

$$\frac{26.84 \text{ g/L of N}}{x \text{ g/L of NH}_4\text{Cl}} = \frac{14.01\mu}{53.49\mu} \quad x = 102.5 \text{ g NH}_4\text{Cl/L stock}$$

Therefore, 102.5 g of NH_4Cl were dissolved in each litre of de-ionized water required to supply the exposure-system with 14.4 L of NH_4Cl -stock per day at a concentration of 57.6 mg/L.

During the 2000 testing-program, changes were made to the nominal $\text{NH}_3\text{-N}$ exposure-concentrations which included reducing the highest total-ammonia concentration from 16.0 mg/L to 8.0 mg/L and adding a 6.0 mg/L exposure-concentration (i.e., dilution factor = 0.25). To accommodate for these changes, the flow rates of ammonia-stock and river water entering the mixing-chamber, the flow rates through the secondary diluters at each test-concentration, and the ammonia- stock concentrations were adjusted accordingly.

Table G-2. Summary of changes made to the test-system between 1999 and 2000.

Variable of test-system	1999	2000
Flow-rate of ammonia-stock entering the mixing-chamber	10 ml/min	12 ml/min
Flow-rate of river-water entering the mixing-chamber	4650 ml/min	5400 ml/min
Flow-rates through diluter at control-table (ammonia-stock; river-water)	no change	no change
Flow-rates through diluter at conc.1-table (ammonia-stock; river-water)	31 ml/min; 3569 ml/min	63 ml/min; 3537 ml/min
Flow-rates through diluter at conc.2-table (ammonia-stock; river-water)	63 ml/min; 3537 ml/min	125 ml/min; 3475 ml/min
Flow-rates through diluter at conc.3-table (ammonia-stock; river-water)	125 ml/min; 3475 ml/min	250 ml/min; 3350 ml/min
Flow-rates through diluter at conc.4-table (ammonia-stock; river-water)	250 ml/min; 3350 ml/min	500 ml/min; 3100 ml/min
Flow-rates through diluter at conc.5-table (ammonia-stock; river-water)	500 ml/min; 3100 ml/min	750 ml/min; 2850 ml/min
Flow-rates through diluter at conc.6-table (ammonia-stock; river-water)	no change	no change
Ammonia-stock NH ₃ -N concentration	26.84 g/L	12.99 g/L
Mass of NH ₄ Cl dissolved in every 20L of deionised water	102.5 g	992 g

APPENDIX H

SAMPLE CALCULATIONS FOR DETERMINING AVERAGE NH₃ CONCENTRATIONS

Sample calculations for determining average NH₃ concentrations

A sample calculation for determining the NH₃ concentration in a single exposure-concentration for one day is described below:

Total ammonia-N concentration = 6.0 mg/L
 pH = 8.4
 Temperature = 20°C

Step 1.

Equation 1 in this thesis is used to calculate the pK at 20 °C.

$$\begin{aligned} \text{Example: } pK &= 0.09018 + 2729.92 / (273.2 + \text{temperature}) \\ &= 0.09018 + 2729.92 / (273.2 + 20) \\ &= 9.400958 \end{aligned}$$

Step 2.

Equation 2 in this thesis is used to calculate the fraction of total ammonia that exists as NH₃ (i.e., f_{NH3}) at a pH of 8.4.

$$\begin{aligned} \text{Example: } f_{\text{NH}_3} &= 1 / (1 + 10^{\text{pK}-\text{pH}}) \\ &= 1 / (1 + 10^{9.400958-8.4}) \\ &= 0.090727 \end{aligned}$$

Step 3.

Multiply the f_{NH3} determined in Step 2 by the measured total ammonia-N value to determine the concentration of NH₃-N.

$$\begin{aligned} \text{Example: } [\text{NH}_3\text{-N}] &= 0.090727 * 6.0 \text{ mg/L} \\ &= 0.544362 \end{aligned}$$

Step 4.

Convert the concentration of $\text{NH}_3\text{-N}$ to NH_3 using the molecular ratio of (14/17).

Example:
$$\frac{0.544362 \text{ mg NH}_3\text{-N/L}}{X} = \frac{14 \text{ u}}{17 \text{ u}}$$

$$X = 0.66 \text{ mg NH}_3\text{/L}$$

End-of-Test (EOT) arithmetic mean NH_3 concentrations are calculated using daily NH_3 concentrations for each exposure-concentrations and control group.

APPENDIX I

SUMMARY OF TEST-ACCEPTABILITY CRITERIA FOR ACUTE- AND CHRONIC-EXPOSURE TESTS

Table I-1. Summary of test-acceptability criteria for acute-exposure testing with fish

Test condition	Recommended	Source	Satisfied (Y=yes; N=no)	Comments
Test Type	Static, semi-static, or flow through	EPA ¹	Y	T1, T2 – static T3A, T3B, T4A, T4B, T5A, T5B – semi-static T6, T7, T8, T9, T10 - flow-through
Test Duration	24, 48 or 96 h	EPA ¹	Y – all tests except T5B	T5B duration = 72 hr
Temperature ²	20+/- 1°C; or 25+/- 1°C	EPA ¹	N	Ambient river water temperatures were used; a key objective of site-specific testing.
Light quality	Ambient lab illumination	EPA ¹	Y	
Photoperiod	16h light, 8h dark	EPA ¹	T1, T2, T3A, T3B, T4A, T4B, T5A, T5B – N T6, T7, T8, T9, T10 - Y	T1, T2, T3A, T3B, T4A, T4B, T5A, T5B - Lights controlled manually therefore photoperiod varied b/w 1-2 h daily; 8h light, 16h dark.
Test chamber size	250 ml (min)	EPA ¹	Y	
Test chamber volume	250 ml (min)	EPA ¹	Y	
Renewal of test solution	Static tests – None Semi-static tests – Daily	EPA ³	Y	
Age of test organisms	1-14 days	EPA ¹	T1, T5A, T5B, T9 – Y T2, T3A, T3B, T4A, T4B, T6, T7, T8, T10 – N	Fish less than 14 days old were not always available for testing. See Appendix D for ages of fish tested.
No. organisms/ test chamber	10 (minimum)	EPA ¹	Y	

Test condition	Recommended	Source	Satisfied (Y=yes; N=no)	Comments
No. replicate chambers/ conc.	2 (minimum)	EPA ¹	Y	
No. organisms per conc.	20 (minimum)	EPA ¹	Y	
Feeding regime	<p>Static: Do not feed during test; feed while holding prior to use in the test</p> <p>Static-renewal: Feed (min) 0.15ml newly hatched brine shrimp nauplii 2X daily</p>	EPA ³	<p>T1, T2 – N</p> <p>T3A, T3B – N T4A – N, T4B – Y T5A – Y, T5B – Y</p> <p>T6, T7, T8, T9, T10 – N</p>	<p>T1, T2 - Fish were fed 1 drop of liquid baby fish food on day 3 of the test because they were losing their yolk sacs. Alternately, they were not fed prior to test start because they had yolk sacs.</p> <p>T3A, T3B – Extra fish from the same batch being tested were not actively feeding and food was therefore not added to test-chambers.</p> <p>T6, 7, 8, 10 – Fish were fed because the tests were extended beyond 96 hours to monitor chronic growth and survival effects.</p> <p>T9 – Fish were fed to minimize the occurrence of cannibalism.</p>
Cleaning of test chambers	Cleaning not required	EPA ¹	Y	
Aeration ³	None unless DO<40%; rate should not exceed 100 ml/min	EPA ¹	Y for all tests except T5A, T5B, and T10	<p>T5A, T5B – Air was added to test chambers at a rate of 45 mL/min because previous tests (i.e., T3 and T4) were at the threshold of acceptability.</p> <p>T10 – Air was added to test chambers at a rate of 310 mL/min as a precaution in the event of a pump failure. The airflow rate was high because the volume of test-solution was also high (i.e., 8L).</p>

