# CENTER FOR DRUG EVALUATION AND RESEARCH

**APPLICATION NUMBER:** 

# 205739Orig1s000

# **ENVIRONMENTAL ASSESSMENT**

#### FINDING OF NO SIGNIFICANT IMPACT

#### NDA 205-739

#### **RLY5016 For Oral Suspension**

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. The Food and Drug Administration (FDA) is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of the regulatory process.

NDA 205-739 requests approval for RLY5016 for Oral Suspension. RLY5016 for Oral Suspension is a non-absorbed polymeric new drug intended for the treatment of hyperkalemia. RLY5016 for Oral Suspension is designed to bind and remove potassium from the gastrointestinal tract by reducing the concentration of free potassium in the gastrointestinal lumen and establishing a gradient favoring further potassium secretion, resulting in a reduction of total body potassium. In support of its application, Relypsa, Inc. prepared an environmental assessment (EA; attached) in accordance with 21 CFR Part 25, which evaluates the potential environmental impact due to the use and disposal of RLY5016.

The Food and Drug Administration, Center for Drug Evaluation and Research, has carefully considered the potential environmental impact due to approval of this application and has concluded that this action is not expected to have a significant effect on the human environment. Therefore, an environmental impact statement will not be prepared.

Attachment: July 28, 2014, Environmental Assessment



#### Environmental Assessment for RLY5016 for Oral Suspension

Prepared for: Relypsa, Inc. Redwood City, California

Prepared by: ENVIRON International Corporation Burton, Ohio

> Date: July 2014

Project Number: 34-30021A



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## Acronyms and Abbreviations

ACR	Acute-Chronic Ratios
CAS	Chemical Abstracts Services
CWNS	Clean Watersheds Needs Survey
EA	Environmental Assessment
EC <sub>10</sub>	10% Effective concentration
EC <sub>20</sub>	20% Effective concentration
EC <sub>25</sub>	25% Effective concentration
EC <sub>50</sub>	Median effective concentration
EEC	Expected environmental concentration
EIC	Expected introduction concentration
ENVIRON	ENVIRON International Corporation
EPA	Environmental Protection Agency
FACR	Final Acute-Chronic Ratio
FAV	Final Acute Value
FDA	Food and Drug Administration
F-ISE	Fluoride ion selective electrode
GLP	Good Laboratory Practice
GMAV	Genus Mean Acute Value
g/mol	Grams per mole
g/L	Grams per liter
kg	Kilogram
kg/day	Kilograms per day
kg/m²	Kilograms per square meter
kg/m³	Kilograms per cubic meter
kg/year	Kilograms per year
LC <sub>50</sub>	Median lethal concentration
LOAEL	Lowest observed adverse effect level
LOEC	Lowest observed effect concentration
L/day	Liters per day
m	Meter
MGD	Million gallons per day

mg/kg	Milligrams per kilogram
mg/kg-day	Milligrams per kilogram per day
mg/L	Milligrams per liter
NDA	New Drug Application
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
pcf	Pounds per cubic foot
рКа	Dissociation constant
POTW	Publicly owned treatment works
ppm	Parts per million
U.S.	United States
USAN	U.S. Adopted Name
USEPA	United States Environmental Protection Agency
wt%	Percent by weight
µg/kg	Micrograms per kilogram
µg/L	Microgram per liter
μm	Micrometer
°C	Celsius

### Definitions

Active Moiety: The molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or other noncovalent derivative (such as a complex, chelate, or clathrate) of the molecule, responsible for the physiological or pharmacological action of the drug substance (21 CFR 314.108(a)). The active moiety is the entire molecule or ion, not the "active site." From: *Guidance for Industry, Environmental Assessment of Human Drugs and Biologics Applications; July 1998, CMC 6, Revision 1.* 

Patiromer Anion or<br/>Polymer Anion:Other names for the active moiety in RLY5016S. "Polymer<br/>Anion" is the designation used throughout NDA 205739.

### 1 Date

July 28, 2014

### 2 Name of Applicant

Relypsa, Inc.

### 3 Address

700 Saginaw Drive Redwood City, CA 94063 Attention: Sarah McNulty Executive Director, Regulatory Affairs Tel: 650-421-9570 Fax: 650-421-9770

### 4 Description of Proposed Action

#### 4.1 Requested Approval

Relypsa, Inc. is filing a New Drug Application (NDA) for RLY5016 for Oral Suspension pursuant to section 505(b) of the Federal Food, Drug, and Cosmetic Act. RLY5016 for Oral Suspension is a powder intended to be administered orally after suspending in water. It is packaged in packets made from five layer laminate web stock. The packets are heat sealed on four sides. The drug product is available in six strengths of patiromer: 4.2, 8.4, 12.6, 16.8, 21.0, and 25.2 grams. This Environmental Assessment (EA) has been submitted in support of the approval of the NDA, pursuant to 21 CFR part 25.

#### 4.2 Need for Action

RLY5016 for Oral Suspension is a non-absorbed polymeric new drug intended for the treatment of hyperkalemia. RLY5016 for Oral Suspension is designed to bind and remove potassium from the gastrointestinal tract by reducing the concentration of free potassium in the gastrointestinal lumen and establishing a gradient favoring further potassium secretion, resulting in a reduction of total body potassium.

#### 4.3 Locations of Use

RLY5016 for Oral Suspension will be used by patients in their homes and in hospitals and clinics throughout the United States (U.S.).

#### 4.4 Disposal Sites

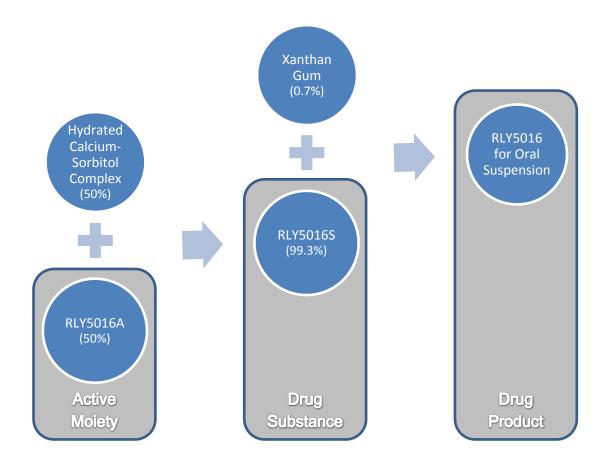
The primary means of disposal will be through patient use of the prescribed product. RLY5016 for Oral Suspension is not absorbed in the gastrointestinal tract and will be excreted by patients, whereupon it will enter the local wastewater treatment system.

At U.S. hospitals, pharmacies, or clinics, empty or partially empty packages will be disposed of according to hospital, pharmacy, or clinic procedures. In the home, empty or partially empty

packages will typically be disposed of by a community's solid waste management disposal system, which may include landfills, incineration, and recycling. Minimal quantities of the unused drug could be disposed of to the sewer system.

### **5** Identification of Substances

The drug product, RLY5016 for Oral Suspension, is the subject of the proposed action. The drug product contains 99.3 percent by weight (wt%) of the drug substance, RLY5016S, and 0.7 wt% of an excipient, xanthan gum (see Figure 1). The drug substance, RLY5016S, consists of a negatively-charged, cross-linked polymer anion, RLY5016A (i.e., the active moiety), with a calcium-sorbitol complex as the counter-ion. RLY5016S is synthesized using a suspension polymerization process that results in spherical beads with a particle size of approximately 100 micrometers ( $\mu$ m). Each bead is a single molecule that is three-dimensionally cross-linked. The beads form a free-flowing powder. For this EA, laboratory testing was conducted on the drug substance, RLY5016S (i.e., testing did not include xanthan gum).



#### Figure 1. Composition of Drug Product, RLY5016 for Oral Suspension.

#### 5.1 Nomenclature

The nomenclature assigned to RLY5016S is described in Table 1.

#### Table 1. RLY5016S Nomenclature

Name	RLY5016S
Established Name (U.S. Adopted Name-USAN)	Applied for
Brand/Proprietary Name /Tradename	To be approved
Chemical Abstracts Index Name (inverted form)	Calcium, hydrolyzed divinylbenzene- Me 2-fluoro-2-propenoate-1,7- octadiene polymer sorbitol complexes
Systematic Chemical Name (uninverted form)	Not available
IUPAC Name	Poly[(D-glucitol-calcium) 2- fluoroacrylate-co-diethenylbenzene-co- octa-1,7-diene]

#### 5.2 Chemical Abstracts Service (CAS) Registration Number

1415477-49-4

#### 5.3 Empirical Formula

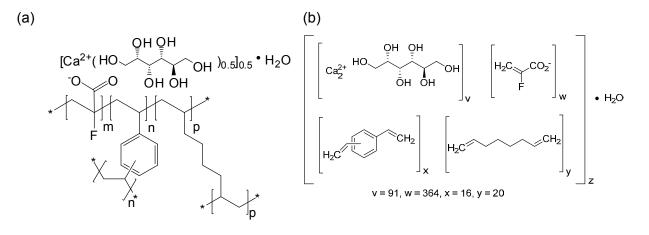
The empirical formula for RLY5016S is expressed as follows:  $C_{613}H_{765}F_{114}O_{399}Ca_{57}$ . This formula is based on the molar ratio of starting materials and the target calcium and sorbitol contents used in the synthesis of the drug substance (see Appendix E [confidential]). The empirical formula does not include associated water.

#### 5.4 Molecular Weight

RLY5016S is synthesized as three-dimensionally cross-linked polymer beads. Each RLY5016S molecule results in a spherical bead due to multiple cross-links between polymer chains. These beads have a narrow size distribution and are approximately 100 µm in diameter. The molecular weight of RLY5016S is not constant as it varies with each bead. The molecular weight of a 100 micrometer RLY5015S bead, calculated using an experimentally-derived value for density and the theoretical calculated value for volume, is estimated as 5.6 x10<sup>17</sup> grams per mole (g/mol).

#### 5.5 Structural Formula

The structural formula of the drug substance, RLY5016S, is shown in Figure 2a. An alternate depiction of the structural formula is shown in Figure 2b.



m = number of 2-fluoro-2-propenoate groups (m=0.91) n, p = number of crosslinking groups n + p = 0.09

•H<sub>2</sub>O = associated water

\* = indicates an extended polymeric network

#### Figure 2. RLY5016S Structural Formula (a) and Alternate Structural Formula (b)

### 6 Environmental Issues

Environmental issues are evaluated below by comparing estimated ecological exposure concentrations with measured or predicted concentrations associated with environmental effects, in accordance with the Food and Drug Administration's (FDA) (1998) *Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications*. An environmental data summary table is provided in Appendix A.

#### 6.1 Environmental Fate of Released Substances

An EA is required for RLY5016 for Oral Suspension based on projected active moiety fifth year annual production for direct use (see Appendix F [confidential]). However, due to its physical characteristics, some of the laboratory tests normally implemented to support an EA are not applicable to RLY5016 for Oral Suspension or are not feasible, and other tests require specific modifications of standard test methods. Testing modifications or omissions are described in the Section 6.1 subsections.

Relypsa proposed an EA testing program which the FDA accepted (see Appendix G [confidential]). Testing conformed to data quality standards associated with Good Laboratory Practice (GLP) as described by the Organisation for the Economic Co-operation and Development (OECD 1998). Reports of test results are provided in Appendix H (confidential).

#### 6.1.1 Identification of Substances of Interest

The environmental risk analysis performed for the EA focuses on the drug substance, RLY5016S (the calcium-sorbitol complex of the polymer), with concentrations of test article expressed as the polymer anion (i.e., the active moiety). Calcium, sorbitol, and the excipient

component of the drug product, xanthan gum, are generally recognized as safe; risks associated with these drug components are not evaluated and are not calculated separately. Degradation products of the polymer are generally assumed to be no more toxic than the polymer itself, consistent with FDA guidance (1998). However, fluoride is slowly eliminated from RLY5016S through degradation, and environmental risks associated with fluoride cannot be estimated based on data for RLY5016S due to the very different physical characteristics (e.g., the molecular size of fluoride versus the polymer, the solubility differences). Therefore, a screening-level environmental risk analysis was performed for fluoride using information available from publicly available scientific literature (see Section 6.2).

#### 6.1.2 Physical and Chemical Characterization

RLY5016S is synthesized as polymer beads. Each bead is a single macromolecule that has multiple covalent cross-links between polymer chains. Due to its cross-linked structure, RLY5016S is not expected to be soluble in any media. Visual observations by Relypsa also indicate that RLY5016S is insoluble in water and in organic solvents. When added to water, the beads are observed to swell slightly, but not dissolve. Nevertheless, formal solubility testing was performed to confirm the lack of solubility, as this characteristic restricts the chemical analyses that can be performed on the drug substance.

#### 6.1.2.1 Water Solubility

The water solubility of RLY5016S was evaluated following testing protocols based on OECD Guideline 105. A gravimetric analytical method was utilized based on methodology developed and validated by a contract testing facility. As expected, the polymer was filtered out of the water at the end of the test, and none of the polymer dissolved. Filtration of undissolved RLY5016S yielded recoveries consistently higher than 100%, which may be due to water absorption, incomplete solubilization of sorbitol, or analytical variability. Water solubility of RLY5016S was calculated to be <0.100 grams per liter (g/L) at  $20 \pm 0.5$  °C.

#### 6.1.2.2 Solubility in Organic Solvents

The solvent solubility of RLY5016S was evaluated following testing protocols based on a modification of OECD Guideline 105 using acetone and hexane as solvents. However, gravimetric analysis of RLY5016S following filtration was not possible because the test material adhered to the glass of the test vessel and could not be removed by rinsing with solvent. Based on these observations, it is apparent that RLY5016S is not sufficiently soluble in solvents to permit conventional solvent extractions or solubilization for analytical purposes.

#### 6.1.2.3 Dissociation Constant (pKa)

The dissociation constant (pKa) of RLY5016S was not determined. Testing was not feasible due to the polyprotic nature of the molecule and the inherent analytical limitations to measure the pKa values for each carboxylate group in RLY5016S.

#### 6.1.2.4 Log octanol/Water Partition Coefficient

RLY5016S is insoluble in water or hydrocarbon solvents. Therefore, the log octanol/water partition coefficient is not meaningful and was not determined.

#### 6.1.2.5 Vapor Pressure

The vapor pressure of RLY5016S is zero due to its very high molecular weight. Analysis of the vapor pressure was not conducted.

#### 6.1.2.6 Physical and Chemical Characteristics of Fluoride

Fluorides are binary compounds or salts of the element fluorine (ATSDR 2003). Many fluoride compounds exist naturally in the environment, and among fluoride compounds, physical and chemical characteristics vary widely (ATSDR 2003). For example, compounds such as sodium fluoride (NaF), hydrogen fluoride (HF), and fluorosilicic acid (H<sub>2</sub>SiF<sub>6</sub>) are soluble, whereas compounds such as calcium fluoride (CaF<sub>2</sub>), magnesium fluoride (MgF<sub>2</sub>), and aluminum fluoride (AIF<sub>3</sub>) are weakly soluble or insoluble. Generally, it is the solubility of these fluoride compounds that makes them biologically available and thus potentially toxic (WHO 2002). While soluble inorganic fluorides have the potential to vaporize or form aerosols, insoluble forms are likely to settle (Drury et al. 1980; Brimblecombe and Clegg 1988).

#### 6.1.3 Environmental Depletion Mechanisms

Environmental depletion mechanisms (i.e., biodegradation, hydrolysis) were not investigated due to analytical limitations. Polymeric degradation products of RLY5016 for Oral Suspension have not been observed. Fluoride, as calcium fluoride, is the only degradation product of RLY5016 for Oral Suspension. Elimination of calcium fluoride from RLY5016S in RLY5016 for Oral Suspension is slow. Relypsa collected data from an experimental lot of the drug substance held at 30 degrees Celsius (°C) in a closed container for 8 days. The validated test method involved extracting a sample of RLY5016S with deionized water and then determining the concentration of dissociated fluoride in the supernatant using a fluoride ion selective electrode (F-ISE). Under these test conditions, calcium fluoride elimination (measured as fluoride ion) was calculated as 5 parts per million (ppm) fluoride per day based on polymer anion content. Assuming that all the fluorine in the polymer is released at the same rate in the environment, it would take 54 years for all of the fluorine to be eliminated as calcium fluoride (see Appendix I [confidential]).

For the purposes of this EA, a conservative approach is utilized to assess exposure concentrations. The following hypothetical depletion scenarios are examined: (1) no environmental depletion occurs and the entirety of the active moiety is present or (2) the active moiety is environmentally depleted and all fluoride is released. Actual environmental exposures will be intermediate between these scenarios. If both of the depletion scenarios are found to be acceptable, then the intermediate conditions would also be acceptable.

#### 6.1.4 Environmental Concentrations

The environmental fate of RLY5016 for Oral Suspension is predicted to be terrestrial. Due to its insolubility, RLY5016S is expected to settle into sludge, rather than effluent, after entering publicly owned treatment works (POTWs). The application of this sludge as biosolids to soils is the pathway by which RLY5016S is expected to enter the environment. RLY5016S exposure concentrations are reported in this EA in terms of polymer anion concentrations.

Although RLY5016S is not expected to be present in treated effluent, hypothetical aquatic ecological risks are included in this assessment. For the purposes of this EA, four exposure scenarios are examined:

- No environmental depletion of RLY5016S occurs, and 100% of the RLY5016S partitions to sludge;
- Environmental depletion of RLY5016S occurs, releasing 100% of the fluoride to sludge;
- No environmental depletion of RLY5016S occurs, and 100% of the RLY5016S partitions to effluent; and
- Environmental depletion of RLY5016S occurs, releasing 100% of the fluoride to effluent.

Each scenario listed above is mutually exclusive of the three other scenarios. However, the examination of each worst-case scenario brackets all hypothetical environmental exposures and provides the most conservative, screening-level risk analysis approach.

#### 6.1.4.1 Terrestrial Exposure Concentrations

Land application of biosolids used to amend soil (e.g., agricultural land, forests, rangelands, or disturbed land in need of reclamation) is the basis for the terrestrial exposure scenario evaluated in this EA. As described above, ecological exposures to the active moiety (i.e., polymer anion) and its degradation product, fluoride, are expected to occur predominantly in the terrestrial environment due to the insolubility of RLY5016S.

#### Active Moiety

The expected introduction concentration (EIC) of the active moiety (i.e., polymer anion) present in biosolids is calculated as follows:

Equation 1:

$$EIC_{biosolids} = \frac{AM}{BS} \times X$$

Whereby:

Variable	Description	Value
EIC <sub>biosolids</sub>	expected introduction concentration in biosolids	calculated above (milligrams per kilogram or mg/kg)
AM	production of active moiety (i.e., polymer anion) for direct use in 5 <sup>th</sup> year	confidential (see Appendix F)
BS	production of biosolids (United States Environmental Protection Agency [USEPA] 1999, projection for 2010)	7.439 x 10 <sup>9</sup> dry kilograms per year (kg/year)
Х	conversion factor	10 <sup>6</sup> mg/kg

 Table 2. Definition of Variables Used in Equation 1.

Using Equation 1, the EIC<sub>biosolids</sub> of the active moiety is calculated as 73.9 mg active moiety/kg dry biosolids. This calculation assumes: (1) the entirety of the annual active moiety production is used throughout the United States and enters the POTW system in proportion to the population; (2) the active moiety is not degraded within the POTW system; and (3) the active moiety loads entirely to biosolids (i.e., does not enter the POTW effluent stream).

The expected environmental concentration (EEC) of the active moiety present in biosolidsamended soil is calculated as follows:

Equation 2:

 $EEC_{amended \ soil} = \frac{EIC_{biosolids} \times AR_{biosolids}}{(ID_{soil} \times \rho_b) + AR_{biosolids}}$ 

Whereby:

Table 5. Deminition of Variables Osed in Equation 2.		
Variable	Description	Value
EECamended soil	expected environmental concentration in biosolids-amended soil	calculated (mg/kg)
EIC <sub>biosolids</sub>	predicted environmental concentration in biosolids	see above (mg/kg)
$AR_{biosolids}$	biosolids application rate (USEPA 2000a)	22.42 dry kilograms per square meter (kg/m <sup>2</sup> )
ID <sub>soil</sub>	depth of incorporation into soils (assumption)	0.15 meters (m)
ρ <sub>b</sub>	dry bulk density of soils (EMEA 2008)	1,500 kilograms per cubic meter (kg/m <sup>3</sup> )

#### Table 3. Definition of Variables Used in Equation 2.

USEPA (2000a) presents multiple biosolid application scenarios for agricultural land, forest land, range land, and reclamation land, ranging from 2 to 100 dry tons per acre. The highest typical application rate for agricultural land is 20 dry tons per acre. For this EA, the biosolid application rate scenario for reclamation land, 100 dry tons per acre (22.42 dry kg/m<sup>2</sup>), is utilized to be most conservative.

Using Equation 2, the EEC<sub>amended soil</sub> of the active moiety is calculated as 6.70 mg active moiety/kg amended soil (dry weight). This calculation relies on the assumptions listed above for EIC<sub>biosolids</sub> and does not consider the accumulation of the active moiety in soil through repeated biosolid applications. The frequency of biosolids application is generally inversely related to the application rate (USEPA 2002a), and biosolids application for land reclamation purposes (as assumed in this EA) would only occur once.

#### Fluoride

Based on the empirical formula of the polymer anion (i.e., the active moiety), fluorine accounts for 18.8% of the polymer anion's molecular weight, as shown in Appendix E (confidential). As such, the maximum concentration of fluoride in biosolids can be estimated as 18.8% of the EIC<sub>biosolids</sub> for the active moiety (73.9 mg/kg), or 13.9 mg fluoride/kg dry biosolids. This approach assumes: (1) the entirety of the annual active moiety production is used throughout the United States and enters the POTW system in proportion to the population; (2) the active moiety is not degraded within the POTW system; (3) the active moiety loads entirely to biosolids (i.e., does not enter the POTW effluent stream); and (4) the active moiety degrades in the biosolids and releases all available fluoride. Since the release of fluoride is expected to occur slowly, the final assumption that 100% of the fluoride contained in the active moiety would be released in surface soil at a given time is unlikely.

For amended soils, the maximum concentration of fluoride can be estimated as 18.8% of the  $EEC_{amended soil}$  for the active moiety (6.70 mg/kg), or 1.26 mg fluoride/kg amended soil (dry weight).

#### 6.1.4.2 Aquatic Exposure Concentrations

The discharge of POTW treated wastewater effluent into aquatic systems is the basis for the aquatic exposure scenario evaluated in this EA. As described above, ecological exposures to the active moiety (i.e., the polymer anion) and its degradation product, fluoride, are expected to occur predominantly in the terrestrial environment due to the insolubility of RLY5016S. However, based on a conservative approach to consider any potential aquatic partitioning, modelled exposure concentrations used to evaluate ecological effects in aquatic systems assumed 100% loading to wastewater effluent following treatment by POTWs.

#### Active Moiety

Based on FDA guidance (1998) the EIC of the active moiety (i.e., polymer anion) entering the aquatic environment from patient use through treated POTW effluent is calculated as follows:

Equation 3:

$$EIC_{effluent} = \frac{AM}{WW} \times Y \times Z$$

Whereby:

Variable	Description	Value
EIC <sub>effluent</sub>	expected introduction concentration entering aquatic systems	calculated above (micrograms per liter or μg/L)
AM	production of active moiety (i.e., polymer anion) for direct use in 5 <sup>th</sup> year	confidential (see Appendix F)
WW	wastewater entering POTWs <sup>a</sup>	1.224 x 10 <sup>11</sup> liters per day(L/day)
Y	conversion factor	year/365 days
Z	conversion factor	10 <sup>9</sup> micrograms per kilogram (µg/kg)

 Table 4. Definition of Variables Used in Equation 3.

<sup>a</sup> The rate of wastewater entering POTWs presented by FDA (1998) has been adjusted to reflect updated data in the USEPA's *Clean Watersheds Needs Survey (CWNS) 2008 Report to Congress*, presented as 32,345 million gallons per day (MGD).

Using Equation 3, the EIC<sub>effluent</sub> for the active moiety is estimated as 12.3  $\mu$ g active moiety/L POTW effluent. This calculation assumes (1) the entirety of the annual active moiety production is used throughout the United States and enters the POTW system in proportion to the population; (2) the active moiety is not degraded within the POTW system; and (3) the active moiety loads entirely to the wastewater effluent (i.e., it does not partition to biosolids).

The EEC of the active moiety present in surface water (EEC<sub>surface water</sub>) is expected to be less than the EIC due to dilution of effluent. The FDA guidance (1998) states that applying a dilution factor of 10 to the EIC<sub>effluent</sub> is normally appropriate based on POTW data available from USEPA. Thus, based on a 10-fold dilution factor, the EEC is calculated as 1.23  $\mu$ g active moiety/L surface water.

#### Fluoride

Based on the empirical formula of the polymer anion (i.e., the active moiety), fluorine accounts for 18.8% of the polymer anion's molecular weight, as in Appendix E (confidential). As such, the maximum amount of fluoride entering aquatic systems can be calculated as 18.8% of the  $EIC_{effluent}$  for the active moiety (12.3 µg/L), or 2.31 µg fluoride/L POTW effluent. This approach assumes (1) the entirety of the annual active moiety production is used throughout the United States and enters the POTW system in proportion to the population; (2) the active moiety is degraded within the POTW system and releases all available fluoride; and (3) the fluoride loads entirely to effluent (i.e., it does not partition to biosolids).

For surface water, the maximum concentration of fluoride can be estimated as 18.8% of the  $EEC_{surface water}$  for the active moiety (1.23 µg/L), or 0.231 µg fluoride/L surface water.

#### 6.1.5 Environmental Fate Summary

Following prescribed patient use and excretion, the drug product, RLY5016 for Oral Suspension, will enter local wastewater treatment facilities. The polymer anion is expected to settle in sludge, which may be applied to land as biosolids. Although the polymer anion is not expected to be present in treated POTW effluent, aquatic ecological risks were also assessed in this EA. In addition, fluoride, a degradation product of the polymer anion, was assessed.

In the preceding sections, environmental exposures to the active moiety (i.e., the polymer anion) and fluoride were estimated first as concentrations at which they would be introduced to the environment as biosolids or effluent (i.e., EIC) and then as concentrations expected to be found in the environment in amended soil and surface water (i.e., EEC). Conservative estimates of terrestrial exposure to the polymer anion and its degradation product, fluoride, are summarized in Table 5.

Chemical	EIC (mg/kg biosolids)	EEC (mg/kg amended soil)
Active Moiety (i.e., Polymer anion)	73.9	6.70
Fluoride	13.9	1.26

#### Table 5. Terrestrial Exposure Summary

Hypothetical, worst-case estimates of aquatic exposure to the polymer anion and its degradation product, fluoride, are summarized in Table 6.

Chemical	EIC (µg/L effluent)	EEC (µg/L surface water)
Active Moiety (i.e., Polymer anion)	12.3	1.23
Fluoride	2.31	0.231

These exposure concentrations are compared to environmental effects concentrations to estimate risks in the next section.

#### 6.2 Environmental Effects of Released Substances

Toxicity data for the active moiety and fluoride in terrestrial and aquatic systems are discussed in the Section 6.2 subsections, and the testing reports are provided in Appendix J (confidential). The significance of estimated ecological exposures is evaluated by comparison to relevant toxicity values.

Relypsa proposed an EA testing program to FDA for the drug product RLY5016 for Oral Suspension (see Appendix G [confidential]), which was accepted by FDA. As part of the

proposed EA testing program, toxicity testing was conducted on the drug substance, RYL5016S to determine activated sludge microbial inhibition and toxicity to representative terrestrial and aquatic receptors. Testing conformed to data quality standards associated with GLP as described by OECD (1998). Toxicity data for the active moiety were collected according to the testing program accepted by the FDA (see Appendix G). Comparisons of the active moiety to ecological exposures were done using one-half the toxicity values for RLY5016S. This is based on the composition of RLY5016S [i.e., 50% active moiety (polymer anion) and 50% calcium-sorbitol complex].

The testing program for RLY5016S included initial acute toxicity testing of algae. Additional aquatic toxicity testing (i.e., fish and invertebrate) would have been required if the margin of safety based on algal toxicity data was <100. Although terrestrial toxicity tests are not typically required in the first tier of testing, a terrestrial exposure scenario of RLY5016S is more realistic than aquatic exposure, and thus terrestrial toxicity testing was also completed.

For fluoride, a screening level risk analysis was conducted using information from publicly available literature.

#### 6.2.1 Activated Sludge Microbial Inhibition

Testing was conducted to determine the toxicity of RLY5016S to microorganisms present in activated sludge (see Appendix J [confidential]). Testing procedures were performed in accordance with OECD Guideline 209. The test exposed activated sludge microorganisms to nominal RLY5016S concentrations of 10, 100, and 1,000 milligrams per liter (mg/L). Effects on respiration inhibition were reported as 10% effective concentration (EC<sub>10</sub>) and median effective concentration (EC<sub>50</sub>) values.

Based on the test results, RLY5016S has no significant adverse effects on activated sludge microorganisms at any concentration tested. Specifically, no significant inhibition of microbial respiration was detected at any treatment level compared to the control data. At the termination of the test, the EC<sub>10</sub> and EC<sub>50</sub> for activated sludge microorganisms were determined to be >1,000 mg/L. For comparison, the concentration of active moiety in the POTW influent and effluent is estimated as 12.3  $\mu$ g active moiety/L (0.0123 mg/L). Therefore, no adverse effects on wastewater treatment processes are expected due to the usage of RLY5016S.

#### 6.2.2 Terrestrial Effects

The drug substance RLY5016S is expected to settle in sludge, which may be applied to land as biosolids. The determination of terrestrial effects concentrations of the polymer anion and its degradation product, fluoride, are described in the following sections.

#### 6.2.2.1 Polymer Anion

Terrestrial toxicity of the polymer anion was determined through a testing program designed for this EA. Details regarding these studies are described below.

#### Invertebrates

Testing was conducted to determine the acute toxicity of RLY5016S to earthworms (*Eisenia fetida*) (see Appendix J [confidential]). Testing procedures were performed in accordance with

OECD Guideline 207. The test exposed earthworms to nominal RLY5016S concentrations of 63, 130, 250, 500, and 1,000 mg/kg, based on the dry weight of soil. Effects on survival were reported as 7- and 14-day no observed effect concentration (NOEC) and median lethal concentration ( $LC_{50}$ ) values.

Earthworms exhibited no significant adverse effects due to RLY5016S at any concentration tested. No treatment-related health effects (e.g., lethargy, absence of burrowing) were observed during testing procedures. No significant reduction in survival was detected at any treatment level compared to the control data. Thus, the NOEC for RLY5016S was determined to be 1,000 mg/kg. The 7- and 14-day LC<sub>50</sub> values for RLY5016S were determined to be >1,000 mg/kg.

Both the terrestrial EIC and EEC were compared to the unbounded  $LC_{50}$  to calculate toxicity margins of safety for the active moiety (Table 7).

Toxicity Value	RLY5016S (mg/kg)	Active Moiety (mg/kg)	EIC (mg/kg biosolids)	EIC-based Margin of Safety	EEC (mg/kg amended soil)	EEC-based Margin of Safety
LC <sub>50</sub>	>1,000	>500	73.9	>6.8	6.70	>75

#### Table 7. Estimated Margin of Safety for RLY5016S on Terrestrial Invertebrates

The margin of safety for terrestrial invertebrates calculated on the basis of active moiety concentration in biosolids is >6.8. However, the safety factor increases to >75 when the  $LC_{50}$  is compared to active moiety concentration in amended soil. Based on the assumptions described in Section 6.1.4.1, this unbounded safety margin for amended soils is conservative, since the biosolids application rate is a conservative estimate. Therefore, no significant impact of RLY5016S on terrestrial invertebrates is expected due to projected usage of the drug product, RLY5016 for Oral Suspension.

#### Plants

Testing was conducted to determine the acute toxicity of RLY5016S to oat (*Avena sativa*), radish (*Raphanus sativus*), and lettuce (*Lactuca sativa*) seeds and seedlings. Testing procedures were performed in accordance with OECD Guideline 208. Testing exposed *A. sativa*, *R. sativus*, and *L. sativa* to nominal RLY5016S concentrations of 4.0, 16, 63, 250, and 1,000 mg/kg, based on dry weight of soil. Effects on percent emergence and fresh shoot weight were reported as 14-day 25% effective concentration (EC<sub>25</sub>), EC<sub>50</sub>, and NOEC values.

Oat and lettuce plants exhibited no significant adverse effects due to RLY5016S at any concentration tested. No treatment related morphological abnormalities (e.g., chlorosis or necrosis of leaves) were observed among these two species during testing procedures. No significant reduction in percent emergence or fresh shoot weight was detected at any treatment level compared to control data. Thus, the 14-day NOECs for oat and lettuce plants were determined to be 1,000 mg/kg. The EC<sub>25</sub> and EC<sub>50</sub> were determined to be >1,000 mg/kg for oats and lettuce.

RLY5016S had no significant adverse effects on the percent emergence of radish plants at any concentration tested (Table 8). However, a significant reduction in fresh shoot weight was observed in radish plants at 1,000 mg/kg. The 14-day NOEC for radish plants was determined to be 250 mg/kg. The EC<sub>25</sub> and EC<sub>50</sub> were determined to be 540 mg/kg and >1,000 mg/kg, respectively.

	NOEC (mg/kg)	EC <sub>25</sub> (mg/kg)	EC₅₀ (mg/kg)
Percent Emergence			
Oats (A. sativa)	1,000	>1,000	>1,000
Radish ( <i>R. sativus</i> )	1,000	>1,000	>1,000
Lettuce (L. sativa)	1,000	>1,000	>1,000
Fresh Shoot Weight			
Oats (A. sativa)	1,000	>1,000	>1,000
Radish ( <i>R. sativus</i> )	250	540	>1,000
Lettuce (L. sativa)	1,000	>1,000	>1,000

 Table 8. 14-day Acute Toxicity to Terrestrial Plants

Both the terrestrial EIC and EEC were compared to the unbounded  $EC_{50}$  values to calculate toxicity margins of safety for the active moiety (Table 9).

Toxicity Value	RLY5016S (mg/kg)	Active Moiety (mg/kg)	EIC (mg/kg biosolids)	EIC-based Margin of Safety	EEC (mg/kg amended soil)	EEC-based Margin of Safety
EC <sub>50</sub>	>1,000	>500	73.9	>6.8	6.70	>75
NOEC	250	125	73.9	1.7	6.70	19

Table 9. Estimated Margin of	of Safety for RLY5016S	on Terrestrial Plants
		•••••••••••••••••••••••••••••••••••••••

The margin of safety for terrestrial plants calculated on the basis of active moiety concentration in biosolids is >6.8. However, the safety factor increases to >75 when the EC<sub>50</sub> is compared to the active moiety concentration in amended soil. Based on the assumptions described in Section 6.1.4.1, this unbounded safety margin for amended soils is conservative since the biosolids application rate is a conservative estimate. Furthermore, the margins of safety, based on both active moiety concentration in biosolids and active moiety concentration in amended soil, remain >1 when compared to the lowest NOEC value (Table 9). Therefore, no significant impact of RLY5016S on terrestrial plants is expected due to projected usage of the drug product, RLY5016 for Oral Suspension.

#### 6.2.2.2 Fluoride

Fluoride toxicity data are available to assess potential effects on plants, soil invertebrates, and terrestrial wildlife (birds and mammals). Each type of organism is discussed below.

#### Plants

Fluoride toxicity to plants has been extensively studied; however, most studies have focused on air exposures or aqueous exposures (i.e., hydroponic exposures or administration of fluoride in irrigation water without soil fluoride analyses). Four studies were identified in which fluoride effects on plants were evaluated based on soil fluoride exposures. Results of three of these studies are summarized in Table 10. Each of these studies provided clearly identifiable NOEC and lowest observed effect concentration (LOEC) values.

Common Name	Species	Effect Level	Endpoint	Toxicity Value (mg/kg)	Reference
Barley	Hordeum vulgare	NOEC, unbounded	Dry matter yield	100	Elrashidi et al. 1998
Spinach	Spinacea oleracea	NOEC	Phytotoxicity	800	Jha et al. 2008
Spinach	Spinacea oleracea	LOEC	Root/shoot biomass	600	Jha et al. 2008
Onion	Allium cepa L.	NOEC	Phytotoxicity	200	Jha et al. 2009
Onion	Allium cepa L	LOEC	Phytotoxicity	400	Jha et al. 2009

 Table 10. Plant Toxicity to Fluoride Exposure in Soil

In addition, Singh et al. (2013) evaluated the effects of 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg sodium fluoride in soil on the growth parameters of *Raphanus sativus L*. All parameters evaluated exhibited a dose-dependent response, but NOEC and LOEC values were not reported. Root length after 45 days was the endpoint showing the greatest response compared to the control, with a difference of 16 and 29 percent at 50 mg/kg and 100 mg/kg of sodium fluoride, respectively.

#### Soil Invertebrates

Applicable data on fluoride toxicity to soil invertebrates are limited to two studies evaluating effects on the earthworm, *Eisenia fetida.* Translations of these two studies from the original German are provided in Appendix B. Vogel and Ottow (1992a) investigated acute effects of fluoride on earthworms, using OECD Guideline 207. Based on 28-day exposures to fluoride-spiked artificial soil, LC<sub>50</sub> values were determined as 4,278 mg/kg for sodium fluoride and 1,861 mg/kg for potassium fluoride. The authors also note that no acute toxicity was observed in preliminary tests with calcium fluoride at concentrations up to 20,000 mg/kg. Potassium fluoride had no significant effect on earthworm biomass, at concentrations up to 2,100 mg/kg (NOEC). The authors report significant differences in earthworm biomass in the sodium fluoride experiment, but NOEC and LOEC values were not reported. However, the lowest soil fluoride concentration associated with a decrease in dry biomass of more than 10% was 3,300 mg/kg.

Vogel and Ottow (1992b) reported chronic (22-week) effects of fluoride on earthworms, with endpoints including weight, sexual maturation, cocoon production, and production of offspring. For sodium fluoride, production of offspring was the most sensitive endpoint, with a significant negative effect reported at the lowest test concentration (unbounded LOEC = 600 mg/kg). For potassium fluoride, no effects on offspring production were observed; for the remaining test endpoints, the NOEC for potassium fluoride was 750 mg/kg and the LOEC was 1,000 mg/kg. No negative effects were observed due to calcium fluoride exposure at soil concentrations up to 16,000 mg/kg.

#### Mammals and Birds

Fluoride toxicity has been observed in livestock exposed to highly elevated concentrations associated with industrial sources, volcanic ash, and long-term phosphate fertilizer applications. Cronin et al. (2000) reviewed the fate, bioavailability, and effects of fluoride in pastures and identified concentration ranges potentially associated with adverse effects in New Zealand livestock. Sheep were considered to be at risk of possible fluorosis when total fluoride concentrations in topsoil were in the range of 372 to 1,461 mg/kg, while cattle were potentially at risk at soil concentrations of 326 to 1,085 mg/kg.

Pascoe et al. (2014) evaluated available mammalian and avian toxicity data to determine riskbased concentrations of fluoride in environmental media protective of terrestrial wildlife. Soil risk-based concentrations were identified for protection of coyotes, deer mice, horned larks, and red-tailed hawks. Coyotes were estimated to be the most sensitive of these four representative wildlife species, while red-tailed hawks were the more sensitive of the two bird species.

To identify toxicity reference values, Pascoe et al. (2014) reviewed studies of fluoride effects on mink, rats, bank voles, mice, red foxes, pigs, rabbits, cows, dogs, white-tailed deer, and owls. Endpoints included reproductive effects, dental and skeletal abnormalities, reductions in growth and survival, and clinical toxicity. Reproductive effects were judged to be the most appropriate for assessing risks to wildlife, and the most sensitive mammalian species to reproductive effects of fluoride was found to be the bank vole. Krasowska (1989) exposed two generations of bank voles to krill-based feed containing either low or high fluoride concentrations. Voles in the high dose group produced significantly fewer litters per female, and offspring mortality in the second generation was also elevated. As determined by Pascoe et al. (2014), these results support a no observed adverse effect level (NOAEL) of 5.3 mg fluoride per kilogram body weight per day (mg/kg-day) and a lowest observed adverse effect level (LOAEL) of 10.98 mg/kg-day. For birds, Pascoe et al. (2014) identified toxicity reference values from a study of Eastern screech owl fertility and hatching success (Pattee et al. 1988), which yielded a NOAEL of 8.02 mg/kg-day and a LOAEL of 32.9 mg/kg-day.

Pascoe et al. (2014) translated the wildlife toxicity reference values to environmental media concentrations based on relationships between soil and small mammal (i.e., prey) concentrations at a fluoride-contaminated site in Idaho. For the coyote, fluoride exposures were estimated to equal the NOAEL of 5.3 mg/kg-day at a soil fluoride concentration of 149 mg/kg dry weight, which corresponded to a small mammal fluoride concentration of 42 mg/kg wet weight. These risk-based concentrations are based on a coyote body weight of 13.6 kilograms (kg), a small mammal ingestion rate of 1.684 kilograms per day (kg/day) wet weight, and a soil

ingestion rate of 0.012 kg/day dry weight. For the red-tailed hawk, fluoride exposures were estimated to equal the NOAEL of 8.02 mg/kg-day at a soil fluoride concentration of 315 mg/kg dry weight, which corresponded to a small mammal fluoride concentration of 54 mg/kg wet weight. The risk-based concentrations for red-tailed hawks were based on a body weight of 1.056 kg, a small mammal ingestion rate of 0.215 kg/day wet weight, and a soil ingestion rate of 0.0017 kg/day. The calculated risk-based soil concentrations are very conservative, because they are based on whole-body fluoride concentrations in small mammals, whereas fluoride accumulates primarily in bone (Shore 1995). Coyotes and hawks do not completely ingest or digest the bones of their prey; thus, the analysis of Pascoe et al. (2014) is more likely to overestimate fluoride-related risks than to underestimate them.

#### **Risk Screening**

Among the plants, invertebrates, birds, and mammals discussed above, the most conservative risk-based soil concentration is selected as the terrestrial effects value for the screening-level analysis of potential environmental risks associated with fluoride. Specifically, the terrestrial effects value for fluoride is selected as 149 mg/kg, based on the risk-based concentration for protection of coyotes.

Both the aquatic EIC and EEC were compared to the terrestrial effects value to calculate toxicity margins of safety for fluoride (Table 11).

Terrestrial Effects Value (mg/kg)	EIC (mg/kg biosolids)	EIC-based Margin of Safety	EEC (mg/kg amended soil)	EEC-based Margin of Safety
149	13.9	10.7	1.26	118

#### Table 11. Estimated Margin of Safety for Fluoride on Terrestrial Receptors

Under the conservative assumptions used in this evaluation, the margin of safety for terrestrial organisms calculated on the basis of fluoride concentration in biosolids is 10.7. However, the safety factor increases to 118 when the terrestrial effects value is compared to fluoride concentration in amended soil. In actuality, it is very unlikely that 100% of the fluorine contained in RLY5016S would occur in surface soil as fluoride at any given time. In addition, the fluoride from RLY5016S is generated as calcium fluoride, which is poorly soluble (0.016 g/L) and poorly bioavailable (Trautner and Einwag 1987, ATSDR 2003). Further, the selected terrestrial effects value does not account for limited bioaccessibility of fluoride contained in the bones of prey. Taken together with the large margin of safety, these factors indicate that no significant impact of fluoride on terrestrial receptors is expected due to projected usage of the drug product, RLY5016 for Oral Suspension.