Test condition	Recommended	Source	Satisfied (Y=yes; N=no)	Comments
Dilution water	Moderately hard synthetic water is prepared using MILLIPORE MILLI-Q ^R or equivalent deionized water and reagent grade chemicals or 20% DMW.	EPA ¹	N	Red River water was used to better represent <i>in situ</i> conditions, a key objective in site-specific testing.
Test Concentrations	5 exposure concentrations (min) plus a control group	EPA ¹	Y	
Dilution Factor	≥ 0.5	EPA ¹	Y – for all tests except T9 and T10	<p>T9 – The dilution factor between the upper three exposure-concentrations was 0.25.</p> <p>T10 – The dilution factor between the upper two exposure-concentrations was 0.25.</p> <p>This dilution factor was used to narrow the concentration margin for effects observed within the upper concentration range.</p>
Endpoint	Survival	EPA ¹	Y	
Test acceptability criterion	90% or greater survival in the controls	EPA ¹	<p>T1, T3A, T3B – N</p> <p>T2, T4A, T4B, T5A, T5B, T6, T7, T8, T9, T10 – Y</p>	<p>T1 had 70% survival in the control group; T3A and T3B had 77% survival in the control group. The ICPIN program smoothes the datasets as described in Section 4.5 then compares exposed fish results with control fish results. Mortality in the control group is accounted for in a way that is similar to using Abbott's formula. Refer to Appendix J.</p>

Notes:

- 1) ¹ Source = US EPA (1993).
- 2) ² Temperatures are reported as test-means +/- test-standard deviations
- 3) ³ Source = US EPA (1991a).
- 4) T1 = 96 hr, static test using walleye fry; treatment: river water (RW) fortified with ammonia
- 5) T2 = 96 hr, static test using white sucker fry; treatment: RW fortified with ammonia
- 6) T3A = 96 hr, semi-static test using white sucker fry; treatment: RW fortified with ammonia
- 7) T3B = 96 hr, semi-static test using white sucker fry; treatment: E fortified with ammonia
- 8) T4A = 96 hr, semi-static test using white sucker fry; treatment: RW fortified with ammonia
- 9) T4B = 96 hr, semi-static test using white sucker fry; treatment: E fortified with ammonia
- 10) T5A = 96 hr, semi-static test using fathead minnow fry; treatment: RW fortified with ammonia
- 11) T5B = 72 hr, semi-static test using fathead minnow fry; treatment: E fortified with ammonia
- 12) T6 = 96 hr, flow-through test using juvenile channel catfish; treatment: RW fortified with ammonia
- 13) T7 = 96 hr, flow-through test using juvenile fathead minnows; treatment: RW fortified with ammonia
- 14) T8 = 96 hr, flow-through test using juvenile fathead minnows; treatment: RW fortified with ammonia
- 15) T9 = 96 hr, flow-through test using northern pike fry; treatment: RW fortified with ammonia
- 16) T10 = 96 hr, flow-through test using juvenile walleye; treatment: RW fortified with ammonia

Table I-2. Summary of test-acceptability criteria for chronic-exposure testing with fish.

Test condition	Recommended	Source	Satisfied (Y=yes; N=no)	Comments
Test Type	Semi-static or Flow-through	EPA ¹	Y	
Test Duration	Semi-static – 7 days	EPA ²	T4A, T4B –N	T4A, T4B – 10 days
	Flow-through – 28-120 days	ASTM ³	T9 –N T6, T7, T8, T10 – Y	T9 – 12 days
Temperature	25 +/- 1°C	EPA ¹	N	Ambient river water temperatures were used; a key objective of site-specific testing.
Light quality	Ambient lab illumination	EPA ¹	Y	
Photoperiod	16h light, 8h dark	EPA ¹	Y – except for T4A and T4B	T4A, T4B – Lights controlled manually so photoperiod varied b/w 1-2 h daily; 8h light, 16h dark.
Test chamber volume	1L (min) for every 10 grams of biological tissue (i.e. fish)	ASTM ³	Y	
Flow rates through test chambers	Semi-static tests – n/a Flow-through tests - 1L (min.) for every 1 gram of biological tissue (i.e. fish); or min. of 10 L per day whichever is less	ASTM ³	Y	
No. organisms/ test chamber	Semi-static: 10 (minimum)	EPA ¹	T4A, T4B – Y	T6- used 15 fish per test chamber; satisfies ASTM recommendation that no more than 10 grams of biological tissue be used per liter of test solution T8 – used 15 fish per test chamber, the maximum number of available fish
	Flow-through: 20 (minimum)	ASTM ³	T6, T8 –N T7, T9, T10 – Y	

Test condition	Recommended	Source	Satisfied (Y=yes; N=no)	Comments
No. replicate chambers/ conc.	Static-renewal: 3 (minimum) Flow-through: (2 minimum)	EPA ¹ EPA ²	Y	
Feeding regime	Static-renewal: Feed (min) 0.15 ml newly hatched brine shrimp nauplii 2X daily Flow-through: Channel Catfish (i.e., brood stock) - food that will support growth/survival; 1x/day Fathead minnow - newly hatched brine shrimp 2x/day Northern pike – live brine shrimp nauplii 3X daily Walleye – no recommended feeding regime	EPA ² ASTM ³ ASTM ³ ASTM ³ ---	Y T6 – Y T7 – N T8 – Y T9 – N T10 – n/a	 T7 – Fed local fathead minnow 1.0-1.5 g of trout chow (i.e., food that will support growth/survival) 1x/day because they were approximately 3 months old and would not have fed as effectively on live brine shrimp. T9 – Fed northern pike 2.6g of live daphnia 3X daily
DO concentra- tion	DO>60% saturation	ASTM ³	Y	
Dilution water	Moderately hard synthetic water is prepared using MILLIPORE MILLI-Q ^R or equivalent deionized water and reagent grade chemicals or 20% DMW.	EPA ¹	N	Red River water was used to better represent <i>in situ</i> conditions, a key component of site-specific testing.

Test condition	Recommended	Source	Satisfied (Y=yes; N=no)	Comments
Test Concentrations	5 exposure concentrations (min) plus a control group	EPA ¹	Y	
Dilution Factor	≥ 0.5	EPA ¹	Y – for all tests except T9 and T10	T9 – The dilution factor between the upper three exposure-concentrations was 0.25. T10 – The dilution factor between the upper two exposure-concentrations was 0.25. This dilution factor was used to narrow the concentration margin for effects observed within the upper concentration range.
Endpoint	Survival	EPA ¹	Y	Growth was also measured (based on dry tissue weights at test-termination).
Test acceptability criterion	Static-renewal: control survival must equal 80% or greater Flow-through: Channel Catfish – control survival must be 65% or greater; Fathead minnow control survival must be greater than 70% Northern pike – control survival must be 70% or greater Walleye – no recommended % control survival	EPA ¹ ASTM ³ ASTM ³ ASTM ³ ---	T4A, T4B – Y T6 – Y T7, T8 – Y T9 – Y T10 – n/a	

Notes:1) ¹ Source = USEPA (1993).2) ² Source = USEPA (1991a).3) ³ Source = ASTM (1996).

4) T4A = 10 day, semi-static test using white sucker fry; treatment: RW fortified with ammonia

- 5) T4B = 10 day, semi-static test using white sucker fry; treatment: E fortified with ammonia
- 6) T6 = 30 day, flow-through test using juvenile channel catfish; treatment: RW fortified with ammonia
- 7) T7 = 30 day, flow-through test using juvenile fathead minnow; treatment: RW fortified with ammonia
- 8) T8 = 29 day, flow-through test using juvenile fathead minnow; treatment: RW fortified with ammonia
- 9) T9 = 12 day, flow-through test using northern pike fry; treatment: RW fortified with ammonia
- 10) T10 = 30 day, flow-through test using juvenile walleye; treatment: RW fortified with ammonia

APPENDIX J

DATA USED TO GENERATE LC50, LC20 AND EC20
VALUES FOR ALL TOXICITY-TESTS

Data used to generate LC50, LC20 or EC20 values

Average NH₃-concentrations and average test-fish responses expressed quantitatively (i.e., percent-mortality or percent-growth inhibition) for replicate test-chambers across each exposure-concentration and control group are tabulated in this appendix and organized as follows:

Table No.	Test-Exposure	Endpoint Calculated
J-1 – J-10, J-23, J-24, J-26	Acute (\leq 96 hr)	LC50
J-11 – J-16	Chronic (>96 hr)	LC20
J-17 - J-22	Chronic (>96 hr)	EC20
J-25	Chronic (> 96 hr)	LC50

Data were analysed via linear interpolation using a computer-based statistical program called ICPIN (version 2.0) as described in Section 4-5 of this report. All ICPIN results are presented in Appendix K.