#### 6.2.3 Aquatic Effects

The drug substance RLY5016S is expected to settle in sludge, which may be applied to land as biosolids. RLY5016S is not expected to be present in treated POTW effluent. Nevertheless, as a conservative screening analysis, this EA includes an aquatic assessment. For simplicity we

make a hypothetical worst-case assumption that all RLY5016S partitions to effluent. The determination of aquatic effects concentrations of the polymer anion and its degradation product, fluoride, are described in the following sections.

#### 6.2.3.1 Polymer Anion

Aquatic toxicity of the polymer anion was determined through preliminary pilot studies and a testing program designed for this EA. Details regarding these studies are described below.

#### **Testing Program**

As part of the proposed EA testing program accepted by FDA, toxicity testing on the drug substance, RYL5016S, was conducted (see Appendix J [confidential]). Testing conformed to data quality standards associated with GLP as described by OECD (1998).

#### Algal Growth Inhibition

Testing was conducted to determine acute toxicity of RLY5016S to the freshwater green alga, *Pseudokirchneriella subcapitata*, formerly *Selenastrum capricornutum*. Testing procedures were performed in accordance with OECD Guideline 201. Testing exposed green algae to nominal RLY5016S concentrations of 5.4, 8.2, 13, 20, and 30 mg/L. Effects on biomass, calculated from cell density counts and expressed as yield and average growth rate, were reported as 72-hour EC<sub>10</sub>, 20% effective concentrations (EC<sub>20</sub>) and EC<sub>50</sub> values, as well as 72-hour NOEC and LOEC values.

Freshwater green algae exhibited no significant adverse effect due to RLY5016S at any concentration tested. No significant reductions to biomass, expressed as yield or average growth rate, were detected at any treatment level compared to control data. Thus, the 72-hour NOEC and LOEC for RLY5016S were determined to be 30 and >30 mg/L, respectively. Likewise, the 72-hour EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values for RLY5016S were all determined to be >30 mg/L.

Both the aquatic EIC and EEC were compared to the unbounded  $EC_{50}$  to calculate toxicity margins of safety for the active moiety (Table 12).

Effect Level	RLY5016S (mg/L)	Active Moiety (mg/L)	EIC (μg/L POTW effluent)	EIC-based Margin of Safety	EEC (µg/L surface water)	EEC-based Margin of Safety
EC <sub>50</sub>	>30	>15	12.3	>1,200	1.23	>12,000

#### Table 12. Estimated Margin of Safety for RLY5016S on Freshwater Algae

The margins of safety for freshwater green algae calculated on the basis of active moiety concentration in effluent and surface water are both >1,200. Therefore, no significant impact of RLY5016S on algae is expected due to projected usage of RLY5016 for Oral Suspension, and further testing of additional aquatic toxicity testing (i.e., fish and invertebrates) was not triggered.

#### Supplemental Data (Pilot Studies)

Pilot testing on RYL5016S was conducted to identify potential method modifications that might be needed in the event fish or aquatic invertebrate tests were required. Pilot tests were preliminary and did not conform to data quality standards associated with GLP. Preliminary algal tests are not described here since they are superseded by GLP-compliant testing described above. Pilot study reports are included as Appendix K (confidential).

#### Acute Toxicity to Water Fleas

Testing was conducted to determine the acute toxicity of RLY5016S to water fleas (*Daphnia magna*) under static conditions. Testing procedures were performed in accordance with OECD Guideline 202. Initial testing exposed *D. magna* to nominal RLY5016S concentrations of 10 and 30 mg/L (i.e., concentrations expected to greatly exceed EECs). No concentration resulted in  $\geq$ 50% immobilization, and so the 48-hour EC50 for RLY5016S was empirically estimated to be >30 mg/L. The NOEC was reported as <10 mg/L. Statistics were not performed, but all results were within 15% of the control.

#### Acute Toxicity to Rainbow Trout

Testing was conducted to determine the acute toxicity of RLY5016S to rainbow trout (*Oncorhynchus mykiss*) under static conditions. Testing procedures were performed in accordance with OECD Guideline 203. Initial testing exposed *O. mykiss* to nominal RLY5016S concentrations of 10 and 30 mg/L (i.e., concentrations expected to greatly exceed EECs). No sub-lethal effects were observed at either concentration. The 96-hour LC<sub>50</sub> was empirically estimated to be >30 mg/L and the NOEC was empirically estimated to be 30 mg/L.

#### 6.2.3.2 Fluoride

No federal water quality criteria are available for fluoride. However, the acute and chronic toxicity of fluoride to aquatic organisms have recently been evaluated by the Illinois Environmental Protection Agency (EPA) to develop revised fluoride water quality criteria for the protection of aquatic organisms (Illinois EPA 2010). These revised standards for fluoride have been incorporated into the *Numeric Standards for Chemical Constituents* (Title 35 of the Illinois Administrative Code, Part 302). Illinois EPA followed USEPA methodology for deriving the numerical water quality criteria (Stephen et al. 1985), which is outlined briefly below. The data used by Illinois EPA for criteria development are shown in Table 13.

Common Name	Species	Duration (hours)	LC₅₀ (mg/L)	Reference
Water flea	Daphnia magna	48	342	Fieser 1985
Water flea	Daphnia magna	48	251	Fieser 1985
Water flea	Daphnia magna	48	187	Fieser 1985
Water flea	Daphnia magna	48	114	Fieser 1985
Water flea	Ceriodaphnia dubia	48	248	Fieser 1985
Water flea	Ceriodaphnia dubia	48	180	Fieser 1985

Table 13. Fluoride Toxicity Data Used by Illinois EPA for Development of Criteria

Common Name	Species	Duration (hours)	LC <sub>50</sub> (mg/L)	Reference
Water flea	Ceriodaphnia dubia	48	182	Fieser 1985
Water flea	Ceriodaphnia dubia	48	122	Fieser 1985
Water flea	Simocephalus vetulus	24	201.5	Hickey 1989
Threespine stickleback	Gasterosteus aculeatus	96	340	Smith et al. 1985
Threespine stickleback	Gasterosteus aculeatus	96	380	Smith et al. 1985
Threespine stickleback	Gasterosteus aculeatus	96	460	Smith et al. 1985
Bluegill	Lepomis macrochirus	96	375.6	USEPA 2000b
Fathead minnow	Pimephales promelas	96	112.2	The Advent Group 2000 <sup>a</sup>
Fathead minnow	Pimephales promelas	96	190	Fieser 1985
Fathead minnow	Pimephales promelas	96	179	Fieser 1985
Fathead minnow	Pimephales promelas	96	134	Fieser 1985
Fathead minnow	Pimephales promelas	96	125	Fieser 1985
Midge	Chironomus tentans	96	124.1	Metcalfe-Smith et al. 2003
Amphipod	Hyalella azteca	96	25.8	Soucek and Dickinson 2010
Wavyrayed lampmussel	Lampsilis fasciola	96	172	Keller and Augspurger 2005
Paper pondshell	Utterbackia imbecillis	96	234	Keller and Augspurger 2005
Appalachian elktoe	Alasmidonta raveneliana	96	303	Keller and Augspurger 2005
Pheasantshell	Actinonaias pectorosa	96	259	Keller and Augspurger 2005
Pheasantshell	Actinonaias pectorosa	96	298	Keller and Augspurger 2005
Net-spinning caddisfly	Ceratopsyche bronta	96	17	Camargo et al. 1992
Net-spinning caddisfly	Hydropsyche occidentalis	96	34.7	Camargo et al. 1992
Net-spinning caddisfly	Hydropsyche bulbifera	96	26.3	Camargo and Tarazona 1990
Net-spinning caddisfly	Hydropsyche exocellata	96	26.5	Camargo and Tarazona 1990
Net-spinning caddisfly	Hydropsyche lobata	96	48.2	Camargo and Tarazona 1990
Net-spinning caddisfly	Hydropsyche pellucidula	96	38.5	Camargo and Tarazona 1990
Net-spinning caddisfly	Chimarra marginata	96	44.9	Camargo and Tarazona 1990
Net-spinning caddisfly	Cheumatopsyche pettiti	96	42.5	Camargo et al. 1992
Rotifer	Brachionus calyciflorus	24	183.3	Calleja et al. 1994
Snail	Physa sp.	96	163.1	The Advent Group 2000 <sup>a</sup>
Annelid	Lumbriculus variegatus	96	93.5	The Advent Group 2000 <sup>a</sup>
Mayfly	Hexagenia limbata	96	32.3	Metcalfe-Smith et al. 2003
Rotifer	Philodina acuticornis	96	212	Buikema et al. 1977

Table 13. Fluoride Toxicity Data Used by Illinois EPA for Development of Criteria

Common Name	Species	Duration (hours)	LC₅₀ (mg/L)	Reference
Grooved fingernailclam	Sphaerium simile	96	62.2	GLEC 2010

Table 42 Elucation Taxial	· Data Haad h	Ulinaia EDA fa	
Table 15. Fluoride Toxicity	y Data Used by		or Development of Criteria

 $LC_{50}$  – median lethal concentration

<sup>a</sup> Documentation from The Advent Group (2000) is provided in Appendix B.

Initially, available fluoride acute toxicity data providing LC<sub>50</sub> values (i.e., the concentration lethal to 50 percent of the tested organisms) were evaluated for inclusion in the standard derivation, based on appropriate organisms, valid test methods, and proper endpoints. Data gaps (i.e., less than the required number of taxonomic groups) were addressed with additional toxicity tests (Great Lakes Environmental Center 2010; Soucek and Dickinson 2010). Because fluoride toxicity is affected by water hardness and the available acute toxicity tests were conducted at varying hardness concentrations, acute toxicity data for each species were normalized to a hardness of 50 mg/L. Genus Mean Acute Values (GMAVs) were then calculated for each genus represented. The GMAVs were ranked from low to high, with the four lowest GMAVs used to calculate the Final Acute Value (FAV), following the formula provided in the guidelines (Stephen et al. 1985). The acute water quality standard for fluoride was calculated as one-half the FAV, or 6.92 mg/L, based on a hardness of 50 mg/L.

A chronic standard for fluoride may be derived from the ratio of the acute and chronic toxicity data available for at least three different families of species (one fish, one invertebrate, and one acutely sensitive species). To support the acutely sensitive species portion of the derivation, additional chronic toxicity data were developed for the amphipod *Hyalella azteca* by Soucek and Dickinson (2010). Ratios of the available acute to chronic toxicity data for each species (or the Acute-Chronic Ratios [ACRs]) were determined, and the geometric mean of the available ACRs was calculated to determine the Final Acute-Chronic Ratio (FACR). The chronic water standard for fluoride was determined by dividing the FAV by the FACR, resulting in a criterion value of 3.48 mg/L, based on a hardness of 50 mg/L.

For the protection of wildlife and livestock, Illinois EPA (2010) places an upper limit on the chronic fluoride standard of 4 mg/L.

Because the hardness of the effluent is unknown in this evaluation, a conservative approach is to use the acute and chronic standards for fluoride based on the relatively low hardness of 50 mg/L. Therefore, the aquatic effects values for fluoride in this evaluation are 6.92 mg/L and 3.48 mg/L for acute and chronic effects, respectively.

Both the aquatic EIC and EEC were compared to acute and chronic aquatic effect values to calculate margins of safety for fluoride (Table 14).

Exposure	Aquatic Effects Value (µg/L)	EIC (µg/L effluent)	EIC-based Margin of Safety	EEC (µg/L surface water)	EEC-based Margin of Safety
Acute	6,290	2.31	2,723	0.231	27,229
Chronic	3,480	2.31	1,506	0.231	15,065

Table 14. Estimated Margin of Safety for Fluoride on Aquatic Receptors

Acute and chronic margins of safety are >1,000. Therefore, no significant impact of fluoride on aquatic receptors is expected due to projected RLY5016 for Oral Suspension usage.

#### 6.2.4 Environmental Effects Summary

The RLY5016S active moiety is insoluble and is therefore expected to partition primarily to sludge. Terrestrial exposure will occur when this sludge is applied to soils as biosolids. For the purpose of the EA, this scenario was evaluated with other less likely scenarios (i.e., aquatic partitioning and/or degradation and release of fluoride) using a worst-case approach. In the preceding sections, the environmental effects of the polymer anion and fluoride were presented. The effects of active moiety were determined through toxicity testing of the drug substance RLY5016S, while its degradation product, fluoride, was screened through an examination of publicly available literature. Risks associated with the use and release of the drug product, RLY5016 for Oral Suspension, were determined through the comparison of these toxicity values to environmental exposure concentrations estimated in Section 6.1.4.

No significant ecological impacts are predicted to occur due to patient use of the drug product, RLY5016 for Oral Suspension, at the predicted rate of production. Conservative estimates of environmental concentrations of the polymer anion and any released fluoride are below biological effects levels. In each scenario, margins of safety are greatest when introduction concentrations (i.e., EICs) are diluted with other material (e.g., soil, surface water) expected to be present in typical exposures situations. In the anticipated terrestrial scenario, conservative estimates of the RLY5016S active moiety concentration in soil will be more than 75 times less than the EC<sub>50</sub> values determined for terrestrial invertebrates and plants. In the hypothetical aquatic scenario, conservative estimates of the RLY5016S active moiety concentration in surface water will be more than 1,500 times less than the EC<sub>50</sub> value determined for freshwater algae. If RLY5016S is found in the environment and breaks down to release all associated fluorides, the margins of safety exceed 100 for both terrestrial and aquatic scenarios using conservative estimates.

### 7 Mitigation Measures

No mitigation measures are needed because no adverse environmental effects have been identified.

### 8 Alternatives to the Proposed Action

Discussion of alternative courses of action is not needed because no adverse environmental effects have been identified.

### 9 List of Preparers

This document was prepared by the following consultants at ENVIRON International Corporation (ENVIRON): Phyllis Fuchsman, Senior Manager; Katrina Leigh, Manager; and Michael Ferguson, Senior Associate. Curriculum vitae for ENVIRON staff are provided in Appendix D. Curriculum vitae of the contract testing laboratory study directors are provided in Appendix L (confidential).

### **10 References**

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### **11 Certification**

The undersigned investigators certify that the information presented in this environmental assessment is true, accurate, and complete to the best of the knowledge of ENVIRON International Corporation.

us Julisman

7/28/14 Date

Phyllis Fuchsman Senior Manager ENVIRON International Corporation

### Appendix A

Data Summary Table (Not Confidential)

## Appendix A: Data Summary Table

RLY5016S	FLUORIDE					
Physical/Chemical Characterization						
Water Solubility						
expected to be insoluble; measured as <0.100 g/L (20 °C)	variable (e.g., CaF <sub>2</sub> = 0.016 g/L [20 °C], NaF = 40.4 g/L [20 °C])					
Dissociation Constant(s)						
not determined	not applicable					
Log Octanol/Water Partit	ion Coefficient (Log Kow)					
not determined	not applicable					
Vapor Pressure						
expected to be zero; not tested	negligible (e.g., NaF = 5.43 x 10 <sup>-26</sup> mm Hg [25 °C])					
Sorption/Desorption (Koc)						
not determined	not applicable					
Depletion Mechanisms						
Biodegradatic	on, Hydrolysis					
not tested due to analytical limitations	not applicable					
Environmental Effects						
Microbial	Inhibition					
EC <sub>10</sub> > 1,000 mg/L EC <sub>50</sub> > 1,000 mg/L	not determined					
Terrestrial Plants						
NOEC = 250 mg/kg EC <sub>25</sub> = 540 mg/kg EC <sub>50</sub> > 1,000 mg/kg	unbounded NOEC = 100 mg/kg NOEC = 200 mg/kg					
Terrestrial Invertebrates						
NOEC = 1000 mg/kg LC <sub>50</sub> > 1,000 mg/kg	unbounded LOEC = 600 mg/kg					
Terrestrial Wildlife						
not evaluated	NOAEL = 149 mg/kg					
Freshwater Algae	Aquatic Effects Value					
NOEC = 30 mg/L LOEC > 30 mg/L EC <sub>10</sub> > 30 mg/L EC <sub>20</sub> > 30 mg/L EC <sub>50</sub> > 30 mg/L	acute = 6.92 mg/L chronic = 3.48 mg/L					

## Appendix B

References (Not Confidential)

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# The Solubility and Behaviour of Acid Gases in the Marine Aerosol

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Abstract. The following Henry's law constants ( $K_{\rm H}$ /mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>) for HNO<sub>3</sub> and the hydrohalic acids have been evaluated from available partial pressure and other thermodynamic data from 0°-40 °C, 1 atm total pressure:  $HNO_3$ , 40 °C – 5.85 × 10<sup>5</sup>; 30 °C – 1.50 × 10<sup>6</sup>; 25 °C – 2.45 × 10<sup>6</sup>; 20 °C – 4.04 × 10<sup>6</sup>; 10 °C – 1.15 × 10<sup>7</sup>; 0 °C – 3.41 × 10<sup>7</sup>. *HF*, 40 °C – 3.2; 30 °C – 6.6; 25 °C – 9.61; 20 °C – 14.0; 10 °C – 32.0; 0 °C – 76. *HCl*, 40 °C – 4.66 × 10<sup>5</sup>; 30 °C – 1.23 × 10<sup>6</sup>; 25 °C – 2.04 × 10<sup>6</sup>; 25 °C – 2.04 × 10<sup>6</sup>; 25 °C – 2.04 × 10<sup>6</sup>; 20 °C – 3.37 × 10<sup>6</sup>; 10 °C – 9.71 × 10<sup>6</sup>; 0 °C – 2.95 × 10<sup>7</sup>. *HBr*, 40 °C – 2.5 × 10<sup>8</sup>; 30 °C – 7.5 × 10<sup>8</sup>; 25 °C – 1.32 × 10<sup>9</sup>; 20 °C – 2.37 × 10<sup>9</sup>; 10 °C – 4.5 × 10<sup>9</sup>; 0 °C – 1.5 × 10<sup>10</sup>. *HI*, 40 °C – 5.2 × 10<sup>8</sup>; 30 °C – 1.5 × 10<sup>9</sup>; 25 °C – 2.5 × 10<sup>9</sup>; 20 °C – 4.5 × 10<sup>9</sup>; 10 °C – 1.5 × 10<sup>10</sup>; 0 °C – 5.0 × 10<sup>10</sup>. Simple equilibrium models suggest that HNO<sub>3</sub>, CH<sub>3</sub>SO<sub>3</sub>H and other acids up to 10× less soluble than HCl displace it from marine seasalt acrosols. HF is displaced preferentially to HCl by dissolved acidity at all relative humidities greater than about 80%, and should be entirely depleted in aged marine acrosols.

Key words. Hydrobromic acid, hydrochloric acid, hydrofluoric acid, hydro-iodic acid, nitric acid, methanesulphonic acid, solubility, Henry's law, seasalt, aerosol, degassing, activity coefficient, Pitzer model, fluoride depletion.

## 1. Introduction

Naturally produced acids such as HNO<sub>3</sub>, methanesulphonic acid (MSA) and particularly  $H_2SO_4$  dissolve into aqueous aerosol droplets and accrete on dry aerosol particles in the atmosphere. This process causes the accumulation of excess sulphate (from  $H_2SO_4$ ) in marine aerosols and the displacement of chloride as HCl (Eriksson, 1960; Chesselet *et al.*, 1972; ten Brink *et al.*, 1982). In atmospheres affected by anthropogenic pollution, chloride may be completely replaced, by NO<sub>3</sub> for example (Martens *et al.*, 1973). Both thermodynamic predictions and ambient studies suggest that most aerosol systems can be regarded as at equilibrium with the surrounding gas phase (Stelson *et al.*, 1979; Tanner, 1982). Thus, soluble aerosols exist as either dry particles or solution droplets whose concentration is controlled by the ambient relative humdity. Very high solute concentrations may occur in the droplets, and modelling acid gas exchange in aerosol systems is therefore complicated by the nonideal behaviour of the solutions.

Clegg and Brimblecombe (1986), and earlier Denbigh (1971), have shown \* To whom all correspondence should be addressed. Present address: The Marine Biological Association of the U.K., The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK.

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that the partitioning of a strong acid HX between aqueous and gas phases is most simply represented as an equilibrium between gaseous HX and its dissolved aqueous ions

$$HX_g = H_{aq}^+ + X_{aq}^-.$$
(1)

The thermodynamic equilibrium constant  $K_{\rm H}$  (mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>) is given by

$$K_{\rm H} = m {\rm H}^+ \cdot m {\rm X}^- \cdot \gamma_{\rm HX}^2 / p {\rm HX}, \qquad (2)$$

where prefix 'm' indicates molal concentrations,  $y_{HX}$  is the mean activity coefficient of H<sup>+</sup> and X<sup>-</sup> ions in solution and pHX the equilibrium partial pressure of HX (Clegg and Brimblecombe, 1986). In this work we refer to  $K_{H}$  as a Henry's law constant, although the term is generally applied only to equilibria involving nondissociating solutes. However, as Denbigh (1971) has pointed out, the quantity  $mH^+ \cdot mX^-/pHX$  tends to a constant value as mHXtends to zero – thus HX exhibits Henry's law behaviour in very dilute solutions when the equilibrium is expressed in the form of Equation (1).

Accurate prediction of the activity coefficient  $\gamma_{HX}$  is essential to calculate partial pressures using Equation (2). Comparison with measurements of pHCland pHNO<sub>3</sub> over concentrated acidified salt solutions has shown that the Pitzer activity coefficient model is suitable (Clegg and Brimblecombe, 1987a, b). This model (e.g. Pitzer, 1979; Harvie and Weare, 1980; Millero, 1982) is based on an expression for the Gibbs excess energy of a solution as a virial expansion of terms in ionic concentration (Pitzer, 1973). Equations for ionic activity and osmotic coefficients are derived from this. A short description of the model is given in Clegg and Brimblecombe (1987a) together with a list of parameter values. Briefly, the model uses three or four parameters to describe osmotic and activity coefficients of single electrolytes. In multicomponent solutions further parameters  $\theta_{ii}$  account for interactions between ions of like sign in solution mixtures, and  $\psi_{ijk}$  for interactions between two ions of one sign and one ion of opposite sign. These parameters are particularly important in concentrated solutions. Activity coefficients at temperatures close to 25 °C may be calculated using the temperature derivatives (for the pure solution parameters) listed by Pitzer (1979). The  $\theta_{ij}$  and  $\psi_{ijk}$  parameters may be treated as constant.

The Pitzer model was developed for strong electrolytes and assumes complete dissociation in solution. This approach is satisfactory for HCl and HNO<sub>3</sub>, so the ion concentrations used in Equation (2) are stoichiometric or total values. For solutions containing  $H_2SO_4$  and weak acids such as  $H_3PO_4$  the interaction parameters must be replaced by, or combined with, the appropriate association constants (Pitzer *et al.*, 1977; Harvie *et al.*, 1984).

The Henry's law constants described by Equation (2) are usually determined from partial pressure measurements of the acids over their pure aqueous solutions, and tabulated mean activity coefficients. However, the partial pressure data are generally sparse and restricted to a small temperature range. They may also lack internal consistency, for example this is true of HCl (Clegg and Brimblecombe, 1986).

In this work we use available partial pressure and other thermodynamic data for MSA, nitric acid and the hydrohalic acids to derive Henry's law constants over the temperature range 0 to 40 °C. Simple modelling calculations are described which relate the solubility of these acids to equilibria involving the seasalt aerosol. The application of Equations (1) and (2) to the solubility of the weak acid HF is discussed, with particular reference to its possible displacement from the marine seasalt aerosol by other dissolved acids.

## 2. Henry's Law Constants of the Acid Gases

Tabulated mean activity coefficients for the acids are readily available, at least at 25 °C, and the data may be found as follows: HNO<sub>3</sub> and the hydrohalic acids, Hamer and Wu (1972); MSA, Covington *et al.* (1973). These are few determinations at temperatures other than 25 °C. Mean activity coefficients for HCl, HBr and HI can be calculated from the Pitzer equations using temperature derivatives of the interaction parameters given in Pitzer (1979). For HCl there exist extensive compilations of values by Harned and Owen (1958), Akerlof and Teare (1937), and Cerquetti *et al.* (1968). Data for HF over a range of temperatures are summarised by Hamer and Wu (1970).

The most commonly used partial pressure data for HCl, HBr, HI and HNO<sub>3</sub> are those compiled in the International Critical Tables (Washburn, 1926) and reproduced extensively elsewhere (e.g. Perry, 1963; Freier, 1978). More recent data for HF and HNO<sub>3</sub> are available (e.g. Brosheer *et al.*, 1947; Davis and DeBruin, 1964).

Henry's law constants can also be calculated indirectly, from the Gibbs free energy change for the reaction described by Equation (1). This approach is sometimes necessary where partial pressure measurements are unavailable or of uncertain accuracy. However, agreement between Henry's law constants calculated by both methods should be interpreted with caution, since it is not always clear that the partial pressure and other thermodynamic data are independent. For example, in a number of cases the activity coefficients of the acids are partly based upon partial pressure data.

It is clear from Equation (2) that the Henry's law constants of the acids depend on the activity coefficients used in the calculations. Throughout this work we have used values compiled by Hamer and Wu (1972) for 25 °C (see Table I), except in the case of MSA where the data of Covington *et al.* (1973) are used. The compilation of Hamer and Wu (1972) is generally consistent with values calculated using the Pitzer equations, which are based on the earlier compilation of Robinson and Stokes (1959). However, values of  $\gamma_{\rm HNO_3}$  given by Hamer and Wu (1972) differ significantly from those of Robinson and Stokes

Table I. Stoichiometric mean activity coefficients of HNO<sub>3</sub> and the hydrohalic acids at 25 °C, compiled by Hamer and Wu (1972). Equations and parameters are given below, where m is total concentration (mol kg<sup>-1</sup>)

т	а	b	С	d	Ι
0.001-0.05	0.76331	$2.7429 \times 10^{-6}$	-1.98157	4.6108	-0.801
0.05-0.5	0.54732	$1.5151 \times 10^{-4}$	-0.0458	0.008825	-1.3543
0.5-4.0	0.54932	$1.5151 \times 10^{-4}$	-0.0488	0.008825	-1.3518
4.0-20.0	0.45868	$1.3351 \times 10^{-2}$	-0.020858	0.017092	-1.3596

HNO<sub>3</sub>, HCl, HBr and HI:

 $\log(\gamma) = -0.5108 \ m^{1/2} / (1 + B^* m^{1/2}) + \beta m + Cm^2 + Dm^3 + Em^4 + Fm^5$ Parameter F is equal to zero for all acids except HCl ( $F = 5.258 \times 10^{-7}$ ).

Acid	т	B*	β	С	D	E
HNO <sub>1</sub> :	0-28	1.5824	$6.2432 \times 10^{-4}$	$-1.3137 \times 10^{-3}$	$-1.2866 \times 10^{-5}$	$4.9168 \times 10^{-7}$
HCl:	0-16	1.525	$1.0494  imes 10^{-1}$	$6.5360  imes 10^{-3}$	$-4.2058  imes 10^{-4}$	$-4.070 \times 10^{-6}$
HBr:	0-11	1.6468	$1.2457 \times 10^{-1}$	$8.8530  imes 10^{-3}$	$2.4750  imes 10^{-5}$	$-3.719 \times 10^{-5}$
HI:	0-10	1.900	$1.6188 \times 10^{-1}$	$-2.8100  imes 10^{-4}$	$1.1544  imes 10^{-5}$	$-8.233 \times 10^{-5}$

(1959), and are also inconsistent with values calculated by Davis and DeBruin (1964). Activity coefficients from these two earlier sources should not be used to calculate pHNO<sub>3</sub> from the Henry's law constants derived in this work.

The variation of Henry's law constants with temperature has also been calculated thermodynamically in the following way. The van 't Hoff equation states the relationship between equilibrium constant and temperature at constant pressure (Stumm and Morgan, 1981) as

$$d\ln(K)/dT = \delta H^{\circ}/(RT^2), \qquad (3)$$

where K is the thermodynamic equilibrium constant, T(K) is temperature, R (J K<sup>-1</sup> mol<sup>-1</sup>) the gas constant, and  $\delta H^{\circ}$  (J mol<sup>-1</sup>) is the enthalpy change for the reaction. For equilibria in the troposphere the relevant temperature range is about 0° to 40 °C. For this small range the change in heat capacity for the reaction,  $\delta C_p^*$  (J mol<sup>-1</sup>), may be regarded as constant. Integration of equation (3) with constant  $\delta C_n^{\circ}$  yields:

$$R \ln(K_2/K_1) = \delta H_1^{\circ}(1/T_1 - 1/T_2) + \delta C_p^{\circ}(T_1/T_2 - (1 + \ln(T_1/T_2)))$$
(4)

symbols with subscript 1 refer to values at 298 K; those with 2 to the temperature of interest. The Henry's law constants of the acids are evaluated below.

## 2.1. Hydrochloric Acid

Six references are given as principal sources for the International Critical Tables data (Washburn, 1926). Partial pressures are listed in the compilation for tem-

peratures between 0 °C and 110 °C and at concentrations from 2 to 46% by weight. However, the data derive essentially from two sets of partial pressure measurements of the acid vapour at 25 °C - that of Bates and Kirschman (1919) over the concentration range 3.24 to 10 mol kg<sup>-1</sup> and Dunn and Rideal (1924) from 0.465 to 5.94 mol kg<sup>-1</sup>. Values at other temperatures were calculated thermodynamically. The data of Dunn and Rideal appear to lack internal consistency and to be somewhat discordant with those of Bates and Kirschman. Dobson and Masson (1924), and more recently Ionin and Kurina (1964), Susarev and Prokof'eva (Hala et al., 1968) and Storonkin et al. (Hala et al., 1968) also provide HCl partial pressure data. Haase et al. (1963) have made partial pressure measurements and obtained values of osmotic and activity coefficients of the hydrohalic acids at very high concentrations. Fritz and Fuget (1956) have used existing thermodynamic data to directly calculate HCl partial pressures over solutions from 0.01 to 15.88 mol kg<sup>-1</sup> from 0 to 50 °C. In addition the authors have recently determined the solubility of HCl using a dynamic method (Clegg and Brimblecombe, 1986). The data at 25 °C, excluding that compiled by Hala et al. (1968), have been evaluated previously by Clegg and Brimblecombe (1986). The most self consistent data set is that of Fritz and Fuget (1956), which yields  $K_{\rm H}$  equal to  $2.04 \times 10^6 \text{ mol}^2 \text{ kg}^{-2}$  atm<sup>-1</sup> at 25 °C. The calculated partial pressures agree well with all measured values at this temperature (Clegg and Brimblecombe, 1986).

Using the activity coefficients of Akerlof and Teare (1937), values of  $K_{\rm H}$  were calculated for temperatures from 0 to 40 °C using the International Critical Tables and Fritz and Fuget (1956) partial pressure values. Results are shown in Figure 1. The International Critical Tables data at 30 and 40 °C yield very variable values of  $K_{\rm H}$ . Recalculation using the activity coefficient data of Cerquetti *et al.* (1968) yields similar results.

The Fritz and Fuget partial pressure data are self consistent and agree well with measurements at 25 °C, and the adoption of  $K_{\rm H}$  values calculated from their data is recommended. Values (at 5 °C intervals) are listed in Table II,

Table II. Recommended values of the Henry's law constants ( $K_{\rm H}/{\rm mol}^2 \, {\rm kg}^{-2} \, {\rm atm}^{-1}$ ) of the acid gases, calculated from available partial pressure and other thermodynamic data, over the temperature range 0 to 40 °C

T/°C	HF	HCI	HBr	HI	HNO <sub>3</sub>	CH <sub>3</sub> SO <sub>3</sub> H
0	76.0	$2.95 \times 10^{7}$	$3.00 \times 10^{10}$	$5.0 \times 10^{10}$	$3.41 \times 10^{7}$	_
5	49.0	$1.68 \times 10^{7}$	$1.54 \times 10^{10}$	$2.7 \times 10^{10}$	$1.95 \times 10^{7}$	-
10	32.0	$9.71 \times 10^{6}$	$8.10 \times 10^{9}$	$1.5  imes 10^{10}$	$1.15 \times 10^{7}$	_
15	21.0	$5.68 \times 10^{6}$	$4.30 \times 10^{9}$	$8.0  imes 10^{9}$	$6.75 \times 10^{6}$	-
20	14.0	$3.37 \times 10^{6}$	$2.37 \times 10^{9}$	$4.5 \times 10^{9}$	$4.04 \times 10^{6}$	
25	9.61	$2.04 \times 10^{6}$	$1.32 \times 10^{9}$	$2.5 \times 10^{9}$	$2.45 \times 10^{6}$	$6.5 \times 10^{13}$
30	6.60	$1.23 \times 10^{6}$	$7.50 \times 10^{8}$	$1.5 \times 10^{9}$	$1.50 \times 10^{6}$	-
35	4.60	$7.54 \times 10^{5}$	$4.30 \times 10^{8}$	$8.8 \times 10^{8}$	$9.33 \times 10^{5}$	_
40	3.20	$4.66 \times 10^{5}$	$2.50 \times 10^{8}$	$5.2 \times 10^{8}$	$5.85 \times 10^{5}$	-

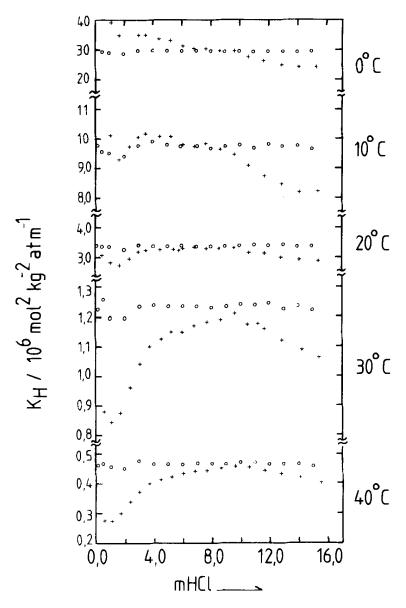


Fig. 1. Henry's law constants  $(K_{\rm H})$  for HCl over the temperature range 0 to 40 °C calculated using the activity coefficients of Akerlof and Teare (1937) and (+), partial pressure data from the International Critical Tables (Washburn, 1926), (°), partial pressures tabulated by Fritz and Fuget (1956).

which summarises the Henry's law constants for all the acids. The variation of  $K_{\rm H}$  with temperature was also calculated using the following thermodynamic data:  $\delta H_{1}^{\circ}$ , -74.88 kJ mol<sup>-1</sup>;  $\delta C_{p}^{\circ}$ , -165.6 J mol<sup>-1</sup> (Stull and Prophet, 1971; Rossini *et al.*, 1961). Values agree with those in Table II to within 1%.

## 2.2. Nitric Acid

Partial pressure data for nitric acid are available from a number of sources:

the International Critical Tables at concentrations from 20 to 100% by weight over the temperature range 0 to 100 °C; Davis and DeBruin (1964) from 2.28 to 37.8 mol kg<sup>-1</sup> at 25 °C; Tang *et al.* (1983) at unspecified low concentrations; Wilson and Miles (Hala *et al.*, 1968) from 0.222 to 0.72 mole fraction at 15 and 20 °C, and Yakimov and Mishin (Hala *et al.*, 1968) from 0.098 to 0.727 mole fraction at 25 °C, 0.0696 to 0.5404 mole fraction at 35 °C and 0.0761 to 0.581 mole fraction at 50 °C. Haase *et al.* (1965) have also made measurements from 2.0 to 28.0 mol kg<sup>-1</sup> at 25°, 50° and 75 °C; and at 20 °C Potier (1956) has measured partial pressures at aqueous phase concentrations from 7.0 to 23 mol dm<sup>-3</sup> and Vandoni and Laudy (1952) from 16 to 80 mole %.

Mean activity coefficients of HNO<sub>3</sub> at 25 °C are available up to concentrations of 28 mol kg<sup>-1</sup> (Hamer and Wu, 1972). This is sufficient to enable six values of partial pressure from the International Critical Tables to be used to calculate  $K_{\rm H}$ . The recent data of Davis and DeBruin (1964) are much more extensive. Davis and DeBruin (1964) have also adjusted the data of Potier (1956) and Vandoni and Laudy (1952) to 25 °C. All partial pressure data at 25 °C, except the unpublished data of Tang *et al.* (1983) are shown in Figure 2 as calculated Henry's law constants. The data of Potier (1956) appears to be discordant with that of the other workers, otherwise agreement is good. It should be noted that the partial pressure and activity coefficient data are not independent of one another; Hamer and Wu (1972) refer to both the Davis and DeBruin

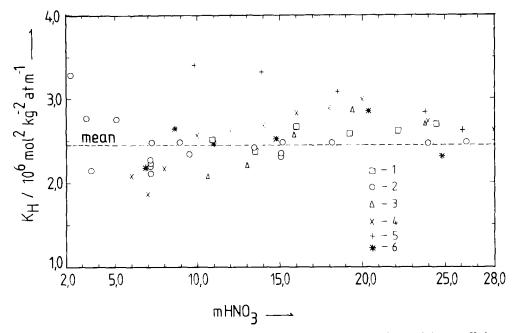


Fig. 2. Henry's law constant  $(K_{\rm H})$  of HNO<sub>3</sub> at 25 °C calcualted from the activity coefficients compiled in Hamer and Wu (1972) and the partial pressure data of: (1) Vandoni and Laudy (1952), (2) Davis and DeBruin (1964), (3) International Critical Tables (1926), (4) Haase *et al.*, (1965), (5) Potier (1956), (6) Yakimov and Mishin (Hala *et al.*, 1968). Both (1) and (5) were corrected to 25 °C.

(1964) and Haase *et al.* (1965) data as sources. The data of both Haase *et al.* (1965) and the International Critical Tables show a trend toward higher  $K_{\rm H}$  with increasing concentration. Because of this only the data of Davis and DeBruin (1964), Vandoni and Laudy (1952) and Yakimov and Mishin (Hala *et al.*, 1968) have been used to calculate a mean  $K_{\rm H}$  at 25 °C of 2.45 × 10<sup>6</sup> mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>, standard deviation 0.18 × 10<sup>6</sup>. All the data points used were given equal weight.

Schwartz and White (1981) used the data of Davis and DeBruin (1964) to calculate an equivalent  $K_{\rm H}$  of  $3.26 \times 10^6 \text{ mol}^2 \text{ dm}^{-6} \text{ atm}^{-1}$ , some 30% higher than that obtained here. This apparent discrepancy is due to the fact that Schwartz and White (1981) used activity coefficients estimated by Davis and DeBruin (1964), which are inconsistent with  $\gamma_{\rm HNO_3}$  derived by Hamer and Wu (1972). It is therefore stressed that the activity coefficients of Hamer and Wu (1972) should be used when calculating partial pressures from the Henry's law constants derived in this work. For calculations involving mixtures the Pitzer model parameters for HNO<sub>3</sub> have been refitted to the Hamer and Wu data (Clegg and Brimblecombe, 1987a).

Three measurements of HNO<sub>3</sub> partial pressures made by the present authors over 2.5, 5.0, and 6.0 mol kg<sup>-1</sup> nitric acid agree closely with the Henry's law constant of  $2.45 \times 10^6$  mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>. Since tabulated partial pressures at lower temperatures are mostly for very high aqueous concentrations where activity coefficients are not readily available, values of  $K_{\rm H}$  at temperatures from 0 to 40 °C were calculated using the following thermodynamic data:  $\delta H_1^{\circ}$ , -73.1 kJ mol<sup>-1</sup>;  $\delta C_p^{\circ}$ , -140.04 J mol<sup>-1</sup> K<sup>-1</sup> (Stull and Prophet, 1971; Rossini *et al.*, 1961). Results are listed in Table II.

## 2.3. Hydrobromic and Hydro-iodic Acids

The only existing partial pressure data for these two gases at aqueous phase concentrations for which activity coefficients are available are those compiled in the International Critical Tables from measurements of Bates and Kirschman (1919), with a few additional values, mostly at extremely high concentration, reported by Haasse *et al.* (1963). Some of these are used here, where consistent with the earlier data. All these data are plotted as values of  $K_{\rm H}$  in Figures 3 and 4. While the self consistency of the Bates and Kirschman data for HCl is excellent (Clegg and Brimblecombe, 1986), that for HI and HBr is less so, probably due to the fact that these two acids are much less volatile. Mean values of  $K_{\rm H}$  at 25 °C are: HBr,  $13.2 \times 10^8$  mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>, standard deviation  $1.45 \times 10^8$ ; HI,  $25 \times 10^8$  mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>, standard deviation  $1.45 \times 10^8$ ; Gare: HBr,  $13.2 \times 10^8$  mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>, standard deviation  $1.45 \times 10^8$ ; Gare: HBr,  $13.2 \times 10^8$  mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>, standard deviation  $1.45 \times 10^8$ ; HI,  $25 \times 10^8$  mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>, standard deviation  $1.45 \times 10^8$  mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup> for HI. The Bates and Kirschman (1919) and Haase *et al.* (1963) partial pressure data are in reasonable agreement therefore the Henry's law constant calculated from their data is probably more nearly correct. The two values of  $K_{\rm H}$  for HI are consistent with one another,

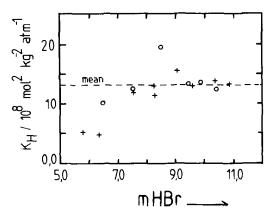


Fig. 3. The Henry's law constant  $(K_{\rm H})$  of HBr at 25 °C calculated using the activity coefficients tabulated in Hamer and Wu (1972) and (+), partial pressure data of Bates and Kirschman (1919); (°), partial pressure data of Haase *et al.* (1963).

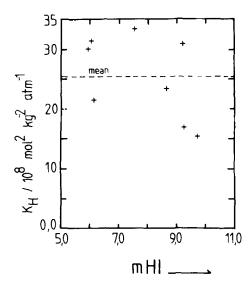


Fig. 4. The Henry's law constant  $(K_{\rm H})$  of HI at 25 °C calculated using the activity coefficients tabulated in Hamer and Wu (1972) and the partial pressure measurements of Bates and Kirschman (1919).

and the value calculated from the partial pressure data is retained here. Equation (4) has been used to calculate the variation of  $K_{\rm H}$  with temperature for both acids, see Table II. Values of  $\delta H_1^{\circ}$  and  $\delta C_p^{\circ}$  at 298 K are as follows for HBr:  $\delta H_1^{\circ}$ , -85.16 kJ mol<sup>-1</sup>;  $\delta C_p^{\circ}$ , -40.87 J mol<sup>-1</sup>; for HI:  $\delta H_1^{\circ}$ , -81.6 kJ mol<sup>-1</sup>;  $\delta C_p^{\circ}$ , -113.2 J mol<sup>-1</sup> (Stull and Prophet, 1971; Rossini *et al.*, 1961).

## 2.4. Hydrofluoric Acid

The partial pressure of HF over aqueous solutions has been evaluated comparatively recently because of its importance in industrial processes: Freden-

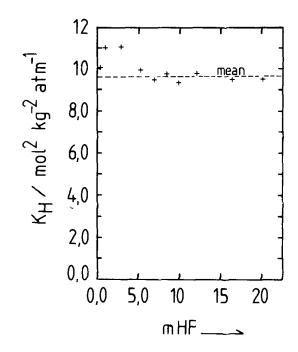


Fig. 5. The Henry's law constant  $(K_{\rm H})$  of HF at 25 °C calculated using the activity coefficients tabulated in Hamer and Wu (1972) and the partial pressure measurements of Brosheer *et al.* (1947). Note that the two values of  $K_{\rm H}$  greater than 11 were ignored in the calculation of the mean.

hagen and Wellman (1932), Brosheer *et al.* (1947), Munter *et al.* (1949). Brosheer *et al.* (1947) measured the partial pressure by a dynamic method similar to that used by Bates and Kirschman (1919) at aqueous concentrations between 2 and 30% HF at the temperatures 25, 40, 60, and 75 °C. At least 10 different concentrations were measured at each temperature. Using tabulated values of the mean activity coefficient of HF (Hamer and Wu, 1972), values of  $K_{\rm H}$  at 25 °C were calculated, see Figure 5. The mean value of  $K_{\rm H}$  is 9.61 mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>, standard deviation 0.244. The internal consistency of the data is good. The data of Munter *et al.* (1949) were mostly obtained for systems at very high temperature and concentration, and were not usable to calculate  $K_{\rm H}$ . The data of Fredenhagen and Wellman (1932) have also not been used, since calculations of  $K_{\rm H}$  at 25 °C show poor precision and yield values inconsistent with the work of Brosheer *et al.* (1947).

Much of the data of Brosheer *et al.* (1947) are for temperatures too high to be relevant to the atmosphere. Therefore, values of  $K_{\rm H}$  from 0 to 25 °C were obtained by plotting measured partial pressures against the reciprocal of temperature and extrapolating the best fit straight lines to 0 °C. The estimated partial pressures were then used with the activity coefficient data of Hamer and Wu (1970) to calculate  $K_{\rm H}$ . These values agree to within about 15% with those listed in Table II, calculated using the experimentally determined  $K_{\rm H}$  at 25 °C and the following thermodynamic data:  $\delta H_{1,}^{\circ}$ , -56.61 kJ mol<sup>-1</sup> and  $\delta C_{p,}^{\circ}$ , -36.46 J mol<sup>-1</sup> K<sup>-1</sup>, (Stull and Prophet, 1971; Rossini *et al.*, 1961).

## 2.5. Methanesulphonic Acid

Activity coefficients of MSA at 25 °C, 0-6.0 mol kg<sup>-1</sup>, can be calculated using the Pitzer equation (Pitzer and Mayorga, 1973) with the following parameters:  $\beta^{(0)}$ , 0.1544;  $\beta^{(1)}$ , 0.4775; C<sup>\$\phi\$</sup>, -0.00409. The original data (up to 40 mol kg<sup>-1</sup>) are given by Covington *et al.* (1973). Clegg and Brimblecombe (1985a) have measured the partial pressure of MSA over concentrated aqueous solutions of the acid at 25 °C, and derived a Henry's law constant of  $6.5 \times 10^{13}$  mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>. However, experimental difficulties and the extrapolation of activity coefficient data mean that this value may be inaccurate by as much as an order of magnitude. Because of this uncertainty and the fact that MSA is so soluble that it will always partition into atmospheric water, no attempt has been made to estimate the variation of  $K_{\rm H}$  with temperature.

## 3. Strong Acids in the Marine Atmosphere

While  $H_2SO_4$  simply accumulates in the marine aerosol because of its high solubility  $HNO_3$ , HCl and the other hydrogen halides are sufficiently insoluble to be transferred between aqueous and gaseous phases in the atmosphere.

A simple box model was constructed to relate the Henry's law constants determined above to real atmospheric behaviour, in particular the displacement of HCl from seasalt aerosols. The system consisted of a 1 m<sup>3</sup> box at 25 °C containing a solution phase of Na<sup>+</sup> and Cl<sup>-</sup> ions (representing seasalt) in a mass W (kg) of water. The gas phase contained water in equilibrium with the solution and an acid gas HX. This was hypothetical in that its Henry's law constant ( $K_{\rm HX}$ ) was made a variable parameter in the equations.

From the equation describing the Henry's law behaviour of the strong acids, and a knowledge of the total amount of each component in the system, the following conservation equations describe the system at equilibrium:

$$m \mathrm{H}^{+}[W + 41.67(m \mathrm{Cl}^{-} \cdot \gamma_{\mathrm{HCl}}^{2}/2.04 \times 10^{6} + m \mathrm{X}^{-} \cdot \gamma_{\mathrm{HX}}^{2}/K_{\mathrm{HX}})] - \mathrm{H}^{T} = 0 \quad (5)$$

for each volatile acid anion:

$$m Cl^{-}[W + 41.67 m H^{+} \cdot \gamma_{HCl}^{2} / K_{HCl}] - Cl^{T} = 0,$$
 (6a)

$$mX^{-}[W + 41.67 mH^{+} \cdot \gamma_{HX}^{2} / K_{HX}] - X^{T} = 0,$$
(6b)

where  $m \pmod{\text{kg}^{-1}}$  denotes aqueous phase concentration and superscript T indicates the total quantity of the component in the system. The factor 41.67 is the reciprocal of the molar volume of an ideal gas at normal temperature and pressure. The equations were solved iteratively for  $m\text{H}^+$ , and the partitioning of the gases HCl and HX determined for varying  $K_{\text{HX}}$ . Activity coefficients used in the calculations (e.g.  $\gamma_{\text{HCl}}$  in aqueous NaCl) were derived directly from experimental data (Hawkins, 1932). The behaviour of HX in the solution phase was considered to be that of a typical strong acid, so values of the mean activity  $\frac{48}{48}$ 

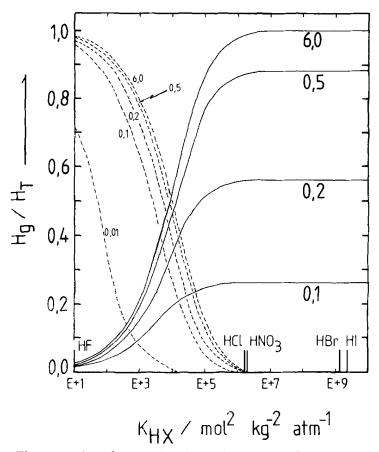


Fig. 6. The proportion of the total hydrogen ion content of the system present in the gas phase as HX (dotted line) and HCl (solid line) for different values of  $K_{HX}$ . The aqueous phase is NaCl, at the following concentrations: 0.01, 0.1, 0.2, 0.5, 1.0, 6.0 mol kg<sup>-1</sup>. H<sub>g</sub>/H<sub>T</sub> for mNaCl equal to 0.01 mol kg<sup>-1</sup> is very small and not shown. Values of the Henry's law constants of HNO<sub>3</sub> and the hydrohalic acids are marked.

coefficient  $\gamma_{HX}$  were set equal to those of HCl. Calculations show that errors, e.g. for  $\gamma_{HNO_3}$ , vary from about +8% (I = 1 mol kg<sup>-1</sup>) to +80% (I = 6 mol kg<sup>-1</sup>). This implies that HNO<sub>3</sub> is more soluble than the model calculations suggest, and will have a greater tendency to displace HCl than the calculations for 'HX' show (see results below).

The total quantity of NaCl in the box was  $40 \times 10^{-8}$  mol, a typical value for the marine troposphere (Blanchard and Woodcock, 1980). The total concentration of HX was set at 1% of the salt content,  $0.4 \times 10^{-8}$  mol m<sup>-3</sup>, comparable to that of HCl and HNO<sub>3</sub> in the marine troposphere (Vierkorn-Rudolph *et al.*, 1984; Kelly *et al.*, 1980).

The salt concentration in the aqueous aerosol phase is determined by the ambient relative humidity. Calculations were made in this study for 0.01 to 6 mol kg<sup>-1</sup> NaCl, which correspond to relative humidities of >99% to about 80% respectively. Figure 6 shows the gas phase speciation of the total acid content of the model box for different ionic strengths. It is clear that HX resides

almost entirely in the aqueous phase for solubilities up to an order of magnitude lower than that of HCl. For example, when  $K_{\rm HX}$  is equal to  $10^5 \text{ mol}^2 \text{ kg}^{-2}$ atm<sup>-1</sup>, less than 15% of total HX is present in the gas phase. This implies that in the real atmosphere gases such as MSA and even HNO<sub>3</sub> will partition strongly into the aqueous phase. For ionic strengths greater than 0.1 mol kg<sup>-1</sup> (relative humidity less than 99.7%) a significant amount of HCl always resides in the gas phase. This may be interpreted as an HCl degassing process. Earlier model calculations have shown that this process is little affected by temperature, and is enhanced for particles of radius less than about  $0.1 \times 10^{-6}$  m (Clegg and Brimblecombe, 1985b).

Acids much less soluble than HCl cause it to degas because chloride concentrations are always very high in a marine aerosol, about 80% of the total ionic strength. Hydrochloric acid is about three orders of magnitude more volatile than HI and HBr. Because of this and the fact that Cl<sup>-</sup> is present in seawater at a higher concentration than both  $Br^-$  and  $I^-$  then loss of  $H^+$  to the gas phase by degassing from the seasalt aerosol will be as HCl. Displacement of HCl is likely to be small only for acids with Henry's law constants less than about 10<sup>3</sup> mol<sup>2</sup> kg<sup>-2</sup> atm<sup>-1</sup>. Formic and acetic acids have Henry's law constants of  $5.6 \times 10^3$  mol dm<sup>-3</sup> atm<sup>-1</sup> and  $8.8 \times 10^3$  mol dm<sup>-3</sup> atm<sup>-1</sup>. respectively (Keene and Galloway, 1986). Considering the fact that they are weak and relatively insoluble acids, it seems unlikely that they will displace HCl significantly. The box model results show that HF should remain in the gas phase, being much less soluble than HCl. This important result appears to confirm the suggestion of Cadle (1980) and Wilkniss and Bressan (1971) that HF might be degassed with HCl from acidified aerosols in the atmosphere. This is investigated in more detail below since these calculations do not take into account the complexation of  $F^-$  ions in real seawater solutions, or the fact that HF is a weak acid.

## 4. The Displacement of HF from Marine Aerosols

The major mechanism for the loss of H<sup>+</sup> from acidified seasalt aerosols is the degassing of HCl. For HF to be displaced it must have a partial pressure comparable with that of HCl in the aerosol droplets. The partial pressures of these acids have therefore been calculated over 'model' acidified seasalt aerosols, which were treated as unbuffered solutions containing the major ions of seawater Na<sup>+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and F<sup>-</sup> in the ratios 0.497 : 0.0655 : 0.569 : 0.0295 : 7.4 × 10<sup>-5</sup>. The partial pressure of HCl in the acidified droplets can be readily calculated using the Pitzer model to estimate  $\gamma_{\text{HCl}}$ . However, this is not the case for HF. The chemistry of fluoride in seawater is complicated by the fact that F<sup>-</sup> is complexed by bivalent metal ions, particularly Mg<sup>2+</sup> and Ca<sup>2+</sup>, and associates with H<sup>+</sup> to form HF<sup>o</sup>. In seawater at normal ionic strength, (with a total fluoride concentration of about  $7 \times 10^{-5}$  mol kg<sub>50</sub><sup>-1</sup>), only about 49% of this is present as

free F<sup>-</sup>, the balance being 49% MgF<sup>+</sup> and about 2% CaF<sup>+</sup> (Millero, 1982).

Strong association such as this cannot be parameterised within the Pitzer model. Following the approach of Whitfield (1975) and Millero (1982, 1983) these association equilibria are treated explicitly and replace the  $H^+-F^-$ ,  $Mg^{++}-F^-$  and  $Ca^{++}-F^-$  interactions in the model. Stability constants for HF° and MgF<sup>+</sup>, and the activity coefficient of F<sup>-</sup> in NaCl media have recently been determined by Clegg and Brimblecombe (1988). The stability constant of CaF<sup>+</sup> is unavailable for ionic strengths greater than about 0.7 mol kg<sup>-1</sup>, but this species is of minor importance and has therefore been ignored.

A preliminary question that was first addressed was whether  $F^-$  might be precipitated in concentrated seasalt solutions as MgF<sub>2</sub> or CaF<sub>2</sub>, both of which are very insoluble. Calculations using published solubility product data and the Pitzer model to estimate ionic activities suggested that this was not the case, Ca<sup>2+</sup> being precipitated as CaSO<sub>4</sub> for example. The partial pressures of HF and HCl over model aerosols were calculated by first solving the following simultaneous equations to determine ion speciation:

$$mF^{-} = mF^{T}/(1 + K^{*} \cdot mMg^{2*} + mH^{+}/K^{1}),$$
(7a)

$$mH^{+} = mH^{T}/(1 + mF^{-}/K^{1}),$$
 (7b)

$$mMg^{2+} = mMg^{T}/(1 + K^{*} \cdot mF^{-}),$$
 (7c)

where  $K^1$  is the stoichiometric dissociation constant of HF° and  $K^*$  the stability constant of MgF<sup>+</sup>. Superscript T indicates a total concentration, and other values are for free ions. The concentration of added  $H^+$  was 0.0001 mol kg<sup>-1</sup>. The Pitzer model was used to estimate the activity coefficients of the free ions  $H^+$ ,  $Cl^-$  and  $F^-$  and equation (2) applied to calculate partial pressures. The results are shown in Figure 7 as partial pressures of HCl and HF over the acidified seasalt aerosols from 84 to 99.5% relative humidity (ionic strength 5.0 to 0.25 mol kg<sup>-1</sup>). Partial pressures were also calculated using values of  $K^1$ measured in seawater by Perez and Fraga (1987) (and corrected for MgF<sup>+</sup> formation). These agreed to within a few percent with those shown in Figure 7. The partial pressure of HF is substantially greater than that of HCl over most of the relative humidity range, and it is clear that HF will be degassed preferentially to HCl. The large difference in partial pressures suggests that this conclusion is unlikely to be affected by errors in the stability constants and their use for solutions containing high concentrations of  $Mg^{2+}$ , and also  $SO_4^{2-}$ . (This anion was included in the model solutions because of its strong interactions with both  $H^+$  and  $Mg^{2+}$ .)

Chesselet *et al.* (1972) estimated that a minimum fract ion of 3% of aerosol chloride is converted to gas phase species (HCl) over the oceans. This implies flux of acidity into the aerosol sufficient to completely remove  $F^-$  as HF, giving an HF flux of up to 0.01 Tmol  $a^{-1}$  from a seasalt production of 500 Tg  $a^{-1}$ 

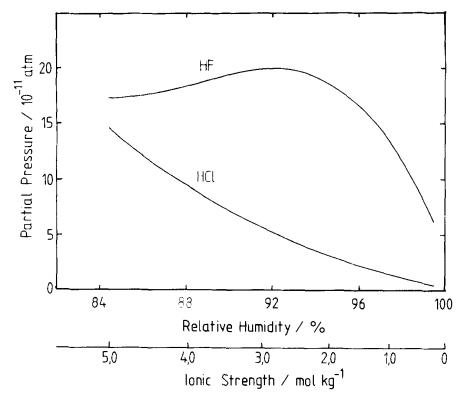


Fig. 7. Calculated equilibrium partial pressures of H F and HCl above a model seasalt aerosol containing the ions Na<sup>+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and F<sup>-</sup>. Ionic strengths in the aerosol solutions range from 0.25 to 5.0 mol kg<sup>-1</sup>. All solutions were acidified by 0.0001 mol kg<sup>-1</sup> H<sup>+</sup>.

(Blanchard, 1985). This is almost 50% of the estimated volcanic flux, the only other natural source of HF (Cadle, 1980).

## 5. Conclusions

The Henry's law constants presented here, together with the Pitzer activity coefficient model, should enable the partial pressures of MSA,  $HNO_3$  and the hydrogen halides to be calculated for concentrated saline solutions over the range of temperatures found in the atmosphere. Model calculations have shown that dissolved MSA and  $HNO_3$  should expel HCl from the marine seasalt aerosol. This occurs despite the fact that  $HNO_3$  has a Henry's law constant very close to that of HCl. The weak acid HF is almost five orders of magnitude less soluble than HCl and calculations suggest HF will be degassed from the aerosol preferentially to HCl. In terms of the total flux of acidity this effect is small. However, the marine aerosol is likely to be totally depleted in fluoride, and could be a significant natural source of HF in the troposphere.

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## Comparative Acute Toxicity of the First 50 Multicentre Evaluation of *In Vitro* Cytotoxicity Chemicals to Aquatic Non-Vertebrates

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Abstract. The acute toxicity data of the first 50 chemicals of the Multicentre Evaluation of In Vitro Cytotoxicity (MEIC) programme is compared for three "cyst-based toxicity tests" (Artoxkit M with Artemia salina, Streptoxkit F with Streptocephalus proboscideus, and Rotoxkit F with Brachionus calyciflorus), and two other tests (the Daphnia magna and the Photobacterium phosphoreum Microtox (19) tests) commonly used in ecotoxicology. The difference in sensitivity for the 50 chemicals was as high as 19 orders of magnitude (on a molecular weight basis) between the most and least sensitive species. Generally, a similar toxicity ranking of the 5 test species was found for most of the chemicals and the interspecies correlations were high. Results from Principal Component Analysis (PCA) and cluster analysis indicated that the groupings are not related to a clear and defined chemical structure. However, the loading plot of the first two principal components may aid in selecting the minimum number and type of tests that have to be included in a battery which encompasses a broad spectrum of toxicity levels. Consequently, this study supports the use of a selected battery of tests to evaluate ecotoxicity and suggests its possible importance for screening of biologically-active compounds from natural sources.

In recent years, acute toxicity tests involving aquatic non-vertebrates have been used not only for ecotoxicological evaluation (Blanck *et al.* 1984; Dawson *et al.* 1975/77; Qureshi *et al.* 1982; Vasseur *et al.* 1984; Calleja *et al.* 1986; Dutka and Kwan 1988; Vittozi and De Angelis 1991), but have also been proposed as alternative teratogen test systems (Kerster and Schaeffer 1983; Goss and Sabourin 1985), and as alternatives to predict human acute toxicity (Stadtlander and Lawohnus 1990; Calleja and Persoone 1992; Calleja *et al.* 1993). Bioassays based on aquatic non-vertebrates have also been utilized for screening of biologically-active compounds isolated from natural sources (Ferrigni *et al.* 1984; Abdel-Hafez *et al.* 1987; el-Maghraby and el-Maraghy 1987; el-Maraghy 1988; Macrae et al. 1988; Bottalico et al. 1990; Combrinck et al. 1991; Wang et al. 1990; Wood et al. 1990; Bories et al. 1991; Del Rayo Camacho 1991; Anderson et al. 1992). A combination of these tests has been recommended by the OECD (cf. Lange 1992) and the EEC (cf. Vosser 1986) to detect adverse effects of xenobiotics to organisms belonging to different trophic levels of an ecosystem. In phytochemical screening, instead of a single bioassay, a set of tests is also used for sorting active fractions of plant extracts (Ferrigni et al. 1984; Macrae et al. 1988; Bories et al. 1991) and fungal strains (Suarez et al. 1981; Diener et al. 1981; Park et al. 1986; Wilson et al. 1986; Stoessl et al. 1989; Faid et al. 1989; Richardson and Hamilton 1990; Savard et al. 1990; Visconti et al. 1992). The latter approach eventually leads to a considerable reduction in cost and time in elucidating the properties and structure of the compound by more sophisticated methods (Harwig and Scott 1971). The use of invertebrates has particularly increased in phytochemical screening due to the simplicity, rapidity and low cost of such bioassays (Meyer et al. 1982; Watson and Lindsay 1982).

Considering the varying degrees of sensitivity among the numerous test criteria and test species available, one is faced with the problem of choosing the type and number of species or test systems to include in a battery with optimal (eco)toxicological information. Persoone and Janssen (1993) have partly addressed this problem by suggesting that the selection of suitable elements for a battery of tests primarily depends on the mode of action of the toxicant. In earlier studies, an approach that was often used is correlation analysis (Kenaga 1978). Interspecies correlation has been used to determine the feasibility of predicting toxicity from one species to another (Kenaga 1978; Doherty 1983; Suter et al. 1983; Nendza and Klein 1990). When toxicities of structurally-related compounds as well as those with similar use are considered, improvement in interspecies correlations have been observed by Kenaga (1978) and in QSAR studies by Cronin et al. (1991). A plausible reason for such findings has been suggested by Cronin et al. (1991), that is, chemicals having similar structure act through a common mode of action.

On the other hand, Gillette (1965) and Ariëns (1971) have underlined the danger in assuming the same mechanism of

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action for different compounds (drugs) evoking similar (pharmacologic) responses (*e.g.*, diuretics, hypotensives) and for structurally-related compounds. Although apparently structurally-related chemicals may interact with common receptors through a common mode of action, *in vivo* situation reveals that compound (drug)-receptor interactions may differ essentially in their pharmacokinetics (Ariëns 1971).

It is also well known and widely accepted that interpretation of correlation between pairs of variables is unsatisfactory, even if significant correlations exist (Box *et al.* 1978). This is especially the case when more than one correlated pair exists.

Since the development of "cyst-based toxkit tests" for costeffective ecotoxicological screening of chemicals and wastes, a comparison of the sensitivities of these tests with the conventional acute Daphnia magna and Microtox metests has not been carried out on a large array of pure and structurally-diverse chemicals. One of the aims of the present study, therefore, is to compare the sensitivity of the aquatic invertebrates used in the Toxkits (Artemia salina, Streptocephalus proboscideus, Brachionus calyciflorus) and the Daphnia magna and Microtox® (Photobacterium phosphoreum) for the 50 chemicals in the priority list of the Multicentre Evaluation of In Vitro Cytotoxicity (MEIC) programme (Bondesson et al. 1989). The majority of these chemicals are of ecotoxicological and pharmacological interest. The organic chemicals, in particular, may represent prototypes of natural active substances such as alkaloids (nicotine, atropine, quinidine, caffeine), glycosides (digoxin, warfarin, salicylate), and microbial by-products (chloramphenicol). This study also aims at determining the use of a multivariate method as an alternative to interspecies correlation, and attempts to examine chemical groupings based on the toxic responses of the aquatic organisms. This may provide insight into the important determinants of species sensitivity and may help in selecting which of the species to include in the battery that can either detect a broad spectrum of (eco)toxicological activities or serve as a guide in phytochemical screening and fractionation.

#### **Materials and Methods**

#### Test Compounds

The tested compounds were the 50 priority chemicals of the MEIC programme (Bondesson *et al.* 1989). The purity was usually greater than 97%, and in many cases, greater than 99%. For some of the chemicals, the purity conforms to pharmacopeial standards.

#### **Experimental Procedure**

The 24-h LC50s of the 50 compounds was determined by the Standard Operational Procedures of the Artoxkit M (*Artemia salina*), the Streptoxkit F (*Streptocephalus proboscideus*) and the Rotoxkit F (*Brachionus calyciflorus*), with slight modifications on the hatching conditions, dilution medium, and test container. The modifications were described in detail by Calleja and Persoone (1992) and were adopted from Vanhaecke *et al.* (1980), Vanhaecke and Persoone (1981), Snell and Persoone (1989), and Centeno *et al.* (1993). The 24-h immobility

test with *Daphnia magna* was performed according to the OECD guidelines (OECD 1984), and the Microtox<sup>®</sup> test utilizing the bioluminescent *Photobacterium phosphoreum* was performed according to the standard assay procedure (Microbics... 1989). In case no EC50s were obtained, the 100% assay procedure of the Microtox<sup>®</sup> test was used. Likewise, a 15-min exposure was used when stable and repeatable EC50s could not be calculated after a 5-min exposure. The pH of the test solutions was not adjusted to the recommended pH range since it affects the toxicity.

The pre-treatment methods for insoluble compounds prior to toxicity testing, and the test container used for volatile chemicals are described by Calleja *et al.* (1993). A minimum of four replicate tests were performed for each chemical, and at least three replicates for the *Daphnia magna* test.

#### Data Analysis

The 24-h L(E)C50s were calculated by the Trimmed Spearman-Karber method (Hamilton *et al.* 1977). The 5- and 15-min EC50s of the Microtox<sup>(1)</sup> test were obtained by the linear regression package provided by the Microbics Corporation (Microbics 1989).

The acute toxicity values expressed in  $\mu$ mol/L were transformed to logarithm prior to correlation or multivariate analyses. Univariate correlation analyses were performed according to Sokal and Rohlf (1981). Principal component analysis (PCA) was used to evaluate all the toxicity data from ecotoxicological tests; PCA reduces the data matrix into a few dimensions containing most of the information obtained from the data (Wold *et al.* 1987) and extracts the underlying dominant structures which are then stored in the loading and score matrices. The loading describes the "variable patterns," while the scores reveal "object patterns," in this case, the ecotoxicological tests and the tested chemicals, respectively. Since PCA in this study will be used as an exploratory tool, the determination of the correct number of significant components by cross-validation is not necessary (Wold *et al.* 1987).

Principal components analysis was carried out by the Unscrambler<sup>®</sup> program (version 2.2, CAMO A/S, Trondheim, Norway) with the logarithm-transformed data which were nelther mean-centered nor scaled to unit variance. Supplementary to the PCA, an average linkage-squared Euclidean cluster analvsis available in the SPSS/PC+® statistical program (version 3.0, SPSS Inc., USA) was used to establish the boundaries of the different groups or the cluster membership. The clustering procedure is initiated by measuring the squared Euclidean distances of all the pairs of samples. The two mutually closest pairs of samples in the distance matrix are then identified, and the average of the squared Euclidean distances between them are calculated. The result obtained then becomes the center of the cluster. This procedure is repeated until all samples form one large cluster. Cluster analysis techniques are described in detail by Willett (1982) and Massart et al. (1988).

#### Results

The mean acute toxicity data expressed in  $\mu$ mol/L are given in Table 1. This table shows that compounds such as diazeparticiphenobarbital, diphenylhydantoin, sodium oxalate, and barium

Table	Aean acute toxicity data (L(E)C50 expressed in µmol/L) of the first 50 MEIC chemicals obtained from aquatic non-vertebrate tests

		Marine tests		Freshwater tests		
No.	Chemical	MX <sup>a</sup>	AS <sup>a</sup>	SP <sup>a</sup>	DM <sup>a</sup>	BC <sup>a</sup>
1	Paracetamol	2,190	3,820	196	367	35,100
2	Acetylsalicylic acid	145	2,120	988	932	785
3	Ferrous sulfate	782	186	1,420	308	230
4	Diazepam	>35,000	230	362	49.5	>35,100
5	Amitriptyline	77.8	133	2.8	20	2 35,100
6	Digoxin	>12,800	>12,800	>12,800	>12,800	>12,800
7	Ethylene glycol	1,810,000	2,910,000	878,000	1,200,000	1,900,000
8	Methanol	916,000	1,360,000	1,020,000	668,000	1,120,000
9	Ethanol	697,000	519,000	409,000	233,000	644,000
10	Isopropanol	380,000	278,000	193,000	159,000	476,000
11	1,1,1-Trichloroethane	342	30,600	9,850	6,860	48,100
12	Phenol	187	1,860	115	82.6	
13	Sodium chloride	563,000	2,730,000	118,000	61,700	12,800
14	Sodium fluoride	632,000	160,000	3,700	15,400	62,700
15	Malathion	488	273,000	195	$8.02 \times 10^{-15}$	9,650
16	2,4-Dichlorophenoxyacetic acid	191	1,820	853	648	3,320
17	Xylene	80.5	1,830	825		663
18	Nicotine	232	3,010	823 707	711	2,920
19	Potassium cyanide	275	258	82.1	35.5	1,350
20	Lithium sulfate	170,000	39,300		15.4	2,400
20	Theophylline	13,800	,	1,020	301	6,480
22	Dextropropoxyphene HCl	15,800	45,800	2,360	2,680	21,800
22	$R-(\pm)$ -propanolol	711	1180	29.2	72.7	16
23	Phenobarbital		1,570	7.2	61.2	10
24 25	Paraquat	12,900	>43,100	5,220	6,300	22,300
26	Arsenic trioxide	2,370 37,500	6,450	3.7	111	75.
20	Cupric sulfate	,	1.3	55.4	41.6	80.
28	Mercuric chloride	10.1	20.4	0.8	1.2	0.
20 29	Thioridazine HCl	0.2	77.2	0.4	0.2	0.
29 30	Thallium sulfate	1.7	39.1	0.9	12.3	0.
30 31	Warfarin	8,810	79	0.8	8.3	18
		219	11,800	1,110	1,540	1,440
32 33	Lindane	21,900	49.6	13,500	5.6	11,700
	Chloroform	12,900	4,740	6,460	2,660	65,400
34 35	Carbon tetrachloride	6,480	14,000	41,800	135,000	37,700
35 36	Isoniazid	79,300	2,350	178	915	22,200
	Dichloromethane	38,100	12,300	13,800	10,700	23,800
37 38	Barium nitrate	>383,000	34,500	2,710	801	2,710
	Hexachlorophene	19.6	$8.1 \times 10^{-3}$	$1.1 \times 10^{-2}$	0.3	$3.1 \times 10^{-1}$
39 40-	Pentachlorophenol	5.1	4.4	1.5	2.2	1.
40 <sup>-</sup>	Verapamil HCl	× 887	725	12.7	113	22.
41	Chloroquine phosphate	1,680	3,960	22.6	84.3	8.
42	Orphenadrine sulfate	1,210	147	14.2	34.6	17.
43 .	Quinidine sulfate	1,120	366	11.1	79.7	11.
14	Diphenylhydantoin	>39,600	>39,600	248	>39,600	>39,600
45	Chloramphenicol	5,310	6,320	943	3,360	6,420
46	Sodium oxalate	>741,000	41,400	3,080	4,030	475
47	Amphetamine sulfate	nt	4,110	148	734	13.
48	Caffeine	3,460	17,800	2,110	822	24,000
49	Atropine sulfate	8,010	22,700	951	513	481
50	Potassium chloride	493,000	70,600	25,100	7,350	22,700

 $^{a}AS = Artoxkit M (Artemia salina) test$ 

SP = Streptoxkit F (Streptocephalus proboscideus) testDM = Daphnia magna test

BC = Rotoxkit F (Brachionus calyciflorus) test

MX = Microtox<sup>(1)</sup> (Photobacterium phosphoreum) test

nitrate did not produce adverse effects to some of the organisms while digoxin was non-toxic to all the organisms. The toxicity ranged from  $10^{-15} \,\mu\text{mol/L}$  (for malathion) to  $2.9 \times 10^6 \,\mu\text{mol/L}$  (for ethylene glycol). Toxicity values obtained with the five tests differed within 1 order of magnitude for 42% of the chem-

icals, and by 3 orders of magnitude for 86% of the chemicals. Analyzing the most and least sensitive tests on a compound to compound basis, the greatest differences in toxicity were observed with malathion and hexachlorophene reaching 19 and 6 orders of magnitude, respectively.

	AS <sup>a</sup>	SP <sup>a</sup>	$DM^{\mathrm{a}}$	$BC^{a}$	MX <sup>a</sup>
AS	1.00	0.82*	0.32*	0.82*	0.66*
115	(n = 47)	(n = 47)	(n = 47)	(n = 46)	$(n = 43)^{\circ}$
SP	0.82*	1.00	0.46*	0.90*	0.73* ´
51	(n = 47)	(n = 49)	(n = 48)	(n = 47)	(n = 44)
DM	0.32*	0.46*	1.00	0.40*	0.47*
Dim	(n = 47)	(n = 48)	(n = 48)	(n = 47)	(n = 44)
BC	0.82*	0.90*	0.40*	1.00	0.69* ´
БС	(n = 46)	(n = 47)	(n = 47)	(n = 47)	(n = 44)
MX	0.66*	0.73*	0.47*	0.69*	1.00
14123	(n = 43)	(n = 44)	(n = 44)	(n = 44)	(n = 44)

Table 2. Matrix of correlation coefficients of the five aquatic non-vertebrate tests

\*p < 0.05

 $^{a}AS = Artoxkit M (Artemia salina) test$ 

SP = Streptoxkit F (Streptocephalus proboscideus) test

DM = Daphnia magna test

BC = Rotoxkit F (Brachionus calyciflorus) test

MX = Microtox<sup>®</sup> (*Photobacterium phosphoreum*) test

n = number of chemicals tested

The freshwater crustaceans (*Daphnia magna* and *Strepto-cephalus proboscideus*) were the most sensitive among the test organisms to 16 and 18 chemicals, respectively, followed by the freshwater rotifer (*Brachionus calyciflorus*) to eight chemicals and the marine bacteria (Microtox<sup>®</sup>) test) to seven chemicals. The least sensitive was the marine crustacean *Artemia salina*. Of the two substances to which the latter test was most sensitive, only arsenic trioxide was 4 orders of magnitude more toxic compared to the least sensitive organism for this compound, and only 1 order of magnitude different from the other organisms.

Digoxin was not included in the correlation, PCA, and cluster analyses due to the absence of toxic effect to all the organisms tested. The results of pairwise correlation analyses in Table 2 showed that the effect data for all test organisms were significantly well-correlated (p < 0.05), the correspondences being the lowest with *Daphnia magna*. The best correlated species were *Brachionus calyciflorus* and *Streptocephalus proboscideus*. Artemia salina in turn was more correlated with the latter two species than with *Photobacterium phosphoreum* (Microtox<sup>GB</sup> test).

The first two components resulting from PCA of the 48 compounds (excluding digoxin) explained 96% of the total toxicity information from the five aquatic non-vertebrates. The extremely toxic malathion and hexachlorophene were detected as outliers, and were excluded from further statistical evaluation. Cluster analysis also showed the same two chemicals (MEIC chemical nos. 15 and 38) as outliers (Figure 1). The groupings displayed in the score plot of the first two principal components (Figure 2) were comparable to those derived from cluster analysis (Figure 3). However, some chemicals, which were not toxic to at least one of the test organisms, were not reflected in both dendrograms (Figures 1 and 3). As can be seen in Figure 2, the first component is solely responsible for the clustering of the chemicals into four groups, mainly according to the magnitude of toxicity. Chemicals which have high scores suggest high values, thus low toxicity. The first group denoted as Group A was the most toxic with values ranging from 0.1 to 10 µmol/L. Most of the heavy metals, sulfur containing- or polychlorinated aromatic compounds were part of this group. For Group B, toxicity of the opioid analgesic, antiarrythmic and antidepressant drugs, a bipyridylium pesticide and other heavy metal compounds ranged from 1 to 100  $\mu$ mol/L. The largest group was Group C for which the toxicity of the compounds ranged from 100 to 10,000  $\mu$ mol/L. Most of the aliphatic alcohols and the essential inorganic compounds belong to Group D. Toxicity for the latter group ranged from 10,000 to 1,000,000  $\mu$ mol/L.

Among the inorganic compounds, the least toxic were generally those essential to life; those having a monovalent cation or anion (MEIC nos. 13, 14 and 50) were more toxic than those having a divalent cation (nos. 3 and 27). For the latter compounds, the atomic weight of the cation influences the degree of toxicity; toxicity increases from alkali earth metals to metalloid, to heavy metals. Lithium sulfate, which belongs to Group C, was positioned close to the essential inorganic chemicals containing a monovalent ion.

For organic chemicals the relationship between toxicity and chemical structure is more difficult to distinguish. The composition of the groupings did not indicate a trend; the clustering of chemicals was based on neither the functional groups nor the type of (pharmacologic) effect. However, moving from one group to another, one can deduce the following: aromatic alcohols or phenolic compounds (nos. 1, 2, 12, and 39) were generally more toxic than aliphatic alcohols (nos. 7, 8, 9, and 10); chlorine-substituted aromatic compounds (nos. 16 and 39) were more toxic than chlorine-substituted aliphatic (nos. 1/2 33, 34, and 36) and substituted alicyclic compound (no. 32) With regard to fused polycyclic compounds consisting of heterocyclic and/or an aromatic nucleus, the chemicals with 3-membered ring (nos. 5 and 29) were also more toxic than compounds with a 2-membered ring (nos. 4, 21, 23, 31, 41, 43 and 48). The xanthine chemicals (nos. 21 and 48), the acidit compounds (nos. 2 and 16), and the one-ring heterocyclic com pounds (nos. 24 and 44) were closely positioned within Group C. The toxicity of nitrogen- and sulfur-containing compounds was higher and between these two types of chemicals, the latter seem to be more toxic.

In Figure 4, Artemia and Photobacterium tests appeared to a more important for the first component while Photobacterium Brachionus, and Streptocephalus tests were of more importance for the second component. As also shown in Figure 4

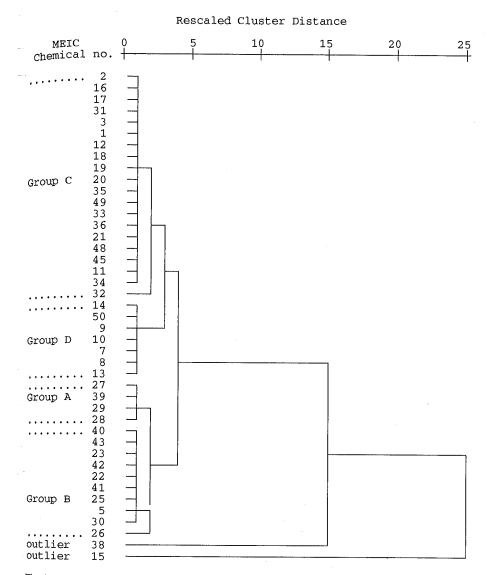


Fig. 1. Dendrogram of the 49 MEIC chemicals using the average linkage-squared Euclidean cluster algorithm. The numbers correspond to the MEIC chemical numbers given in Table 1

## <sup>-</sup>Discussion

Since it is not possible to use humans in toxicity testing nor to perform tests on more than a small percentage of organisms that abound in nature, one is mostly limited to a few test species for which standardized testing protocols have been established for comparing the toxicity of various chemicals to biota. Many comparative toxicity studies have indicated that sensitivity is dependent not only on the chemical but also on the species, a finding which is corroborated again by the present study. In most investigations, the results in ranking sensitivity have revealed phylogeny rather than habitat as the determining factor. As pointed out by Cairns (1986), sensitivity is not only dependent on the species and the chemical but also on the endpoint or criterion used. It must be noted in our study that the tests using sublethal endpoints (*e.g.*, *Daphnia* and Microtox<sup>®</sup> tests) did not always exhibit greater sensitivity than those using a lethal criterion (*e.g.*, cyst-based tests).

Despite differences in sensitivity among aquatic organisms, the relative toxicity of most chemicals is similar to all the organisms, except malathion, extremely toxic to *Daphnia magna*,

Artemia salina was more correlated with the Photobacterium phosphoreum than with the other three tests. Daphnia magna, Brachionus calyciflorus, and Streptocephalus proboscideus likewise were well correlated with each other.

Considering the first component, the two tests (Daphnia magna and Streptocephalus proboscideus) nearest the origin shown in the loading plot (Figure 4) appeared to be the most sensitive; the other two tests farthest from the origin (Artemia salina and Photobacterium phosphoreum) were generally the least sensitive. The sensitivity of Brachionus calyciflorus test was intermediate to that of the four other tests.

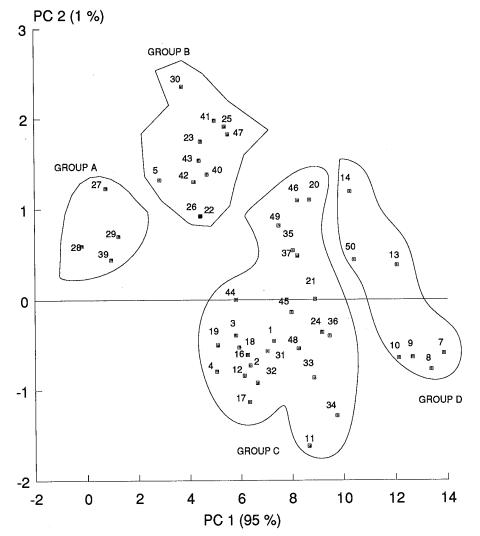
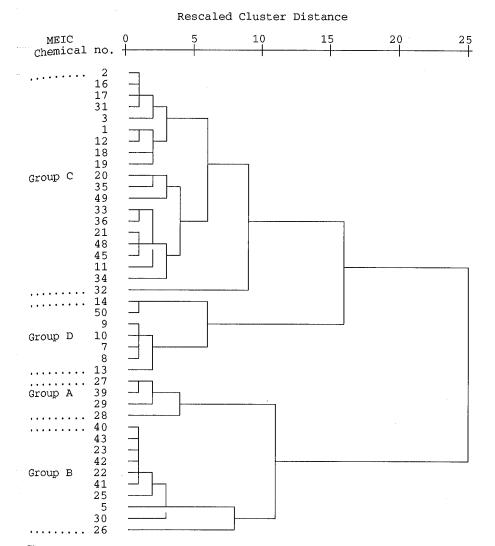
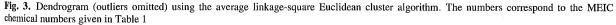


Fig. 2. Score plot of the first two principal components (PCs). The numbers correspond to the MEIC chemical number given in Table 1

and hexachlorophene, to Brachionus calyciflorus. On the other hand, the composition of the most or the least toxic group of chemicals as well as of chemicals with intermediate toxicity was not based on a clear and defined chemical structure. Evaluation of 267 compounds with six aquatic and one terrestial species displayed the same undefined class composition (Kaiser and Esterby 1991). This may be explained by the nature of toxicity or biological activity, which Miller (1990) has reported to be dependent on more than just one functional group. With regard to trace elements being required by all living organisms for their normal metabolic processes, it is not surprising to find that, generally, the essential metals (Na, K) and non-metals (Cl, F) were the least toxic inorganic chemicals. However, not all essential elements were always less toxic than non-essential inorganic chemicals. For instance, copper sulfate and ferrous sulfate were more toxic than the non-essential barium nitrate. Toxicity of metallic compounds to aquatic organisms increases from alkali metals, alkali earth metals, metalloids, to heavy metals. Khangarot and Ray (1989) have suggested that the periodic table, which reflects the physico-chemical properties of the elements, can serve as a guide to determine the toxicity ranking among metals.

For organic chemicals, toxicity is dependent on both the substituent and its nucleus, whether aliphatic, aromatic, of alicyclic. Organic compounds containing nitrogen, sulfur and/or phosphorus were more toxic than those having only carbon and hydrogen with or without oxygen. This confirms the findings of Eastmond et al. (1984) for a series of polycyclic aromatic sulfur heterocycles (PASHs) generally being more toxic than their analogous polyaromatic hydrocarbons (PAHs) For digoxin which is highly toxic to humans, the absence of acute toxicity to the aquatic non-vertebrates may either be at tributed to low aqueous solubility (Calleja and Persoone 1992) or to low sensitivity of specific receptors (if present) in the test species used. To determine whether aquatic non-vertebrates are indeed not sensitive to steroidal compounds, and to determine the usefulness of such organisms in detecting the presence of steroidal compounds in plants and animals and/or in environa





\_mental samples, other chemicals of similar structure should be tested.