For presentational purposes data have been smoothed manually using the method described in Section 4-5, adjusted for control mortality and plotted on graphs 5-1 to 5-16 and 5-23 to 5-26, Sections 5-1, 5-3, and 5-5. The adjustment uses Abbott's formula:

$$p_i^a = (p_i^s - p_o^s) / (1 - p_o^s)$$

where:

- p_i^a = adjusted percent-mortality at concentration i
- i = stressor concentration
- p_i^s = the smoothed percent-mortality at concentration i
- p_o^s = the smoothed percent-mortality for the control

Sample calculation for T1A (Table J-1):

$$p_i^s = .400$$

$$p_o^s = .233$$

$$p_i^a = (0.400 - 0.233) / (1 - .233)$$
$$= .217 \text{ or } 21.7\%$$

ICP values calculated by the ICPIN program for each test are also plotted on graphs 5-1 to 5-16 and 5-23 to 5-26, Sections 5-1, 5-3 and 5-5. This process was repeated for all tests designed to test sublethal toxicity except that adjustments to the data, using Abbott's formula, were unnecessary. These data are plotted in graphs 5-17 to 5-22, Section 5-3.

Table J-1. Data used to generate the LC50 for T1

Test-Description: 96-hr acute-exposure test; static

Test-Species: walleye fry

Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C				
control	0.059	30.0	30.0	30.0	30.0	0.0	23.3	0.0
conc.1	0.169	10.0	0.0	40.0	16.7	20.8	23.3	0.0
conc.2	0.276	40.0	40.0	40.0	40.0	0.0	40.0	21.7
conc.3	0.393	80.0	100.0	70.0	83.3	15.3	83.3	78.2
conc.4	0.580	100.0	100.0	100.0	100.0	0.0	100.0	100.0
conc.5	0.720	100.0	100.0	100.0	100.0	0.0	100.0	100.0
conc.6	1.284	100.0	100.0	100.0	100.0	0.0	100.0	100.0
conc.7	1.345	100.0	100.0	100.0	100.0	0.0	100.0	100.0
conc.8	2.313	100.0	100.0	100.0	100.0	0.0	100.0	100.0

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-1 are highlighted in bold-type.

Table J-2: Data used to generate the LC50 for T2

Test-Description: 96-hr acute-exposure test; static

Test-Species: white sucker fry

Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality
		Rep.A	Rep.B	Rep.C		
control	0.055	0.0	0.0	0.0	0.0	0.0
conc.1	0.190	0.0	0.0	0.0	0.0	0.0
conc.2	0.323	0.0	0.0	0.0	0.0	0.0
conc.3	0.431	0.0	40.0	0.0	13.3	23.1
conc.4	0.499	60.0	100.0	0.0	53.3	50.3
conc.5	0.793	100.0	100.0	100.0	100.0	0.0
conc.6	1.102	100.0	100.0	100.0	100.0	0.0
conc.7	1.179	100.0	100.0	100.0	100.0	0.0
conc.8	2.048	100.0	100.0	100.0	100.0	0.0

Notes: a SD = standard deviation

* Data plotted on Figure 5-2 are highlighted in bold-type.

Table J-3. Data used to generate the LC50 for T3

Test-Description: 96-hr acute-exposure test; semi-static
 Test-Species: white sucker fry
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C				
control	0.024	30.0	20.0	20.0	23.3	5.8	21.1	0.0
conc.1	0.047	60.0	0.0	10.0	23.3	32.1	21.1	0.0
conc.2	0.074	40.0	10.0	0.0	16.7	20.8	21.1	0.0
conc.3	0.108	70.0	20.0	0.0	30.0	36.1	30.0	11.3
conc.4	0.178	40.0	60.0	40.0	46.7	11.5	46.7	32.4
conc.5	0.272	80.0	70.0	90.0	80.0	10.0	80.0	74.6
conc.6	0.521	100.0	100.0	100.0	100.0	0.0	100.0	100.0
conc.7	0.690	100.0	100.0	100.0	100.0	0.0	100.0	100.0

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-3 are highlighted in bold-type.

Table J-4. Data used to generate the LC50 for T4A

Test-Description: 96-hr acute-exposure test; semi-static
 Test-Species: white sucker fry
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C				
control	0.016	10.0	0.0	0.0	3.3	5.8	3.3	0.0
conc.1	0.093	0.0	10.0	30.0	13.3	15.3	13.3	10.3
conc.2	0.178	10.0	30.0	20.0	20.0	10.0	20.0	17.3
conc.4	0.318	10.0	40.0	30.0	26.7	15.3	26.7	24.2
conc.5	0.413	11.1	50.0	20.0	27.0	20.4	27.0	24.5
conc.6	0.532	20.0	80.0	60.0	53.3	30.6	53.3	51.7
conc.7	0.868	100.0	90.0	100.0	96.7	5.8	96.7	96.6
conc.3	0.233	10.0	0.0	0.0	3.3	5.8		

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-4 are highlighted in bold-type.

* Concentration 3 was removed from dataset because the observed response did not follow the response-gradient.

Table J-5. Data used to generate the LC50 for T5

Test-Description: 96-hr acute-exposure test; semi-static

Test-Species: fathead minnow fry

Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)				Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C	Rep.D				
control	0.008	10.0	20.0	30.0	0.0	15.0	12.9	15.0	0.0
conc.1	0.081	10.0	50.0	50.0	40.0	37.5	18.9	37.5	26.5
conc.2	0.164	60.0	30.0	60.0	30.0	45.0	17.3	45.0	35.3
conc.3	0.446	63.6	70.0	70.0	50.0	63.4	9.4	63.4	56.9
conc.4	1.048	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0
conc.5	2.150	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0
conc.6	3.361	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-5 are highlighted in bold-type.

Table J-6. Data used to generate the LC50 for T6

Test-Description: 96-hr acute-exposure test; flow-through

Test-Species: juvenile channel catfish

Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)				Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality
		Rep.A	Rep.B	Rep.C	Rep.D			
control	0.007	0.0	0.0	0.0	0.0	0.0	0.0	
conc.1	0.019	0.0	0.0	0.0	0.0	0.0	0.0	
conc.2	0.045	0.0	0.0	0.0	0.0	0.0	0.0	
conc.3	0.067	0.0	0.0	20.0	6.7	6.7	9.4	
conc.4	0.207	13.3	6.7	0.0	0.0	5.0	6.4	
conc.5	0.501	6.7	40.0	20.0	33.3	25.0	14.8	
conc.6	0.748	46.7	66.7	73.3	46.7	58.3	13.7	

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

* Data plotted on Figure 5-6 are highlighted in bold-type.

Table J-7. Data used to generate the LC50 for T7

Test-Description: 96-hr acute-exposure test; flow-through

Test-Species: juvenile fathead minnow

Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)				Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C	Rep.D				
control	0.008	0.0	5.0	0.0	5.0	2.5	2.9	1.9	0.0
conc.1	0.026	0.0	0.0	5.0	0.0	1.3	2.5	1.9	0.0
conc.2	0.041	5.0	0.0	5.0	0.0	2.5	2.9	2.0	0.1
conc.3	0.082	0.0	0.0	5.0	0.0	1.3	2.5	2.0	0.1
conc.4	0.175	0.0	5.0	0.0	5.0	2.5	2.9	2.0	0.1
conc.5	0.428	0.0	0.0	5.0	5.0	2.5	2.9	2.0	0.1
conc.6	0.758	0.0	5.0	0.0	0.0	1.3	2.5	2.0	0.1

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-7 are highlighted in bold-type.

Table J-8. Data used to generate the LC50 for T8

Test-Description: 96-hr acute-exposure test; flow-through

Test-Species: juvenile fathead minnow

Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)				Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality
		Rep.A	Rep.B	Rep.C	Rep.D			
control	0.008	0.0	0.0	0.0	0.0	0.0	0.0	
conc.1	0.040	0.0	0.0	0.0	0.0	0.0	0.0	
conc.2	0.060	0.0	0.0	0.0	0.0	0.0	0.0	
conc.3	0.116	0.0	0.0	5.0	25.0	7.5	4.0	
conc.4	0.161	0.0	0.0	0.0	0.0	0.0	4.0	
conc.5	0.361	0.0	0.0	0.0	0.0	0.0	4.0	

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

* Data plotted on Figure 5-8 are highlighted in bold-type.

Table J-9. Data used to generate the LC50 for T9

Test-Description: 96-hr acute-exposure test; flow-through

Test-Species: northern pike fry

Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)				Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C	Rep.D				
control	0.024	0.0	5.0		10.0	5.0	5.0	0.0	
conc.1	0.051	0.0	15.0	20.0	15.0	12.5	8.7	5.3	
conc.2	0.099	5.0	5.0	15.0	5.0	7.5	5.0	5.3	
conc.3	0.201	10.0	25.0	20.0	15.0	17.5	6.5	13.2	
conc.4	0.399	35.0	25.0	30.0	30.0	30.0	4.1	26.3	
conc.5	0.592	80.0	65.0	60.0	60.0	66.3	9.5	64.5	
conc.6	0.659	95.0	90.0	95.0	75.0	88.8	9.5	88.2	

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-9 are highlighted in bold-type.

Table J-10. Data used to generate the LC50 for T10

Test-Description: 96-hr acute-exposure test; flow-through

Test-Species: juvenile walleye

Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C				
control	0.028	10.0	5.0	0.0	5.0	5.0	3.8	0.0
conc.1	0.035	0.0	5.0	5.0	3.3	2.9	3.8	0.0
conc.2	0.088	0.0	10.0	0.0	3.3	5.8	3.8	0.0
conc.3	0.180	5.0	5.0	0.0	3.3	2.9	3.8	0.0
conc.4	0.368	25.0	5.0	0.0	10.0	13.2	7.5	3.9
conc.5	0.484	5.0	0.0	10.0	5.0	5.0	7.5	3.9

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-10 are highlighted in bold-type.

Table J-11. Data used to generate the LC20 for T4A

Test-Description: 10 day chronic-exposure test; semi-static
 Test-Species: white sucker fry
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C				
control	0.015	40.0	0.0	10.0	16.7	20.8	16.7	0.0
conc.1	0.060	20.0	10.0	40.0	23.3	15.3	21.1	5.3
conc.2	0.129	10.0	30.0	50.0	30.0	20.0	21.1	5.3
conc.3	0.183	20.0	0.0	10.0	10.0	10.0	21.1	5.3
conc.4	0.267	30.0	40.0	30.0	33.3	5.8	31.9	18.2
conc.5	0.351	11.1	50.0	30.0	30.4	19.5	31.9	18.2
conc.6	0.505	20.0	90.0	70.0	60.0	36.1	60.0	52.0
conc.7	0.868	100.0	100.0	100.0	100.0	0.0	100.0	100.0

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-11 are highlighted in bold-type.

Table J-12. Data used to generate the LC20 for T6

Test-Description: 30 day chronic-exposure test; flow-through
 Test-Species: juvenile channel catfish
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)				Avg. Mortality (%)	SD ^a of % Mortality
		Rep.A	Rep.B	Rep.C	Rep.D		
control	0.007	0.0	0.0	0.0	0.0	0.0	0.0
conc.1	0.021	0.0	0.0	0.0	0.0	0.0	0.0
conc.2	0.039	0.0	0.0	0.0	0.0	0.0	0.0
conc.3	0.059	0.0	0.0	20.0	26.7	11.7	13.8
conc.4	0.205	13.3	40.0	6.7	33.3	23.3	15.9
conc.5	0.326	20.0	46.7	40.0	73.3	45.0	22.0
conc.6	0.616	93.3	100.0	100.0	100.0	98.3	3.3

Notes: a SD = standard deviation

* Data plotted on Figure 5-12 are highlighted in bold-type.

Table J-13. Data used to generate the LC20 for T7

Test-Description: 30 day chronic-exposure test; flow-through
 Test-Species: juvenile (three-month-old) fathead minnow
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Percent Mortality				Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C	Rep.D				
control	0.007	0.0	10.0	0.0	10.0	5.0	5.8	5.0	0.0
conc.1	0.022	*	5.0	5.0	5.0	5.0	0.0	5.0	0.0
conc.2	0.039	5.0	5.0	10.0	5.0	6.3	2.5	5.2	0.2
conc.3	0.061	10.0	0.0	10.0	0.0	5.0	5.8	5.2	0.2
conc.4	0.198	0.0	5.0	5.0	13.3	5.8	5.5	5.2	0.2
conc.5	0.310	5.0	0.0	5.0	5.0	3.8	2.5	5.2	0.2
conc.6	0.575	0.0	10.0	10.0	0.0	5.0	5.8	5.2	0.2

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-13 are highlighted in bold-type.

* (*) removed from dataset because of accidental ammonia spike in test-solution.

Table J-14. Data used to generate the LC20 for T8

Test-Description: 29 day chronic-exposure test; flow-through
 Test Species: juvenile (one-month-old) fathead minnow
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Percent Mortality				Avg. Percent Mortality	SD ^a of % Mortality	Smoothed ^b %-Mortality	Adjusted ^c %-Mortality
		Rep.A	Rep.B	Rep.C	Rep.D				
control	0.006	21.4	0.0	7.1	26.7	13.8	12.4	8.0	0.0
conc.1	0.019	*	7.1	6.7	6.7	6.8	0.3	8.0	0.0
conc.2	0.036	7.1	0.0	0.0	6.7	3.5	4.0	8.0	0.0
conc.3	0.057	6.7	0.0	14.3	46.7	16.9	20.7	12.0	4.3
conc.4	0.193	13.3	13.3	6.7	16.7	12.5	4.2	12.0	4.3
conc.5	0.303	13.3	6.7	6.7	0.0	6.7	5.4	12.0	4.3

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-14 are highlighted in bold-type.

* (*) removed from dataset because of accidental ammonia spike in test-solution.

Table J-15. Data used to generate the LC20 for T9

Test-Description: 12 day chronic-exposure test; flow-through
 Test-Species: northern pike fry
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)				Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b %-Mortality	Adjusted ^c %-Mortality
		Rep.A	Rep.B	Rep.C	Rep.D				
control	0.030	15.0	18.8		27.8	20.5	6.6	20.5	0.0
conc.1	0.072	14.3	52.9	53.8	29.4	37.6	19.2	35.7	19.2
conc.2	0.127	26.3	15.8	60.0	33.3	33.9	18.9	35.7	19.2
conc.3	0.210	21.1	65.0	55.0	76.5	54.4	23.9	54.4	42.6
conc.4	0.410	65.0	52.6	45.0	60.0	55.7	8.7	55.7	44.3
conc.5	0.588	95.0	95.0	95.0	100.0	96.3	2.5	96.3	95.3
conc.6	0.661	100.0	95.0	100.0	100.0	98.8	2.5	98.8	98.5

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-15 are highlighted in bold-type.

Table J-16. Data used to generate the LC20 for T10

Test-Description: 30 day chronic-exposure test; flow-through
 Test-Species: juvenile walleye
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b %-Mortality	Adjusted ^c %-Mortality
		Rep.A	Rep.B	Rep.C				
control	0.012	11.8	20.0	16.7	16.2	4.1	12.8	0.0
conc.1	0.025	5.6	5.3	17.6	9.5	7.0	12.8	0.0
conc.2	0.048	14.3	*	15.8	15.1	1.1	15.1	2.6
conc.3	0.089	15.8	11.1	23.5	16.8	6.3	16.8	4.6
conc.4	0.177	26.7	38.9	12.5	26.0	13.2	26.0	15.1
conc.5	0.243	50.0	31.3	27.8	36.4	11.9	36.4	27.0

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-16 are highlighted in bold-type.

* (*) Removed from dataset due to cannibalism of all but one fish.

Table J-17. Data used to generate the EC20 for T4A

Test-Description: 10 day chronic-exposure test; semi-static
 Test-Species: white sucker fry
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Dry-weight per fish (mg)			Avg.dry- weight per fish (mg)	SD ^a of % Weight (mg)	% weight change compared to controls
		Rep.A	Rep.B	Rep.C			
control	0.015	1.67	1.39	1.92	1.66	0.27	
conc.1	0.060	2.06	1.81	1.88	1.92	0.13	15.5
conc.2	0.129	1.71	1.80	2.64	2.05	0.51	23.5
conc.3	0.183	1.53	1.81	1.77	1.70	0.15	2.6
conc.4	0.267	2.00	2.03	2.31	2.11	0.17	27.3
conc.5	0.351	1.66	2.24	1.87	1.92	0.29	15.9
conc.6	0.505	1.83	1.20	2.83	1.95	0.82	17.7
conc.7	0.868						

Notes: a SD = standard deviation

* Data plotted on Figure 5-17 are highlighted in bold-type.

Table J-18. Data used to generate the EC20 for T6

Test-Description: 30 day chronic-exposure test; flow-through
 Test-Species: juvenile channel catfish
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Dry-weight per fish (mg)				Avg.dry- weight per fish (mg)	SD ^a of % Weight (mg)	% weight change compared to controls
		Rep.A	Rep.B	Rep.C	Rep.D			
control	0.007	938	1232	1104	1030	1076	124.4	
conc.1	0.021	849	1097	1018	1088	1013	115.0	-5.9
conc.2	0.039	1003	1072	1019	1015	1027	30.6	-4.5
conc.3	0.059	963	1110	917	784	943	134.3	-12.3
conc.4	0.205	977	942	999	1098	1004	66.9	-6.7
conc.5	0.326	916	1111	1132	1040	1050	97.3	-2.4
conc.6	0.616	1514				1514		40.7

Notes: a SD = standard deviation

* Data plotted on Figure 5-18 are highlighted in bold-type.