Aside from the similarity in toxicity ranking, significant interspecies correlations were also found. The latter results support the findings of Kenaga (1978), Doherty (1983), and Nendza and Klein (1990), who compared acute toxicities of chemicals among aquatic species belonging to different trophic levels, and Suter et al. (1983) at different taxonomic levels among fish species. Furthermore, the relatively lower correlations of toxicity values for Daphnia magna with those of other species found after paired species comparisons were in agreement with the findings of other investigators (Kenaga 1978; LeBlanc 1984; Mayer and Ellersieck 1986). The reason for such good interspecies correlation and similar toxicity ranking of diverse classes of chemicals to the organisms may be due to the non-specific mode of toxicity to the cell, which is the basic structure of all living matter, whether animal, plant, or microorganism. The same mechanism has been suggested to explain the good predictability of human lethal dosage by *in vitro* cultures of non-differentiated cells for about 80% of a random selection of chemicals (Ekwall and Ekwall 1988).

Marked differences in biochemical reactions occurring in the various tissues within a single organism (Albert 1985) and in the toxicokinetic processes: absorption, distribution, metabolism/biotransformation, and excretion (Smith 1977; Bend and James 1978; Foster and Crosby 1987; Barron and Plakas 1991) could account for the selective toxicity to some organisms, although all living organisms have the same "basic" structure and a common biochemistry. The use of a battery of single species tests is more practical to encompass a broad spectrum of toxicity levels either to protect the environment or to improve detection of active substances in plants, animals or microorganisms. Considering the two most sensitive species (*Streptocephalus proboscideus* and *Daphnia magna*), toxicity differs within 1 order of magnitude for 92% of the chemicals and within 2 orders of magnitude for 98% of the chemicals.

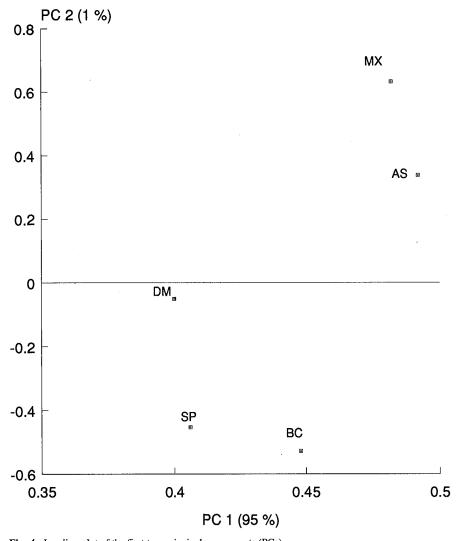


Fig. 4. Loading plot of the first two principal components (PCs)

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Furthermore, with the addition of the next sensitive freshwater species (Brachionus calyciflorus) to the combination Streptocephalus proboscideus-Daphnia magna (all three tests were nearest the origin in Figure 4), the toxicity levels were within 1 order of magnitude of the lowest L(E)C50 for all the chemicals. On the other hand, when the Photobacterium phosphoreum test was taken together with Streptocephalus proboscideus and Daphnia magna tests in place of the equally sensitive Brachionus calyciflorus tests, the toxicity levels of 96% of the chemicals could be covered within 1 order of magnitude, and that for all the chemicals within 4 orders of magnitude difference. These findings confirm the statement of Kimerle et al. (1985) that improvement in toxicity detection of chemicals within 1 and 2 orders of magnitude could be achieved from a combination of tests rather than using only one single species test. In the latter study, the improvements have been more pronounced with a combination consisting of at least one species belonging to another taxon. The selection of the tests for inclusion in the

battery could be based on the importance of each test using similar PCA plot as illustrated by the present work.

#### Conclusions

The results are encouraging in showing that it is meaningful we pursue the use of a battery of tests rather than a single test either for ecotoxicological or for phytochemical screening. While I may be a potential guide in the isolation of novel compounds with various pharmacological activity from extracts of biological materials, there is a need to further investigate the concell tration-effect relationships of extracts from plants, animals, of microorganisms using the test organisms. Industrial effluents and leachates from sediment samples merit similar analysis Enlarging the toxic concentration-effect data base of man-mad chemicals by inclusion of representatives of other chemical standardized and cost-effective microbiotests are deemed approximate. propriate. A cost-effective approach of dealing with the latter could be through a multicenter type of evaluation in analogy to the MEIC programme, wherein each participating laboratory tests a defined set of chemicals with its own microbiotests. In addition, the use of a PCA, particularly with cross-validation techniques in the appraisal of a larger data base, could provide a more reliable way of determining the model or the best combination of tests, which is considered as one of the urgent priorities in ecotoxicology. Principal components analysis assesses all the variables (in this case, the ecotoxicological tests) simultaneously, thus giving an advantage of optimizing available information contained in the data necessary for the identification of the most suitable elements of a battery of tests.

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#### Environmental Contamination and Toxicology

## Acute Toxicity to Freshwater Benthic Macroinvertebrates of Fluoride Ion (F-) in Soft Water

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Fluorine is the most electronegative of all elements. It does not occur naturally as a free element and only appears with a valence of -1. The most abundant fluorine-containing minerals are fluorite (CaF<sub>2</sub>), fluorapatite (Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>F) and kryolite (Na<sub>3</sub>AlF<sub>6</sub>).

The fluoride concentration in sea water normally ranges from 1.2 to 1.4 mg/l (Dobbs 1974). Most fresh waters contain less than 0.2 mg F-/L, although total concentrations can be considerably higher if the fluoride is bound to small suspended particles (Dave 1984). However, fluoride concentration in surface waters is increasing as a result of industrial pollution (Martin and Salvadori 1983).

Toxic effects of fluorine compounds have been described in aquatic animals like *Daphnia magna* (Le Blanc 1980; Dave 1984), *Artemia salina* (Pankhurst et al. 1980), *Penaeus indicus* (McClurg 1984) and *Oncorhynchus mykiss* (Pimentel and Bulkley 1983; Smith et al 1985). However, very little is known about fluoride toxicity in benthic macroinvertebrate community of fresh waters.

In this paper is described the fluoride acute toxicity for five species of aquatic insect larvae which are ordinary benthic macroinvertebrates in rivers from The Iberian Peninsula.

## MATERIALS AND METHODS

Last instars of trichoptera aquatic larvae were collected from fluoride unpolluted areas of Spanish rivers: *Chimarra marginata* Linnaeus, *Hydropsyche lobata* MacLachlan and *Hydropsyche bulbifera* MacLachlan from Río Aulencia (Madrid), *Hydropsyche exocellata* Dufour from Río Jarama (Madrid), and *Hydropsyche pellucidula* Curtis from Río Duratón (Segovia). In the laboratory, test organisms were randomly selected and placed into test aquaria.

Laboratory bioassays were conducted in glass aquaria each with a volume of 10 L dechlorinated tap water. Necessary water oxygenation and turbulence were produced by two air pumps per aquarium. Chamber environmental temperature and natural photoperiod were utilized. Test fluoride solutions were made from sodium fluoride (NaF pro analysi, Merck, FRG).

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883 66 Methods for these static acute toxicity bioassays were those recommended for standardized laboratory toxicity tests (US Environmental Protection Agency 1975; American Public Health Association 1980). Five fluoride tests were designated as A, B, C, D, and E bioassays. A control and 5 to 6 fluoride concentrations were used per bioassay. Two species were tested simultaneously in each bioassays with 10 larvae per species, excepting *H. pellucidula* with 12 larvae in the A test.

Test organisms were acclimatized to water quality conditions for 4-5 days prior to tests and were not fed during acclimatization nor during toxicity bioassays. During the acclimatization there were no dead animals and during toxicity tests dead animals were removed every day.

Hardness, alkalinity, chlorine, chloride, sodium, potassium, ammonia, nitrite, pH, water temperature, dissolved oxygen and conductivity, were analysed at the start and at the end of each toxicity bioassay using analytical methods described by American Public Health Association (1980) and Rodier (1981).

Fluoride concentration were monitored daily using an Orion-USA model 94-09 specific ion electrode and an Orion-USA model 90-02 calomel reference electrode. Water samples were analysed at pH 5.5 after adding total ionic strength adjustment buffer (TISAB-III) with cyclohexanediamine tetra acetic acid (CDTA) as complexing agent for total fluoride ion analysis. The specific ion electrode was calibrated using analytical method described by Orion Research (1983).

The 96-hr median lethal concentration (96-hr LC50), its 95% confidence limits (95% cl), and  $X^2$  values, were calculated by the method of Litchfield and Wilcoxon (1949), using simultaneously the fluoride ion median concentrations of duplicate tests for each species. The death was defined as test larvae floating upside down and not moving.

To verify whether the toxicity of sodium fluoride was due to fluoride ion fundamentally, sodium and conductivity toxicity controls were conducted parallel to fluoride toxicity bioassays, using sodium chloride (NaCl pro analysis, Merck, FRG). For that, 10-15 larvae of each test species were exposed in soft water (13-18 mg CaCO<sub>3</sub>/L) for 96 hr to high sodium concentrations (255-313 mg Na<sup>+</sup>/L) and conductivities (650-740  $\mu$ mhos/cm) which were higher than those values measured in fluoride bioassays. Sodium concentration and conductivity were measured using an Orion-USA model 97-11 specific ion electrode and a Yellow Springs-USA model 33 conductivity meter, respectively. The possible mortality was checked daily.

## **RESULTS AND DISCUSSION**

There were no dead animals in sodium and conductivity controls after 96-hr exposure to sodium chloride. Mean values and their standard deviations of water quality parameters analysed during fluoride toxicity bioassays are shown in Table 1. Chlorine was not detected.

All mean values of those parameters are within water quality criteria for aquatic organisms (US Environmental Protection Agency 1986). The variation of obtained values between different bioassays and between aquaria for a same test (Table 1) was probably due to slight shifts in the tap-water quality and the

influence of test larvae on physico-chemical characteristics of water during each fluoride toxicity test.

Fluoride mean concentrations and mortality percentages of fluoride five static acute toxicity bioassays are presented in Table 2. Standard deviations were lower than 10% of their respective mean values. There was no mortality in control aquaria. The mortality percentage increased with regard to the sodium fluoride concentration. Mean values of sodium concentration and conductivity ranged from 6.65 to 244 mg Na+/L and from 55 to 665  $\mu$ mhos/cm respectively, depending on the NaF concentration into each aquarium.

The 96-hr median lethal concentrations, their 95% confidence limits and  $X^2$  values obtained for each test species are shown in Table 3. All  $X^2$  values are lower than  $X^2$  values (P=0.05), indicating that data are not significantly heterogeneous.

The present study has demonstrated that the toxicity of sodium fluoride is due to fluoride ion (F-) principally, since in sodium and conductivity toxicity controls there was no mortality of test species.

From a simple comparison of median lethal concentrations for five species, we can infer that H. bulbifera and H. exocellata are the most sensitive species to fluoride, since their 96-hr LC50s are smallest and their 95% confidence limits do not significantly overlap with the 95% confidence limits of the other species tested.

Compared with other aquatic invertebrates, trichoptera test larvae appear to be more sensitive to fluoride. McClurg (1984) obtained a 96-hr LC50 of 1,118 mg F-/L for the estuarine prawn *Penaeus indicus*, and Le Blanc (1980), in tests with NaF in hard water, found 24 and 48-hr EC50s and a "no discernible effect concentration" of 680, 340 and 110 mg NaF/L, respectively, in *Daphnia magna*.

This may be due to the formation of innocuous complexes with one or more ions of seawater (Oliveira et al. 1978), and the precipitation of insoluble calcium fluoride from hard water.

Thus, Smith et al. (1985) have deduced that the acute toxicity of fluoride ion to *Gasterosteus aculeatus, Pimephales promelas,* and juvenile *Oncorhynchus mykiss* varied with fish species and initial water hardness due to the precipitation of CaF. The smallest 96-hr LC50 obtained directly by them was of 200 mg F-/L to 23-62 mg CaCO<sub>3</sub>/L of initial hardness in rainbow trout.

On the other hand, Pimentel and Bulkley (1983) found a 96-hr LC50 of 51 mg F-/L to 17 mg CaCO<sub>3</sub>/L of hardness in *Oncorhynchus mykiss*, and Prochnow (1978) observed that 25 mg NaF/L did generate no acute toxictity in *Cyprinus carpio*.

All this could indicate that some freshwater benthic macroinvertebrates like H. bulbifera and H. exocellata can be more sensitive than freshwater fish to fluoride ion. Water quality criteria, based on the more sensitive species, should provide adequate protection to fluoride pollution.

	A	В	С	D	Ε
Water temperature (°C)	15.15	14.10	13.25	17.30	15.95
	(0.36)	(0.31)	(0.16)	(0.93)	(0.26)
Dissolved oxygen (mg/L)	10.15	10.16	10.25	9.95	10.03
	(0.08)	(0.10)	(0.09)	(0.22)	(0.06)
pH	7.84	7.49	7.46	7.43	7.41
~	(0.07)	(0.11)	(0.12)	(0.14)	(0.15)
Hardness (mg CaCO3/L)	17.60	18.77	16.28	12.04	13.18
,	(3.61)	(2.84)	(1.59)	(1.98)	(1.79)
Alkalinity (mg CaCO3/L)	33.24	24.07	24.67	22.94	22.18
	(2.83)	(0.93)	(0.90)	(1.29)	(1.67)
Chloride (mg/L)	3.54	7.27	15.64	8.91	4.50
	(2.08)	(2.01)	(3.46)	(1.52)	(0.66)
Potassium (mg/L)	0.09	0.09	0.08	0.08	0.07 <sup>´</sup>
	(0.02)	(0.01)	(0.01)	(0.02)	(0.01)
Ammonia (mg N/L)	0.04	0.05	0.05	0.04	<b>`</b> 0.03 <sup>´</sup>
	(0.04)	(0.05)	(0.05)	(0.04)	(0.03)
Nitrite (mg N/L)	0.02	0.04	0.03	0.02	0.02
	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)

Table 1. Mean values and standard deviations of water quality pa	parameters analysed in static
fluoride acute toxicity bioassays (A, B, C, D and E).	-

Table 2. Results of A, B, C, D and E toxicity bioassays after 96-hr exposure to fluoride ion.

	Cont	rol	A1	A2	A3	A4	A5
Fluoride mean concentration (mg/L)	0.	03	2.51	7.64	21.34	64.98	194.13
Mortality of <i>H. pellucidula</i> (%)	0.	0	0.0	0.0	16.6	75.0	100.0
Mortality of H. bulbifera (%)	0.	0	0.0	10.0	30.0	90.0	100.0
	Control	B1	B2	B3	B4	В5	B6
Fluoride mean concentration (mg/L)	0.05	12.18	19.12	30.16	48.52	77.12	121.8
Mortality of <i>H. pellucidula</i> (%)	0.0	0.0	20.0	40.0	70.0	80.0	100.0
Mortality of <i>H. lobata</i> (%)	0.0	0.0	10.0	30.0	40.0	60.0	100.0
	Control	C1	C2	C3	C4	C5	C6
Fluoride mean concentration (mg/L)	0.06	11.78	18.90	29.78	48.56	77.06	122.6
Mortality of <i>H. lobata</i> (%)	0.0	0.0	10.0	30.0	60.0	70.0	100.0
Mortality of <i>H. bulbifera</i> (%)	0.0	10.0	40.0	60.0	70.0	90.0	100.0
· · · · · · · · · · · · · · · · · · ·	Control	D1	D2	D3	D4	D5	D6
Fluoride mean concentration (mg/L)	0.06	12.40	19.44	30.08	49.90	77.18	124.9
Mortality of <i>H. exocellata</i> (%)	0.0	20.0	40.0	50.0	80.0	100.0	100.0
Mortality of Ch. marginata (%)	0.0	0.0	10.0	30.0	60.0	70.0	90.0
	Control	E1	E2	E3	E4	E5	E6
Fluoride mean concentration (mg/L)	0.07	12.46	20.52	30.80	50,42	78.38	121.8
Mortality of <i>H. exocellata</i> (%)	0.0	20.0	30.0	50.0	70.0	90.0	100.0
Mortality of Ch. marginata (%)	0.0	0.0	20.0	30.0	50.0	80.0	100.0

	96-hr LC50 (mg F-/L)	95% cl (mg F-/L)	$\chi^2$
Hydropsyche bulbifera	26.30	18.8-36.7	1.87
Hydropsyche exocellata	26.50	20.4-34.4	3.63
Hydropsyche lobata	48.20	37.9-61.2	4.80
Hydropsyche pelluciduld	a 38.50	29.9-49.5	2.79
Chimarra marginata	44.90	35.2-57.3	4.08

Table 3. 96-hr LC50s, their 95% confidence limits and  $X^2$  values.

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## The Relative Sensitivity of Competing Hydropsychid Species to Fluoride Toxicity in the Cache la Poudre River (Colorado)

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Abstract. The influence of increased fluoride concentrations generated by a wastewater treatment plant on the spatial distribution and abundance of benthic larvae of Hydropsychidae (Insecta, Trichoptera) species in the Cache la Poudre River (Colorado) was examined. Acute lethal concentrations of fluoride ion (F<sup>-</sup>) to these species were determined in soft water (average value of hardness 40.2 mg CaCO<sub>3</sub>/L) by static toxicity bioassays. The wastewater treatment plant caused a significant (P < 0.05) increase in the fluoride concentration at three downstream sampling sites (mean values 1.17, 0.84, and 0.56 mg  $F^{-}/L$  at 0.1, 1.6, and 9.2 km downstream sites, respectively) compared with the upstream reference station (0.31 mg F<sup>-</sup>/L). The 48, 72, 96, 120, and 144-h LC50s (mg F<sup>-</sup>/L) were 52.6, 25.8, 17.0, 13.4, and 11.5 for *Hydropsyche* bronta Ross, 102.0, 53.5, 34.7, 27.0, and 21.4 for Hydropsyche occidentalis Banks, and 128.0, 73.2, 42.5, 31.9, and 24.2 for Cheumatopsyche pettiti (Banks). LC50 values for *H. bronta* were significantly (P < 0.05) lower than LC50 values for the other two test species. Abundance and biomass of all hydropsychid species were significantly (P <0.05) lower at the 0.1 km downstream site than at the upstream reference site. H. occidentalis was usually the most abundant species at the reference and 9.2 km downstream stations, with highest abundances at the 9.2 km downstream site. C. pettiti was dominant at 0.1 and 1.6 km downstream \_sites, showing higher abundances at the 1.6 km downstream site than at the upstream reference site. H. bronta was never collected at 0.1 and 1.6 km downstream sites, but was abundant at the upstream reference site. It is concluded that the fluoride pollution is not a major factor in determining the spatial distribution and abundance of competing hydropsychid species in the Cache la Poudre River. However, the greater sensitivity of H. bronta larvae to fluoride ions could result in decreased abundances of this species at downstream sampling sites.

Fluoride is universally present in varying amounts in water, soils and tissues of plants and animals. The fluoride ion con-

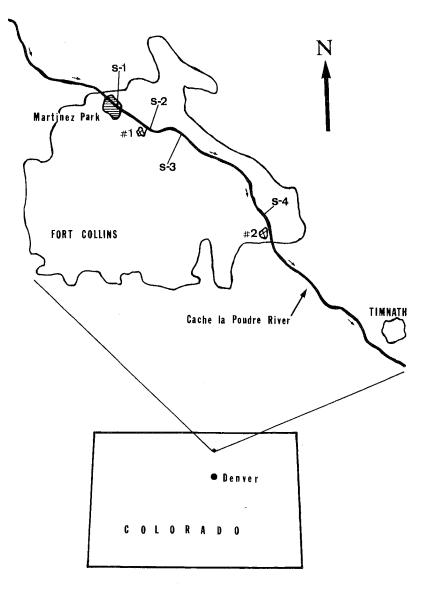
centration in sea waters normally ranges from 1.2 to 1.4 mg/L (Dobbs 1974). In contrast, most fresh waters contain less than 0.2 mg F<sup>-</sup>/L, although total fluoride concentrations may be considerably higher if the fluoride is bound to small suspended particles (Dave 1984). However, the concentration of the fluoride ion in many river systems is significantly increasing as a result of man's activities (Britton *et al.* 1983; Martin and Salvadori 1983).

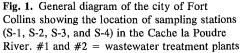
The exposure of living organisms to above-normal concentrations of fluoride may result in an alteration of the organism's biochemistry and morphology; vertebrates and invertebrates accumulate fluoride in their skeleton and exoskeleton during chronic exposures (Wright and Davidson 1975; Henny and Burke 1990). Moreover, freshwater organisms may be far more sensitive to fluoride pollution than those living in sea waters because the toxicity of fluoride ions is decreased by the formation of innocuous complexes with one or more ions of sea water (Oliveira *et al.* 1978; McClurg 1984).

Toxic effects of fluoride compounds have been described in aquatic invertebrates such as Daphnia magna (Le Blanc 1980), Penaeus indicus (McClurg 1984) and Palearctic larvae of Hydropsyche species (Camargo 1989; Camargo and Tarazona 1990). Species tested in soft water appear more sensitive to fluoride than those tested in hard or sea water. In fish, the fluoride toxicity is influenced not only by the usual factors such as size (Hemens and Warwick 1972), species (Smith et al. 1985) and physiological state (Pillai and Mane 1984), but also by the physicochemical characteristics of the water. The tolerance of Oncorhynchus mykiss to fluoride is increased by low temperatures (Angelovic et al. 1961) and high levels of calcium hardness (Pimentel and Bulkley 1983; Smith et al. 1985). On the other hand, Camargo and Tarazona (1990, 1991) demonstrated that the toxicity of sodium fluoride (NaF) to aquatic animals is fundamentally due to fluoride ions.

Little is known about the toxicity of fluoride ions to Nearctic species of freshwater benthic macroinvertebrates. Caddisflies (Trichoptera) are an important group of aquatic insects in running waters. Larvae of the caddisfly family Hydropsychidae are sedentary and construct fixed retreatnets on the bottom of rivers and streams to strain food particles from the current (Wiggins 1984). In general, larvae of

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hydropsychid species have similar ecological requirements with regard to net-spinning sites and strained food particles (Hildrew and Edington 1979; Lapchin and Neveu 1979), and therefore the interspecific competition within hydropsychid guilds appears to be very significant.

The present investigation evaluates the influence of the increased fluoride concentration generated by the Fort Collins wastewater treatment plant #1 on the spatial distribution and abundance of benthic larvae of three competing hydropsychid species in the Cache la Poudre River (Colorado). Field and laboratory studies were performed in order to achieve this goal.

#### **Methods and Materials**

#### Study Area

The Cache la Poudre River has its origins in the Poudre Ponds (3,290 m elevation) near the Continental Divide in Rocky Mountain National Park. The stream flows northeastward for about 32 km and then eastward through the Poudre Canyon, most of which is, in

Roosevelt National Forest. Several mountain reservoirs are used to retain water from spring snow melt and the periodic summer storms for later use. There are no known domestic sewage additions to the Cache la Poudre River above the city of Fort Collins, although there are two small communities located on the river several kilometers up Poudre Canyon.

The city of Fort Collins (Figure 1) removes water from the Cache la Poudre River as part of its drinking water supplies and fluoridates the treated water in order to improve the dental health of citizens. State drinking water standards dictate a maximum contaminant level for fluoride of 1.4 to 2.4 mg/L depending on the water temperature (Boyd *et al.* 1986). Thus, some of this exogenous fluoride will be found as a component of domestic wastewater discharges. Boyd *et al.* (1986) showed that the concentration of fluoride ion in the Cache la Poudre River increases significantly as a consequence of discharges from Fort Collins wastewater treatment plants, the trend over a six year period (1980–1985) being upwards (Figure 2).

The outfall from wastewater treatment plant #1 is located about 1.5 km downstream from Martinez Park and 10 km upstream from wastewater treatment plant #2. Four sampling stations were selected along the study area (Figure 1). An upstream unpolluted station (S-1) located at Legacy Park, next to Martinez Park, was used as a reference site. The second (S-2), third (S-3), and fourth (S-4)

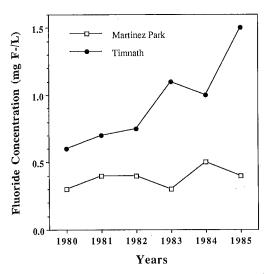


Fig. 2. 1980–1985 yearly mean fluoride ion concentrations upstream (Martinez Park) and downstream (Timnath) from Fort Collins wastewater treatment plants. Data from Boyd *et al.* (1986)

sampling sites were placed approximately 0.1, 1.6, and 9.2 km downstream from wastewater treatment plant #1. The stream bottom was mainly stony with cobbles and pebbles at all sampling stations.

#### Field Studies

Alkalinity, conductivity, dissolved oxygen, fluoride, hardness, nitrate, pH, sulfate and water temperature were analyzed four times at each sampling site during 1990, according to standardized methods described by American Public Health Association (1989). Dissolved oxygen, conductivity, water temperature and pH were analyzed *in situ*.

In order to determine the abundance of hydropsychid species at each station, sampling surveys were undertaken in May, August and November of 1990 using a Surber square foot sampler, which enclosed a sampling area of 930 cm<sup>2</sup> and was equipped with a 1.1 m net with a mesh size of 250  $\mu$ m. Three riffle macrobenthic samples were collected at each station on each sampling date, and were preserved in 80% alcohol until laboratory analysis. Following identification and counting, quantitative samples were dried in an oven at 60°C for 24 h in order to estimate the total biomass of hydropsychid guilds.

Significant differences (P < 0.05) between the upstream reference station (S-1) and downstream sites (S-2, S-3, and S-4) were determined by a two-sample t-test in accordance with Elston and Johnson (1987). It has been assumed that physicochemical and biological parameters were normally distributed, each parameter exhibiting the same variance along the sampling area.

#### Laboratory Studies

Static short-term toxicity bioassays were performed in duplicate using glass aquaria, each containing 0.5 L of Fort Collins filtered tap water and a few granitic pebbles. The chlorine was removed during filtration. Water oxygenation and turbulence were produced with air pumps. Chamber environmental temperature and a 12-h photoperiod were utilized. A control (average fluoride concentration of 0.6 mg F<sup>-</sup>/L) and 5 different fluoride concentrations were used per replicate (6.0, 12.3, 24.5, 49.0, and 94.8 mg F<sup>-</sup>/L for the first replicate, and 10.0, 18.5, 33.1, 59.0, and 105.0 F<sup>-</sup>/L for the second

replicate), with 10 animals of each test species (N = 30) per aquarium. Test solutions were made from sodium fluoride (NaF fluormetric, Matheson Coleman & Bell, USA), and fluoride concentrations were monitored daily.

Last instar larvae of *Hydropscyhe occidentalis* Banks, *Hydropsyche bronta* Ross, and *Cheumatopsyche pettiti* (Banks) were hand-collected from the reference sampling station (S-1). No animal died during transportation. In the laboratory, test organisms were randomly distributed into test aquaria and acclimatized for three days prior to fluoride toxicity tests. All larvae built their retreat and capture nets within the early hours of their acclimatization, although *Cheumatopsyche* larvae were generally the last animals in finding a net-spinning site because of the aggressive behavior of *Hydropsyche* larvae. *H. bronta* larvae exhibited two different head colour patterns, the striped Appalachian form and the checkerboard Central form (Schuster and Etnier 1978; Schefter and Wiggins 1986). The first form was used in the second replicate and the second form in the first replicate.

Animals were not fed during their acclimatization nor during the tests, and during toxicity tests dead larvae were removed every day. Average values of water quality conditions during fluoride toxicity bioassays were 7.8 for pH, 9.5 mg/L for dissolved oxygen, 18°C for water temperature, 31.7 mg CaCO<sub>3</sub>/L for alkalinity, and 40.2 mg CaCO<sub>3</sub>/L for hardness. Toxicity tests were carried out in soft water in order to avoid the loss of fluoride ions by precipitation of CaF<sub>2</sub> and MgF<sub>2</sub> (Smith *et al.* 1985). No precipitation of fluoride salts was observed during these toxicity bioassays.

The 48, 72, 96, 120, and 144-h LC50 values, their 95% confidence limits and  $\chi^2$  values were calculated by the method of Litchfield and Wilcoxon (1949), using mortalities and mean fluoride ion concentrations obtained in duplicate for each test species. Death was defined as test larvae not moving and not reacting to gentle prodding. In addition, the formula of factors (Litchfield and Wilcoxon 1949; American Public Health Association 1989) was applied in order to determine significant (P < 0.05) differences between test species with regard to their fluoride sensitivity.

#### Results

#### Water Physico-Chemistry

Mean (n = 4) values of water physicochemical parameters analyzed at each sampling station during 1990 are presented in Table 1. There were no significant (P > 0.05; t-test) differences between the reference station (S-1) and downstream sampling sites for conductivity, water temperature, total hardness, total alkalinity, dissolved oxygen, pH and sulfate. However, mean values of fluoride and nitrate concentrations were significantly (P < 0.05; t-test) higher at S-2, S-3, and S-4 than at S-1, decreasing downstream with distance from wastewater treatment plant #1. The highest and lowest fluoride concentrations estimated at each sampling station were 0.40-0.24, 1.48-0.95, 0.95-0.80 and 0.62-0.50 (mg F<sup>-</sup>/L) for S-1, S-2, S-3 and S-4, respectively. All nitrate concentrations were within water quality criteria for aquatic organisms (US Environmental Protection Agency 1986).

#### Hydropsychid Guild

Mean (n = 3) densities of hydropsychid species at each station during sampling surveys are presented in Table 2. All species had significantly (P < 0.05; t-test) lower abundances just below the wastewater treatment plant #1 (S-2) than at 73

Table 1. Mean values (n = 4) and standard deviations of physicochemical parameters analyzed at sampling sites (S-1, S-2, S-3, and S-4) in the Cache la Poudre River (Colorado) during 1990

	S-1	S-2	S-3	S-4
Conductivity (µmhos/cm)	$350 \pm 61.3$	418 ± 92.9	415 ± 86.0	387 ± 82.2
Dissolved oxygen (mg $O_2/L$ )	$9.2 \pm 1.47$	$8.4 \pm 1.35$	$9.4 \pm 1.18$	$9.7 \pm 1.6$
Fluoride (mg F <sup>-</sup> /L)	$0.31 \pm 0.08$	$1.17 \pm 0.27$	$0.84 \pm 0.11$	$0.56 \pm 0.0^{\circ}$
pH	$7.8 \pm 0.18$	$7.8 \pm 0.25$	$7.9 \pm 0.19$	$7.9 \pm 0.10$
Sulfate (mg $SO_4^{-}/L$ )	$79.2 \pm 16.2$	$94.0 \pm 20.6$	$84.7 \pm 15.1$	$82.1 \pm 18.8$
Total alkalinity (mg CaCO <sub>3</sub> /L)	$114 \pm 32.5$	118 ± 28.6	$116 \pm 26.3$	$124 \pm 25.1$
Total hardness (mg CaCO <sub>3</sub> /L)	$161 \pm 55.4$	$193 \pm 53.2$	$207 \pm 84.6$	$213 \pm 79.3$
Nitrate (mg N/L)	$0.18 \pm 0.10$	$2.13 \pm 0.76$	$1.38 \pm 0.35$	$1.03 \pm 0.3$
Water temperature (°C)	$12.3 \pm 5.85$	$12.5 \pm 5.61$	$12.8 \pm 6.22$	$12.9 \pm 6.2$

**Table 2.** Mean (n = 3) densities (individuals/m<sup>2</sup>) of each hydropsychid species and mean (n = 3) values of total density (individuals/m<sup>2</sup>) and total biomass  $(g/m^2)$  estimated at sampling sites (S-1, S-2, S-3, and S-4) in the Cache la Poudre River (Colorado) during May (M), August (A), and November (N) of 1990. Standard deviations in parentheses

	S-1	S-1					S-3			S-4		
	М	А	N	M	Α	N	M	Α	N	M	A	N
Hydropsyche												
occidentalis	527 (70.5)	387 (64.5)	631 (91.4)	0 (0.0)	0 (0.0)	25 (22.1)	204 (49.3)	172 (53.8)	254	577	623	878
Hydropsyche	(1010)	(0415)	()1.4)	(0.0)	(0.0)	(22.1)	(49.5)	(33.6)	(61.1)	(64.8)	(70.5)	(103)
bronta	176	237	405	0	0	0	0	0	0	0	79	122
	(61.1)	(69.9)	(80.7)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(45.2)	(37.8)
Cheumatopsyche					. ,	• • •		()	(0.0)	(010)	(1212)	(37.0)
pettiti	505	452	283	82	122	118	480	516	620	381	416	276
	(75.3)	(70.5)	(48.5)	(43.4)	(27.1)	(38.8)	(69.9)	(67.1)	(125)	(77.8)	(64.8)	(48.5)
Total density	1208	1076	1319	82	122	143	684	688	874	958	1118	1276
	(119)	(96.2)	(144)	(43.4)	(27.1)	(35.2)	(50.7)	(62.4)	(85,2)	(107)	(90.3)	(115)
Total biomass	3.70	3.53	4.02	0.17	0.25	0.40	1.40	1.54	2.27	3.41	3.65	3.98
	(0.41)	(0.29)	(0.44)	(0.10)	(0.08)	(0.11)	(0.12)	(0.18)	(0.20)	(0.37)	(0.33)	(0.41

the upstream reference station (S-1). Hydropsyche occidentalis was usually the most abundant species at S-1 and S-4, with highest abundances at the lowest downstream sampling site (S-4). Cheumatopsyche pettiti was dominant at S-2 and S-3, showing higher abundances at S-3 than at the reference station (S-1). Hydropsyche bronta was never collected at S-2 and S-3, and had higher abundances at S-1 than at S-4. H. occidentalis and C. pettiti were the dominant species at S-1 during May and August sampling surveys, whereas H. bronta was more abundant than C. pettiti at S-1 during the November sampling survey.

Mean (n = 3) values of total density and total biomass for the hydropsychid guild at each sampling site are presented in Table 2. These values were significantly (P < 0.05; t-test) lower at S-2 and S-3 than at the upstream reference site (S-1), increasing downstream with distance from the wastewater treatment plant #1. However, differences in the values of these two biological parameters between S-1 and S-4 were not significant (P > 0.05; t-test).

#### Toxicity Tests

Mortality percentages and mean fluoride ion concentrations for each test species are shown in Figure 3. Standard deviations were lower than 10% of their respective mean fluoride

values. The mortality increased with increasing fluoride concentrations and exposure times. In general, larvae migrated from their retreat and capture nets and protruded their anal papillae before dying, and most of protruded anal papillae were darkened in larvae exposed to the highest concentrations of sodium fluoride. These same sublethal effects have been reported in Palearctic larvae of Hydropsyche species exposed to sodium fluoride (Camargo 1989) and residual chlorine (Camargo 1991). Only larvae of H. bronta died in the aquarium with the lowest test concentration (6.0 mg  $F^{-1}$ L) (Figure 3). There were no dead animals in control aquaria, and no sublethal effect was observed in control larvae. Test hydropsychid species had mean individual dry weights, after fluoride toxicity bioassays, of  $6.2 \pm 0.44$  mg for C. pettiti,  $6.6 \pm 0.52$  mg for H. bronta, and  $7.1 \pm 0.68$  mg for H. occidentalis.

The 48, 72, 96, 120 and 144-h LC50 values and their 95% confidence limits for each test species are presented in Table 3. All  $\chi^2$  values were lower than those for P = 0.05, indicating that the data are not significantly heterogeneous (Litchfield and Wilcoxon 1949). *H. bronta* appears to be the most sensitive test species to fluoride toxicity because its LC50 values are significantly (P < 0.05; formula of factors) lower than LC50 values for *H. occidentalis* and *C. pettiti*. In addition, both head colour patterns of *H. bronta*, the Appalachian form and the Central form, showed a similar sensi-

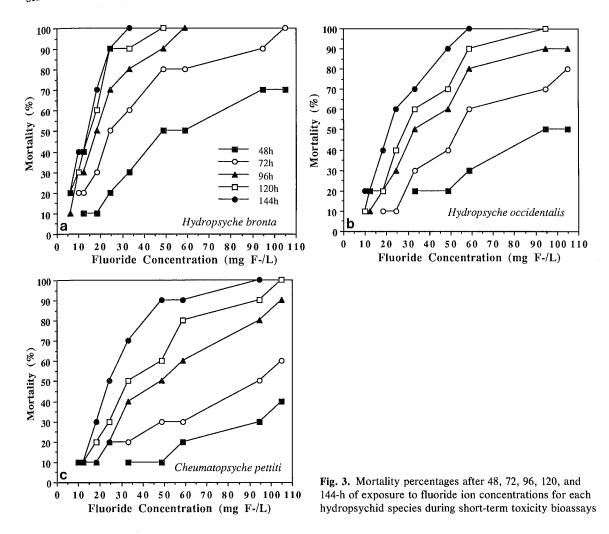


Table 3. LC50 values (mg  $F^{-}/L$ ) and their 95% confidence limits (mg  $F^{-}/L$ ) estimated for each hydropsychid species after fluoride toxicity bioassays

	Hydropsyche bronta	Hydropsyche occidentalis	Cheumatopsyche petti	
48-h	52.6 (37.1–74.6)	102.0 (70.0–148.9)	128.0 (83.6–196.1)	
72-h	25.8 (19.7-33.7)	53.5 (41.2-69.6)	73.2 (50.9–105.3)	
96-h	17.0 (12.8–22.5)	34.7 (25.5-47.2)	42.5 (29.2-61.8)	
120-h	13.4 (10.1–17.6)	27.0 (21.6-32.8)	31.9 (23.3-43.6)	
144-h	11.5 (8.9–14.8)	21.4 (16.9–27.1)	24.2 (17.7–33.2)	

tivity to fluoride ions. However, differences in LC50 values between *H. occidentalis* and *C. pettiti* were not significant (P > 0.05; formula of factors). All 144-h LC50s were much higher than fluoride concentrations measured at downstream sampling sites during field studies.

#### Discussion

Although safe criteria of fluoride ion for aquatic organisms in river systems have not yet been determined (US Environmental Protection Agency 1986), and predicting the potential chronic effects of pollutants on survival, growth and reproduction from short-term toxicity bioassays is questionable because biochemical and physiological different responses and differences in sensitivity of individuals within a population have been observed during acute and chronic exposures (Giesy and Graney 1989), the much higher LC50 values obtained in laboratory studies compared to fluoride concentrations measured in field studies would indicate that the fluoride pollution, generated by the wastewater treatment plant #1, is not a major factor in determining the spatial distribution and abundance of hydropsychid species along the study area. Moreover, *Hydropsyche occidentalis* was the dominant species at S-4, whereas *Cheumatopsyche pettiti* was dominant at S-2 and S-3, despite the fact that differences in sensitivity to fluoride between these two hydropsychid species were not significant. However, the greater sensitivity of  $\frac{1}{75}$  *Hydropsyche bronta* larvae to fluoride ions could result in decreased abundances of this species at downstream sampling sites. Other environmental factors rather than or in addition to fluoride pollution would determine the relative abundance and spatial distribution patterns of hydropsychid species in the Cache la Poudre River.

The net migration of hydropsychid larvae exposed to sodium fluoride may be interpreted as a useful adaptation in running waters to escape from unfavorable environmental conditions, and the protrusion of anal papillae may be a physiological response for increasing and improving the elimination of harmful ions (mainly  $F^-$ ). It has been reported that anal papillae can act as important ion regulatory organs in other caddisfly species (Nuske and Wichard 1972; Komnick 1977). In addition, high fluoride concentrations in waters around protruded anal papillae could generate biochemical changes in the cellular tissues of these ion regulatory organs, resulting in darkening.

Sensitivities of Nearctic and Palearctic larvae of Hydropsychidae species to fluoride toxicity appear to be similar. Camargo and Tarazona (1990) estimated 96-h LC50 values in soft water of 26.3, 26.5, 38.5 and 48.2 mg  $F^{-}/L$  for Hydropsyche bulbifera, H. exocellata, H. pellucidula, and H. lobata, respectively. Both Nearctic and Palearctic species of hydropsychids appear more sensitive to fluoride than trout fingerlings. Camargo and Tarazona (1991) have estimated 120, 144, 168 and 192-h LC50 values in soft water of 92.4, 85.1, 73.4 and 64.1 mg F<sup>-/</sup>L for Oncorhynchus mykiss and 135.6, 118.5, 105.1 and 97.5 mg F<sup>-/</sup>L for Salmo trutta fario. This greater sensitivity of hydropsychid species to fluoride may occur because fluoride ions can form stable complexes with calcium in blood and bone of fish (Sigler and Neuhold 1972), whereas stable complexes could not be formed in freshwater insect larvae. However, marine invertebrates exposed to fluoride compounds tend to accumulate fluoride in their exoskeleton during chronic exposures (Wright and Davidson 1975).

Studies on fluoride toxicity to freshwater organisms should be conducted in water quality conditions of highest toxicity (e.g., soft water) in order to determine safe criteria of the fluoride ion for aquatic life, and chronic toxicity bioassays should be performed in order to improve these quality criteria. The data presented in this work may provide a suitable background for future long-term fluoride toxicity studies with freshwater benthic invertebrates.

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## Fluoride: A review of its fate, bioavailability, and risks of fluorosis in grazed-pasture systems in New Zealand

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Abstract Fluoride (F) is an essential element for animal growth, not readily taken up by plants from soils, yet cases of acute fluorosis in grazing animals caused by ingestion of phosphatic fertilisers, volcanic ash, and industrial wastes remind us of its potential hazard. Fluoride concentrations in topsoils slowly increase where annual inputs through atmospheric pollution and phosphatic fertilisers exceed losses. This paper reviews information on the fate of F in grazed pasture systems with the aim of assessing the potential toxicity of accumulating soil F. A preliminary F-cycling model for grazed pastures, based on the review of international literature and F concentrations in selected New Zealand pasture soils, indicated that grazing sheep and cattle obtain over 50% of their dietary F (and this may be >80% during winter) from soil ingestion. The model suggests that at the extremes of the ranges of the measured winter soil ingestion  $(143-300 \text{ g d}^{-1} \text{ for sheep and } 900-1600 \text{ g d}^{-1} \text{ for}$ cattle) and dietary F absorptivity (bioavailability) of soil F (20-38%), total topsoil F concentrations in the range of 372–1461  $\mu$ g F g<sup>-1</sup> could cause chronic fluorosis in sheep and 326–1085  $\mu$ g F g<sup>-1</sup>

in cattle. We recommend that research is undertaken to measure F accumulation rates and soil F dietary absorptivity for a range of intensively managed New Zealand pasture soils.

Keywords cattle; fluoride; pasture; fluoride bioavailability; fluorosis; phosphate fertilisers; sheep; soil ingestion; volcanic ash

## **INTRODUCTION**

Fluorine is widely dispersed in nature and is estimated to be the 13th most abundant element on our planet (Mason & Moore 1982). While being distributed universally throughout soils, plants, and animals. F is assumed to be an essential element in animals. However, no environment has been described where F is limiting (National Research Council 1980). By contrast, the effects of excess F are only too well known in many parts of the world, where endemic fluorosis affects grazing animals and humans alike. In parts of India, Australia, and Africa, groundwater with high F concentrations causes fluorosis in grazing animals, while in parts of North Africa chronic fluorosis is caused by contamination of plant foliage and water by F-rich phosphatic dust blown from phosphate rock deposits (Underwood 1981). Chronic and acute fluorosis is also common in Iceland during and following volcanic eruptions, where F-rich gases and ashes contaminate surrounding pastures (Roholm 1937).