Table J-19. Data used to generate the EC20 for T7

Test-Description: 30 day chronic-exposure test; flow-through
 Test-Species: juvenile (three-month-old) fathead minnow
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Dry-weight per fish (mg)				Avg.dry- weight per fish (mg)	SD ^a of % Weight (mg)	% weight change compared to controls
		Rep.A	Rep.B	Rep.C	Rep.D			
control	0.007	330	305	307	326	317	13.0	
conc.1	0.022	312	331	300	364	327	27.7	3.1
conc.2	0.039	304	284	307	289	296	11.3	-6.7
conc.3	0.061	272	320	297	322	303	23.5	-4.5
conc.4	0.198	290	317	302	290	300	12.7	-5.5
conc.5	0.310	284	314	295	277	292	16.3	-7.8
conc.6	0.575	243	235	235	279	248	20.7	-21.8

Notes: a SD = standard deviation

* Data plotted on Figure 5-19 are highlighted in bold-type.

Table J-20. Data used to generate the EC20 for T8

Test-Description: 29 day chronic-exposure test; flow-through
 Test Species: juvenile (one-month-old) fathead minnow
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Dry-weight per fish (mg)				Avg.dry- weight per fish (mg)	SD ^a of % Weight (mg)	% weight change compared to controls
		Rep.A	Rep.B	Rep.C	Rep.D			
control	0.006	8.3	11.1	10.9	14.2	11.1	2.4	
conc.1	0.019	10.5	12.8	11.5	17.0	13.0	2.9	16.4
conc.2	0.036	11.2	11.2	12.4	14.7	12.4	1.7	11.2
conc.3	0.057	12.0	8.6	14.3	20.6	13.9	5.1	24.7
conc.4	0.193	11.5	13.5	14.1	14.2	13.3	1.3	19.8
conc.5	0.303	9.4	14.8	10.3	11.9	11.6	2.4	4.3

Notes: a SD = standard deviation

* Data plotted on Figure 5-20 are highlighted in bold-type.

Table J-21. Data used to generate the EC20 for T9

Test-Description: 12 day chronic-exposure test; flow-through
 Test-Species: northern pike fry
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Avg. dry weight per organism (mg)				Avg. dry weight per fish (mg)	SD ^a of % Weight (mg)	% change in weight compared to controls
		Rep.A	Rep.B	Rep.C	Rep.D			
control	0.030	3.6	5.9		5.2	4.9	1.2	
conc.1	0.072	6.9	8.5	8.3	9.2	8.2	1.0	67.9
conc.2	0.127	6	8.1	7.1	5.9	6.8	1.0	38.3
conc.3	0.210	5.7	5.4	6.3	11.9	7.3	3.1	49.5
conc.4	0.410	5.4	5.2	4.6	5.3	5.1	0.4	4.6
conc.5	0.588	1.8	6.8	9.9		6.2	4.1	25.9
conc.6	0.661							

Notes: a SD = standard deviation

* Data plotted on Figure 5-21 are highlighted in bold-type.

Table J-22. Data used to generate the EC20 for T10

Test-Description: 30 day chronic-exposure test; flow-through
 Test-Species: juvenile walleye
 Ammonia Source: NH₄Cl

	Avg. NH ₃ (mg/L)	Avg. dry weight per organism (mg)			Avg. dry weight per fish (mg)	SD ^a of % Weight (mg)	% change in weight compared to controls
		Rep.A	Rep.B	Rep.C			
control	0.012	35.7	34.6	34.5	34.9	0.7	
conc.1	0.025	40.0	31.6	39.4	37.0	4.7	5.9
conc.2	0.048	34.9	*	39.6	37.3	3.3	6.6
conc.3	0.089	28.9	35.3	32.9	32.4	3.2	-7.3
conc.4	0.177	40.9	38.3	30.5	36.6	5.4	4.7
conc.5	0.243	45.2	30.4	29.1	34.9	8.9	-0.1

Notes: a SD = standard deviation

* Data plotted on Figure 5-22 are highlighted in bold-type.

* (*) Removed from dataset due to cannibalism of all but one fish.

Table J-23. Data used to generate the LC50 for T3B

Test-Description: 96 hr acute-exposure test; semi-static

Test-Species: white sucker fry

Ammonia Source: NH₄Cl plus effluent

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C				
control	0.024	30.0	20.0	20.0	23.3	5.8	16.8	0.0
conc.1	0.045	0.0	22.2	22.2	14.8	12.8	16.8	0.0
conc.2	0.067	20.0	40.0	20.0	26.7	11.5	16.8	0.0
conc.3	0.091	0.0	20.0	0.0	6.7	11.5	16.8	0.0
conc.4	0.124	18.2	0.0	20.0	12.7	11.1	16.8	0.0
conc.5	0.159	10.0	50.0	50.0	36.7	23.1	36.7	23.8
conc.6	0.176	50.0	60.0	40.0	50.0	10.0	50.0	39.9
conc.7	0.197	90.0	100.0	100.0	96.7	5.8	96.7	96.0

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-23 are highlighted in bold-type.

Table J-24. Data used to generate the LC50 for T4B

Test-Description: 96 hr acute-exposure test; semi-static

Test-Species: white sucker fry

Ammonia Source: NH₄Cl plus effluent

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C				
control	0.016	10.0	0.0	0.0	3.3	5.8	3.3	0.0
conc.1	0.055	0.0	30.0	0.0	10.0	17.3	10.0	10.0
conc.2	0.100	30.0	20.0	0.0	16.7	15.3	15.1	15.1
conc.3	0.135	10.0	10.0	20.0	13.3	5.8	15.1	15.1
conc.4	0.180	20.0	10.0	20.0	16.7	5.8	15.1	15.1
conc.5	0.233	11.1	0.0	30.0	13.7	15.2	15.1	15.1
conc.6	0.282	10.0	30.0	20.0	20.0	10.0	20.0	20.0
conc.7	0.331	70.0	50.0	60.0	60.0	10.0	60.0	60.0

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-24 are highlighted in bold-type.

Table J-25. Data used to generate the LC50 for T4B

Test-Description: 10-day chronic-exposure test; semi-static

Test-Species: white sucker fry

Ammonia Source: NH₄Cl plus effluent

	Avg. NH ₃ (mg/L)	Mortality (%)			Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C				
control	0.015	40.0	0.0	10.0	16.7	20.8	16.7	0.0
conc.1	0.049	0.0	30.0	22.2	17.4	15.6	17.4	17.4
conc.2	0.084	50.0	40.0	0.0	30.0	26.5	23.3	23.3
conc.3	0.124	20.0	20.0	20.0	20.0	0.0	23.3	23.3
conc.4	0.167	20.0	10.0	30.0	20.0	10.0	23.3	23.3
conc.5	0.222	22.2	20.0	60.0	34.1	22.5	34.1	34.1
conc.6	0.263	40.0	30.0	40.0	36.7	5.8	36.7	36.7
conc.7	0.293	80.0	80.0	80.0	80.0	0.0	80.0	80.0

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-25 are highlighted in bold-type.

Table J-26. Data used to generate the LC50 for T5B

Test-Description: 72-hr acute-exposure test; semi-static

Test-Species: fathead minnow fry

Ammonia Source: NH₄Cl plus effluent

	Avg. NH ₃ (mg/L)	Mortality (%)				Avg. Mortality (%)	SD ^a of % Mortality	Smoothed ^b % Mortality	Adjusted ^c % Mortality
		Rep.A	Rep.B	Rep.C	Rep.D				
control	0.010	0.0	0.0	10.0	0.0	2.5	5.0	1.3	0.0
conc.1	0.084	0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.3
conc.2	0.127	0.0	0.0	10.0	10.0	5.0	5.8	5.0	5.0
conc.5	0.206	100.0	100.0	100.0	100.0	100.0	0.0	58.8	58.8
conc.3	0.210	10.0	10.0	20.0	30.0	17.5	9.6	58.8	58.8
conc.6	0.260	100.0	100.0	100.0	100.0	100.0	0.0	97.5	97.5
conc.4	0.361	100.0	90.0	100.0	90.0	95.0	5.8	97.5	97.5

Notes: a SD = standard deviation

b Smoothed means that any adjacent %-mortality values that did not increase monotonically were replaced with their average.

c Adjusted means that smoothed %-mortality data was corrected for control-group mortality using Abbott's formula.