Human activities have extended the areas affected by fluorosis. Factories producing fertiliser, aluminium, steel, bricks, and glass, as well as coalfired power stations, produce F-rich emissions that can seriously affect surrounding plants and animals (Roholm 1937). Supplements containing phosphate rock materials are fed out to ruminants in amounts that have sometimes also produced chronic and acute fluorosis (Suttie 1969; Jubb et al. 1993; Schultheiss & Godley 1995). Also, the extraction of deep F-rich groundwaters to maintain production

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during droughts and summer periods has caused numerous cases of fluorosis (Botha et al. 1993).

In addition to these point-source F polluters, vast areas of land around the world have F added on a yearly basis with the application of phosphatic fertilisers (Robinson & Edgington 1946). Fluoride added in this way persists in most soils and has slowly accumulated over the last century. In New Zealand for the year ended 30 June 1994, 1.4 million tonnes of single superphosphate (SSP) was applied to c. 13.5 million ha of land (Registrar-General 1996). With an average 1.5% F in the SSP (Evans et al. 1971; P. Loganathan et al. unpubl. data), this equates to c. 21 000 tonnes of F added in only one vear (c. 1.6 kg ha<sup>-1</sup>). Assuming similar rates of SSP application over the last 50 or more years, there has probably been >1 000 000 tonnes of F added to pastoral soils in New Zealand. What does not appear to be known, is just what are the likely repercussions of this continual addition of F to our food-producing soils. How will it affect our crops, grazing animals, water supplies, and soil organisms? How long will it take before signs of excess F in our pastoral and crop systems show?

In this review we compile the information currently known and identify what needs to be researched, in order to answer these questions. The answers are drawn from many fields of research, including geochemistry, soil science, plant science, animal science, and veterinary science. Our review is an attempt at integrating published work from these diverse areas and from this to develop models for predicting the effects of accumulating F on New Zealand pastoral systems.

#### **FLUORIDE IN SOILS**

#### Natural abundance of F in soils

Native F in soils is generally contained within the minerals apatite (specifically fluorapatite  $(Ca_5(PO_4)_3F))$ , fluorite  $(CaF_2)$ , cryolite  $(Na_3AlF_6)$ , forms of topaz  $(Al_2(SiO_4)F_2)$ , and within micaeous clay minerals. Fluoride can also be present in soil as specifically and non-specifically adsorbed ions and compounds (Bowen 1966; Robinson 1978; Pickering 1985). Native soil F content is highly dependent on soil parent material. It can be very high in phosphate-derived soils in North Africa, USA, and Russia, and on some Pacific and Caribbean Islands, where values of 35 000-42 000  $\mu g g^{-1}$  soil are reported (Manley et al. 1975). Soil F can also be high in some volcanic areas that have  $_{79}$  USA were 0–1.5 µg ml<sup>-1</sup> (Fleischer & Robinson

regular inputs of fresh F-rich volcanic ash; the best example of this is in Iceland (Roholm 1937; Óskarsson 1980). However, in most areas, where soils are developed in quartzo-feldspathic rocks and sediments, phosphate-free carbonate rocks, and older igneous rocks, native soil F contents are substantially lower (Table 1).

In almost all studies of total soil F, high variability is reported between soils that are apparently uncontaminated (Table 1). Many authors correlate this variability with particle size of the soil; in particular, increasing total F is associated with increasing clay content (Robinson & Edgington 1946; Eysinga 1974; Manley et al. 1975; Omueti & Jones 1977a). Under natural conditions it is also common for total soil F to increase with depth in the profile (Robinson & Edgington 1946). This may be due to low affinity of F for organic matter (Omueti & Jones 1977a), increasing clay content with increasing soil depth, and the longterm downward movement of F through the profile.

Labile forms of soil F are variously estimated by water soluble, acid-extractable, and resinextractable F concentrations. The most common method of estimating water soluble F is by shaking soil with a 0.01 M CaCl<sub>2</sub> solution (Larsen & Widdowson 1971). Labile soil F estimated by any one of these methods is often one to three orders of magnitude lower than the total soil F (Table 1).

Fluoride forms its most stable bonds with Fe. Al, and Ca, and labile F is held by soil components that contain these elements, including clay minerals, calcium and magnesium compounds, and iron and aluminium compounds (Bower & Hatcher 1967; Omueti & Jones 1977b; Häni 1978). At low concentrations, iron and aluminium oxides and hydroxides have the greatest ability to adsorb F and, among the layer-lattice clay minerals, kaolinite and halloysite have the greatest capacity for adsorption (Bower & Hatcher 1967; Omueti & Jones 1977b; Morshina & Fanaskova 1987). The degree of F adsorption is also controlled by soil pH and is greatest in non-calcareous soils, which generally contain higher Al levels (Larsen & Widdowson 1971; Omueti & Jones 1977b; Pickering 1985; Barrow & Ellis 1986).

Natural soil solution F concentrations are normally a small proportion of labile soil F, and are normally  $<1 \ \mu g \ ml^{-1}$ . Almost all soils hold F strongly, and only in coarse, clay and Fe/Al oxidepoor soils is F weakly held (Pickering 1985). Measured normal ranges of soil solution F in the 1963), and in Sweden 0.3–0.4  $\mu$ g ml<sup>-1</sup> (Noemmik 1953). In a suite of industrially polluted soils, total soil F was 616–2700  $\mu$ g g<sup>-1</sup>, the distilled-water extractable fraction was 10–292  $\mu$ g g<sup>-1</sup>, and the soil solution F was 0.3–8.2  $\mu$ g ml<sup>-1</sup> (Polomski et al. 1982a). Manoharan et al. (1996) reported soil solution F concentrations ranging from 0.23 to 0.49  $\mu$ g ml<sup>-1</sup> in New Zealand pasture topsoils fertilised for 8 years with superphosphate at a rate of 30–60 kg P ha<sup>-1</sup> yr<sup>-1</sup>.

In most developed and developing countries native soil F contents are strongly affected by application of fertilisers and by deposition of industrial air-borne pollutants. Labile and watersoluble F concentrations appear to be affected most by industrial pollution (Polomski et al. 1982a; Haidouti 1991) and superphosphate application (Larsen & Widdowson 1971; Manoharan et al. 1996). However, prolonged application of superphosphate can have marked effects on total soil F (e.g., Kudzin & Pashova 1970; P. Loganathan et al. unpubl. data).

### Mobility and adsorption of F in soils

Many of the common F compounds are only sparingly soluble, e.g.,  $CaF_2$  (0.016 g l<sup>-1</sup>), MgF<sub>2</sub>

(0.13 g  $l^{-1}$ ), and Na<sub>3</sub>AlF<sub>6</sub> (0.42 g  $l^{-1}$ ), although some others are quite soluble, e.g., HF, SiF<sub>4</sub> (hydrates), and NaF (40 g  $l^{-1}$ ) (Pickering 1985). Many investigations (detailed below) have been made to gauge the F adsorption of soils and to determine which soil components are responsible for this.

Early studies concluded that F is retained by finer textured soils, particularly those with a significant clay component (Robinson & Edgington 1946; Brewer 1966). Later work revealed that the sorption of F also depended strongly on soil pH. At a pH of 6, F sorption was at a maximum and it decreased dramatically (by a factor of 2) at one unit higher or lower pH (Larsen & Widdowson 1971; Gilpin & Johnson 1980). This behaviour was parallel with that of the solubility of natural fluorapatite under changing pH. Barrow & Ellis (1986) later predicted that at low pH, complexes between Al and F formed in soil solution and that little was present as free F(F) ions. At high pH an increasingly unfavourable electrostatic potential decreases retention of F on the soil and increases the F<sup>-</sup> concentration in soil solution. It is also due to displacement of adsorbed F<sup>-</sup> by the increased concentration of OH<sup>-</sup> in soil solution at the higher pH (Larson & Widdowson 1971).

**Table 1** The total and labile native F concentrations (range, and mean within parenthesis;  $\mu g g^{-1}$  soil) of various soils. Water soluble F is measured by extraction with differing concentrations of CaCl<sub>2</sub> as well as with water.

Location and number of samples	Total F concentration	Water– soluble F	Resin extractable F	Source
Argentina – La Pampa	24-1220	0.53-8.33		Lavado & Reinaudi (1979)
Canada – New Foundland		1.0-2.7		Sidhu (1979)
China	186-388	0.8-2.7		Fung et al. (1999)
England	110, 230			Agate et al. (1949)
England (100)	(300)	0-1.6 (0.25)	0-50 (20)	Larsen & Widdowson (1971)
France	~ /	0.1-8		Bertrand & Wolf (1970)
Greece	95-108			Haidouti (1991)
Netherlands (102)	39-679			Eysinga (1974)
New Zealand			20-30	Stewart et al. (1974)
New Zealand – Bluff (25)	22-230 (134)			Manley et al. (1975)
New Zealand (28)	68-540 (200)			Gemmell (1946)
New Zealand (3)	116,133,214			P. Loganathan et al. (unpubl. data)
Russia (46)	30-320 (200)			Vinogradov (1954)
Scotland	8, 13, 15, 82			Agate et al. (1949)
Sweden		5.3-20.3		Noemmik (1953)
Switzerland	166-288	11-12		Polomski et al. (1982a)
USA – Illinois (201)	70-618 (271)			Omueti & Jones (1977a)
USA – Pennsylvania (55)	136–990 (377)	0.05 - 1.5(0.4)	7.7-66.8 (21.7)	Gilpin & Johnson (1980)
USA – Tennessee	80–3650		· · ·	MacIntire (1952)
USA (30)	20-590 (290)			Robinson & Edgington (1946)
USA (clay subsoil)	45, 83, 411			McHargue & Hodgkiss (1939)
Various tropical areas (37)	68-216 (131)			Hall & Cain (1972)
<u></u>		80		

Bower & Hatcher (1967) investigated the retention of F by alkaline soils, an acid soil, common hydroxyl-containing soil minerals, freshly precipitated Al(OH)<sub>3</sub>, and Al(OH)<sub>3</sub> precipitated onto bentonite. Adsorption of F over a range of 2-16 mg  $1^{-1}$  was well described by Langmuir isotherms and pH of the solution phase increased, although the increase in OH<sup>-</sup> was only a small fraction of F adsorbed. The order in the ability of the various materials to adsorb F was: Al(OH)<sub>3</sub> precipitate on bentonite  $> Al(OH)_3 >> hydrated$ halloysite and dehydrated halloysite > a weakly acidic soil >> kaolinite > gibbsite > alkaline soils > goethite > bentonite and vermiculite. Their main findings were that (1) adsorption of F on hydrated halloysite was not higher than on its dehydrated form (with much less lattice OH exposed), and (2) that Al(OH)<sub>3</sub> had an extremely high F adsorption capacity. From this, Bower & Hatcher (1967) concluded that F adsorption occurs primarily by exchange with OH groups from Al(OH)<sub>3</sub> and basic Al polymers adsorbed on mineral surfaces, rather than by exchange with crystal lattice OH groups of clay minerals.

Later, Flühler et al. (1982), investigated F adsorption and desorption rates in an acid soil and two calcareous soils with solutions over the range of 0–5000 mg F  $1^{-1}$ . Adsorption isotherms in these soils were not linear over the range of solution concentrations and the Langmuir equation provided the best fit, especially at the low end of the concentration range. Adsorption and desorption in the calcareous soils are considerably slower than in the acid soil, appearing to be limited by the rate of formation and dissolution of CaF2. In calcareous soils F retention is mostly in the form of  $CaF_2$  and  $Ca_5F(PO_4)_3$  (Brewer 1966; Eysinga 1972). The percolation experiments of Flühler et al. (1982) showed that although adsorption was effectively instantaneous in acid soils, in the calcareous soils (particularly under high flow rates) slow rates of F adsorption led to its rapid leaching.

The ability of non-calcareous soils (even those that are highly disturbed and strongly leached) to strongly retain F has been demonstrated by MacIntire et al. (1948, 1955) and Murray (1983, 1984). MacIntire et al. (1948) reported that even with application of 5529 kg ha<sup>-1</sup> of CaF<sub>2</sub>, 98% of the F added was retained over 10 years. Murray (1984) added up to 10 kg F m<sup>-2</sup> soil surface in the form of NaF and Na<sub>3</sub>AlF<sub>6</sub> to a highly leached and disturbed soil and found that only 2.6–4.6% of it leached after a year. In addition, Murray (1984) demonstrated that the link between soil components and F strengthened over time, resulting in lowering of water-soluble F concentrations.

From the observations of Bower & Hatcher (1967), Barrow (1986), Murray (1984), and Barrow & Ellis (1986), it appears that F is adsorbed specifically by displacing  $OH^-$  from soil surfaces and that the initial adsorption is followed by diffusive penetration of F into the soil particles.

Histeretic desorption in calcareous soils was attributed to the rate-limiting effect of  $CaF_2$ dissolution (Flühler et al. 1982). However, similar irreversible adsorption was exhibited in soils containing amorphous Al hydroxides as the dominant sorption sites (Peek & Volk 1985). This behaviour and that reported by Murray (1984) indicate that F sorption is more complex than a simple ligand exchange reaction.

More recent work has examined the presence and role of fluoro-aluminium complexes as exchange-phase components in natural acid soils and F-contaminated soils (Anderson et al. 1991; Wenzel & Blum 1992; Manoharan et al. 1996). The cationic  $AIF^{2+}$  and  $AIF_2^+$  species exist at pH < 6 and play an important role in adsorption/desorption of F, increasing the apparent solubility of F under acidic conditions.

# Accumulation of F in soils from industrial sources

The F pollution sources of greatest magnitude are those associated with industrial sites, including manufacturers of bricks, iron, fertilisers, and glass, coal-fired power stations, and, particularly, aluminium smelters (Israel 1974; Polomski et al. 1982a; Pickering 1985; Haidouti 1991; Gritsan et al. 1995). These pollution sources release gaseous (e.g., HF, SiF<sub>4</sub>) and particulate fluorides (e.g., AIF<sub>3</sub>, Na<sub>3</sub>AlF<sub>6</sub>, CaF<sub>2</sub>), and can raise soil F concentrations by between 2 and 20 times (e.g., Polomski et al. 1982a; Gritsan et al. 1995). However, the effect of these F pollution sources is generally restricted to within 10–20 km downwind (e.g., Haidouti 1991) and becomes most serious where the density of Femitters is high (e.g., Gritsan et al. 1995).

Soils highly polluted by industrial F emissions tend to have their highest total F concentrations at the surface of the profile (opposite to the "natural" situation, e.g., Robinson & Edgington 1946), whereas water-extractable F tends to increase with depth (Polomski et al. 1982a; Haidouti 1991).

# Accumulation of F in soils from long-term fertilisation

A more widespread source of F pollution in agricultural soils is the long-term application of phosphatic fertilisers (McLaughlin et al. 1996). Most phosphate rocks contain around 3-4% F (Table 2), which is reduced by the acidulation process during manufacture of phosphate fertilisers. With the addition of other nutrients such as S in superphosphate (SSP), and NH<sub>4</sub> in ammoniated phosphates, the F content is typically diluted to between 1 and 3% (Table 2) depending on the type of phosphate rock used. Environmental concern has forced HF, released during acidulation, to be removed from stack gases by lime scrubbers. The scrubber effluent is often used in the granulation process. In this case all phosphate rock F ends up in the SSP fertiliser, and F/P ratios of SSP will be similar to those of the parent phosphate rock. In the years prior to 1980 the scrubber effluent was mostly discharged elsewhere and, therefore, SSP made during that period would have had a lower F content.

Single superphosphate applications of 10–30 kg P ha<sup>-1</sup> yr<sup>-1</sup> are likely to add at least 2–5 kg F ha<sup>-1</sup> yr<sup>-1</sup> to pastoral soils. Where phosphate fertilisers containing higher F-contents are used (e.g., ammoniated phosphates and phosphate rock), the

same application rates of P could add as much as 12 kg F ha<sup>-1</sup> yr<sup>-1</sup>. Given the strong retention of F demonstrated in all but calcareous soils, F is likely to accumulate over years of application. There have been few detailed studies of such F accumulation.

Robinson & Edgington (1946) examined the effects of fertiliser F accumulation in five different soil types in New Jersey by analysing soils from adjacent areas with or without past fertiliser applications. Total soil F concentrations in cultivated soils that had been fertilised for 23-40 years were 36-96% higher in the top c. 150 mm compared with their unfertilised equivalents. The largest increases occurred in the finer-textured soils (loams). In the depth range of c. 150-350 mm, total F concentrations were elevated by 13-39% compared with their unfertilised equivalents. The largest increases at this depth were in the coarsertextured soils (sandy loams), indicating greater F movement to depth in soils with lower particulate specific surface areas. Robinson & Edgington (1946) calculated that the finest textured soils (loams) retained as much as 60% of the fertiliserapplied F in the top 400 mm, whilst the coarsertextured soils (sandy loams and gravelly sandy loams) retained only between 7 and 20% of applied F in the top 350 mm. In a later study, Kudzin &

Material	F concentration (%)	Reference
Phosphate rocks		
Arad (Israel)	4.0	Syers et al. (1986)
Christmas Island –A	2.2	Evans et al. (1971)
Chatham Rise phosphorite	3.0	Syers et al. (1986)
Gafsa (Tunisia)	4.1	Syers et al. (1986)
Jordan	3.8	Syers et al. (1986)
Makatea Island	3.2	Syers et al. (1986)
Mexican	4.1	Syers et al. (1986)
Khouribga (Morocco)	4.0	Anon. (1990)
Nauru Island	3.0	Syers et al. (1986)
North Carolina	3.5	Syers et al. (1986)
North Florida	4.0	Syers et al. (1986)
Sechura (Peru)	3.4	Syers et al. (1986)
Fertilisers		
Single superphosphate	1.08-1.84	Evans et al. (1971); P. Loganathan et al. unpubl. data; McLaughlin et al. (1997)
Triple superphosphate	1.3-2.4	Mordvedt & Sikora (1992); Evans et al. (1971)
Monoammonium phosphate	1.6-2.2	Mordvedt & Sikora (1992); Evans et al. (1971)
Diammonium phosphate	1.2-3.0	Mordvedt & Sikora (1992); Manoharan et al. (1996)

 Table 2
 Fluoride concentration of a range of phosphate rocks and fertilisers that have been used in New Zealand.

Pashova (1970) reported a 22-25% increase in total soil F after single superphosphate (SSP) was applied at the rate of c. 26 kg P ha<sup>-1</sup> yr<sup>-1</sup> to Chernozem soils for 35 years.

Larsen & Widdowson (1971) reported a measurable increase in water-soluble soil F in field trials where 38 kg P ha<sup>-1</sup> yr<sup>-1</sup> was applied for seven vears as SSP. Control plots had water-soluble F concentrations of 0.22–0.40  $\mu g g^{-1}$ , while treated plots contained 0.31–0.60  $\mu$ g g<sup>-1</sup>; increases between 40 and 70% of initial values.

## Fertiliser effects on soluble F concentration in New Zealand pastoral soils

Significant changes have been recorded in soil solution F concentration after eight years of varying rates and types of phosphatic fertiliser application on a silt loam pastoral soil (Manoharan et al. 1996). SSP was applied at rates of 15-60 kg P ha<sup>-1</sup> yr<sup>-1</sup> (supplying 2.4–9.0 kg F ha<sup>-1</sup> yr<sup>-1</sup>), North Carolina and Jordan phosphate rocks at a rate of 30 kg P  $ha^{-1} yr^{-1}$  (7 kg F  $ha^{-1} yr^{-1}$ ), and diammonium phosphate at a rate of 30 kg P ha<sup>-1</sup> yr<sup>-1</sup> (1.8 kg F ha<sup>-1</sup> yr<sup>-1</sup>). Increasing rates of SSP application resulted in a linear increase in the soil solution F concentration from the control plot (0 kg P ha<sup>-1</sup> yr<sup>-1</sup>) value of 0.05  $\mu$ g ml<sup>-1</sup> to 0.49  $\mu$ g ml<sup>-1</sup> for the 60 kg P ha<sup>-1</sup> yr<sup>-1</sup> SSP application rate. However, although more F was added in the phosphate rock applications, soil solution F concentrations rose only to  $0.10-0.32 \ \mu g \ ml^{-1}$  due to incomplete dissolution (48-68% dissolution) of the phosphate rocks over the eight-year trial (Manoharan et al. 1995). The diammonium phosphate treatment resulted in an increase to  $0.21 \,\mu g \, ml^{-1}$ . Manoharan et al. (1995) also noted that total soil solution F concentrations were not significantly higher in the upper 30 mm of soil compared with samples from the 30-75-mm depth. The free F concentration, however, was higher and Al-F complex concentration lower in the upper depth, due to a higher surface soil pH.

These few studies demonstrate that the application of phosphate fertilisers, including SSP, over long periods of time increases the concentration of total and soluble F in the soil. The greatest elevation of existing total soil F probably occurs in finetextured acidic soils, and is highest in the upper 150 mm of soil for all but very coarse-textured soils (e.g., gravelly sandy loams). It also appears that the greater solubility of SSP compared with some phosphate rocks releases more F into the soil solution.

## Preliminary assessment of total F accumulation rates in fertilised New Zealand pastoral soils

Soils from two sets of existing SSP fertiliser trials were used to make a preliminary assessment of the rates of F accumulation. The first is a trial on sheepgrazed farmlets at AgResearch Ballantrae Hill Research Station, and the second a grazing/mowing trial at Massey University. Total soil F was measured by fusing soil with NaOH, followed by dissolution of the fused cake in water, adjusting the pH to  $8.5 \pm 0.1$  with conc. HCl, filtering the solution (Frankenberger et al. 1996), and determining F in the filtrate using an F-ion selective electrode (Larsen & Widdowson 1971; Manoharan et al. 1996).

#### The AgResearch Ballantrae Hill Research Station

Soils collected were Brown Soils (New Zealand classification system; Hewitt 1992) or Typic Dystrochrepts (US soil taxonomy) formed from quartzo-feldspathic sandstone and siltstone, under an average annual rainfall of 1200 mm, 20 km northeast of Palmerston North, at an altitude of 250-350 m. The P retention of the soils was <27% (Lambert et al. 1988). Soil pH (H<sub>2</sub>O) was between 5.1 and 5.9 within the top 75 mm. Further soil details are reported by Loganathan et al. (1995). Three sets of fertilised and unfertilised soils were collected from low slope areas (0-12°) facing east, south-west, and north-west. The fertilised soils have received 761 kg P ha<sup>-1</sup> (corrected for slope) over 20 years of SSP application (Loganathan et al. 1995). Using the mean of the F concentration in SSP (1.46% F) reported by Evans et al. (1971) and P. Loganathan et al. (unpubl. data), a total application of 123 kg F ha<sup>-1</sup> is calculated for these soils over 20 years.

The Ballantrae data (Table 3) indicate that 20 years of SSP application has increased topsoil F concentrations by 60-120%. On these soils, between 42 and 54% of F applied in fertiliser is retained in the top 75 mm of soil, depending on the slope and aspect. The same retention patterns for P and S on the three aspects were found in these trial areas and attributed to animal transfer and leaching patterns (Saggar et al. 1990). Sheep grazing these areas spend proportionally more time on the eastfacing slopes, sheltering from the prevailing NW wind. Hence, proportionally greater returns of F from urine and dung may occur in these east-facing areas.

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### Number 4 Dairy Unit, Massey University

The soil at this location was a Pallic Soil (New Zealand classification system; Hewitt 1992) or an Aeric Fragiagualf (US Soil Taxonomy) formed from quartzo-feldspathic loess, located on flat land near Palmerston North, and receiving an average annual rainfall of 1000 mm. The soil pH (H<sub>2</sub>O) was 5.70 for 0-75 mm depth (Manoharan et al. 1996), and P retention is c. 22% (Saunders 1965). Plots at this site received 0, 30, and 60 kg P ha<sup>-1</sup> yr<sup>-1</sup> for 8 years as SSP, adding a total of 0, 38.9, and 77.9 kg F ha<sup>-1</sup>. respectively (Manoharan et al. 1996). The No. 4 Dairy Unit data (Table 4) indicate that 8 years of SSP application at rates of 30 and 60 kg P ha<sup>-1</sup> yr<sup>-1</sup> have increased total topsoil F concentrations by 37-48%. The proportion of F retained in the top 30 mm of soil is higher than in the Ballantrae soils, which may be in part due to a lack of animal tread-induced mixing of the upper part of the soil on the mowing/ grazing No. 4 Dairy Unit trial and lower rainfall. For the rates of SSP application at 30 and 60 kg P ha<sup>-1</sup> yr<sup>-1</sup> the percentage of applied F retained is similar in the 0-30-mm depth, although at 60 kg P ha<sup>-1</sup> yr<sup>-1</sup> F retention is lower in the 30-75-mm depth increment. This may reflect greater competition of F with P for adsorption sites at 30-75 mm in the SSP(60) treatment.

Both of these data sets indicate that retention of F elevates topsoil F concentrations, even in soils with low P-retention and clay mineral fractions dominated by crystalline layer-lattice clay minerals (New Zealand Soil Bureau 1968; Pollock 1975). Our results obtained for 0–75 mm depth cannot be directly compared with the whole profile values reported by Robinson & Edgington (1946), MacIntire et al. (1948), and Murray (1984). In soils containing appreciable Al- and Fe-hydroxide minerals (e.g., allophanic soils), F retention is likely to be much higher.

In addition to the above data sets, lower retention values were obtained in a 40-year, sheep-grazed pasture irrigation trial at Canterbury, New Zealand

Total soil F concentration  $(\mu g g^{-1})$ Concentration Increase in Soil depth soil F storage % of applied F increase Control Fertilised  $(\mu g g^{-1})$  $(kg ha^{-1})$ retained (%) Aspect (mm) 109.7 0-30 93.9 203.6 26.3 21 190.8 83.2 39.7 East 30-75 107.6 0-75 66 54 0 - 3096.7 186.9 90.2 21.6 18 South-west 30 - 75113.9 191.3 77.4 36.9 0-75 58.5 48 0 - 30109.7 178.3 68.6 16.5 13 30-75 120.4 193.0 72.6 34.5 North-west 42 0-75 51

**Table 3**Calculation of the accumulation of soil F from 20 years of SSP application on grazed farmlets at Ballantrae(providing a total of 123 kg F ha<sup>-1</sup>).Values for total soil F concentration are means of two replicate analyses.Bulkdensity of 900 kg m<sup>-3</sup> is assumed for calculation of increase in soil F storage.

Table 4         Calculation of F retention following 8 years of SSP application on a mowing/grazing trial at No. 4 Dairy
Unit, Massey University. SSP(30) and SSP(60) represent fertiliser treatments of 30 and 60 kg P ha <sup>-1</sup> yr <sup>-1</sup> as SSP,
respectively. <sup>†</sup> Means of five replicate samples. <sup>‡</sup> Means of four replicate samples. Bulk density of 900 kg m <sup>-3</sup> is
assumed for calculation of increase in soil F storage.

Coll doub	Total soil F concentration $(\mu g g^{-1})$			Concen increase		Increase in soil F storage (kg ha <sup>-1</sup> )		% of applied F retained (%)	
Soil depth (mm)	Control	SSP(30)	SSP(60)	SSP(30)	SSP(60)	SSP(30)	SSP(60)	SSP(30)	SSP(60)
0-30 30-75	90.7† 90.3±	122.2† 113.2±	154.1† 116.7±	31.5 22.9	63.4 26.4	8.5 10.3	17.1	22	22
0-75	>0.04					18.8	29.0	48	37

(P. Loganathan et al. unpubl. data). In this trial, 32 and 37% of F applied in SSP at rates of 376 and 188 kg SSP ha<sup>-1</sup> yr<sup>-1</sup>, respectively, remained in the 0–75-mm depth. This low recovery may have been due to leaching of F as a result of the irrigation treatment.

#### Critical levels of F in soils

Increased F uptake is toxic to plants and animals (see below), thus it is desirable to minimise the rates of F accumulation in soils. Establishing single upper threshold F concentrations is a difficult task. This is because soils differ considerably in their ability to retain F. Hence, high concentrations of F strongly retained in fine-textured, amorphous Alhydroxide-rich, slightly acidic soils may not cause any problems for F leaching into groundwater or being taken up by plants. However, even much lower concentrations within calcareous or very coarse textured soils, low in amorphous compounds, may prove critical for affecting groundwater or plant life. Further, plants take up F to differing degrees (Brewer 1966), and some may show little effect from very large available-F pools (MacIntire et al. 1942; Braen & Weinstein 1985; Singh 1990). Of greatest importance for plant uptake and groundwater pollution are probably the critical levels of the more available forms of F in soils, particularly that in soil solution or in the water-soluble fraction. In soil solution, F exists in various species. In acid soils a significant fraction of F exists as  $AIF^{2+}$  and  $AIF_{2}^{+}$  (Manoharan et al. 1996). Although recent studies indicated that Al-F species are non-toxic to plants at lower concentrations, they are toxic at higher concentrations and vary between species (Manoharan 1997; McLaughlin et al. 1997). Stevens et al. (1997) showed that Al-F species are less toxic than  $Al^{3+}$ ,  $Al(OH)^{2+}$ , and  $Al(OH)_2^+$  species to tomato and oats. Presently no standard values for critical concentrations of the various F species in soil solution appear to exist. Further research in this area is warranted, because with increasing acidification of pastoral soils, the concentrations of Al-F species in soil solution increase (Stevens et al. 1998a, 1998b).

However, for grazing animal health, the total F levels of soil may also be critical if soil is ingested at high rates (e.g., in winter in New Zealand, Healy 1968; Lee et al. 1996), and if a significant fraction of soil F can be extracted by digestive fluids (see below).

### Effects on soil organisms

Rao & Pal (1978) reported increasing organic matter contents in a series of surface soils progressively closer to an aluminium factory as the concentrations of F in soil and litter rose (soil total F from 380 to 1803  $\mu$ g g<sup>-1</sup>). They attributed this to inhibition of growth and activity of soil micro-organisms by the elevated F levels, thus reducing organic matter breakdown. However, an additional and opposing effect of F addition to soils is the increased mobilisation of organic matter by F and its subsequent leaching (Polomski et al. 1982b). Subsequent laboratory studies have shown that soil amendments of  $<200 \ \mu g \ F \ g^{-1}$  inhibited soil respiration and dehydrogenase activity, but amendments over this level and up to 2000  $\mu$ g g<sup>-1</sup> did not affect respiration or redox level but influenced denitrification (Ottow & Kottas 1984; Becker & Ottow 1985). Wilke (1987) recorded increased leaching of organic matter, Fe, Al, and P on application of F to acid soils but not in the case of a calcareous soil. After treatments of up to 3700  $\mu$ g F g<sup>-1</sup>, soil respiration was not affected, although activities of dehydrogenase and other enzymes, as well as nitrification, were inhibited at much lower F concentrations in the acid soils. The microbiological activity of the calcareous soil was unaffected. Wilke (1987) proposed that the soil respiration was not affected by addition of F because the negative effects of F on micro-organisms were balanced by the F-induced dissolution of organic matter, desorption of P, and increasing soil pH.

A recent study of the effects of airborne F accumulation on grassland (Tscherko & Kandeler 1997) reported more detailed threshold concentrations of water-extractable F that affect microbiological activity. In a series of sites at increasing distances from an Al smelter, water-extractable F concentrations decreased from 189 to 10  $\mu$ g g<sup>-1</sup>, and microbiological activities showed a strong inverse trend. In the most polluted soils, microbiological activities were only 5-20% of those in the uncontaminated soils and more organic matter had accumulated. When water-extractable F concentration was >100  $\mu$ g g<sup>-1</sup>, microbial biomass and dehydrogenase activity decreased substantially, while at 20  $\mu$ g g<sup>-1</sup> the arylsulphatase activity was inhibited.

### Soil quality regulations for agricultural soils

There are currently no global regulations or recommendations that apply to permissible levels Cronin et al.—Fluoride in New Zealand grazed pastures

of F in soils. This is probably due in part to the paucity of data on the concentrations of various forms of F in natural soils world-wide, and the huge variation of reported F levels in the limited data set in existence (Table 1). In parts of Europe a maximum permissible level of 45  $\mu$ g g<sup>-1</sup> waterextractable F is applied to soils (Fiedler & Rösler 1993), which is considerably higher than most natural soil levels (Table 1).

#### **EFFECTS OF SOIL F ON PLANTS**

Most plant material in the absence of any pollution sources contains between 2 and 20  $\mu$ g F g<sup>-1</sup> on a dry weight basis (Robinson & Edgington 1946; Brewer 1966; Weinstein 1977; Robinson 1978). Reported F concentrations in pastures range between 0.7 and 16  $\mu$ g F g<sup>-1</sup> (Blakemore et al. 1948; Agate et al. 1949; Compton et al. 1953; Allcroft et al. 1965; Oelschläger et al. 1968; Farrier & Pullen 1973; Manley et al. 1975). McLaughlin et al. (1997) reported that F concentrations in clover collected from a P fertiliser trial were extremely low (<10 µg  $g^{-1}$ ), often near the detection limit for the analysis technique (1  $\mu$ g g<sup>-1</sup>). F concentrations in mixed herbage from pasture lands in New Zealand were  $<10 \ \mu g \ g^{-1}$ , most samples being  $<2 \ \mu g \ F \ g^{-1}$ . However, some plants are known accumulators of F, such as tea (Camellia sinensis) (Fung et al. 1999) and species of Camellia, Dichapetalum, Gastrolobium, Oxylobium, and Palicoure (Zimmerman 1952; Brewer 1966; Hall 1972; Vickery & Vickery 1976). Some of these species can accumulate up to 4000  $\mu$ g F g<sup>-1</sup> and not show signs of toxicity (Jacobson et al. 1966). Most other plants show signs of toxicity at much lower concentrations (c. 30-300  $\mu$ g F g<sup>-1</sup>; Brewer 1966), while some species are extremely sensitive to concentrations <20 µg F  $g^{-1}$ , e.g., *Gladiolus* and *Freesia* (Jacobson et al. 1966; Istas & Alaerts 1974).

Table 5Pasture F concentrations following eight yearsof SSP application on a mowing/grazing trial at No. 4Dairy Unit, Massey University.†Mean of three replicateplots; others are means of four replicate plots.

Treatment	Mean F concentration (µg g <sup>-1</sup> )	Standard error (µg g <sup>-1</sup> )
Control	5.3	0.5
30 kg P ha <sup>-1</sup> yr <sup>-1</sup> as SSP 60 kg P ha <sup>-1</sup> yr <sup>-1</sup> as SSP	5.7 7.1+	1.1 2.1

Fluoride toxicity in plants is normally indicated by marginal necrosis (tip-burn, scorching, or lesions) on foliage, which begins on the margins or tips of the leaves and moves inward (Brewer 1966). Chronic F toxicity may also be indicated by interveinal chlorosis, before marginal necrosis develops (Thomas 1951; Brewer 1966). Both of these conditions lead to decreased photosynthetic efficiency and lower plant yields (Brewer et al. 1967; Robinson 1978). Other effects include reduction in flower production (Koster 1972) and flower quality (Bruyn & Hulsman 1972).

Many studies have revealed that, adjacent to industrial areas and active volcanoes, airborne contaminants are the most important source of excess F in plants (Thomas 1951; Adams 1956; Treshaw 1971; Istas & Alaerts 1974; Gritsan et al. 1995). Atmospheric F is absorbed by the plant leaves and remains within the foliage (Brewer 1966). Irrigation water can also be an important source of F, and Singh et al. (1995) have demonstrated its uptake by plant roots grown in sand, although much of the F remained within the roots.

Some studies have suggested that F complex ions, such as Al-F complexes (Takmaz-Nisancioglu & Davison 1988; MacLean et al. 1992) and Si-B-F complexes (Collet 1969), may be taken up by plants to a greater degree than free  $F^-$ . Davison (1983) suggested that low pH may drive increased F uptake by plants. This is probably due to low pH encouraging formation of Al-F species (Barrow & Ellis 1986) and, at very low pH (<4.5), possible formation of the highly permeable HF species (Kronberger 1988). In nutrient solution studies. Stevens et al. (1998a, 1998b) have shown that F as HF is much more readily taken up by oats and tomato than F<sup>-</sup> and Al-F complexes. Takmaz-Nisancioglu & Davison (1988) demonstrated that F given as AlF<sub>3</sub> to plants in solution culture caused root and new shoot F concentrations to be more strongly elevated than when NaF was applied.

# Availability of soil and fertiliser F to crops and pastures

As discussed above, most natural soils have a very high affinity for F and retain it strongly. Hence, plant uptake (like leaching loss) of excess F from soils is generally minimal. The amount of F taken up by plants appears to be unrelated to the total F concentration of the soil, and instead depends on soil type, pH, organic matter, and Ca and P content (MacIntire et al. 1942; Prince et al. 1949; Hurd-Karrer 1950; Treshow 1971; Hall & Cain 1972; Cooke et al. 1976; McClenahen 1976). Adding large quantities of soluble F (50  $\mu$ g g<sup>-1</sup> soil, as HF and NaF) to acid soils (pH 5.0) can produce increased uptake of F by plants, but for well-limed soils or the application of less soluble compounds (e.g., CaF) increase in F uptake was minimal (Prince et al. 1949; Hurd-Karrer 1950; MacIntire et al. 1951; Singh et al. 1995). However, Keerthisinghe et al. (1991) demonstrated in a pot trial that addition of NaF, at rates of 25–100  $\mu$ g F g<sup>-1</sup> soil, to a soil limed to pH 5.4 increased clover shoot concentrations of F by 50–360%. Formation and uptake of A1-F complexes were thought to be responsible for the increasing plant F concentrations.

On the basis of results in the literature, Brewer (1966) suggested that water-soluble F levels in soils may be better predictors of plant uptake than total soil F. Eysinga (1974) found a corresponding relationship of increasing F uptake by plants with water-soluble F concentrations in soils, but other studies found no such relationship (Hall & Cain 1972; Cooke et al. 1976). However, such relationships are likely to be complicated by plants that accumulate F in their roots rather than leaves (Cooke et al. 1976; Singh et al. 1995) and plants with mechanisms that can impede F uptake (Koster 1972). Later, Davison (1982) found that watersoluble F concentration was highly correlated with plant F levels in sodic soils, but not in mildly acidic soils.

Hart et al. (1934) observed that F in fertilisers did not significantly increase the F content of plants. Later, MacIntire et al. (1942) reported that the addition of superphosphate and CaF<sub>2</sub>-bearing slag to soils, increasing their F concentration by as much as 2300  $\mu$ g F g<sup>-1</sup>, caused only very slight increases to the F content of grass and clover. Kudzin & Pashova (1970) reported increases in the F content of maize of 23% (whole plant), 65% (leaves), 15% (stems), and 11% (grain) after application of superphosphate (60 kg  $P_2O_5$  ha<sup>-1</sup> yr<sup>-1</sup>) for 35 years. However, later studies have revealed little change in plant F content due to application of P fertilisers. In a two-year greenhouse experiment Singh (1990) noticed only slight increases in F content of oats and rape caused by P fertiliser application during the first year and no increases in the second year, presumably due to increasing retention of F with time within the soil. However, these levels were well within the normal range of plant F contents (2–20  $\mu$ g g<sup>-1</sup>; Brewer 1966). McLaughlin et al. (1992) found that on a pasture soil to which superphosphate had been added at a

rate of >4000 kg of P ha<sup>-1</sup> over 80 years, pasture herbage F contents were not significantly higher than pasture on adjacent unfertilised soil. Similar results have also been found for F in herbage from pasture lands in New Zealand where SSP had been applied for 20–40 years (P. Loganathan et al. unpubl. data).

We measured F concentrations (using the method of Frankenberger et al. 1996) in pasture collected from the previously described No. 4 Dairy Unit mowing/grazing fertiliser trial. Eight years of SSP application in the No. 4 Dairy Unit trial caused substantial increases in total soil F concentrations (Table 4). However, the same total application of 38.9 or 77.9 kg F ha<sup>-1</sup> over 8 years did not cause significant (P = 0.05) increases in pasture F concentrations (Table 5). The plots with fertiliser applied showed a greater variability between replicates.

Our results and those of previous plant studies, together with results from soil F mobility studies, indicate that, within the pH range of 5.5-6.5, increasing soil F levels are not likely to cause substantial increases in pasture or crop F concentrations. Increases to levels causing plant toxicity or toxicity to animals fed with the plants are unlikely, providing soil ingestion is excluded (see below). However, at lower soil pH some studies indicate that more F may be available for plant uptake as A1-F complexes. Factors controlling F uptake in acidic soils require further clarification, particularly in areas subject to soil acidification.

#### **EFFECTS OF SOIL F ON WATER QUALITY**

In most instances, water with high F concentrations  $(5-45 \ \mu g \ g^{-1})$  is sourced from deep aquifers in areas of phosphatic or recent volcanic geology (Walker & Milne 1955; Bower & Hatcher 1967; Paliwal et al. 1969; Somani 1974). Other shallower aquifers with varying geology as well as land surface drainage waters are generally much lower in F  $(0.08-0.22 \ \mu g \ g^{-1}; K \ddot{o} p f et al. 1968; Eysinga 1972;$ Manley et al. 1975). The F leaching and adsorption studies described above indicate that in most instances soil F is not likely to degrade the quality of ground water. This is borne out by soil solution F concentrations, which are generally  $<1 \ \mu g \ g^{-1}$ (Pickering 1985). Conversely, the opposite may be true, and many types of soils may be useful filters to purify groundwater containing high F concentrations (Bower & Hatcher 1967; Gilpin & Johnson 1980).

## EFFECTS OF SOIL F ON GRAZING ANIMALS

# Accumulation and effects of dietary F on grazing animals

Fluorine occurs throughout bones, teeth, soft tissue, and fluids of animals but, despite its ubiquitous presence, its function is unknown and its necessity not unequivocally proven (Underwood 1981). Some studies have demonstrated various symptoms that were attributed to F deficiency in laboratory animals in artificially F-depleted environments (Schwarz & Milne 1972; Milne & Schwarz 1974; Messer et al. 1974), while others could find no such effects (Maurer & Day 1957; Doberenz et al. 1964; Weber & Reid 1974; Tao & Suttie 1976). However, the toxic effects of F in animals are well proven. The earliest records of acute and chronic fluorosis in farm animals are in Iceland, during and after volcanic eruptions (Roholm 1937). In many other areas of the world, chronic fluorosis in grazing farm animals has been identified from 1931 onward (Underwood 1981).

In humans and in rats, F is absorbed in both the stomach and small intestine (Cerklewski 1997). At the low pH within the stomach, formation of highly permeable HF is encouraged and absorption is rapid (Whitford & Pashley 1984). Studies of rats indicate that most F is absorbed in the small intestine, where F is absorbed by non-pH-dependent diffusion (Nopakun et al. 1989; Nopakun & Messer 1990). If the animal is in a fasted state, greater F absorption occurs rapidly in the stomach (up to 100% absorption), whereas in the presence of food, absorption is 50–80% efficient (Cerklewski 1997). Passive absorption of F occurs predominantly in the rumen of ruminants (National Research Council 1980).