* Data plotted on Figure 5-26 are highlighted in bold-type.

APPENDIX K

ICPIN PRINTOUTS FOR ALL TOXICITY-TESTS

ICPIN PRINTOUT FOR T1 – survival data

Test-Description: 96-hour acute-exposure test; static

Test-Species: larval walleye

DATA FILE: T1asurv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.059	70.000	0.000	76.667
2	3	0.169	83.333	20.817	76.667
3	3	0.276	60.000	0.000	60.000
4	3	0.393	16.667	15.275	16.667
5	3	0.580	0.000	0.000	0.000
6	3	0.720	0.000	0.000	0.000
7	3	1.284	0.000	0.000	0.000
8	3	1.345	0.000	0.000	0.000
9	3	2.313	0.000	0.000	0.000

The Linear Interpolation Estimate: 0.3345 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated

The Bootstrap Estimates Mean: 0.3353 Standard Deviation: 0.0118

Original Confidence Limits: Lower: 0.3169 Upper: 0.3638

Expanded Confidence Limits: Lower: 0.2976 Upper: 0.3959

Resampling time in Seconds: 0.16 Random_Seed: -358871734

ICPIN PRINTOUT FOR T2 – survival data

Test-Description: 96-hour acute-exposure test; static

Test-Species: larval white sucker

DATA FILE: T1bsurv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.055	100.000	0.000	100.000
2	3	0.190	100.000	0.000	100.000
3	3	0.323	100.000	0.000	100.000
4	3	0.431	86.667	23.094	86.667
5	3	0.499	46.667	50.332	46.667
6	3	0.793	0.000	0.000	0.000
7	3	1.102	0.000	0.000	0.000
8	3	1.179	0.000	0.000	0.000
9	3	2.048	0.000	0.000	0.000

The Linear Interpolation Estimate: 0.4933 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated

The Bootstrap Estimates Mean: 0.5205 Standard Deviation: 0.0553

Original Confidence Limits: Lower: 0.4574 Upper: 0.6355

Expanded Confidence Limits: Lower: 0.4180 Upper: 0.7919

Resampling time in Seconds: 0.16 Random_Seed: -1561201784

ICPIN PRINTOUT FOR T3 – survival data

Test-Description: 96-hour acute-exposure test; semi-static
 Test-Species: larval white sucker

DATA FILE: T2Asurv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.024	76.667	5.774	78.889
2	3	0.047	76.667	32.146	78.889
3	3	0.074	83.333	20.817	78.889
4	3	0.108	70.000	36.056	70.000
5	3	0.178	53.333	11.547	53.333
6	3	0.272	20.000	10.000	20.000
7	3	0.521	0.000	0.000	0.000
8	3	0.690	0.000	0.000	0.000

The Linear Interpolation Estimate: 0.2172 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.2122 Standard Deviation: 0.0164
 Original Confidence Limits: Lower: 0.1780 Upper: 0.2346
 Expanded Confidence Limits: Lower: 0.1349 Upper: 0.2538
 Resampling time in Seconds: 0.17 Random_Seed: 423227536

ICPIN PRINTOUT FOR T4A – survival data

Test-Description: 96-hour acute-exposure test; semi-static
 Test-Species: larval white sucker

DATA FILE: T3A96sur.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.016	96.667	5.774	96.667
2	3	0.093	86.667	15.275	86.667
3	3	0.178	80.000	10.000	80.000
4	3	0.318	73.333	15.275	73.333
5	3	0.413	72.967	20.381	72.967
6	3	0.532	46.667	30.551	46.667
7	3	0.868	3.333	5.774	3.333

The Linear Interpolation Estimate: 0.5245 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.5324 Standard Deviation: 0.0573
 Original Confidence Limits: Lower: 0.4527 Upper: 0.6347
 Expanded Confidence Limits: Lower: 0.3737 Upper: 0.7559
 Resampling time in Seconds: 0.11 Random_Seed: 186986288

ICPIN PRINTOUT FOR T5 – survival data

Test-Description: 96-hour acute-exposure test; semi-static
 Test-Species: larval fathead minnow

DATA FILE: T6A96sur.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.008	85.000	12.910	85.000
2	4	0.081	62.500	18.930	62.500
3	4	0.164	55.000	17.321	55.000
4	4	0.446	36.600	9.429	36.600
5	4	1.048	0.000	0.000	0.000
6	4	2.150	0.000	0.000	0.000
7	4	3.361	0.000	0.000	0.000

The Linear Interpolation Estimate: 0.3556 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.3484 Standard Deviation: 0.0855
 Original Confidence Limits: Lower: 0.1512 Upper: 0.4962
 Expanded Confidence Limits: Lower: 0.0286 Upper: 0.5805
 Resampling time in Seconds: 0.17 Random_Seed: -1047492623

ICPIN PRINTOUT FOR T6 – survival data

Test-Description: 96-hour acute-exposure test; flow-through
 Test-Species: juvenile channel catfish

DATA FILE: t896hr.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.007	100.000	0.000	100.000
2	4	0.019	100.000	0.000	100.000
3	4	0.045	100.000	0.000	100.000
4	4	0.067	93.332	9.428	94.166
5	4	0.206	95.000	6.382	94.166
6	4	0.501	75.000	14.780	75.000
7	4	0.748	41.665	13.741	41.665

The Linear Interpolation Estimate: 0.6860 Entered P Value: 50

Number of Resamplings: 280 261 Resamples Generated
 Those resamples not used had estimates above the highest concentration/ %Effluent.
 The Bootstrap Estimates Mean: 0.6819 Standard Deviation: 0.0305
 No Confidence Limits can be produced since the number of resamples generated is not a multiple of 40.
 Resampling time in Seconds: 0.17 Random_Seed: 1627700384

ICPIN PRINTOUT FOR T7 – survival data

Test-Description: 96-hour acute-exposure test; flow-through
 Test-Species: juvenile fathead minnow

DATA FILE: t996hr.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.008	97.500	2.887	98.125
2	4	0.026	98.750	2.500	98.125
3	4	0.041	97.500	2.887	98.125
4	4	0.082	98.750	2.500	98.125
5	4	0.175	97.500	2.887	97.917
6	4	0.428	97.500	2.887	97.917
7	4	0.758	98.750	2.500	97.917

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 50% of the control response mean.

ICPIN PRINTOUT FOR T8 – survival data

Test-Description: 96-hour acute-exposure test; flow-through
 Test-Species: juvenile fathead minnow

DATA FILE: T1196hr.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.008	100.000	0.000	100.000
2	4	0.040	100.000	0.000	100.000
3	4	0.060	100.000	0.000	100.000
4	4	0.116	92.500	11.902	97.500
5	4	0.160	100.000	0.000	97.500
6	4	0.361	100.000	0.000	97.500

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 50% of the control response mean.

ICPIN PRINTOUT FOR T9 - survival data

Test-Description: 96-hour acute-exposure test; flow-through

Test-Species: larval northern pike

DATA FILE: t1896hr.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.024	95.000	5.000	95.000
2	4	0.051	87.500	8.660	90.000
3	4	0.099	92.500	5.000	90.000
4	4	0.201	82.500	6.455	82.500
5	4	0.399	70.000	4.082	70.000
6	4	0.592	33.750	9.465	33.750
7	4	0.659	11.250	9.465	11.250

The Linear Interpolation Estimate: 0.5187 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated

The Bootstrap Estimates Mean: 0.5220 Standard Deviation: 0.0153

Original Confidence Limits: Lower: 0.4935 Upper: 0.5490

Expanded Confidence Limits: Lower: 0.4783 Upper: 0.5672

Resampling time in Seconds: 0.17 Random_Seed: -319232476

ICPIN PRINTOUT FOR T10 - survival data

Test-Description: 96-hour acute-exposure test; flow-through

Test-Species: juvenile walleye

DATA FILE: t2196hr.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.028	95.000	5.000	96.250
2	3	0.035	96.667	2.887	96.250
3	3	0.088	96.667	5.774	96.250
4	3	0.180	96.667	2.887	96.250
5	3	0.368	90.000	13.229	92.500
6	3	0.484	95.000	5.000	92.500

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 50% of the control response mean.

ICPIN PRINTOUT FOR T4A – survival data

Test-Description: 10-day chronic-exposure test; semi-static
 Test-Species: larval white sucker

DATA FILE: T3A10sur.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.015	83.333	20.817	83.333
2	3	0.060	76.667	15.275	76.667
3	3	0.129	70.000	20.000	70.000
4	3	0.267	66.667	5.774	68.150
5	3	0.351	69.633	19.453	68.150
6	3	0.505	40.000	36.056	40.000
7	3	0.868	0.000	0.000	0.000

The Linear Interpolation Estimate: 0.3591 Entered P Value: 20

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.2607 Standard Deviation: 0.1547
 Original Confidence Limits: Lower: 0.0463 Upper: 0.5527
 Expanded Confidence Limits: Lower: -0.2978 Upper: 0.7656
 Resampling time in Seconds: 0.17 Random_Seed: 1825091252

ICPIN PRINTOUT FOR T6 – survival data

Test-Description: 30-day chronic-exposure test; flow-through
 Test-Species: juvenile channel catfish

DATA FILE: T8surv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.007	100.000	0.000	100.000
2	4	0.021	100.000	0.000	100.000
3	4	0.039	100.000	0.000	100.000
4	4	0.059	88.325	13.756	88.325
5	4	0.205	76.675	15.858	76.675
6	4	0.326	55.000	22.013	55.000
7	4	0.616	1.675	3.350	1.675

The Linear Interpolation Estimate: 0.1634 Entered P Value: 20

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.1622 Standard Deviation: 0.0520
 Original Confidence Limits: Lower: 0.0575 Upper: 0.2379
 Expanded Confidence Limits: Lower: -0.0061 Upper: 0.2826
 Resampling time in Seconds: 0.17 Random_Seed: 307455177

ICPIN PRINTOUT FOR T7 – survival data

Test-Description: 30-day chronic-exposure test; flow-through

Test-Species: juvenile (three-month-old) fathead minnow

DATA FILE: T9surv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.007	95.000	5.774	95.000
2	3	0.022	95.000	0.000	95.000
3	4	0.039	93.750	2.500	94.835
4	4	0.061	95.000	5.774	94.835
5	4	0.198	94.175	5.513	94.835
6	4	0.310	96.250	2.500	94.835
7	4	0.575	95.000	5.774	94.835

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 80% of the control response mean.

ICPIN PRINTOUT FOR T8 – survival data

Test-Description: 29-day chronic-exposure test; flow-through

Test-Species: juvenile (one-month-old) fathead minnow

DATA FILE: T11surv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.006	86.200	12.376	91.864
2	3	0.019	93.167	0.231	91.864
3	4	0.036	96.550	3.987	91.864
4	4	0.057	83.075	20.692	87.967
5	4	0.193	87.500	4.186	87.967
6	4	0.303	93.325	5.430	87.967

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 80% of the control response mean.

ICPIN PRINTOUT FOR T9 – survival data

Test-Description: 12-day chronic-exposure test; flow-through
 Test-Species: larval northern pike

DATA FILE: Y2K6surv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.030	79.500	6.587	79.500
2	4	0.072	62.400	19.206	64.275
3	4	0.127	66.150	18.859	64.275
4	4	0.210	45.600	23.875	45.600
5	4	0.410	44.350	8.738	44.350
6	4	0.588	3.750	2.500	3.750
7	4	0.661	1.250	2.500	1.250

The Linear Interpolation Estimate: 0.1301 Entered P Value: 20

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.1127 Standard Deviation: 0.0446
 Original Confidence Limits: Lower: 0.0561 Upper: 0.2172
 Expanded Confidence Limits: Lower: 0.0117 Upper: 0.2694
 Resampling time in Seconds: 0.17 Random_Seed: -8215220

ICPIN PRINTOUT FOR T10 – survival data

Test-Description: 30-day chronic-exposure test; flow-through
 Test-Species: juvenile walleye

DATA FILE: Y2K9surv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.012	83.833	4.126	87.167
2	3	0.025	90.500	7.016	87.167
3	2	0.048	84.950	1.061	84.950
4	3	0.089	83.200	6.260	83.200
5	3	0.177	73.967	13.213	73.967
6	3	0.243	63.667	11.957	63.667

The Linear Interpolation Estimate: 0.2039 Entered P Value: 20

Number of Resamplings: 280 228 Resamples Generated
 Those resamples not used had estimates
 above the highest concentration/ %Effluent.
 The Bootstrap Estimates Mean: 0.1931 Standard Deviation: 0.0260

No Confidence Limits can be produced since the number of resamples
 generated is not a multiple of 40.

Resampling time in Seconds: 0.11 Random_Seed: -108782898

ICPIN PRINTOUT FOR T4A – growth data based on final dry weights/fish

Test-Description: 10-day chronic-exposure test; semi-static

Test-Species: larval white sucker

DATA FILE: T3A10grw.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.015	1.660	0.266	1.903
2	3	0.060	1.919	0.129	1.903
3	3	0.129	2.050	0.513	1.903
4	3	0.183	1.701	0.154	1.903
5	3	0.267	2.116	0.173	1.903
6	3	0.351	1.925	0.292	1.903
7	3	0.505	1.953	0.824	1.903

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 80% of the control response mean.

ICPIN PRINTOUT FOR T6 - growth data based on final dry weights/fish

Test-Description: 30-day chronic-exposure test; flow-through

Test-Species: juvenile channel catfish

DATA FILE: T8grow.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.007	1075.950	124.411	1075.950
2	4	0.021	1012.750	114.955	1020.075
3	4	0.039	1027.400	30.625	1020.075
4	4	0.059	943.375	134.287	973.613
5	4	0.205	1003.850	66.916	973.613
6	4	0.326	1039.900	100.556	995.708

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 80% of the control response mean.

ICPIN PRINTOUT FOR T7 – growth data based on final dry weights/fish

Test-Description: 30-day chronic-exposure test; flow-through

Test-Species: juvenile (three-month old) fathead minnow

DATA FILE: T9grow.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.007	317.050	13.044	321.938
2	4	0.022	326.825	27.664	321.938
3	4	0.039	295.825	11.346	299.400
4	4	0.061	302.850	23.501	299.400
5	4	0.198	299.525	12.670	299.400
6	4	0.310	292.475	16.271	292.475
7	4	0.575	247.925	20.742	247.925

The Linear Interpolation Estimate: 0.5176 Entered P Value: 20

Number of Resamplings: 280 242 Resamples Generated

Those resamples not used had estimates above the highest concentration/ %Effluent.

The Bootstrap Estimates Mean: 0.4967 Standard Deviation: 0.0374

No Confidence Limits can be produced since the number of resamples generated is not a multiple of 40.

Resampling time in Seconds: 0.17 Random_Seed: -216123574

ICPIN PRINTOUT FOR T8 – growth data based on final dry weights/fish

Test-Description: 29-day chronic-exposure test; flow-through

Test-Species: juvenile (one-month-old) fathead minnow

DATA FILE: T11grow.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.006	11.125	2.414	12.730
2	4	0.019	12.950	2.859	12.730
3	4	0.036	12.375	1.650	12.730
4	4	0.057	13.875	5.058	12.730
5	4	0.193	13.325	1.255	12.730
6	4	0.303	11.600	2.371	11.600

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 80% of the control response mean.

ICPIN PRINTOUT FOR T9 – growth data based on average dry weight/fish at test-termination

Test-Description: 12-day chronic-exposure test; flow-through
Test-Species: larval northern pike

DATA FILE: Y2K6grow.icp

Conc. ID	Number Replicates	Concentration	Response Means	Response Dev.	Std. Response	Pooled Response Means
1	3	0.030	0.005	0.001	0.007	
2	4	0.072	0.008	0.001	0.007	
3	4	0.127	0.007	0.001	0.007	
4	4	0.210	0.007	0.003	0.007	
5	4	0.410	0.005	0.000	0.006	
6	3	0.588	0.006	0.004	0.006	

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 80% of the control response mean.

ICPIN PRINTOUT FOR T10 – growth data based on average dry weight/fish at test-termination

Test-Description: 30-day chronic-exposure test; flow-through
Test-Species: juvenile walleye

DATA FILE: Y2K9grow.icp

Conc. ID	Number Replicates	Concentration	Response Means	Response Dev.	Std. Response	Pooled Response Means
1	3	0.012	0.035	0.001	0.036	
2	3	0.025	0.037	0.005	0.036	
3	2	0.048	0.037	0.003	0.036	
4	3	0.089	0.032	0.003	0.035	
5	3	0.177	0.037	0.005	0.035	
6	3	0.243	0.035	0.009	0.035	

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 80% of the control response mean.