Absorption of F by ruminants is affected by other dietary factors; Al salts protect against high F intakes by reducing its absorption in the intestinal tract (Becker et al. 1950; Hobbs & Merriman 1959; Krishnamachari 1987), as do Ca salts in rats (Weddel & Muhler 1954). Increasing levels of dietary fat seem to exacerbate toxicity of F (McGown & Suttie 1974) by increasing F absorption.

Chronic fluorosis has been reported in grazing animals due to volcanic gas and ash (Roholm 1937; Araya et al. 1990, 1993; Shanks 1997), ingestion of commercial phosphorus licks and supplements (Shupe et al. 1992; Jubb et al. 1993; Schultheiss & Van Niekerk 1994; Schultheiss & Godley 1995; Singh & Swarup 1995), ingestion of phosphate fertiliser residues (O'Hara & Cordes 1982; Clark & Stewart 1983), ingestion of forage or waters polluted by industrial emissions (e.g., Karstard 1967; Kay et al. 1975; Singh & Swarup 1995; Kierdorf et al. 1996), and intake of F-rich groundwater or geothermal waters (Harvey 1952; Shupe et al. 1984; Botha et al. 1993).

The effects of chronic F toxicity usually take weeks or months to manifest themselves while excess F is excreted in urine and deposited within bones (Underwood 1981). The skeleton of animals normally contains the greatest proportion of F within the animal and normal whole bone F concentrations range between 300 and 600  $\mu g g^{-1}$ (fat-free, dry basis), the highest concentrations being within cancellous bones such as ribs and vertebrae (Underwood 1981). Teeth contain around half the F concentration of bone, and soft tissue around 204  $\mu g g^{-1}$  on a dry basis in ruminants (Underwood 1981). The kidney has the highest F concentration of soft tissue because F is lost mainly in urine (National Research Council 1974). Plasma F concentrations reflect short-term changes in F uptake with levels of  $< 0.1 \, \mu g \, g^{-1}$  in normal animals and 1  $\mu$ g g<sup>-1</sup> indicating a high F uptake (Suttie et al. 1972). Fluoride does not readily pass the mammary barrier, and milk and milk products contain less F than soft tissue (Underwood 1981; Miller et al. 1991). Milk from cows fed with 10, 19, 55, and 109  $\mu g$  F g<sup>-1</sup> from the age of 3 months to 7.5 years, contained 0.06, 0.10, 0.14, and 0.20  $\mu$ g F g<sup>-1</sup> on a fresh weight basis, respectively (Greenwood et al. 1964). Suttie et al. (1957) reported fresh milk F concentrations of 0.1-0.4 µg  $g^{-1}$  in fluorotic cattle, and values up to 0.64  $\mu g g^{-1}$ have also been reported by others (Krishnamachari 1987).

When animals are continually exposed to large amounts of F, build-up in bones occurs up to a saturation point of 15 000–20 000  $\mu$ g F g<sup>-1</sup> (30–40× normal levels), at which point soft tissues are flooded with F, resulting in metabolic breakdown and death (Underwood 1981).

In chronic fluorosis under lesser doses of F, rises in skeletal F concentrations are accompanied by small rises in tissue and blood F levels (Suttie et al. 1972; Underwood 1981) and the activities of various enzymes are affected (Sievert & Phillips 1959; Shupe et al. 1962; Zebrowski et al. 1964). In young animals, tooth development is hindered by uptake of F, resulting in mottling and erosion of enamel and excessive tooth wear (National Research Council 1974; Shupe 1980; Milhaud et al. 1987). Other symptomatic effects include lameness, skeletal deformity, reduced feed and water intake, and lower weight gain and milk production (National Research Council 1980; Shupe 1980; Singh & Swarup 1995).

In dairy cattle, fluorosis is associated with F concentrations of >5500  $\mu$ g g<sup>-1</sup> and >7000  $\mu$ g g<sup>-1</sup> in compact and cancellous bone, respectively (Suttie et al. 1958). In sheep, F concentrations of 2000- $3000 \,\mu g \, g^{-1}$  (compact bone) and  $4000-6000 \,\mu g \, g^{-1}$ (cancellous bone) are consistent with fluorosis (Jackson & Weidmann 1958). Sheep and cattle urine normally contains <10  $\mu$ g F g<sup>-1</sup>. In long-term experiments with dairy cows, urine F concentrations were  $<5 \ \mu g \ g^{-1}$  for normal animals, 20–30  $\ \mu g \ g^{-1}$ in animals on the borderline of fluorosis, and >35 $\mu g g^{-1}$  in animals with signs of fluorosis (Suttie et al. 1961; Shupe et al. 1963a). Plasma F concentrations >0.2  $\mu$ g g<sup>-1</sup> are normally associated with dental lesions in young cattle; below this concentration, few adverse effects are evident (Suttie et al. 1972).

## Critical levels of F intake for grazing animals

Doses of >100  $\mu$ g F g<sup>-1</sup> of body weight are acutely lethal in most mammals (National Research Council 1974). Doses of >100  $\mu$ g g<sup>-1</sup> of dietary F normally induce immediate systematic toxic effects in grazing animals (Underwood 1981). Tolerance to lower doses of F depends on the animal species (Table 6), animal condition, age at exposure, form of F uptake, duration and continuity of uptake, and the amount of F being consumed (Underwood 1981; National Research Council 1988). Cattle are less tolerant of F toxicity than other livestock (Phillips & Suttie 1960). In long-term experiments with beef cattle,  $30 \ \mu g \ g^{-1}$  of dietary F caused excessive wear and staining of teeth (Hobbs & Merriman 1959). In another study, beginning with young calves and lasting 7 years, the tolerance for soluble F was also  $30 \ \mu g \ g^{-1}$  of the dry diet (Shupe et al. 1963b). Lactating cows, however, could tolerate  $30 \ \mu g \ g^{-1}$  dietary F, with  $40 \ \mu g \ g^{-1}$  being marginal and a rate of  $50 \ \mu g \ g^{-1}$  causing fluorosis within 3–5 years (Suttie et al. 1957). Finishing cattle can tolerate up to  $100 \ \mu g \ g^{-1}$ , due to a short feeding period (National Research Council 1988).

For New Zealand standards, forage must not exceed 40  $\mu$ g F g<sup>-1</sup> of dry matter (DM) for more than one year, 60  $\mu$ g F g<sup>-1</sup> DM for more than two consecutive months, or 80  $\mu$ g F g<sup>-1</sup> DM for more than a month (Farrier & Pullen 1973).

The chemical form of dietary F is important. The availability of F in the form of CaF<sub>2</sub> and raw phosphate rock has been estimated to be 50% of that in NaF, which was 75% available (Clay & Suttie 1985). In a study of dairy cattle, animals were fed a diet containing 65 µg F g<sup>-1</sup>, in the form of NaF, CaF<sub>2</sub>, and atmospherically contaminated hay. Examination of F retention in bones and urinary F indicated that F as CaF<sub>2</sub> was only around half as available as the other two sources, which were 75% bioavailable (Shupe et al. 1962). Harkins et al. (1963) estimated that NaF administered in solution to rats was rapidly absorbed to a level of 79%; from solid form CaF<sub>2</sub>, 60–70% of F was absorbed.

Sheep have a higher tolerance to dietary F than cattle (Table 6), but its effects are also strongly dependent on the continuity of F uptake. In the hot

Tolerance Definitely unsafe ( $\mu$ g F g<sup>-1</sup> dry diet) ( $\mu$ g F g<sup>-1</sup> dry diet) Animal Beef or dairy calves ≥40 (<4 months old) 30-40 Beef or dairy heifers ≥50 (4 months-2 years old) Mature beef or dairy cattle 40-50 ≥60 (>3 years old) Finishing cattle 100 ≥120 ≥70 60 Breeding ewes Feeder lambs 150 ≥170

**Table 6** Dietary F tolerance for grazing animals, adapted from the data ofSuttie (1977), Thompson (1978), and National Research Council (1980). Valuesassume an otherwise adequate diet and the ingestion of soluble F (e.g., NaF).

and dry Queensland climate, bore water containing 5  $\mu$ g F ml<sup>-1</sup> has been enough to induce fluorosis symptoms in sheep (Harvey 1952). However, water containing 20 µg F ml<sup>-1</sup> did not cause ill effects in sheep in cool and moist conditions in South Australia, where water intake was variable and seasonally low (Pierce 1954). Some of the F stored within the skeleton is exchangeable and is excreted in urine during periods of low F uptake, hence, more F can be immobilised during a later period of high uptake (Suttie et al. 1972). Rapidly alternating periods of high and low F uptake can be more damaging to animals than an equivalent continuous intake due to rapid increases and decreases of skeletal F concentrations (Suttie et al. 1972). This is exacerbated with short-term exposures of high intakes, e.g., in dairy cattle with short-term intakes of 90  $\mu$ g g<sup>-1</sup>, dietary F induced weight loss and decreased appetite (Suttie et al. 1972). The effects of chronic fluorosis are also exacerbated in animals that are undernourished (Suttie & Faltin 1973).

#### Sources of F intake for grazing animals

#### Pasture and other feeds

As previously described, most pasture species contain between 0.7 and 16  $\mu$ g F g<sup>-1</sup>, do not accumulate large amounts of F, and absorb only very limited amounts from the soil. Even where large quantities of F-bearing, phosphatic fertilisers are added to soils, pasture F concentrations are not strongly affected (MacIntire et al. 1942; Singh 1990; McLaughlin et al. 1992). Other feeds such as lucerne hay are also low in F (Suttie 1969); 107 samples of lucerne hay from throughout the USA contained a mean of 3.6  $\mu$ g F g<sup>-1</sup> (range 0.8–36.5  $\mu$ g F g<sup>-1</sup>). Cereals and other grains usually contain lesser amounts of F than plant leaves, usually only 0.5-6  $\mu$ g F g<sup>-1</sup> (McClure 1949; Ammerman & Henry 1983). From the evidence of Shupe et al. (1962), F within hay appears to be just as available to animals as within the form of NaF.

Due to their low F concentration if uncontaminated, pastures and other common plant feeds are not likely to be a major factor in the incidence of fluorosis. This is supported by the overwhelming number of reports of fluorosis citing F-sources other than pastures. However, numerous cases of fluorosis result from contamination of plant leaves by airborne industrial pollutants (Walton 1988; Singh & Swarup 1995; Vikøren & Stuve 1996), superphosphate residues (O'Hara & Cordes 1982; O'Hara et al. 1982), evaporation of F-rich irrigation water (Botha et al. 1993), and volcanic gases (Araya et al. 1990).

Other types of feeds and animal supplements. particularly those that contain phosphate or those produced from animal bone, can be high in F and have been incriminated as causes of fluorosis. Ten percent of the dairy feed supplements used in the 1960s in 7 states in the USA exceeded 30  $\mu$ g F g<sup>-1</sup> and some have contained over 200  $\mu$ g F g<sup>-1</sup> (Suttie 1969), while some meat and bone meals may also contain as much as 200  $\mu$ g F g<sup>-1</sup> (Underwood 1981). The F contained within these phosphate additives or bone meal products has been estimated to be only one to two thirds as available as soluble NaF (Underwood 1981). Jubb et al. (1993) described severe chronic fluorosis in a herd of cattle in Northern Australia after they were fed a supplement containing monoammonium phosphate and diammonium phosphate as well as meat meal. Estimated dietary F intake of the cattle was approximately 100  $\mu g F g^{-1}$ , with the mineral supplement containing 2000  $\mu$ g F g<sup>-1</sup> and the meat meal 130  $\mu$ g F g<sup>-1</sup>. In South Africa, a phosphate rock-based commercial lick containing 1400  $\mu$ g F g<sup>-1</sup> caused seasonal chronic fluorosis symptoms in a herd of adult cattle (Schultheiss & Godley 1995). During winter, stock were ingesting an estimated 63.5  $\mu$ g g<sup>-1</sup> of dietary F and showing signs of fluorosis; symptoms went into remission during summer where dietary intake rates of F reduced to 28  $\mu$ g g<sup>-1</sup>. Pasture and water F concentrations were 0.3  $\mu$ g g<sup>-1</sup> and 0.03  $\mu$ g g<sup>-1</sup>, respectively, hence the supplement supplied the bulk of the animals' diet. The level of F intake compared with the tolerance level for adult cattle (Table 6) indicates that, in this case, F within the phosphate rock was as available to animals as that in NaF. Schultheiss & Van Niekerk (1994) described a case of fluorosis in a flock of sheep fed a phosphate rock-bearing lick for 20 months. During this time sheep were ingesting at least 91  $\mu$ g g<sup>-1</sup> dietary F from the lick, affecting 17-37% of various groups of animals depending on their age and condition.

#### Water supplies

Water supplies enriched in F have often been the cause of fluorosis in grazing animals. As described above, water enriched in F is usually found only in deep bores, particularly in areas with underlying phosphatic geology where F concentrations can reach as high as 45  $\mu$ g ml<sup>-1</sup>. The tolerance levels for F in drinking water for cattle are 4–8  $\mu$ g ml<sup>-1</sup>

(Shupe & Olsen 1987). Botha et al. (1993) reported two outbreaks of fluorosis in cattle and sheep whose main water sources contained 19.8 and 26.6  $\mu$ g F ml<sup>-1</sup>. Other cases have occurred in parts of India, Australia, and Africa where bore water concentrations of up to 40  $\mu$ g F ml<sup>-1</sup> may occur (e.g., Harvey 1952; Zumpt 1975; Underwood 1981). Water concentrations as low as 5  $\mu$ g F ml<sup>-1</sup> have caused fluorosis in sheep in hot and dry climates (Harvey 1952).

However, outside such F-enriched areas, and in surface drainage waters, F concentrations are typically very low (<0.22  $\mu$ g ml<sup>-1</sup>). Hence, normal water sources are likely to contribute very little to the F intake of grazing animals. In New Zealand, F concentrations in lakes and streams are all <0.5  $\mu$ g ml<sup>-1</sup> (Chamberlain 1944; Denmead 1946; Hewat & Eastcott 1955; Manley et al. 1975), with the exception of mineral springs and thermal pools and lakes which can reach up to 5200  $\mu$ g F ml<sup>-1</sup> (Mahon 1964).

### Fertiliser residue

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Residues of phosphatic fertilisers or basic slags on pastures have caused numerous cases of poisoning of sheep and cattle (Swan & McIntosh 1952; Crowley & Murphy 1962; Jones & Jones 1962; Rosney 1962; Clark et al. 1976). In New Zealand, O'Hara & Cordes (1982) reported 37 outbreaks of superphosphate poisoning in sheep between 1965 and 1975. They stated that outbreaks typically occurred in late winter and spring, mostly affecting pregnant and lactating ewes which were already under nutritional stress. Most outbreaks occurred during fine weather when sheep grazed short pastures within a week of topdressing with superphosphate.

Clark et al. (1976) considered F to be the toxic component of superphosphate. Stewart et al. (1974) described temporarily elevated urine F concentrations in an experiment with sheep grazing pastures recently dressed with superphosphate. O'Hara et al. (1982) compared experimental poisoning of sheep with NaF and superphosphate and concluded that the course of the poisoning was very similar, although superphosphate poisoning was more protracted. O'Hara et al. (1982) found NaF doses of 45–135  $\mu$ g F g<sup>-1</sup> of body weight to be toxic compared with toxic superphosphate doses of 70–90  $\mu$ g F g<sup>-1</sup>. The cause of the superphosphate poisoning was probably more protracted than with NaF, due to lower availability of CaF<sub>2</sub> in super-

phosphate F (Shupe et al. 1962), reducing the effective F dose of superphosphate by up to 50%. O'Hara et al. (1982) considered that phosphate plays a contributing role in superphosphate poisoning, but that the role of F is dominant.

## Volcanic ash

The first recorded cases of acute and chronic animal fluorosis were caused by excessive F concentrations in volcanic ash and gases in Iceland (Roholm 1937). Tens of thousands of sheep, cattle, and horses died, mostly from fluorosis, following the 1783 eruption of Lakagigar in Iceland (Thorarinsson 1979). In another Icelandic eruption, F concentrations of ash-covered pastures exceeded 4000  $\mu$ g g<sup>-1</sup> and 7500 deaths ensued. Óskarsson (1980) reported that F was adhering to the outside of volcanic ash grains and was in a highly soluble form as the salts CaSiF<sub>6</sub>, NaF, and AlF<sub>3</sub>.

Chronic fluorosis in cattle also occurred during and following the 1988–1989 eruption of Lonquimay in southern Chile (Araya et al. 1990, 1993). Pastures contained 240–315  $\mu$ g F g<sup>-1</sup> on a dry weight basis and the affected animals had elevated bone, urine, and blood F levels (means of 10 707, 87, and 1.30  $\mu$ g F g<sup>-1</sup>, respectively). The animals began showing signs of fluorosis 10 weeks after the eruption began. In the two years following the eruption, pasture F concentrations ranged between 7 and 34  $\mu$ g g<sup>-1</sup>, which was enough to maintain fluorosis symptoms in cattle that lived during the eruption and cause some tooth wear in bulls that were not exposed during the eruption (Araya et al. 1993).

Following the October 1995 eruption and ash fall of Ruapehu volcano in New Zealand, sheep deaths in the Rangitaiki plains area were attributed to ingestion of ash and resultant fluorosis (Shanks 1997; Cronin et al. 1998). Approximately 2.5% of pregnant or lactating ewes died in the area; the only other stock that suffered comparable effects were a flock of 1-2-year-old sheep that had been kept in yards for some time before the ash fall. Hence, in all cases the animals most affected were those having the greatest energy demands, grazing on short pastures covered by 2-3 mm of ash. Pasture samples collected 10 days after ash fall (after heavy rain) contained 20–90  $\mu$ g F g<sup>-1</sup>, but the F concentration was probably much higher in the first few days after ash fall considering the fact that the ash had total F concentration ranging from 350-850 µg  $g^{-1}$  (S. J. Cronin et al. upubl. data). Elevated rumen F concentrations of 360–520  $\mu g g^{-1}$  were found (normally 5–40  $\mu g g^{-1}$ ; Shanks 1997), and, as well as classic symptoms of fluorosis, proton microprobe analysis of teeth indicated a zone of pitting of the enamel indicative of high F intake.

#### Soil ingestion

Ingestion of soil has not yet been implicated as a cause of chronic fluorosis but, given the accumulation of F in most soils from various pollution sources (including phosphatic fertilisers), soil must be considered as an additional source of dietary F at present and future levels.

Under normal conditions, pastoral grazing animals, including sheep and cattle, can ingest large amounts of soil along with pasture (Field & Purves 1964; Healy & Ludwig 1965; Arnold et al. 1966; Healy 1967, 1968). Soil splash adheres to leaves of pasture, particularly during wet periods, and soil is also ingested via worm casts and when root crops are grazed to ground level (Healy & Drew 1970; Healy 1973). Healy (1968) reported that dairy cattle in various areas of New Zealand ingested between 100 and 450 kg of soil per year (rates were mostly  $>180 \text{ kg yr}^{-1}$ ). Dewes (1996) reported that a typical soil ingestion rate may be as much as  $670 \text{ kg yr}^{-1}$ in some areas. Factors such as grazing root crops, over-grazing, break-feeding, feeding on sacrifice areas, and abundant worm casts may substantially increase soil ingestion rates. Healy (1968) found that ingestion rates were highest in winter and autumn, and when stocking rates were higher. Annual soil ingestion rates for stocking rates of 3.7 cattle ha<sup>-1</sup> were 200-300% of those for stocking rates of 2–2.5 cattle ha<sup>-1</sup>. For stocking rates of 2– 2.5 cattle ha<sup>-1</sup>, winter soil ingestion rates ranged between 900 and 1600 g d<sup>-1</sup>, and summer rates between 220 and 470 g  $d^{-1}$ . Variations in these rates reflect the different parts of the southern North Island of New Zealand where measurements were made. From Healy's (1968) results, an overall average soil ingestion rate for cattle is c. 700 g  $d^{-1}$ , with c. 350 g  $d^{-1}$  in summer and c. 1200 g  $d^{-1}$  in winter.

Sheep in New Zealand are reported to ingest soils at rates of up to 75 kg yr<sup>-1</sup> (Healy & Drew 1970), with similar seasonal and stocking rate effects as cattle leading to a maximum intake of >300 g d<sup>-1</sup>. A later study by Lee et al. (1996) found soil ingestion rates for New Zealand sheep of 11– 30 g d<sup>-1</sup> in summer, 92–102 g d<sup>-1</sup> in autumn, and 264–275 g d<sup>-1</sup> in winter. For sheep in Scotland, Field & Purves (1964) found a maximum uptake of 200 g of soil d<sup>-1</sup> in winter. In Ireland, McGrath et al. (1982) reported that sheep ingested up to 400 g of soil kg<sup>-1</sup> of body weight during a grazing season of May to November. During this period, higher rainfall and stocking rates increased amounts of soil uptake. McGrath et al. (1982) also found that silt and clay was preferentially ingested from the soil. Vaithiyanathan & Singh (1994) found total soil ingestion by sheep in India to be 39 kg yr<sup>-1</sup>, ranging between 71 and 163 g d<sup>-1</sup>, the highest rates being in winter. In climates such as in Ireland, Scotland, and New Zealand, the average rate of soil ingestion by sheep is probably around 100 g d<sup>-1</sup> or 10% of total dry matter intake (Field & Purves 1964; McGrath et al. 1982; Lee et al. 1996).

It has been recognised for some time that ingested soil may be an important source of micronutrients in animals. Rigg & Askew (1934) prevented Co-deficiency symptoms in sheep by drenching them with 20 g of soil per week for 6 months. Later, Andrews et al. (1958) attributed increased liver Co concentrations in sheep grazing short pastures to soil ingestion. Healy et al. (1970) dosed soil with radioisotopes of micronutrients to determine the proportions of these absorbed by sheep ingesting the soil. They found minimum animal absorption rates of 34% for <sup>75</sup>Se, 14% for  $^{65}$ Zn, 1% for  $^{60}$ Co, and 0.4% for  $^{54}$ Mn. In another study, Healy (1972) used ruminal duodenal/ abomasal and ileal liquors collected from sheep to extract each of 17 different soils. Soil addition increased the ruminal liquor concentrations of Mg, Al, Fe, Mn, Se, and Zn. Duodenal/abomasal liquors were elevated in Ca, Mg, Al, Mn, and Se, and ileal liquor concentrations of Al, Cu, Fe, Mn, and Se increased upon addition of soil. The amount of elemental absorption from the soils by the liquors varied considerably between soils, mostly depending on the available amounts present within the soil. Hence, the composition or type of soil ingested as well as the quantity has an effect on elements absorbed. Using an average soil ingestion rate of 100 g  $d^{-1}$  (of Brown Soil) Grace et al. (1996) demonstrated changes in the storage of various micronutrients in the liver, blood plasma, and digestive fluids of sheep. Soil ingestion increased plasma and liver Se and vitamin  $B_{12}$  (related to Co intake) concentrations, but not those of Cu, Mn, Fe, Zn, and Cd.

The above studies have demonstrated that soil ingestion contributes significantly to animal uptake of various micronutrients, particularly Se, Co, and possibly Zn. These elements are apparently available to the animal in proportions of 1 to 34% of their total content in the soil.

### Bioavailability of soil F in grazing animals

There have been very few studies on the bioavailability of soil F in grazing animals. Milhaud et al. (1989) conducted a balanced feeding trial in which sheep were given a concentrated feed mixture containing 30% soil by weight. Seven soils were used covering a range of types (including those derived from schist and limestone), from sites polluted by Al smelter emissions and also from unpolluted sites. Total F concentrations in the soils ranged from 235 to 1030  $\mu$ g g<sup>-1</sup>, and waterextractable F concentrations from 0.4 to 15.9 µg  $g^{-1}$ . They found from 28 animals (4 for each soil type) that apparent digestive absorption of F ranged between 5 and 23% (averaged for the four animals in each treatment), and four of the groups had F absorption in the range 18-23%. Fluoride absorption was positively correlated with total soil F, but not correlated to extractable F. Water-extractable F was significantly higher in the smelter-polluted soils but F absorption by sheep from these soils was not significantly higher than uncontaminated soils with similar total F levels. In lambs, Clay & Suttie (1985) found that in comparison to NaF, F added as phosphate rock was absorbed by a proportion of 65%, defluorinated phosphate by 20%, and CaHPO<sub>4</sub> by 50%. Also in lambs, Chaso et al. (1991) estimated that the bioavailability of F within sepiolite (a clay mineral in the palygorskite group) was 3% of that as NaF, based on monitored blood plasma concentrations.

In a study of cattle, Wöhlbier et al. (1968) dosed animal feed for almost a year with a soil containing 718  $\mu$ g g<sup>-1</sup> total F and 16.5  $\mu$ g g<sup>-1</sup> water-extractable F. Absorbed F ranged between 30 and 41% (38% average). Shupe et al. (1962) found that with dairy heifers, F within hay and as NaF was 75% absorbed, but in the form of  $CaF_2$  only around half as much was absorbed (i.e., c. 38%). The National Research Council (1980) reported that in ruminants, F absorption from dietary bone meal ranges from 37 to 54% and in phosphate rock 50% of the F is absorbed.

From the study of Wöhlbier et al. (1968) it appears that the bioavailability of F in natural and polluted soils is similar or slightly lower than that of rock phosphate, dietary bone meal, and CaF<sub>2</sub>. However, the results of Milhaud et al. (1989) indicate soil F absorption of half or less than the other sources, and that F absorption is positively correlated to total soil F concentrations. Hence, it appears that soil F bioavailability is extremely variable between different soil types, and probably depends on the species of F that occurs within different soils (e.g., CaF<sub>2</sub> in calcareous soils, AlF<sub>x</sub> complexes in acidic soils).

### A PRELIMINARY MODEL OF SHEEP AND CATTLE F INTAKE

The following calculations are made to determine potential effects of accumulation of soil F on grazing ruminants in New Zealand. The cases of both cattle and sheep are considered, initially under "normal" conditions. An average total F soil concentration of 200  $\mu$ g F g<sup>-1</sup> is considered for New Zealand soils (Gemmel 1946), and an average pasture concentration of 5  $\mu$ g F g<sup>-1</sup> (Table 5). Soil and dry matter ingestion rates of sheep (Table 7) are based on the data of Lee et al. (1996), and dry matter intake of cattle on the data of Coop (1965) (Table 8). Cattle soil ingestion rates (Table 8) are based on the data of Healy (1968) under the lower stocking rates reported (2-2.5 cattle ha<sup>-1</sup>). The absorption of pasture F is assumed to be 75%, based on the data of Shupe et al. (1962) and Clay & Suttie (1985). Absorption of soil F is more difficult to

Table 7Calculation of the daily available dietary F intake of sheep under an average New Zealand pastoral system;source of input parameters is described in the text. Low soil bioavailable soil F assumes 20% of soil F is absorbed.High soil bioavailability assumes 38% of soil F is absorbed.

Season	Total dry matter intake (g d <sup>-1</sup> )	Soil intake (g d <sup>-1</sup> )	Soil F concentration (µg g <sup>-1</sup> )			Bioavailable soil F (low) (µg d <sup>-1</sup> )	(high)	Absorbed dietary F (low) (µg g <sup>-1</sup> d <sup>-1</sup> )	Absorbed dietary F (high) (µg g <sup>-1</sup> d <sup>-1</sup> )
Summer	1800	30	200	5	6638	1200	2280	4.4	5.0
Winter	1000	250	200	5	2813	10000	19000	12.8	21.8
Average	1400	100	200	5	4875	4000	7600	6.3	8.9

quantify, but based on the studies previously described, 20% was chosen as a low value and 38% as a high value (based on the data of Wöhlbier et al. 1968). The other assumption made in these calculations is that intake of F in drinking water is negligible. Since most natural waters contain <0.22  $\mu$ g F ml<sup>-1</sup>, drinking water is expected to make no difference to the calculations for New Zealand sheep and only small differences for cattle. Using these input variables, calculations of the available F dietary intake for sheep and cattle under normal conditions are presented in Tables 7 and 8.

From these calculations it appears that under normal conditions (i.e., without any human addition of F to the soils) during winter months sheep can ingest a NaF-equivalent dose (i.e., accounting for only 75% absorption of NaF) of up to 17  $\mu$ g F g<sup>-1</sup> d<sup>-1</sup> (12.8/0.75; using the low soil F bioavailability), or 29  $\mu$ g F g<sup>-1</sup> dry matter d<sup>-1</sup> (21.8/0.75; using the high soil F bioavailability). These doses are 28 and 48% of the daily dietary NaF tolerance (60  $\mu$ g F g<sup>-1</sup> dry diet), respectively (Table 6). The other feature shown by these calculations is that 45–61% of average daily dietary F is derived from soil ingestion, and that this may reach levels of 78–87% in winter.

Cattle during winter months can ingest NaFequivalent doses of up to 14  $\mu$ g F g<sup>-1</sup> d<sup>-1</sup> (10.6/ 0.75; low soil F bioavailability), or 23  $\mu$ g F g<sup>-1</sup> d<sup>-1</sup> (17.3/0.75; high soil F bioavailability). These doses are 31 and 51% of the daily dietary NaF tolerance (45  $\mu$ g F g<sup>-1</sup> dry diet), respectively. Of the average daily dietary F of dairy cattle, soil ingestion appears to supply 47-63%, which may increase up to 71– 82% in winter.

Using the same model parameters, we can calculate the threshold soil F concentration at which the daily dietary F tolerances of sheep and cattle are reached with the relationship:

$$pastureF\left[\mu gFd^{-1}\right]$$

$$+ \begin{pmatrix} Soilintake\left[gd^{-1}\right] \\ \times bioavailability\left[\%/100\right] \\ \times thresholdsoilF\left[\mu gFg^{-1}\right] \end{pmatrix}$$

$$DMintake\left[gd^{-1}\right]$$

Where:

$$PastureF[\mu gFd^{-1}] = pasture intake[gd^{-1}]$$
$$\times pastureF concentration[\mu gFg^{-1}]$$
$$\times 0.75$$

Tolerance = the tolerance limit of absorbed dietary F pastureF = daily bioavailable pasture F intake bioavailability = soil F bioavailabity thresholdsoilF = threshold soil F concentration DMintake = daily dry matter intake.

This equation rearranges to:

$$ThresholdsoilF = \frac{\{(Tolerance \times DMintake) - pastureF\}}{soilintake \times bioavailability}$$

In Table 9, threshold soil F concentrations required to reach a NaF tolerance level of 60  $\mu$ g F g<sup>-1</sup> for sheep (tolerance limit of absorbed dietary F of 60 × 0.75 = 45  $\mu$ g F g<sup>-1</sup>) and 45  $\mu$ g F g<sup>-1</sup> (tolerance limit of absorbed dietary F of 45 × 0.75 = 33.75  $\mu$ g F g<sup>-1</sup>) for cattle during the winter months are calculated for low (20%) and high (38%) soil F bioavailablities (Wöhlbier et al. 1968) and low (143 g d<sup>-1</sup> for sheep, Healy & Drew 1970; 900 g d<sup>-1</sup> for cattle, Healy 1968) and high (300 g d<sup>-1</sup> for sheep, Healy 1967; 1600 g d<sup>-1</sup> for cattle, Healy 1968) soil ingestion rates. The soil F concentrations ranged from 372 to 1461  $\mu$ g F g<sup>-1</sup> for sheep and 326 to 1085  $\mu$ g F g<sup>-1</sup> for cattle.

**Table 8** Calculation of the daily available dietary F intake of dairy cattle under an average New Zealand pastoral system; sources of input parameters are described in the text. Low bioavailable soil F assumes 20% of soil F is absorbed. High soil bioavailability assumes 38% of soil F is absorbed.

Season	Total dry matter intake (g d <sup>-1</sup> )	Soil intake (g d <sup>-1</sup> )	Soil F concentration (µg g <sup>-1</sup> )	Pasture F concentration (µg g <sup>-1</sup> )		Bioavailable soil F (low) (µg d <sup>-1</sup> )	(high)	Absorbed dietary F (low) (µg g <sup>-1</sup> d <sup>-1</sup> )	Absorbed dietary F (high) (µg g <sup>-1</sup> d <sup>-1</sup> )
Summer Winter	11600 6400	350 1200	200 200	5	42188 19500	14000 48000	26600 91200	4.8	5.9
Average	9000	700	200	5	31125	28000	53200	6.6	9.4

Regular application of phosphatic fertilisers is likely to increase the total F concentrations of soils as described above. Our results indicate that New Zealand Brown and Pallic Soils can retain between 40 and 60% of applied F in the top 75 mm of soil (Tables 3 and 4). The corresponding retention in Allophanic soils is expected to be much higher (70-90%). The following calculations are made to determine the effects of a regular maintenance application of 20 or 40 kg P ha<sup>-1</sup> yr<sup>-1</sup> to pastoral soils, on cattle and sheep dietary F intake. Given the parameters of a SSP fertiliser containing c. 9% P, and 1.46% F (Evans et al. 1971; P. Loganathan et al. unpubl. data), maintenance application rates are likely to add 3 or 6 kg F ha<sup>-1</sup> yr<sup>-1</sup>. For a phosphate rock fertiliser containing c. 13-14% P. and 3.2-3.4% F (e.g., Jordan or North Carolina phosphate rock, Manoharan et al. 1996), maintenance application is likely to add 4.9 kg F ha<sup>-1</sup> vr<sup>-1</sup>. If we assume that 80% of applied F is retained within the top 75 mm of an Allophanic Soil, and an average dry bulk density of the soil of  $0.9 \text{ Mg m}^{-3}$ . then maintenance applications of SSP and phosphate rock fertilisers at 40 kg P ha<sup>-1</sup> yr<sup>-1</sup> will increase total soil F concentrations by 7.6  $\mu$ g g<sup>-1</sup>  $yr^{-1}$  and 11.6  $\mu g g^{-1} yr^{-1}$ , respectively. In Pallic and Brown Soils (20 kg P ha<sup>-1</sup> yr<sup>-1</sup>) with an average Fretention of 50%, soil F concentrations will probably rise by 2.4  $\mu$ g g<sup>-1</sup> yr<sup>-1</sup> and 4.1  $\mu$ g g<sup>-1</sup> yr<sup>-1</sup>, respectively.

Given these F accumulation rates from phosphatic fertiliser application, the time taken for total soil F concentrations in an average pastoral soil to reach the thresholds for F intake of sheep and cattle during winter can be calculated. For the case of Allophanic Soils with present soil F concentration of 200  $\mu$ g g<sup>-1</sup> and a low soil F bioavailability, depending on the soil ingestion rate 55–166 years of superphosphate fertilisation is required, or 36– 109 years of phosphate rock application. However, if the high rate of soil F bioavailability applies, only 17–75 years of superphosphate, or 11–49 years of phosphate rock fertilisation are required. Many of the higher P-fixing volcanic ash pastoral soils of New Zealand may have already received this amount of fertilisation.

A whole series of factors may influence these calculated predictions. The F content of SSP, and site-specific factors including soil type, soil pH, pasture management (and renewal), grazing management, and drainage, can markedly change local rates of soil F accumulation and rates of soil ingestion. In many Allophanic Soils (e.g., in Taranaki), high P-fixation occurs, and phosphate fertiliser application rates can be 45–60 kg P ha<sup>-1</sup> yr<sup>-1</sup> on dairy farms (Cornforth & Sinclair 1984). With an application rate of 60 kg P ha<sup>-1</sup> yr<sup>-1</sup> as SSP, F deposition rates could be as high as 10 kg F ha<sup>-1</sup> yr<sup>-1</sup> and the tolerance soil F levels would be reached in 36–76 years (low soil F bioavailability) or 11–32 years (high soil F bioavailability).

The use of phosphate rock fertilisers may lead to greater dietary F uptakes than those predicted so far. The low solubility of these fertilisers may lead to substantial amounts of undissolved phosphate rock incorporated in the surface soil. Manoharan et al. (1995) demonstrated that only 48% of Jordan phosphate rock, and 68% of North Carolina

**Table 9** Calculation of soil F concentration required to reach daily dietary intake tolerances for sheep and cattle in winter. Assuming 75% of F is absorbed from NaF doses of  $60 \,\mu g F g^{-1} d^{-1}$  (sheep) and  $45 \,\mu g F g^{-1} d^{-1}$  (cattle), from Shupe et al. (1962). Low soil bioavailability assumes 20% of soil F is absorbed. High soil bioavailability assumes 38% of soil F is absorbed.

Animal	Tolerance limit of absorbed dietary F (µg g <sup>-1</sup> )	Total dry matter intake (g d <sup>-1</sup> )	Soil intake (g d <sup>-1</sup> )	Pasture F concentration $(\mu g g^{-1})$		Required bioavailable soil F (μg d <sup>-1</sup> )	Threshold soil F concentration (low) (µg g <sup>-1</sup> )	Threshold soil F concentration (high) (µg g <sup>-1</sup> )
Sheep	45	1000	143	5	3214	41786	1461	
Sheep	45	1000	300	5	2625	42375	706	
Sheep	45	1000	143	5	3214	41786		769
Sheep	45	1000	300	5	2625	42375		372
Cattle	33.75	6400	900	5	20625	195375	1085	
Cattle	33.75	6400	1600	5	18000	198000	619	
Cattle	33.75	6400	900	5	20625	195375		571
Cattle	33.75	6400	1600	5	18000	198000		326

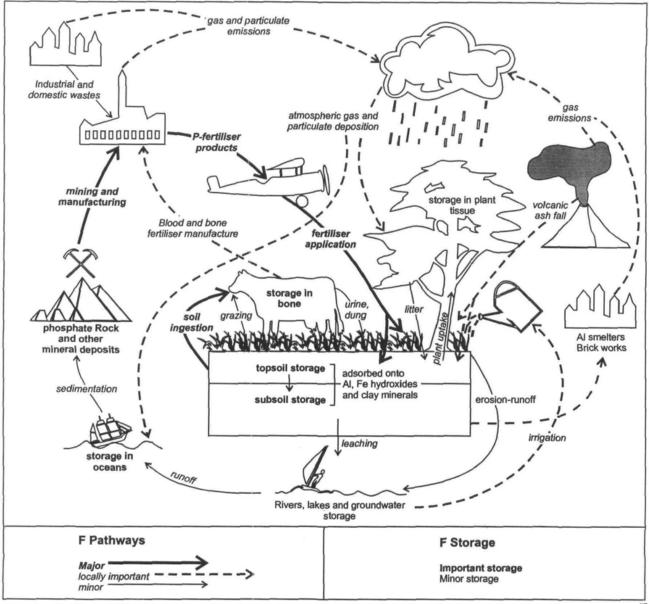


Fig. 1 Cycling of fluoride in grazed pasture systems.

phosphate rock dissolved during an 8-year trial. Fluoride within phosphate rock is around 38% bioavailable in grazing ruminants (Clay & Suttie 1985). Hence, on the basis of an average daily dry matter intake (Tables 7 and 8), sheep could obtain their daily tolerance level of bioavailable dietary F by ingesting just 5.1 g of phosphate rock residue with soil, and cattle by ingesting 24 g.

Accumulation of F in soil within the pH range 5.5–6.5 (general pH range of New Zealand pastoral soils) may lead to slightly elevated pastoral F contents over long periods, but, as discussed above, these increases are likely to be minor and should not provide much additional dietary F. However, at

lower or higher pH, greater uptake of F may occur, enriching pasture in F and leading to substantially higher dietary F intakes.

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### CONCLUSIONS AND FUTURE DIRECTIONS

This review allows the preparation of an F cycle (Fig. 1), identifying important F-transfer pathways and F-storage locations. Fluoride is accumulating in most topsoils through atmospheric pollution and fertiliser addition. Rates of F input exceed losses because F is strongly held by many specific and non-specific adsorption sites in soils. Soils rich in

amorphous Al and Fe hydroxides and other clay minerals can retain up to 80% of applied F within the top 75 mm. Although F accumulation increases the soil solution and soluble and labile pools of F in soils, due to its strong adsorption, these comprise only a minor fraction of total soil F. Hence, the effect of F accumulation in soils does not appear to pose a threat to groundwater supplies. A combination of the strong adsorption of F in soils and its low rate of uptake by most plants means that normal pastoral plants are not likely to be greatly affected by increasing soil F concentrations. However, at low soil pH (<5), greater plant uptake of F may occur due to a possible preference for AlF<sub>x</sub>, Si-FB, and HF species. At high pH (>6.5), desorption of nonspecifically adsorbed F may also result in higher plant F uptake. The activity of soil micro-organisms is likely to be negatively affected as soil F concentrations rise. Data on the critical soil F concentration affecting soil microbial activity is scarce and inconclusive. In one study the activity of some microbial enzymes has been reported to have been inhibited at water-extractable soil F concentrations of  $\geq 20 \ \mu g \ F \ g^{-1}$ .

Although soil F is not likely to be passed on to grazing animals via pasture, it can be directly taken up by soil ingestion during grazing. Our model indicates that under normal conditions, soil ingestion probably provides more than 50% of the average dietary F of grazing sheep and cattle, and this may rise to >80% during the winter months. Ingestion of soil containing 326–1461  $\mu$ g F g<sup>-1</sup> is likely to lead to chronic fluorosis in sheep and cattle. depending on the soil ingestion rate and bioavailability of F within the soil. Some New Zealand topsoils (0-75 mm) may already be within this F concentration range (Gemmel 1946) or close to this range (P. Loganathan unpubl. data), and certainly many agricultural soils around the world contain even higher concentrations of F. In another 17-166 years of SSP fertilisation and 11-109 years of phosphate rock fertilisation (depending on the application rate and F content of fertilisers, soil ingestion rate, and bioavailability of soil F), most New Zealand dairy and sheep farm soil F levels could rise into this potentially toxic concentration range. Currently, no alternative source of P fertilisers with low F content is available for use in our pastoral lands to reduce this rate of F accumulation.

Research is required to establish present F concentrations in New Zealand pastoral soils and accurately quantify the rate of F accumulation. From

this work, areas of greatest risk from chronic fluorosis problems can be identified, based on their soil, fertiliser, and pasture management characteristics. In addition, research is needed to determine the rates of dietary absorption of soil F within grazing ruminants. Particular attention must be paid to dietary F absorption from different soil types, e.g., those containing high allophane contents (which require high doses of P fertiliser), and those containing layer-lattice clay mineral assemblages (e.g., Pallic Soils). Further research also needs to ascertain rates of plant uptake of F under differing pH and consequent F speciation conditions, and potential impacts of elevated F levels on microbiological processes such as nitrogen fixation and nitrification.

Only once this additional information is obtained can adequate recommendations be made for mitigating the risks of increasing F levels in our pastoral systems, and developing practices that are sustainable in the future.

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## Effect of Fluoride and Phosphate on Yield and Mineral Composition of Barley Grown on Three Soils

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## ABSTRACT

In a greenhouse experiment, the effect of fluoride (F) and phosphorus (P) addition on the growth and mineral composition of barley (*Hordeum vulgare* L.) was studied in three different soils, Cahaba sandy loam (acid), Weld loam (neutral), and Haverson silty loam (alkaline calcareous). Four levels of F [0, 100, 400, and 1,000 mg kg<sup>-1</sup> soil as hydrogen fluoride (HF)] and three levels of P [50, 150, and 550 mg kg<sup>-1</sup> soil as phosphoric acid ( $H_3PO_4$ )] were used. The effect of P addition on the native soil F and the capacity of soil to sorb added F was investigated. Addition of P released some of the native F from the soil samples that did not receive any F. The amount of F released ranged from 0.135 to 1.860 mg kg<sup>-1</sup> soil. The amount of F released from the soils decreased with increasing P addition. Most of F added was sorbed by the soil solid phase. The amount of F sorbed ranged between 74.0% and 96.3% of the added F. For both the acid and neutral soils, increasing P addition increased

F sorption at all F levels. In the case of alkaline soil, however, this effect was only clear at the low F level. The formation of insoluble F minerals may be enhanced by the addition of P to the soils. In the greenhouse experiment, F addition had a negative effect on dry matter yield (DMY) of barley grown on the acid and neutral soil while no effect was observed for the alkaline soil. These results may reflect the effect of F addition on the solubility of aluminum (Al) and other metals in the soils. On the other hand, increasing F addition from 50 to 550 mg kg<sup>-1</sup> soil had no clear effect on DMY of plants in the three soils. The study also included the effect of F and P addition on their uptake by plants. Generally, addition of P depressed F uptake by plants grown on the three soils. On the other hand, increasing F addition depressed P uptake for the acid soil while no clear trend was observed for the neutral and alkaline soil. A significant positive effect of F addition on Al uptake was observed for both the acid and neutral soils. A highly significant correlation of 0.87 and 0.60 was obtained between soil extractable F and Al uptake for the acid and neutral soil, respectively. Addition of F to the alkaline soil resulted in minor increases in Al uptake. The effect of P addition, at different levels of F, on Al uptake was investigated for the three soils. For the acid soil, significant decrease in Al uptake was observed only at the highest F level. The effect of P addition on Al uptake was not clear in the other two soils.

## INTRODUCTION

Relative to the enormous hazard of F contamination in the environment, little is known about the effect of F on the mobility of chemical species in the contaminated soils. The work of Polomski et al. (1982) was one of the few studies done on this area of research. In their study, various F solutions were leached through soil columns at a constant flow rate of 4 mL hr<sup>-1</sup>. They concluded that F induced mobilization of Al, iron (Fe), and organic matter (OM) in the soils investigated. However at the flow rate used in the work of Polomski et al. (1982), the dissolved chemical species may not be in equilibrium with the soil phase especially in calcareous soils (Fluhler et al., 1982). Huang and Jackson (1965) studied the mechanism of reaction of F in solution with layer silicates and oxides of soils. They indicated that F forms Al and Fe complexes which disrupt the mineral surfaces. Similar studies on soils and soil minerals have been conducted by Semmens and Meggy (1966), Perrott et al. (1976), and Omueti and Jones (1977).

The ability of F ions to extract P from soils was first postulated by Dickman and Bray (1941). This led to the development of the Bray tests which employ acid fluoride reagents for extracting available P from soils (Bray and Kurtz, 1945). Later, Chang and Jackson (1957) used the differences in the extent of Al and Fe fluoride complex formation to develop the P fractionation technique for soils.

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The interaction between F and P applied to soils was investigated in few studies. Prince et al. (1949) reported that addition of F at 360 mg  $L^{-1}$  as sodium fluoride (NaF) was injurious to buckwheat. They also noted that the toxicity, as reflected in yield, was reduced by increasing the rate of P application. Singh et al. (1979a) studied the effect of five levels of F (as NaF) and four levels of P [as potassium dihydrogen phosphate  $(KH_2PO_4)$ ] on the yield and chemical composition of rice grown on two sodic soils of very high pH values. They concluded that increasing F above 50 mg  $L^{-1}$  decreased the yield of rice. The adverse effect of added F was eliminated by greater application of P. In another study, Singh et al. (1979b) concluded that application of both F and P resulted in higher extractability of each other in soils. They also reported that while there was a positive effect of P on soil extractable F, it had a negative effect on its uptake by wheat. The experimental findings of the above studies suggest that addition of P decreases or eliminates F toxicity in plants. However, these studies were conducted either in acid soils (Prince et al., 1949) or sodic soils of very high pH (Singh et al., 1979a, 1979b). No studies have been reported on naturally occurring neutral or calcareous soil. There is a need to study and compare these interactions simultaneously in various soils of wide range of properties.

The objectives of this study were i) to investigate how the interaction between added F and P affect the yield and chemical composition of barley grown in three soils differ greatly in their properties and ii) to determine the effect of added P on the release and sorption of F in these soils.

## MATERIALS AND METHODS

Three soil samples of Cahaba (fine-loamy siliceous, thermic Typic Hapludults), Weld (fine, montmorillonitic, mesic Aridic Paleustolls) and Haverson [fine-loamy, mixed (calcareous) mesic Ustic Tortifluvents] series were selected for this experiment. The characteristics of these samples are as follows: Cahaba sandy loam (pH=4.75 and OM%=0.86), Weld loam (pH=6.57 and OM%=0.74), and Haverson silty loam [pH=7.50, and OM%=1.27, and calcium carbonate (CaCO<sub>3</sub>) %=6.4]. Both Cahaba and Weld soils have no carbonates. More properties of these soils were given in previous reports (Elrashidi and Lindsay, 1986b, 1987).

In a previous study, Elrashidi and Lindsay (1987) concluded that additions of high concentration of F increased significantly the solubility of Al and manganese (Mn) for both the acid and neutral soils. Meanwhile, minor effects were observed for the alkaline soil. Therefore in this experiment, F treatments included four levels of F (0, 100, 400, and 1,000 mg kg<sup>-1</sup> of soil) for the alkaline soil. For both the acid and neutral soils, the highest level of F was not used. Fluoride was added to soils as diluted hydrofluoric acid (HF) solution (100 mg F L<sup>-1</sup>). The use of dilute HF simulated more nearly the conditions in the field where crops are subject to fumes from industrial plants. Ten separate increments were used to supply the amount of F required for each treatment. The study also included three levels of

P (50, 150, and 550 mg kg<sup>-1</sup> of soil) added as diluted  $H_3PO_4$  solution (10 mg P L<sup>-1</sup>). All treatments were replicated four times. In total 144 plastic pots were used, each of 2-liter capacity.

One kg of air-dried soil (ground to pass through a 2-mm sieve) was added to each pot. A basal fertilizer application of 50 mg nitrogen (N) as ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), 50 mg P and 63 mg potassium (K) as KH<sub>2</sub>PO<sub>4</sub>, 40 mg calcium (Ca) as calcium chloride (CaCl<sub>2</sub>·2H<sub>2</sub>O), 20 mg magnesium (Mg) as magnesium chloride (MgCl<sub>2</sub>·6H<sub>2</sub>O), 20 mg Fe as ferrous sulfate (FeSO<sub>4</sub>·7H<sub>2</sub>O), and 5 mg zinc (Zn) as zinc sulfate (ZnSO<sub>4</sub>·7H<sub>2</sub>O) was used for each pot. The solutions containing the amount of H<sub>3</sub>PO<sub>4</sub> required along with the basal fertilizer applications were mixed thoroughly with the soil. Fifteen barley (*Hordeum vulgare* L) seeds were sown in each pot. After seven days the plants were thinned to 10 plants pot<sup>-1</sup>. During the experiment, soil moisture was kept at field capacity by daily addition of the required amount of distilled water. When the plants were 20 days old, additions of dilute HF were made in irrigation water at 2-day intervals.

Thirty days after sowing, 25 mg of N as  $NH_4NO_3$  was added in irrigation water to each pot. The tops were harvested when the plants were 60-day-old. The tops were rinsed with dilute hydrochloric acid (HCl) followed by several rinses with distilled water. The plant samples were dried at 60°C for 48 hours, weighed, and ground for analysis. Total F in plants was determined by a sodium hydroxide (NaOH) fusion selective ion electrode technique described by McQuaker and Gurney (1977). Phosphorus, Al, and Mn in plants were determined after digestion with a nitric acid (HNO<sub>3</sub>), perchloric acid (HClO<sub>4</sub>), and sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) acid mixture (Jackson, 1967). Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to measure the concentration of elements in the plant digest (Soltanpour et al., 1982).

After harvesting, the soil in each pot was air-dried, mixed thoroughly, and crushed to pass through a 2-mm sieve. Precautions were taken to separate plant roots from soil. The soil was extracted by shaking 20 grams of soil and 60 mL  $CaCl_2$  solution in a 125-mL plastic bottle for 24 hours. The suspensions were centrifuged and filtered through Whatman 42 filter paper into plastic bottles. Fluoride was measured in the filtrate with a combination F electrode (Orion Instruction Manual, 1982). Phosphorus was determined by the chloromolybdic boric acid method after Peterson and Corey (1966). The pH of the solution was measured with a combination pH electrode.

The experimental data were subject to analysis of variance (ANOVA) to study the effect of soil, F and P addition, and their interactions on various dependent variables. Duncan's Multiple Range Test and regression analysis were used to examine means within soils.

The effect of P addition on native and added F was investigated for the three soils. The amount of native F released as mg kg<sup>-1</sup> soil was calculated as follows:

amount of F released = 
$$F_{ex} + F_{ab}$$

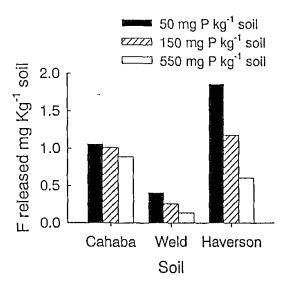


FIGURE 1. Effect of P addition (50, 150, and 550 mg P kg<sup>-1</sup> soil) on the amount of native F released (mg F kg<sup>-1</sup> soil) from the three soils.

where:  $F_{ex}$  is the amount of F (mg kg<sup>-1</sup> soil) extracted with 0.01M CaCl<sub>2</sub> solution from the soil sample which received no F addition. The  $F_{ab}$  is the amount of F (mg kg<sup>-1</sup> soil) absorbed by plant from the soil. For each F treatment, the amount of F sorbed by the soil was calculated as a percentage of added F as follows:

F sorbed % = {
$$[F_{ad} - (F_{ax} + F_{ab})]/(F_{ad})$$
} x 100

where:  $F_{ad}$  is the amount of F (mg kg<sup>-1</sup> soil) added. The effect of P addition on the amount of native F released and the percentage of F sorbed by soils are shown in Figures 1 and 2, respectively.

### **RESULTS AND DISCUSSION**

Significant effects of soil, and the F and P additions were found for most of the dependent variables investigated, namely dry matter yield, soil pH, total available F and P in soil, and F, P, Al, and Mn uptake by the plants (Table 1). Significant effects were also obtained for the interactions: soil x F, soil x P, F x P, and soil x F x P. Therefore, responses to F and P addition were measured by Duncan's Multiple Range Test and regression analysis within each of the three soils investigated (Tables 2, 3, and 4).

#### Soil pH

Addition of F decreased the pH of the three soils (Table 2). The magnitude of pH decreases followed this order: neutral soil > acid soil > alkaline soil. When

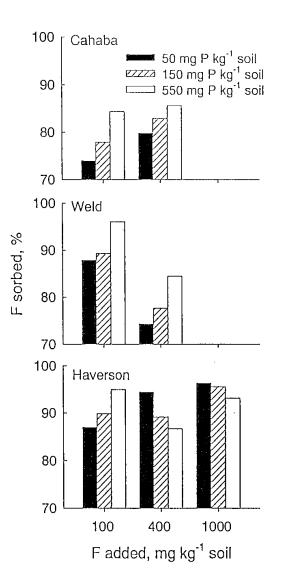


FIGURE 2. Effect of P addition at different levels of added F (100, 400, and 1,000 mg F kg<sup>-1</sup> soil) on the percentage of F sorbed by the three soils.

HF was added to soil, HF and F ions acted oppositely on pH. The pH decreased with increasing hydrogen (H<sup>+</sup>) ion concentration while F<sup>-</sup> ions addition increased pH. The substitution of F<sup>-</sup> for hydroxyl (OH<sup>-</sup>) ions in various hydroxyl minerals such as clays and amorphous hydroxides has been suggested by many investigators (Huang and Jackson, 1965; Perrott et al., 1976).

Highly acid soils normally contain high amounts of Al and Fe hydroxides (Jackson, 1969). When F is added to these soils, OH ions are released in relatively higher concentrations than from soils containing less amounts of these hydroxyl minerals. Accordingly, HF addition is expected to be less effective in decreasing the pH of these acid soils. On the other hand, alkaline calcareous soils have high

	-		Mean	squares	
Source	DF <sup>†</sup>	Dry matter	Soil pH	F total	P total
		g pot <sup>-1</sup>		extractable	mg kg <sup>-1</sup> soil
Soil	2	147.6***	76.4***	2281.2***	10892.5***
Fluoride (F)	2	18.7***	1.07***	39780.0***	58.87***
Phosphate (P)	2	0.61	2.17***	421.0***	15702.4***
Soil x F	4	5.65***	0.42***	2121.0***	238.0***
Soil x P	4	2.14**	0.15***	454.8***	5767.8***
FxP	4	1.02	0.08***	97.47***	102.0***
Soil x F x P	8	0.73	0.07***	454.7***	242.3***
ERROR	81	0.47	0.01	10.45	3.27
		F	Р	Al	Mn
			Plant uptak	e mg pot <sup>-1</sup>	5 <b>6 11 % = 2 %</b> 4 4 6 <b>a</b> 2 5 4 6 <b>a</b> 5
Soil	2	432.0***	1519.2***	516.7***	927.7***
Fluoride (F)	2	174.9***	90.67***	913.7***	144.6***
Phosphate (P)	2	1.36	1473.0***	9.96	210.9***
Soil x F	4	91.35***	100.9***	425.1***	157.6***
Soil x P	4	12.22***	48.04*	78.37***	184.7***
F x P	4	7.06***	60.50***	35.28**	13.5
Soil x F x P	8	9.26***	33.85	96.99***	15.09***
ERROR	81	1.41	6.17	7.66	1.95

TABLE 1. Analysis of variance for soil, fluoride, and phosphate addition effects on various dependent variables.

\*, \*\*, \*\*\*Indicate significance at 0.05, 0.01, and 0.001 level of probabililty, respectively.

†DF=degree of freedom.

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Revelied	Ca	haba soil (ac	id)	W	eld soil (neut	ral)	Haven	son soil (alka	aline)
F applied mg kg <sup>-1</sup> soil		+		P aj	pplied mg kg	-1 soil			
	50	150	550	50	150	550	50	150	550
				Dr	y matter g po	t <sup>-1</sup>			
0	3.53cx	4.13cx	3.70bx	3.33ax	4.38bx	4.68bx	6.70ax	6.50ax	6.90ax
100	2.00bx	3.03by	3.38by	4.38bx	3.73abxy	4.90by	7.28ax	6.03ax	6.00ax
400	1.00axy	1.10ay	0.75ax	3.30ax	2.93ax	3.23ax	6.95ax	5.75ax	6.35ax
1000	-	-	-	-	•	•	6.38ax	6.60ax	6.40ax
					Soil pH				
0	4.75bz	4.41ay	4.25bx	6.55cz	5.91cy	5.39cx	7.49ay	7.45cy	7.25cx
100	4.64abz	4.41ay	4.21bx	5.83bz	5.56by	5.16bx	7.45ay	7.45cy	7.17cx
400	4.53ay	4.51ay	4.13ax	5.33az	5.23ay	5.00ax	7.34ay	7.29by	6.97bx
1000	-	-	•	-	-	, <b>-</b>	7.24az	7.11ay	6.78ax
				Soil ext	ractable F mg	g kg <sup>-1</sup> soil			
0	1.01ay	0.95ay	0.83ax	0.38az	0.23ay	0.11ax	1.75az	1.10ay	0.55ax
100	25.88by	21.93by	15.50bx	11.93by	10.56by	3.85ax	12.80aby	9.99by	4.91ax
400	79.00cz	67.50cy	57.13cx	100.38cz	88.13cy	61.13bx	22.00bx	43.18cy	52.98bz
1000	-	-	-	-	-		36.53cx	43.95cy	67.75cz
				Soil extr	actable P mg	kg <sup>-1</sup> soil			
0	0.20ax	3.40by	23.35cz	5.00ax	15.18ay	95.73bz	1.55ax	6.95ay	8.43az
100	0.15ax	2.13ay	15.85bz	5.79ax	17.73ay	105.00cz	1.50ax	8.60by	16.50bz
400	0.10ax	3.66by	11.39az	7.28bx	18.08ay	71.70az	1.29ax	9.65bcy	27.20cz
1000	-	-	-	-	-	-	1.28ax	10.58cy	35.95dz

TABLE 2. Effect of fluoride and phosphate addition on dry matter yield of barley, soil pH, extractable fluoride, and phosphate in the three soils.\*

\*In each soil, means within columns with the same letter (a,b,c, or d) and means within rows with the same letter (x,y, or z) are not significantly different at the 5% level according to Duncan's Multiple Range Test.

content of carbonates which can neutralize the added  $H^+$  ions. This may explain why the magnitude of pH decreases was relatively higher for the neutral soil than for both the acid and alkaline soils.

Expectedly, the addition of P decreased the pH for the three soils (Table 2). It appears that  $H_3PO_4$  addition along with HF has an accumulative effect on H<sup>+</sup> concentration in these soils. This suggests the absence of any interaction between P and F in soils that may enhance or inhibit each one effect on the pH.

## Fluoride Release and Sorption by Soils

Phosphorus addition released a part of the native F from the three soils (Figure 1). The amount of F released ranged from 0.135 to 1.860 mg kg<sup>-1</sup> soil. The magnitude of F released from the soils followed this trend: alkaline soil > acid soil > neutral soil. This trend could be explained by the effect of P addition on the pH of the three soils. The data in Figure 1 also show that the amount of F released is decreasing with the increase in P addition. This effect could be explained by reactions between P and F and the formation of insoluble F minerals.

The results given in Table 2 show that most of F added to the soils was sorbed by the solid phase. The amount of F sorbed ranged between 74.0 and 96.3%. The alkaline soil shows relatively higher F sorbing capacity than the acid and neutral soil. For both the acid and neutral soils, increasing P addition has increased F sorption at all F levels. For the alkaline soil, however, this effect was clear only at the low F level. The formation of insoluble F minerals may be enhanced by the addition of P to the soils. But the formation of a mineral such as fluoroapatite is unlikely because precipitation reactions are very slow in soils. MacIntire et al. (1947), Jung (1953), and Scharrer et al. (1953) reported that the precipitation of fluoroapatite following F application to soils requires a long time.

### Dry Matter Yield (DMY)

Highly significant effects of soil, F addition and their interactions on DMY of barley plants were observed (Table 1). Similar results were obtained by Prince et al. (1949) for tomato and by Singh et al. (1979a, 1979b) in their study on rice and wheat.

Fluoride addition appeared to have a significant negative effect on DMY for barley grown on the acid and neutral soil (Table 2). The reduction of DMY due to F addition can be attributed to: a) accumulation of toxic amounts of F in soils, b) increasing the mobility of other toxic elements in soils (e.g., Al), and/or c) the effect on nutrient balance in the plant.

For the alkaline soil, F addition had no effect on DMY. This result may reflect the minor effect of F addition on both the pH and the solubility of Al in alkaline soils (Elrashidi and Lindsay, 1987). On the other hand, increasing P addition from 50 to 550 mg kg<sup>-1</sup> soil at all levels of added F had no clear effect on DMY for plants grown on the three soils (Table 2).

F applied	Ca	ahaba soil (ac	id)	We	eld soil (neut	ral)	Haver	son soil (alk	aline)
mg kg <sup>-1</sup> soil	P applied mg kg <sup>-1</sup> soil								
	50	150	550	50	150	550	50	150	550
				F	uptake mg p	ot <sup>-1</sup>			
0	0.048ax	0.063ax	0.050ax	0.025ax	0.028ax	0.025ax	0.110az	0.078ay	0.055a
100	0.128ax	0.173by	0.130ax	0.190by	0.150axy	0.120ax	0.210ay	0.158bx	0.128b
400	1.858by	1.060cx	0.578bx	2.555cz	1.345by	1.058bx	0.240ax	0.235cx	0.288cz
1000	•	-	-	-	-	-	0.408bx	0.545dx	0.675dz
			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		P uptake m	ig pot <sup>-1</sup>			
0	6.32bx	10.91bx	28.45by	8.34ax	10.99ax	22.28aby	19.25bx	24.20ay	31.40bz
100	3.03ax	8.29ay	23.58bz	12.05bx	14.15bx	23.30by	19.25bx	24.50axy	26.30ay
400	2.76ax	6.21ay	9.17ay	11.93bx	16.55by	18.93ay	16.48ax	23.05ay	29.40ab
1000	-	-	-	-	-		17.78ax	25.05ay	30.90b
				A	l uptake mg p	oot <sup>-1</sup>			
0	5.74ax	7.71ay	7.76ay	4.50ax	5.12ax	6.49ay	7.38ax	7.12ax	7.99a
100	6.52ax	8.11ay	8.08ay	6.68bx	7.03bx	7.42ax	6.68ax	6.55ax	5.70a
400	40.63by	22.33bx	19.73bx	6.80bx	11.24cy	16.30bz	7.61ax	7.97ax	7.17a)
1000	-	-	-	-	-		7.21ax	8.82ax	8.26ax
				Mn	uptake mg p	ot <sup>-1</sup>			
0	14.88bx	16.73cx	29.85by	3.04ax	3.42ax	6.74ay	10.55az	9.26ay	8.32abx
100	9.69ax	13.08bx	26.55by	4.38bx	5.16bx	7.01ay	10.58ay	9.14axy	7.72ax
400	6.59ax	6.62ax	11.93ay	3.92abx	5.07bxy	6.16ay	10.21ax	8.61ax	8.81bx
1000	-	-	-	-	-	-	10.53ay	9.27ax	8.28abx

TABLE 3. Effect of fluoride and phosphate addition on F, P, Al, and Mn uptake by barley grown on the three soils.\*

\*In each soil, means within columns with the same letter (a,b,c, or d) and means within rows with the same letter (x,y, or z) are not significantly different at the 5% level according to Duncan's Multiple Range Test.

A multiple regression equations (Table 4), including soil extractable F and P along with soil pH as independent factors, gave highly significant correlations with DMY for both the acid and neutral soil. No relation was observed for the alkaline soil.

## Fluoride Uptake

The results indicate that soil F addition and their interactions together and with P had highly significant effects on F uptake by barley plants (Table 1). The data on F uptake within each soil (Table 3) indicate that F addition had the highest effect on the neutral soil, followed by the acid soil while the least effect on F uptake was observed for the alkaline soil.

The data in Table 3 also indicate generally that addition of P depressed the F uptake by plants growing on these soils. Similar results were obtained by Prince et al. (1949) and Singh et al. (1979a, 1979b). Phosphorus addition may enhance a formation of insoluble F minerals in the soils.

Highly significant correlations of 0.89, 0.95, and 0.86 were obtained between soil extractable F and F uptake by plants for the acid, neutral, and alkaline soil, respectively. Including soil extractable F and P along with pH in a multiple regression equation (Table 4) could predict most of the variations in the values of F uptake by plants.

### **Phosphorus Uptake**

Highly significant effects of soil, the F and P additions and their interactions on P uptake were obtained (Table 1). Analysis of the data within each soil (Table 3) shows that increasing F addition depressed P uptake by plants grown on the acid soil. No clear trend for the effect of F addition on P uptake was observed for the neutral and alkaline soils.

Expectedly in all the soils, addition of P increased significantly P content of plants. For both the acid and neutral soils the magnitude of that increase decreased with increasing F addition. For the alkaline soil, however, The relation between P addition and P uptake was not much affected by F addition.

Highly significant correlations of 0.92, 0.87, and 0.73 were observed between soil- extractable P and P uptake for the acid, neutral, and alkaline soil, respectively. A multiple regression analysis (Table 4) including soil-extractable P and F along with soil pH accounted for 89.5, 78.9, and 60.8% of the variations in the values of P uptake for the acid, neutral, and alkaline soil, respectively.

## **Aluminum Uptake**

Soil F and P additions and their interactions have highly significant effects on Al uptake by barley plants (Table 1). Fluoride addition had significant effects on increasing Al uptake for both the acid and neutral soil (Table 3). For instance, the

<u> </u>		С	ahaba soil (acid)	)	
Dependent variables <sup>†</sup>	Intercept _	$R^2$			
	- <u>1</u>	Soil F	Soil P	pH	
Dry matter	1.931	-0.038***	0.011	0.365	0.81***
F-uptake	-1.611	0.018***	0.001	0.342	0.80***
P-uptake	-7.217	-0.065***	1.023***	2.952	0.90***
Al-uptake	-19.490	0.356***	0.153	4.916	0.76***
Mn-uptake	-12.011	-0.114***	0.846***	5.527*	0.88***
	_	Weld soil (neutral)			
Dry matter	7.905	-0.016**	0.001	-0.650	0.37***
F-uptake	-0.639	-0.021***	0.0004	0.105	0.91***
P-uptake	19.240	0.007	0.106***	-1.410	0.79***
Al-uptake	16.098	0.040*	0.022	-1.822	0.59***
Mn-uptake	7.571	-0.001	0.026***	-0.631	0.76***
	_		Haverson soil	(alkaline)	
Dry matter	4.863	-0.006	-0.013	0.279	0.091
F-uptake	1,466	0.007***	-0.004	-0.182	0.752***
P-uptake	29,886	-0.090**	0.483***	-1.272	0.608***
Al-uptake	7.725	0.017	-0.043	0.001	0.036
Mn-uptake	3.552	0.017	-0.077**	0.858	0.301***

TABLE 4. Multiple regression equations relating soil pH, soil extractable F and P to dry matter yield and various elements absorbed by barley grown on the three soils.

\*, \*\*, \*\*\*Indicate significance at 0.05, 0.01, and 0.001 level of probabilility, respectively.

 $^{+}$ All variables are expressed as mg pot<sup>-1</sup> except dry matter, which is expressed as g pot<sup>-1</sup>.

addition of 400 mg F kg<sup>-1</sup> soil, at the highest P treatment, increased Al uptake from 7.76 to 19.7 mg pot<sup>-1</sup> and from 6.49 to 16.3 mg pot<sup>-1</sup> for the acid and neutral soil, respectively. On the other hand, addition of F to the alkaline soil resulted in only minor increases in Al uptake. Addition of 1,000 mg F kg<sup>-1</sup> of soil, at 550 mg P kg<sup>-1</sup> of soil, increased Al uptake from 7.99 to 8.26 mg pot<sup>-1</sup>.

In earlier study in this laboratory, Elrashidi et al. (1987) concluded that addition of F increased considerably the solubility of Al in acid soils whereas it had only a minor effect on alkaline calcareous soils. Polomski et al. (1982) reported that F addition increased the solubility of Al, Fe, and OM in soils. Elrashidi and Lindsay 116 (1986a) studied F solution complexes in soils. They found that Al-F complexes contribute significantly to the total soluble F in acid soils. In the current study, a highly significant correlation of 0.96 was obtained between F and Al uptake by barley plants grown on the acid soil.

For both the acid and neutral soils, the extractable F is the most important parameter influencing Al uptake. Highly significant simple correlations of 0.87 and 0.60 were obtained between extractable F and Al uptake for the acid and neutral soil, respectively. No relation was obtained for the alkaline soil.

The effect of P addition, at different levels of F, on Al uptake was investigated for the three soils. Only at the highest F level in the acid soil that P addition decreased significantly Al uptake (Table 3). For both the neutral and alkaline soils, the effect of P addition on Al uptake was not clear. Extractable F and P along with soil pH were able to predict 76.1 and 58.9% of the variations in Al uptake for the acid and neutral soils, respectively.

## **Manganese** Uptake

In general, soil, the F and P additions and their interactions had a highly significant effect on Mn uptake by barley plants (Table 1). Increasing the F addition to the acid soil decreased significantly the uptake of Mn by plants (Table 3). The effect of F addition was not clear for both the neutral and alkaline soils. Singh et al. (1979a) found that increasing F addition decreased the macro-nutrient content of rice plants in alkaline soils.

For both the acid and neutral soils, the data given in Table 3 suggest that increasing P additions increased significantly the Mn content of the plants at all F levels. On the contrary, P additions decreased Mn uptake by plants grown on the alkaline soil. No reasonable explanation could be offered for the decrease in Mn uptake.

Similar responses, as to the data discussed above for Mn, were found for the uptake of other elements (e.g., Fe, Zn, Cu, K, Ca, and Mg) as a result of the F and P additions. No data for these elements are given in this report.

## CONCLUSIONS

- (1) Most of the soluble F added to soils is converted into insoluble chemical forms which are not available to plants. The addition of P appears to enhance the conversion process, particularly under neutral and acidic environments.
- (2) Addition of soluble F to soils of low pH can result in serious damage to vegetation. However, plants growing in alkaline calcareous soils are able to maintain a normal growth even with addition of high amounts of soluble F.
- (3) In general, addition of P to soils depresses F uptake by plants. The addition of P may enhance the formation of insoluble F minerals in soils.
- (4) The addition of F increases Al uptake particularly for plants grown on acid soils.

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TOXICITY OF FLUORIDES TO AQUATIC ORGANISMS: MODELING FOR WATER HARDNESS AND TEMPERATURE

by

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of

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#### ABSTRACT

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TOXICITY OF FLUORIDES TO AQUATIC ORGANISMS: MODELING FOR WATER HARDNESS AND TEMPERATURE

> Arthur H. Fieser, Ph.D. University of Pittsburgh

In addition to naturally occurring fluorides, major industries such as steel, aluminum and glass discharge fluorides into public waters. Fluorides in sufficient concentration are known to be toxic to aquatic organisms. Research to date has, with few exceptions, not followed modern methodologies; the EPA has not yet established a criterion. This project was designed to expand the data base and provide toxicity models relative to the parameters of water hardness and temperature.

Acute (48 hour) and chronic (21 day) survival and reproduction experiments were conducted with <u>Daphnia magna</u>, and acute (48 hour) and chronic (7 day) experiments with <u>Ceriodaphnia affinis/dubia</u>. Temperatures ranged from 15 to  $25^{\circ}$ C, water hardnesses ranged from 69 to 292 mg/L as CaCO<sub>3</sub>, and sodium fluoride concentrations ranged from 6 to 575 mg F<sup>-</sup>/L. Acute (96 hour) tests were similarly run with <u>Pimephales promelas</u> at  $20^{\circ}$ C. Methodologies essentially followed guidelines of EPA and Standard Methods.

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 $LC_{50}$  for <u>D. magna</u> were established at 109 to 354 mg F<sup>-</sup>/L, and for <u>C.</u> <u>affinis/dubia</u> at 120 to 340 mg F<sup>-</sup>/L. For both species,  $LC_{50}$  increased with increasing hardness and decreasing temperature.  $LC_{50}$  for <u>P. prome-</u> <u>las</u> ranged from 124 to 194 mg F<sup>-</sup>/L at 20<sup>o</sup>C; the  $LC_{50}$  varied inversely relative to water hardness. Mathematical models were established for these relationships.

Chronic studies of the daphnids allowed chronic/acute ratios to be calculated. For the specific experimental environments, <u>D. magna</u> application factors ranging from 10% to 21% were determined, with MATC ranging from 26 to 48 mg F<sup>-</sup>/L, depending upon hardness. For <u>C. affinis/dubia</u>, AF and MATC were calculated at 8 to 15% and 10 to 26 mg F<sup>-</sup>/L, respectively. Mathematical models were established.

A three-tiered mathematical model is suggested as a tentative criterion; the safe levels based on this logarithmically curved model vary from a minimum of 2.4 mg  $F^{-}/L$  at 46 mg/L hardness to 16.6 mg  $F^{-}/L$  at 300 mg/L hardness.

In order to better understand toxicity effects on daphnid reproduction, individual egg production was studied during the chronic tests, together with hatchability and neonate survival rate. At low fluoride levels, <u>D. magna</u> experience a reproduction stimulation effect; egg production increases but hatchability rate decreases for a net increase in neonate production. This effect was not observed in the <u>C. affinis/dubia</u> experiments. Recommendations for future research are included.

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## DESCRIPTORS

Acute Toxicity

<u>Ceriodaphnia</u> <u>affinis/dubia</u>

Daphnia magna

Fluoride

Modeling

Sodium Fluoride

Toxicity

Aquatic Organisms

Chronic Toxicity

Fathead Minnows

Hardness

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Pimephales promelas

Temperature

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#### 1.0 INTRODUCTION

Fluorine, the most electronegative and reactive element, ranks thirteenth among the elements in order of abundance. It commonly occurs in mineral form such as fluorspar (CaF<sub>2</sub>), cryolite (Na<sub>3</sub>AlF<sub>6</sub>), and fluorapatite (Ca<sub>10</sub>F<sub>2</sub>(PO<sub>4</sub>)<sub>6</sub>),<sup>(1)\*</sup> all of which are important to American process industries.

Fluoride minerals are mined, transported to industrial centers, and concentrated in the environment as a result of industrial process operations. This occurs in several major industries; as described by Zubban and Helwick:<sup>(2)</sup>

- (1) Aluminum production: the presence of fluoride in wastewater is attributed to the wet scrubbing of emissions from reduction furnaces in which aluminum is separated from bauxite ore in the presence of cryolite.
- (2) Steel production: the presence of fluoride is usually attributed to pickling of stainless steel with mixtures of nitric and hydrofluoric acid, or to scrubbing of emissions from steel production furnaces in which fluorspar is used as a slag conditioner.

<sup>\*</sup>Parenthetical references placed superior to the line of text refer to the bibliography.

(3) Glass manufacturing: fluoride wastes originate either from chemical frosting, etching, or polishing operations, as well as from wet scrubbing processes. 2

- (4) Phosphate fertilizer production: fluoride which is initially present in the phosphate mineral in the form of fluorapatite, is liberated by acidulation to silicon tetrafluoride and then hydrolyzed to fluosilicic acid, of which part is discharged as a plant effluent.
- (5) Other industries: electroplating, coke ovens, manufacture of certain organic chemicals where boron trifluoride is used as a catalyst, and others.

According to Angelovic et al.<sup>(3)</sup>, fluorides also occur naturally in many waters, especially in the western United States. These waters generally contain low concentrations of fluoride except in some instances such as the Firehole and Madison Rivers in Yellowstone National Park, which contain from 1 to 14 mg/L and Walker and Pyramid Lakes in Nevada which contain up to 13 mg/L.

Fluoride concentrations in drinking water from 0.7 mg/L to 1.5 mg/L can reduce the incidence of tooth decay in humans.<sup>(4,5)</sup> Higher concentrations can cause mottling or discoloration of teeth,<sup>(6)</sup> or even loss of teeth at an early age.<sup>(7)</sup> EPA standards<sup>(8)</sup> for drinking water range from 1.4 mg/L to 2.4 mg/L depending upon the annual average maximum

daily air temperature. According to the Safe Drinking Water Act, all waters that contain more than the allowable maximum fluoride concentration must be defluoridated prior to use as public water supplies.<sup>(9)</sup> It has been estimated that more than a thousand public water supplies utilize water that exceeds the recommended maximum fluoride level.<sup>(10)</sup>

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Few defluoridation plants have been constructed, and no single treatment technique has emerged as the best approach.  $^{(9)}$  Defluoridation processes include precipitation of fluoride with calcium salts,  $^{(10)}$  by lime precipitation and alum and polyelectrolyte coagulation,  $^{(12)}$  adsorption with activated alumina  $^{(13)}$  and ion exchange systems.  $^{(14)}$  Because of technical considerations (e.g., inability to effectively reduce fluoride concentration to desired level) and cost considerations (capital and operating costs), defluoridation units have not been installed, and communities have either arranged for alternate water supplies or accepted the higher fluoride levels.

As a result of the above considerations, there has been a tendency to set ambient water quality standards at or below the drinking water standards. Such a standard voids the need for defluoridation equipment. However, a penalty is involved in that industrial plants might be held to an overly-conservative level, especially if their effluent is subject to significant dilution upstream of the intake of the drinking water treatment plant. In this case, ambient standards should more properly be based on toxicity to aquatic life in the body of water under considera-

tion.

The establishment of standards with regard to aquatic life has been incorporated into federal legislation for twenty years. The Water Quality Act of 1965 provided for the establishment of water quality standards for interstate waters.<sup>(15)</sup> Section 10 of the Act reads in part as follows:

". . .Standards of quality established pursuant to this subsection shall be such as to protect the public health or welfare, enhance the quality of water and serve the purposes of this act. In establishing such standards. . . shall take into their consideration their use and value for public water supplies, propagation of fish and wildlife, recreational purposes, and agricultural, industrial and other legitimate uses. . ."

Although the Act has been amended by legislation, and interpreted by the National Technical Advisory Committee and subsequent governmental organizations, courts, etc., the basic purposes as described in the above quote still hold forth two decades later.

For example, the Federal Register for November 28, 1980,<sup>(16)</sup> in providing extensive notice of water quality criteria documents prepared by the Environmental Protection Agency, reads in part:

". . .EPA is required to periodically review and publish criteria for water quality accurately reflecting the latest scientific knowledge. . .on the kind and extent of effects on health and welfare including, but not limited to, plankton, fish, shellfish, wildlife, plant life, shorelines, beaches, esthetics, and recreation. . ."

The EPA has established water quality criteria for many substances; criteria have been published for 129 toxic pollutants, including the following twenty examples:<sup>(16)</sup>

Arsenic	Lead
Asbestos	Mercury
Benzene	Napthalene
Cadmium	Nickel
Carbon tetrachloride	Nitrobenzene
Chlorinated phenols	Nitrosamines
Chromium	Phenol
Copper	PCBs
Cyanides	Vinyl Chloride
Halomethanes	Zinc
Halomethanes	Zinc

Interestingly, no criterion has been established by the EPA for fluorides; this has been confirmed by the EPA – Environmental Criteria and Assessment Office and Environmental Research Laboratory in correspondence of March, 1984. (17, 18)

It has been known for many years that fluorides in sufficient concentrations can be toxic to aquatic life; references to this toxicity will be detailed in Chapter 2. Despite many experiments performed over a number of years, the data generated is insufficient to establish a fluoride toxicity criterion under present-day EPA guidelines. In the absence of such a criterion, many states will, as a conservative action, establish or continue a standard centered about the drinking water standard. This action could possibly cause undue technical and economic hardship on many of our basic industries, if a significantly higher concentration could be safely accommodated by aquatic life.

This project, then, is designed to study the effects of fluoride toxicity on several different forms of freshwater aquatic organisms. It is planned to establish models of acute toxicity for parameters such as hardness of water and temperature of water; models for chronic toxicity is an additional goal. If such experiments are successfully completed, we can expect to establish recognized factors such as acute/chronic ratios, minimum acceptable toxicant concentrations, and similar values. From these base models, it is hoped that an overall toxicity model can be established which would be a major contribution towards establishing a fluoride criterion. Although completion of the minimum data base required by the EPA would require a formidable amount of experimental projects, it is planned that the data generated in this project will act as a major building block in constructing a criterion, and as a minimum, would allow a "tentative" criterion or standard to be established which would provide a realistic guide to industry in planning treatment facilities for their fluoride effluents.

#### 2.0 BACKGROUND

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The toxic and health effects of fluorides have been studied in varying degrees of intensity for approximately forty years, mostly with regard to mammals (especially humans), and certain agricultural crops. Aquatic organisms have received only minor attention.

This chapter summarizes some of the more applicable research done to date; it is subdivided into fluoride effects on microorganisms, invertebrates, vertebrates, and plants in the aquatic habitat in sections 2.1, 2.2, 2.3, and 2.4, respectively. An overall evaluation of subject data is presented in section 2.5. The description of background literature that follows is adapted and updated from an unpublished literature review conducted in 1983 by Dr. J. L. Sykora at the University of Pittsburgh<sup>(19)</sup>

### 2.1 Effect of Fluorides on Aquatic Microorganisms

Research has been conducted by Eisenberg et al.<sup>(20)</sup> and Streckfuss et al.<sup>(21)</sup> into uptake of fluoride by bacteria, but with little attention to toxicity to the bacteria. Other studies with bacteria (Baranova et al.)<sup>(22)</sup> were primarily concerned with biochemical changes in bacterial cultures in the presence of fluorides and chlorides.

Toxic activity to <u>Escherichia</u> <u>coli</u> was studied by Bringmann and Kuehn<sup>(23)</sup>, in which it was found that a threshold concentration of 180

mg/L of fluoride inhibited fermentation reactions. Grune and Sload<sup>(24)</sup> determined that 0.5 to 5.0 ppm of fluoride did not measurably affect sewage digestion by bacteria.

Smith and Woodson<sup>(25)</sup> showed that fluorides inhibited growth of cultures of the freshwater alga <u>Chlorella pyrenoidosa</u>, with greatest effect at concentrations higher than 42 mg/L. No mathematical relationship was established between fluoride concentration and inhibition of growth.

Malewicz et al., (26), in experiments with the green algae <u>Chlorella</u> <u>vulgaris</u>, <u>Scenedesmus quadricauda</u> and <u>Dyctiosphaerium pulchellum</u>, determined that at 50 mg/L, fluoride ions had no measurable effect on the rate of CO<sub>2</sub> assimilation, but at the 500 mg/L level, a 36% reduction in biomass occurred, accompanied by a reduction in cellular chlorophylls and karotenoids.

Experiments with <u>Chlorella pyrenoidosa</u> by Sargent and Taylor<sup>(27)</sup> showed that a 7% reduction in respiration rate was effected at a fluoride concentration of 950 mg/L, but no measurable effect was observed in fluoride concentrations of 760 mg/L.

Bringmann and Kuehn<sup>(23)</sup> determined that a threshold concentration of 45 mg/L of fluoride caused replication inhibition in <u>Scenedesmus</u> <u>quadricauda</u>.

## 2.2 Effect of Fluorides on Aquatic Invertebrates

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Jones<sup>(28)</sup> reported that 46 mg/L of NaF was lethal to the planaria <u>Polycelis nigra</u>. Anderson<sup>(29)</sup> determined that 504 mg/L of NaF was the threshold concentration needed for immobilization of <u>D. magna</u> and <u>D.</u> <u>pulex</u>, and determined that fluorides affected the development of the males, with the effect on <u>D. magna</u> greater than that on <u>D. pulex</u>.

Test of <u>D. magna</u> neonate response to 50 Hz electroacoustical stimulus by Bringmann and Kuehn<sup>(23)</sup> indicated a threshold concentration of 270 mg/L fluoride during a 48 hour period.

Fluoride effluents from an aluminum plant substantially reduced the species diversity of mollusks in a river, according to Lisicky<sup>(31)</sup>. Malakocoenosis in stagnant water and woodland areas were most affected and eurytopic species dominated in contaminated areas. Fluorides may affect the mollusks either directly by corroding their shells or indirectly, by binding the calcium in the water and forcing the shellfish to consume their own shell to satisfy their CaCO<sub>3</sub> requirments.

Buikema<sup>(32)</sup> analyzed the interaction of toxicity of selected inorganic compounds to the rotifer <u>Philodina acuticornis</u>; additive interactions were observed for mixtures of chromium and fluorides, whereas antagonistic responses were obtained for individual combinations of fluoride with iron, zinc, and chlorine. No interactions were observed for copper and fluoride.

Protozoa and rotifers survived and reproduced in water containing 100 mg/L of NaF, but were killed at 1700 mg/L, according to Wantland (33).