ICPIN PRINTOUT FOR T3B – survival data

Test-Description: 96-hour acute-exposure test; semi-static
 Test-Species: larval white sucker

DATA FILE: T2Bsurv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.024	76.667	5.774	83.160
2	3	0.045	85.200	12.817	83.160
3	3	0.067	73.333	11.547	83.160
4	3	0.091	93.333	11.547	83.160
5	3	0.124	87.267	11.064	83.160
6	3	0.159	63.333	23.094	63.333
7	3	0.176	50.000	10.000	50.000
8	3	0.197	3.333	5.774	3.333

The Linear Interpolation Estimate: 0.1798 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.1795 Standard Deviation: 0.0019
 Original Confidence Limits: Lower: 0.1749 Upper: 0.1828
 Expanded Confidence Limits: Lower: 0.1696 Upper: 0.1861
 Resampling time in Seconds: 0.17 Random_Seed: 2004449424

ICPIN PRINTOUT FOR T4B – survival data

Test-Description: 96-hour acute-exposure test; semi-static
 Test-Species: larval white sucker

DATA FILE: T3B96sur.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.016	96.667	5.774	96.667
2	3	0.055	90.000	17.321	90.000
3	3	0.100	83.333	15.275	85.000
4	3	0.135	86.667	5.774	85.000
5	3	0.180	83.333	5.774	84.817
6	3	0.233	86.300	15.168	84.817
7	3	0.282	80.000	10.000	80.000
8	3	0.331	40.000	10.000	40.000

The Linear Interpolation Estimate: 0.3208 Entered P Value: 50

Number of Resamplings: 280 266 Resamples Generated
 Those resamples not used had estimates above the highest concentration/ %Effluent.
 The Bootstrap Estimates Mean: 0.3204 Standard Deviation: 0.0047
 No Confidence Limits can be produced since the number of resamples generated is not a multiple of 40.
 Resampling time in Seconds: 0.17 Random_Seed: -188859724

ICPIN PRINTOUT FOR T4B – survival data

Test-Description: 10-day chronic-exposure test; semi-static
 Test-Species: larval white sucker

DATA FILE: T3B10sur.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	3	0.015	83.333	20.817	83.333
2	3	0.049	82.600	15.565	82.600
3	3	0.084	70.000	26.458	76.667
4	3	0.124	80.000	0.000	76.667
5	3	0.167	80.000	10.000	76.667
6	3	0.222	65.933	22.486	65.933
7	3	0.263	63.333	5.774	63.333
8	3	0.293	20.000	0.000	20.000

The Linear Interpolation Estimate: 0.2780 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.2761 Standard Deviation: 0.0033
 Original Confidence Limits: Lower: 0.2696 Upper: 0.2819
 Expanded Confidence Limits: Lower: 0.2603 Upper: 0.2861
 Resampling time in Seconds: 0.17 Random_Seed: 231424600

ICPIN PRINTOUT FOR T5B – survival data

Test-Description: 96-hour acute-exposure test; semi-static
 Test-Species: larval fathead minnow

DATA FILE: T6Bsurv.icp

Conc. ID	Number Replicates	Concentration	Response Means	Std. Dev.	Pooled Response Means
1	4	0.010	97.500	5.000	98.750
2	4	0.084	100.000	0.000	98.750
3	4	0.127	95.000	5.774	95.000
4	4	0.206	0.000	0.000	41.250
5	4	0.210	82.500	9.574	41.250
6	4	0.260	0.000	0.000	2.500
7	4	0.361	5.000	5.774	2.500

The Linear Interpolation Estimate: 0.1941 Entered P Value: 50

Number of Resamplings: 280 280 Resamples Generated
 The Bootstrap Estimates Mean: 0.1939 Standard Deviation: 0.0028
 Original Confidence Limits: Lower: 0.1888 Upper: 0.1993
 Expanded Confidence Limits: Lower: 0.1857 Upper: 0.2024
 Resampling time in Seconds: 0.16 Random_Seed: -130453521

APPENDIX L

DATA USED TO CALCULATE SITE-SPECIFIC CRITERIA FOR ACUTE- AND CHRONIC-EXPOSURE TESTS

Table L-1. Data used to calculate site-specific criteria for acute tests.

Test no.	Species - Age class	LC50 (mg NH ₃ /L)	LC50 (mg total NH ₃ -N/L) ^a	LC50 (mg total NH ₃ -N/L) @ pH = 8.0 ^b	Substitution into Equation 3* or 6* ^c @ pH = 7.8 (mg total NH ₃ -N/L)	Substitution into Equation 3* or 6* ^c @ pH = 8.4 (mg total NH ₃ -N/L)
1	Walleye - larval	0.335	5.10	9.09	6.57 *	2.10 *
2	White sucker - larval	0.493	7.56	13.48	7.33 ^d *	2.34 ^d *
3A	White sucker - larval	0.217	4.14	6.08		
4A	White sucker - larval	0.525	8.69	12.75		
5A	Fathead minnow - larval	0.356	2.05	6.51	4.70 *	1.50 *
6	Channel catfish - juvenile	0.686	12.45	26.94	19.46 *	6.22 *
7	Fathead minnow - juvenile	>0.758	>14.17	>30.67	>22.15 ^e	>7.09 ^e
8	Fathead minnow - juvenile	>0.361	>5.59	>14.66	>10.59 ^e	>3.39 ^e
9	Northern pike - larval	0.519	4.77	12.52	9.04 *	2.89 *
10	Walleye - juvenile	>0.484	>5.06	>10.95	>7.91 ^f	>2.53 ^f
MSWQO					12.14	3.88

Notes:

*** Site-specific criteria plotted on Figure 5-27.**

a Calculated using Equations 1 and 2 (see Section 2.1).

b Calculated using Equation 6 (see Section 3.3).

c Equations 3* and 6* are equivalent to Equation 10 (see Sections 3.3.1 and 3.4).

d The geometric mean of 13.48 mg/L, 6.08 mg/L and 12.75 mg/L (i.e., 10.15 mg/L) was used to determine the site-specific criteria for larval white sucker.

e This value is not plotted on Figure 5-27 because a point estimate is not known. Also, Test 5A produced a more sensitive LC50 for this species.

f This value is not plotted on Figure 5-27 because a point estimate is not known. Also, Test 1 produced a more sensitive LC50 for this species.

Table L-2. Data used to calculate site-specific criteria for chronic tests.

Test no.	Species - Age class	LC20 or (EC20) (mg NH ₃ /L)	LC20 or (EC20) (mg NH ₃ -N/L) ^a	LC20 or (EC20) @ pH = 8.0 ^b (mg NH ₃ -N/L)	Substitution into Equation 2* ^c @ temp. = 0°C; pH = 7.8 (mg NH ₃ -N/L)	Substitution into Equation 2* ^c @ temp. = 25°C; pH = 8.4 (mg NH ₃ -N/L)	Substitution into Equation 5* ^d @ temp. = 0°C; pH = 7.8 (mg NH ₃ -N/L)	Substitution into Equation 5* ^d @ temp. = 25°C; pH = 8.4 (mg NH ₃ -N/L)
4A	White sucker – larval	0.359	5.98	8.13	22.69 *	9.20 *	n/a ^e	n/a ^e
6	Channel catfish – juvenile	0.163	3.29	6.21	17.34 *	7.03 *	17.34 *	7.03 *
7	Fathead minnow – juvenile	(0.518)	(10.85)	(20.48)	57.17 *	23.17 *	57.17 *	23.17 *
8	Fathead minnow – juvenile	>0.303	>6.96	>13.14	>36.68	>14.87	>36.68 ^f	>14.87 ^f
9	Northern pike – larval	0.130	1.19	2.67	7.45 *	3.02 *	n/a ^e	n/a ^e
10	Walleye – juvenile	0.204	5.14	5.15	14.38 *	5.83 *	14.38 *	5.83 *
MSWQO					7.96	1.64	12.92	1.64

Notes:

* **Site-specific criteria plotted on Figure 5-27.**

a Calculated using Equations 1 and 2 (see Section 2.1).

b Calculated using Equation 7 (see Section 3.3).

c Equation 2* is equivalent to Equation 11 (see Sections 3.3.2 and 3.4).

d Equation 5* is equivalent to Equation 12 (see Sections 3.3.2 and 3.4).

e Chronic data for this species can not be substituted into Equation 5* because early life stage (ELS) fish were used and the equation applies only when ELS-organisms are absent.

f This value not plotted on Figure 5-27 because a point estimate is not known. Also, Test 7 (conducted using the same fish species) produced an EC20 for a sensitive endpoint, growth.