## 2.3 Effect of Fluorides on Aquatic Vertebrates

In 1937, Ellis<sup>(34)</sup> experimented with the effects of fluorides on goldfish. At 100 mg/L, no mortalities were experienced in 4 days. In soft water, 1000 mg/l killed the goldfish in 12 to 29 hours, but in hard water, the time to death was extended to 60 to 102 hours. By comparison, DeRoos<sup>(35)</sup> attributed death to goldfish in 4 days to a concentration of 120 mg/l of fluoride. Later studies by Ellis<sup>(36)</sup> showed that trout eggs were delayed in hatching when subjected to 1.5 mg/L of fluorides.

Wallen et al.<sup>(37)</sup> tested toxicity of selected pure chemicals to <u>Gambusia affinis</u> in turbid waters, including NaF. Pond water was used as the test medium, with alkalinities lower than 100 mg/L as CaCO<sub>3</sub> and turbidity at 25 - 150 mg/L. At 560 mg/L of NaF, all fish appeared normal; at 1000 mg/L, partial mortalities were observed at 24 and 48 hours; and at 1800 mg/L, all fish were dead within 24 hours. The calculated LC<sub>50</sub> for 24, 48, and 96 hours was 1240, 925, and 925 mg NaF per liter, respectively.

Extensive experiments were performed by Neuhold and Sigler<sup>(38)</sup> on the effects of NaF on rainbow trout and carp. Experiments were conducted in very soft water; temperature for the cold water fish were conduc-

ted at 53 - 57 $^{\rm O}$ F and for the warm water fish at 60 - 70 $^{\rm O}$ F. Water from the municipal water supply system was used, and passed through a softening unit to yield a very soft water (less than 3 ppm of calcium or magnesium). LC $_{50}$  for the rainbow trout varied between 2.7 and 4.7 ppm in a 480 hour experiment, in which the last recorded mortality was at 218 LC<sub>50</sub> for the carp was between 75 and 91 ppm fluoride; the time hours. limits were not indicated. Rainbow trout eggs were tested at three different temperatures during a variable time period of 167 to 424 hours;  $LC_{50}$  was estimated to be between 222 and 273 mg/L of fluoride (424 hours). Experiments with trout embryos and fry were conducted at  $60^{\circ}F$ for 825 hours;  $LC_{50}$  for larval survival was 61 to 85.3 mg/L of fluoride. In addition, experiments on fluoride uptake were conducted, in which it was determined that uptake in the various parts of the fish body were dependent upon fluoride concentration in the medium and time in the medium.

Rainbow trout were also studied by Angelovic et al.<sup>(39)</sup>. In soft water,  $LC_{50}$  at  $45^{0}F$  was estimated at 5.9 to 7.5 mg/L of fluorides; at  $55^{0}F$ , at 2.6 to 6.0 mg/L, and at  $65^{0}F$ , 2.3 to 7.3 mg/L. The experiments were terminated at 240 hours.

Neuhold and Sigler in a later report<sup>(40)</sup> indicated that tempering of rainbow trout to NaCl reduced their response to fluoride;  $LC_{50}$  was 6 mg/L fluoride for non-tempered fish and 22 mg/L for tempered fish, in a 120 hour experiment.

Vallin's<sup>(41)</sup> experiments with Atlantic salmon and salmon trout indicated that fish were more susceptible to fluoride poisoning in soft water than in hard water.

Rainbow trout were exposed to fluorides in soft and hard water by Herbert and Shurben<sup>(42)</sup>. In soft water of 12 mg/L as  $CaCO_3$ , threshold concentrations were determined to be 8.5 mg/L fluoride and 4.0 mg/L fluoride for 50% and 5% mortality, respectively. In water of 320 mg/L as  $CaCO_3$ , 75 mg/L of fluoride had no measurable effect. From graphs presented, a 96 hour  $LC_{50}$  could be calculated at approximately 22 mg/L fluoride.

In studies with brown trout by Wright<sup>(43)</sup> in tap water of 73 mg/L as  $CaCO_3$ , mortality rates eventually reached 100% at fluoride concentrations of 20 mg/L, 30 mg/L, and 60 mg/L. Below 20 mg/L, mortality rates declined; from data presented, a 48 hour  $LC_{50}$  of approximately 30 mg/L fluoride can be estimated.

Sigler and Neuhold<sup>(44)</sup> indicate that the response of fish to moderate fluoride concentration (1.5 to 5.0 mg/L) is related to environmental acclimatization and is species dependent. They also indicate that healthy, growing populations of trout exist in the Firehole River in Yellowstone National Park where fluoride concentrations reach 14 ppm, and at Pyramid and Walker Lakes in Nevada, where the concentrations reach 13 ppm. Pimentel and Bulkley<sup>(45)</sup> reported on static bioassays conducted to determine effects of water hardness on toxicity of sodium fluoride to rainbow trout. Ninety-six hour  $LC_{50}$  values increased from 51 to 193 mg/L(F) as water hardness levels rose from 17 to 385 mg/L as CaCO<sub>3</sub>. It was concluded by the authors that tests of chronic toxicity at different water hardness levels are needed before fluoride standards for aquatic life can be set.

 $McKim^{(46)}$  presented a table of life-cycle toxicity tests with fish for various toxicants, in which reference is made to fluoride toxicity to fathead minnows and a maximum acceptable toxicant concentration (MATC) of 6.8 to 13.6 mg/L is listed. This information is also presented by Rand and Petrocelli<sup>(47)</sup>. Reference for the data is listed as "D. Olson - personal communication, Environmental Research Laboratory, Duluth, Minn." Mr. D. Olson is deceased; conversations with personnel at subject laboratory have not yielded any additional information in the form of publications, laboratory files, or other sources that would provide details for the MATC value. Hardness, temperature, and other parameters are not known.

### 2.4 Effect of Fluorides on Aquatic Plants

The uptake of fluoride by water hyacinth (<u>Eichhornia crassipes</u>) was studied by Rao et al.<sup>(48)</sup>. Although capable of uptake of fluorides, consideration of the plant as a defluoridation agent for natural water was deemed not practical because the uptake is appreciable only in high-

er concentration ranges, possibly above 10 mg/L fluoride.

### 2.5 Discussion of Previous Research

Previous research performed over the last several decades has provided much knowledge relative to fluoride toxicity to aquatic organisms. For example, insights into parameters affecting toxicity (e.g., hardness, temperature) would not exist without the important work of Neuhold, Sigler, Vallin, and others. However, over the last decade, stringent guidelines have been established by the EPA for the conductance of toxicity tests<sup>(16)</sup>; most of the research described above do not meet these modern guidelines, the experiments of Pimentel and Bulkley<sup>(45)</sup> being a notable exception.

In his review<sup>(19)</sup> of the literature and research of the last several decades, Prof. J. L. Sykora at the University of Pittsburgh concluded:

- A. The data base on acute toxicity of fluorides to freshwater organisms is inadequate because:
  - it is limited only to a few species which are not representative of the freshwater community,
  - was developed using sometimes unacceptable species, and variable, non-standard methodology,
  - does not conform to EPA Guidelines and Standards for toxicity testing.

B. No data are available on chronic toxicity.

- C. Insufficient data are available on bioaccumulation of fluoride.
- D. No definite criteria or standards could be developed using this data base for fluorides which would compare with criteria or standards for other toxicants already published or promulgated by EPA or PENNDER (Pennsylvania Department of Environmental Resources).
- E. Very little information is available on the distribution and survival of fish and aquatic organisms exposed to elevated fluoride levels in field situations.

The author recommended:

A. A new data base for toxicity of fluorides to aquatic life be developed.

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- B. The testing should include acute as well as chronic experiments with several animals, and follow standard methods and guidelines formulated either by EPA or PENNDER.
- C. The criteria and/or relevant standards should be related to water quality characteristics such as hardness and temperature.
- D. Different criteria should be developed for warm water and cold water environments.
- E. The models of fluoride toxicity to aquatic organisms based on laboratory experiments should be verified in the field.

### 3.0 RESEARCH PLAN

Toxicity testing of aquatic organisms should comprehend the following parameters:

- Species of experimental animals.
- Life stage of animals and exposure conditions.
- Environmental conditions:
- Water quality
- Temperature
- Dissolved oxygen
- Daylight hours
- Feeding regimen of animals
- Toxicant concentration

The research plan adopted for this project comprehends the above parameters, and is based on the general conditions and restrictions described below. Details of equipment and materials utilized to achieve and maintain the research parameters are described in Chapter 4.

### 3.1 Species of Experimental Organisms

Rand and Petrocelli<sup>(49)</sup> list six criteria for selection of test organisms:

"1. Since sensitivities vary among species, species representing a broad range of sensitivities should be used whenever possible.

2. Widely available and abundant species should be considered.

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3. Whenever possible, species should be studied which are indigenous to or representative of the ecosystem that may receive the impact.

4. Species that are recreationally, commercially, or ecologically important should be included.

5. Species should be amenable to routine maintenance in the laboratory and techniques should be available for culturing and rearing them in the laboratory so that chronic toxicity tests can be conducted.

6. If there is adequate background information on a species (i.e., it physiology, genetics, and behavior) the data from a test may be more easily interpreted."

Organisms that meet these criteria and are also recommended by the  $EPA^{(50,51,52)}$  include the fathead minnow (<u>Pimephales promelas</u>) as a warm freshwater vertebrate, and Cladocera (various species of <u>Daphnia</u>) as a warm freshwater invertebrate. The species of Cladocera selected were <u>Daphnia</u> nia magna and Ceriodaphnia affinis/dubia.

The initial culture of <u>Daphnia magna</u> was obtained from a commercial biological supply house (Ward's\*), and the initial culture of <u>Ceriodaphnia affinis/dubia</u> was obtained from the EPA regional laboratory at Wheeling, West Virginia. These daphnid cultures were subsequently maintained in our laboratories.

\* Ward's Natural Science Establishment, Inc., Rochester, New York

<u>D. magna</u> were maintained in 5 liter aquaria containing gently aerated (20 cc/min.) hard water (170 mg/L as  $CaCO_3$ ) using 16 hour photoperiods at temperatures ranging from 19-21°C. The daphnids were fed three times a week, Monday, Wednesday, and Friday, with a water suspension of a mixture of trout chow, alfalfa, yeast and commercial fish food as described in detail below. The water in the aquaria was changed approximately every 10 days and thinning of the culture was performed at the same time as necessary.

<u>C. affinis/dubia</u> were maintained in 2 liter aquaria in hard water without aeration at 16 hour photoperiods and temperatures ranging from 19-21<sup>0</sup>C. Feeding consisted of a fermented mix of chow, alfalfa, yeast, ceraphyl, and algae as described below.

### 3.2 Life Stage of Organisms and Exposure Conditions

Both acute (short-term) and chronic (long-term) exposure of the daphnids, and acute exposure of the fathead minnows was selected so as to provide a comprehensive group of toxicity tests that could be considered for potential establishment of environmental control criteria.

Tests were either of the "static" or "renewal" type. In a "static"  $test^{(53)}$ ". . . the organisms are exposed in still water. The test material is added to the dilution water to produce the desired test concentrations. The control and test organisms are then placed in test chambers and there is no change of water for the duration of the test."

A "renewal" test<sup>(53)</sup> ". . . is similar to a static test because it is conducted in still water, but the test solutions and control water are renewed periodically by transferring the test organisms to chambers with freshly prepared material or by removing and replacing the material in the original containers."

General exposure conditions and age of the animals are indicated in Table 1. Tests generally followed the guidelines of the EPA<sup>(54,55)</sup>.

### 3.3 Environmental Conditions

Environmental conditions that were monitored and controlled included water quality, temperature, daylight hours, feeding regimen, and toxicant concentration.

#### 3.3.1 Water Quality

Test water used in all of the experiments and in the culture tanks, was "reconstituted water" prepared in accordance with Standard Methods<sup>(56)</sup> and the EPA<sup>(57)</sup>. Reconstituted water is prepared by adding reagent-grade chemicals to double-distilled water.

As indicated in Chapter 2, hardness of water can significantly influence the toxicity of chemicals to aquatic animals. "Hardness" as used herein refers to the concentration of polyvalent cations such as  $Ca^{++}$  and  $Mg^{++}$  and expressed in "mg/L as  $CaCO_2$ ."

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## TEST EXPOSURE AND ANIMAL AGES

			Age of Animals				
Organism	Type of Test	Exposure System	Upon Collection	Upon Initial Insertion Into Test Chamber			
D. magna	Acute: 48 hours	Static	0-24 hours	< 28 hours			
<u>D. magna</u>	Chronic: 21 days	Renewal: 48-72 hours	0-24 hours	< 28 hours			
<u>C. affinis/dubia</u>	Acute: 48 hours	Static	0-2 hours	< 6 hours			
<u>C. affinis/dubia</u>	Chronic: 7 days	Renewal: 48-72 hours	0-2 hours	5-6 hours			
<u>P. promelas</u>	Acute: 96 hours	Static	0-24 hours	14-18 days			

 $Mg^{++}$  and expressed in "mg/L as  $CaCO_3$ ."

The basic hardness selected was "hard" water (approximately 170 mg/L as  $CaCO_3$ ). Harder and softer waters were formulated on a logarithmic scale relative to the hard water to provide four water types as shown in Table 2:

#### TABLE 2

### TYPES OF RECONSTITUTED WATER

Water Type	Symbol*	Nominal Hardness Level (mg/L as CaCO <sub>3</sub> )
Very Hard	VHRW	250 - 270
Hard	HRW	160 - 180
Moderately Hard	MHRW	100 - 120
Semi-Soft	SSRW	60 - 80
* RW stands for	r reconstitut	ed water

The various reconstituted waters were prepared by adding reagent grade chemicals (as listed in Appendix B) weighed to 0.1 mg, to double distilled water in amounts shown in Table 3. Typical analyses are shown in Table 3; the specific data are that recorded for the acute <u>Daphnia</u> <u>magna</u> tests. The hardness, alkalinity, and pH fall in a range comparable to that commonly found in North American rivers and streams.

# TYPICAL COMPOSITION OF RECONSTITUTED WATER

	Nominal Hardness Level (Nominal mg/L as CaCO <sub>3</sub> )									
Parameter	Very Hard (260 mg/L)	Hard (170 mg/L)	Moderately Hard (110 mg/L)	Semi-Soft (70 mg/L)						
alts added to double distilled water: NaHCO <sub>3</sub>	303 mg/L	192 mg/L	121 mg/L	76 mg/L						
$CaSO_4 \cdot 2H_2O$	190 "	120 "	76 "	48 "						
MgSO <sub>4</sub>	190 "	120 "	76 <sup>ii</sup>	48 <sup>.</sup>						
ксі	13 "	8"	3 "	3 "						
easured water quality:*										
Hardness (mg/L as CaCO <sub>3</sub> )	265 (261 - 268)	169 (167 - 171)	109 (105 - 113)	69 (67 - 72)						
Alkalinity (mg/L as CaCO <sub>3</sub> )	174 (168 - 179)	111 (107 - 114)	71 (67 - 74)	46 (43 - 49)						
рH	8.3 (8.2 - 8.5)	8.1 (8.0 - 8.3)	8.0 (7.8 - 8.1)	7.8 (7.7 – 7.9)						

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#### 3.3.2 Temperature

Temperatures for the various tests were selected on the basis of providing sufficient differences for modeling purposes and yet be in the viability range of the organism involved.

Acute tests of <u>D. magna</u> and <u>C. affinis/dubia</u> were conducted at  $15^{\circ}$ C,  $20^{\circ}$ C, and  $25^{\circ}$ C. Chronic tests of the <u>D. magna</u> were conducted at  $20^{\circ}$ C, per recommendation of the EPA<sup>(51)</sup>, whereas chronic tests of <u>C. affinis/dubia</u> were conducted at  $25^{\circ}$ C. The acute test of <u>P. promelas</u> was conducted at  $20^{\circ}$ C.

Temperature was maintained at the designated temperature within a range of  $\pm 1^{\circ}$ C via use of a constant temperature water bath as described in the following chapter. Actual temperature variation was small; all experiments recorded a 95% C.I. of design temperature  $\pm 1^{\circ}$ C or less.

### 3.3.3. Dissolved Oxygen

The reconstituted water and toxicant utilized, together with the biological systems involved, were found to have no significant effect on dissolved oxygen (D.O.) concentration. No aeration was utilized during the tests. In all tests, at all temperatures, all D.O. measurements at the beginning, intermediate, and end points were at 95-100% of saturation concentration.

#### 3.3.4 Daylight Hours

Following EPA guidelines, a basic 16-hour photoperiod was utilized. It consisted of a 10-hour fluorescent light period at 65 footcandles, preceeded and followed by a 3-hour period of incandescent light at 4 footcandles. This basic lighting regimen was utilized for the testing of <u>D. magna and P. promelas</u>; a constant 3 footcandle fluorescent photoperiod of 16 hours was utilized in the case of the <u>C. affinis/dubia</u>.

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3.3.5 Feeding Regimen

Acute tests were conducted on the basis of no feeding during the test period.

For the chronic <u>D. magna</u> tests, feeding consisted of 0.4 mg of "prepared food" plus 10 million cells of algae per organism, following each water change (48-72 hours).

For the chronic <u>C. affinis/dubia</u> tests, daily feedings consisted of 0.4 mg. of prepared food plus 15 million cells of algae and 0.4 mg algal nutrient water.

The formulation of the specific foods are presented in Chapter 4; they were arrived at after tests of different formulations so as to provide continued viability of the organism in the zero-toxicant controls, as measured by a minimum of mortality and a high reproduction rate.

#### 3.3.6 Toxicant Concentration

Reagent-grade sodium fluoride (NaF) was used as the toxicant in concentrations based on a logarithmic expansion, viz., 0, 6.3, 10, 16, 25, 40, 63, 100, 158, 251, 316, 398, 447, 501 mg  $F^/L$ . Appendix B provides the detailed analysis of the toxicant. The indicated  $F^-$  levels are "total" fluoride levels, as measured by additions of NaF weighed to 0.1 mg.

"Ionic" F<sup>-</sup> of all solutions was measured by a selective ion electrode. The "ionic" F<sup>-</sup> level will vary from the total F<sup>-</sup> level at various fluoride and hardness concentrations, because of such factors as formation of complexes of fluoride anion with the polyvalent cations of hard water constituents, elevated activities, solubility limits of fluoride salts, etc.

Further, the concentration of "total fluoride" will vary during the experiment, due to the factors of evaporation from the test chambers during the test period, and dilution of the test water during transfer of organisms or food additions. Thus, the term "nominal" fluoride will be used in this report, as an indicator of original total fluoride concentration prior to the indicated concentration and dilution factors. Calculations will be made on the basis of the actual measured fluoride.

In the field, it is important from a pollution control viewpoint to know the fate of fluoride contaminants as they enter a river or stream of

a given hardness, and thus, "total" as well as "measured" fluoride concentrations are of interest. From the ecological viewpoint, the "measured" or "ionic" form of fluoride concentration is of interest as being immediately available for uptake by the organisms.

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### 4.0 MATERIALS, EQUIPMENT AND PROCEDURES

Chapter 3 described the general research plan; this chapter describes details of the materials, equipment and procedures used to achieve subject plan.

### 4.1 Organism Supply

In order to assure proper age of neonates for subject experiments, adults were set out separately from the stock culture in advance, and neonates collected within the time frame previously indicated in Table 1. For example, in the acute <u>D. magna</u> test, neonates 24 hours or younger were desired for testing. On day "1", adults were set out in 250 ml beakers in water quality equal to that to be used in the test (HRW, MHRW, etc., but without toxicant), five adults to a beaker. They were fed the standard food recipe as described below at time of insertion. On day "2", not more than 24 hours after initial adult insertion, neonates were removed and inserted into the test chambers on a random basis. Daphnids were transferred using pipettes.

### 4.2 Test Chambers and Organism Loading

For acute <u>D. magna</u> and <u>C. affinis/dubia</u> experiments, and for chronic <u>D. magna</u> experiments, 100 ml KIMAX glass beakers were used and filled with 80 ml of test solution. For <u>C. affinis/dubia</u> experiments, 50 ml beakers were used, filled with 30 ml test solution. <u>P. promelas</u> experiments were conducted in 1.5 liter glass containers containing 1.1 liters

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of test water. Loading of the chambers and number of chambers used are indicated in Table 4.

### TABLE 4

Organism	Organisms per Chamber	No.of Chambers for Each Toxicant Concentration Tested	Total No. of Organisms per Concentration
<u>D. magna</u> Acute	5	4	20
<u>D. magna</u> Chronic	1	10	10
<u>C. affinis/dubia</u> Acute	5	4	20
<u>C. affinis/dubia</u> Chronic	1	10	10
<u>P. promelas</u> Acute	10	2	20

## LOADING OF TEST ANIMALS

A total of approximately 4,100 animals were used in testing as shown in Table 5.

# 4.3 Water Quality

Reconstituted water was prepared in accordance with Table 3. Analytical testing of renewal water and wastewater was performed following the guidelines of Standard Methods as follows:

# NUMBER OF ANIMALS EXPOSED

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Test	No. of Hardnesses Tested	No. of Temperatures Tested	Average No. of Concentrations Tested	No. of Organisms per Concentration	Total No. Organisms Used
<u>D. magna</u> Acute	4	3	6.4	20	1,536
<u>D. magna</u> Chronic	3	1.	6.3	10	189
<u>C. affinis/dubia</u> Acute	4	3	6.8	20	1,632
<u>C. affinis/dubia</u> Chronic	4	1	6.2	10	248
P. promelas Acute	4	1	6.2	20	496
	1	L	· · · · · · · · · · · · · · · · · · ·	Total	4,101

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- Hardness: EDTA titrimetric method using "Calgon 20"\* titrant, buffer, and indicator.<sup>(58)</sup>
- Alkalinity: potentiometric titration with 0.02N HCl through a pH of 4.6.<sup>(59)</sup>
- pH: direct measurement with a Corning Model 12 pH meter.<sup>(60)</sup>
- Dissolved oxygen: direct measurement with a Yellow Springs Model 57 oxygen meter. (61)
- Fluoride: sample mixed 1:1 with a total ionic strength adjustment buffer (TSIAB), and measured with an Orion Model
   94-09 selective ion electrode, Orion Model 90-01 reference electrode, and Orion Model 601A Millivolt meter.<sup>(62)</sup>

Appendix C lists major equipment utilized. All equipment and meters were kept clean and in adjustment; calibrations were made using either purchased or laboratory standards.

#### 4.4 Fluoride Analyses

Because fluoride content is central to all of the experiments, details of fluoride measurement are presented herewith.

Selective ion electrodes have been used in analytical methods at an ever-increasing rate for a number of reasons<sup>(63)</sup>:

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<sup>\*</sup> Calgon Corporation, Pittsburgh, PA

- direct measurement of a large number of cations and anions is possible

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- simpler to use than most other analytical techniques
- sensitivity of electrodes, often measuring down to parts per
   billion
- measurements are independent of sample volume
- relatively low cost of purchase and maintenance.

The specific electrode utilized was an Orion Model 94-09 instrument, described by the manufacturer as follows<sup>(64)</sup>:

"... the electrode consists of a single-crystal lanthanum fluoride membrane, and an internal reference, bonded into an epoxy body. The crystal is an ionic conductor in which only fluoride ions are mobile. When the membrane is in contact with a fluoride solution, an electrode potential develops across the membrane. This potential, which depends on the level of free fluoride ions in solution, is measured against an external constant reference potential with a digital pH/mv meter or specific ion meter. The measured potential corresponding to the level of fluoride ions in solution is described by the Nernst equation:

$$E = E_0 - S \log A$$

where:

E = measured electrode potential E<sub>o</sub> = reference potential (a constant) A = fluoride level in solution S = electrode slope

The level of fluoride, A, is the activity of "effective concentration" of free fluoride ions in solution. The total fluoride concentration,  $C_t$ , may include some bound or complexed ions as well as free ions. The electrode responds only to the free ions, whose concentration is:

$$C_f = C_t - C_b$$

where  $\mathbf{C}_{\mathbf{b}}$  is the concentration of fluoride ion in all bound or complexed forms.

 $A = T C_{f}$ 

Ionic activity coefficients are variable and largely depend on total ionic strength.

Ionic strength is defined as:

where:  $C_i = \text{concentration of ion i}$  $Z_i = \text{charge of ion i}$ 

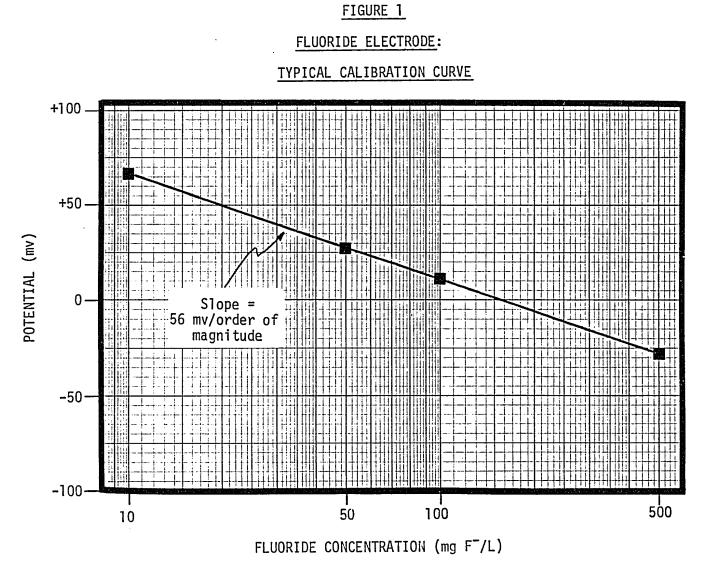
If the background ionic strength is high and constant relative to the sensed ion concentration, the activity coefficient is constant and activity is directly proportional to concentration."

A comprehensive description of the design and operation of ion-selective electrodes has been published by Koryta and Stulik<sup>(65)</sup>

The fluoride analytical system was calibrated utilizing laboratoryprepared solutions of 10, 50, 100, and 500 mg/L, and was periodically checked against purchased standards obtained from Orion\*. Calibrations were made each time a new set of fluoride determinations were made, unless a previous calibration had been made within the previous hour.

Graphing of millivolts versus fluoride concentration on semi-log paper gave a straight line with a slope of 56 mv per order of magnitude change in mg/L - see Figure 1 for a typical calibration curve.

<sup>\*</sup> Orion Research, Inc., Cambridge, MA





Typical electrode response time (66) is shown in Figure 2. The actual response time at the concentrations being measured was determined to be 45 seconds or less; a standard timed measurement of 50 seconds was therefore adopted for all measurements.

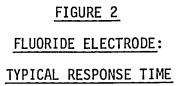
Free fluoride measurement will depend on  $pH^{(67)}$  as shown in Figure 3. All water samples were mixed 1:1 with TISAB obtained from Orion. TISAB (total ionic strength adjustment buffer) adjusts the sample pH to 5.0 - 5.5 so as to avoid complexation of fluoride by hydrogen ions (below pH of 5.0) and by hydroxide interference (above pH of 7.0); it also preferentially complexes iron and aluminum, breaking up any complexes that may have been formed with these ions by the fluoride<sup>(68)</sup>.

TISAB is prepared by mixing 57 ml glacial acetic acid, 58 g NaCl and 4 g 1,2 cyclohexanediaminetetraacetic acid (CDTA), adjustment of the pH with sodium hydroxide to 5.0 - 5.5, and dilution to 1 liter<sup>(69)</sup>.

The reference electrode utilized (Orion 90-01) was a sleeve-type Ag/ÅgCl electrode filled with a solution containing Na<sup>+</sup>, K<sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and Cl<sup>-</sup> ions, saturated with AgCl, and matching potential characteristics of a KCl calomel electrode (70).

During experiments based on higher concentrations of fluorides and hardness, the complexing of ions and precipitation of salts must be anticipated in the measurements. The polyvalent cations of the hardness (especially calcium) will combine with the fluoride as follows:<sup>(66)</sup>

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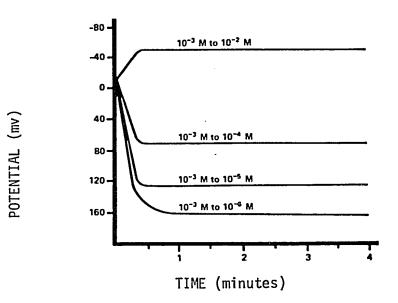
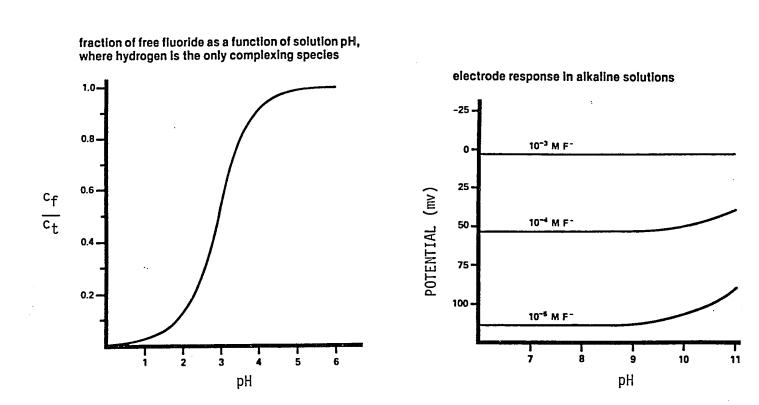


FIGURE 3

# FLUORIDE ELECTRODE: RESPONSE VS. pH

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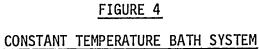
$$Ca^{++} + 2F^{-} \longrightarrow CaF_{2}$$
  
but  $[Ca^{++}][F^{-}] = K_{sp} = Constant$   
 $K_{sp} = 3.4 \times 10^{-11} @ 18^{\circ}C$   
 $= 3.95 \times 10^{-11} @ 26^{\circ}C$ 

where:

Because the solubility product is constant, the product of the calcium and fluoride concentration must be equal to  $K_{sp}$  at all times. Consequently, as the concentration of either the cation or anion is increased to a point where the salt solubility limit is reached, precipitation will occur. In later chapters as analyses of solutions are reported, the reduction in hardness/fluoride concentrations can be observed.

### 4.5 Temperature Control

Temperatures of the test solutions were closely maintained through the use of a constantly recirculating water bath, designed and assembled in the laboratory as shown in Figure 4. Tank A measured 914 mm length, 305 mm width, and 381 mm depth; water depth was 229 mm. Tank B measured 1143 mm length, 305 mm width, and 165 mm depth; water depth was 51 mm. The cooling unit required for testing at  $15^{\circ}$  and  $20^{\circ}$ C, had a flow rate of approximately 0.65 LPM and a cooling capacity of approximately 40 kilocalories/hour. Because the cooling unit operated constantly without benefit of a thermostat, 100 watt fish tank heaters were installed as shown to maintain the required temperature. This relatively simple system maintained the temperature within the EPA recommended temperature range of  $\pm$  $1^{\circ}C^{(71)}$ ; all experiments were conducted at a temperature range in the 95%



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# Notes:

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- (1) Drawing not to scale, dimensions in mm.
- (2) Fish tank heaters.
- (3) Siphon.
- (4) Test chamber.

C.I. of design temperature  $\pm 1^{\circ}$ C or less.

4.6 Materials of Construction and Cleaning

All equipment used in contact with experimental solutions was constructed of glass, tygon, or teflon. New glassware was washed with "Sparkleen" detergent (Fisher\*), soaked 24 hours in 25% HCl and rinsed successively with tap water and distilled water. Old glassware was washed with "Sparkleen", 25% HCl, and rinsed with tap water and distilled water.

#### 4.7 Food Preparation

As indicated in Chapter 3, none of the animals were fed during the acute test. During the chronic tests, daphnids were fed as follows:

<u>D. magna</u> were fed every two or three days upon renewal of test water; each daphnid received 0.4 mg of "basic" food plus 10 million cells of algae (<u>Selenastrum capricornutum</u>). The "basic" food was prepared by mixing 3 g trout chow, 3.3 g commercial fish food (Tetramin\*\*), 2.6 g dried yeast, and 0.5 g dried alfalfa with 500 ml double-distilled water in a blender and mixed at high speed for 7 minutes. The mixture was refrigerated for one hour; the top 300 ml of the suspension were decanted and <sup>1</sup> frozen in 20 ml polyethylene bottles for future use, and the sediment was discarded. This recipe generally follows that proposed by the EPA<sup>(72)</sup>

<sup>\*</sup> Fisher Scientific Co., Pittsburgh, PA

<sup>\*\*</sup> Tetramin Staple Food, obtainable in local pet shops, manufactured in West Germany by TetraWerke.

except that Tetramin has been substituted for a portion of the trout chow.

The <u>Selenastrum</u> <u>capricornutum</u> was cultured in the laboratory along the guidelines of the EPA<sup>(73)</sup> utilizing a start agar slant of the algae (UTEX-1648) obtained from the University of Texas\*. Cell count was performed using a hemacytometer.

<u>C. affinis/dubia</u> were each fed daily 0.1 mg of "prepared" food and 0.03 ml of "algal solution". The "prepared" food was mixed similarly to the <u>D. magna</u> basic food, but 3.3 g trout chow was substituted for the Tetramin; this mixed food was then allowed to ferment at room temperature for seven days before being frozen. The "algal solution" was the growing medium of the <u>S. capricornutum</u> culture, containing macro and micro nutrients and approximately 50 million cells/ml of <u>S. capricornutum</u>.

These recipes were determined to be the best of several tests for providing acceptable viability and reproduction in the daphnids.

Although not fed during the acute tests, the <u>P. promelas</u> stock cultures were maintained with a 50/50 mix of newly-hatched brine shrimp and ground (mortar and pestle) Tetramin fish food.

<sup>\*</sup> Department of Biology, University of Texas at Austin, Austin, Texas.

### 5.0 DAPHNIA MAGNA ACUTE EXPERIMENTS

Acute (48 hour) experiments utilizing <u>D. magna</u> were conducted at 15, 20, and  $25^{\circ}$ C in very hard, hard, moderately hard, and semi-soft water, at fluoride concentrations ranging from 0 to 447 mg F<sup>-</sup>/L. These experiments, involving approximately 1,500 daphnids, were conducted in accordance with the plan, procedures, and equipment described in Chapters 3 and 4.

### 5.1 Experimental Data

Table 6 indicates method of preparation and the median analyses of the reconstituted waters used for all of the <u>D. magna</u> acute experiments.

The analysis of the major measured parameter of "hardness" will vary from the beginning of the test to the end of the test primarily because of complexing of the hardness polyvalent cations with the fluoride anion, and evaporation of water from the test solutions, especially at the elevated temperatures. Table 7 lists the measured hardness of the control solutions for each temperature as the median value of the analyses performed at the beginning and end of the experiments.

Tables 8, 9, 10, and 11 summarize the fluoride concentrations tested and daphnid mortality rate for the experiments in very hard, hard, moderately hard, and semi-soft waters, respectively.

# COMPOSITION OF RECONSTITUTED WATER

D. magna ACUTE EXPERIMENTS

	· Nomi	nal Hardness Level	(Nominal mg/L as C	aCO <sub>3</sub> )
Parameter	Very Hard (260 mg/L)	Hard (170 mg/L)	Moderately Hard (110 mg/L)	Semi-Soft (70 mg/L)
Salts added to double distilled water: NaHCO <sub>3</sub> CaSO <sub>4</sub> °2H <sub>2</sub> O MgSO <sub>4</sub> KCl	303 mg/L 190 " 190 " 13 "	192 mg/L 120 " 120 " 8 "	121 mg/L 76 " 76 " 3 "	76 mg/L 48 " 48 " 3 "
Measured water quality:* Hardness (mg/L as CaCO <sub>3</sub> ) Alkalinity (mg/L as CaCO <sub>3</sub> ) pH	$265 \\ (261 - 268) \\ 174 \\ (168 - 179) \\ 8.3 \\ (8.2 - 8.5)$	169     (167 - 171)     111     (107 - 114)     8.1     (8.0 - 8.3)	$ \begin{array}{r} 109\\(105 - 113)\\71\\(67 - 74)\\8.0\\(7.8 - 8.1)\end{array} $	$ \begin{array}{r} 69\\ (67 - 72)\\ 46\\ (43 - 49)\\ 7.8\\ (7.7 - 7.9) \end{array} $
*First number is medi	an values; number	in parentheses inc	licates 95% C.I.	I

# MEDIAN HARDNESS OF CONTROL SOLUTIONS

# DAPHNIA magna ACUTE EXPERIMENTS

Water Type	Nominal Test Temperature ( <sup>O</sup> C)	Hardness (mg/L as CaCO <sub>3</sub> )
Very Hard	15 20 25	265 266 272
Hard	15 20 25	170 169 184
. Moderately Hard	15 20 25	113 110 122
Semi-Soft	15 20 25	69 70 NA

## SUMMARY OF FLUORIDE CONCENTRATIONS AND DAPHNID MORTALITY

D. magna ACUTE EXPERIMENTS IN VERY HARD WATER

	Exp	nt at	15 <sup>0</sup> C:	Exp	nt at	20 <sup>0</sup> C:	Experiment at 25 <sup>0</sup> C:					
Nominal Fluoride (mg/L)	Ionic	asure Fluo entra End	ride tion	Percent Mortality	Measured Ionic Fluoride Concentration Start End Avg.		Percent Mortality	Measured Ionic Fluoride Concentration Start End Avg.		Percent Mortality		
0	0	0	0	0	0	0	0	0	· 0	0	0	0
158	-	-	-	-	-	-	-	-	148	120	134	5
251	230	200	215	0	222	192	207	0	224	200	212	0
316	290	260	275	5	336	270	303	10	286	266	276	95
398	380	320	350	40	440	366	403	100	370	340	355	100
447	446	360	403	95	480	380	430	100	436	390	413	100
501	500	420	460	100	520	440	480	100	-	-	-	-
Notes:				l	l			1	<u> </u>			I

All Ionic fluoride concentrations as measured by selective ion electrode, reported as F

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### SUMMARY OF FLUORIDE CONCENTRATIONS AND DAPHNID MORTALITY

D. magna ACUTE EXPERIMENTS IN HARD WATER

Nominal Fluoride (mg/L)	Exp	nt at	15 <sup>0</sup> C:	Experiment at 20 <sup>0</sup> C:				Experiment at 25 <sup>0</sup> C:				
	Ionic	asure Fluo entra End	ride	Percent Mortality	Me Ionic Conce Start	ntrat	ide	Percent Mortality	Ionic	easure Fluo centra End	ride	Percent Mortality
0	0	0	0	0	0	0	0	0	0	0	0	0
0	-	-	-	-	-	-	-	-	55	50	52	0
63	-		-	-	96	90	93	0	86	84	85	15
158	123	126	124	0	144	136	140	10	136	134	135	25
251	210	210	210	20	224	210	217	15	230	240	235	25
316	286	290	288	35	284	290	287	80	300	280	290	100
398	374	350	362	100	380	376	378	100	-	-	-	-
447	416	410	413	100	-	-	-	-	-	-	-	-

Notes:

All Ionic fluoride concentrations as measured by selective ion electrode, reported as F<sup>-</sup>

## SUMMARY OF FLUORIDE CONCENTRATIONS AND DAPHNID MORTALITY

## D. magna ACUTE EXPERIMENTS IN MODERATELY HARD WATER

Nominal Fluoride (mg/L)	Exp	erime	nt at	15 <sup>0</sup> C:	Exp	erime	nt at	20 <sup>0</sup> C:	Experiment at 25 <sup>0</sup> C:				
	Ionic	easure Fluo entra End	ride	Percent Mortality	Me Ionic Conce Start	ntrat	ide	Percent Mortality	Ionic	easure Fluo entra End	ride	Percent Mortality	
0	0	0	0	0	0	0	0	0	0	0	0	. 0	
25	-	-	-	-	-	-	-	-	24	25	24	0	
40	-	-	-	-	29	35	32	0	37	44	40	15	
63	-	-	-	-	56	58	57	0	61	63	62	25	
100	91	89	90	0	92	83	87	0	100	100	100	75	
158	140	146	143	0	136	138	137	5	152	154	153	90	
251	230	236	233	55	220	224	222	75	250	270	260	100	
316	310	320	315	100	290	300	295	100	-	-	-	-	
398	380	390	385	100	-	-	-	-	-	-	-	-	

All Ionic fluoride concentrations as measured by selective ion electrode, reported as F<sup>-</sup>

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#### SUMMARY OF FLUORIDE CONCENTRATIONS AND DAPHNID MORTALITY

D. magna ACUTE EXPERIMENTS IN SEMI-SOFT WATER

	Experiment at 15 <sup>0</sup> C:		Experiment at 20 <sup>0</sup> C:			20 <sup>0</sup> C:	Experiment at	25 <sup>0</sup> C:		
Nominal Fluoride (mg/L)	Ionic	easure Fluo entra End	ride tion	Percent Mortality	Ionic	entrat	ide ion	Percent Mortality	Measured Ionic Fluoride Concentration Start End Avg.	Percent Mortality
0	0	0	0	0	0	0	0	0		
16	-	-	-	-	14	14	14	0		
25	-	-	-	-	23	23	23	0		
40	35	38	36	10	38	38	38	0	Inconsistent resu	lts
63	58	61	5 <del>9</del>	0	50	60	60	0	obtained at this	low
100	95	100	97	20	100	94	97	15	hardness and r	ıigh
158	140	150	145	40	143	144	143	100	temperature	
251	238	236	237	85	-	-	-	-		
	320	306	313	100	-	-	-	-		

Of the twelve experiments conducted, eleven were judged to be valid on the basis of no mortality in the controls, no mortality at a low fluoride concentration, 100% mortality at a high fluoride concentration, and partial mortality rates at intermediate fluoride concentrations. In the case of the semi-soft experiment at  $25^{\circ}$ C, inconsistent results were obtained (including multiple deaths in the controls) despite the fact that the experiment was repeated twice. The combination of low hardness coupled with the elevated temperature (thus increasing the organisms metabolic rate) apparently stressed the daphnids beyond the level where meaningful results could be obtained relative to fluoride toxicity.

For all experiments in this group temperature was closely maintained within  $\pm 1^{\circ}$ C as specified by the EPA<sup>(71)</sup>. Median temperatures were 15.0, 20.0, and 24.9°C, with a 95% C.I. within design temperature  $\pm 1^{\circ}$ C. For convenience, the "nominal" temperature of 25°C is utilized in the discussions; in the calculations for modeling, the actual mean value of 24.9°C is utilized.

Dissolved oxygen in all samples tested ranged from 95% to 100% of saturation concentration. Saturation concentration for the laboratory conditions averaged 9.0 mg/L.

#### 5.2 Data Analysis

Median lethal concentrations (LC $_{50}$ ) were calculated from the data by both the log concentration versus percent mortality method  $^{(74)}$  (LCPM) and

the "moving-average-angle" method<sup>(75)</sup> (MAAM) for the eleven valid experiments; the results are shown in Table 12. There is substantial agreement in  $LC_{50}$  values when calculated by the two methods. The MAAM has the advantage of comprehending several values above and below the median, and also providing a 95% C.I. Figure 5 depicts the  $LC_{50}$  values versus hardness at different temperatures calculated by the MAAM.

In general, the experiments indicate increasing median lethal concentrations with increasing hardness and decreasing temperatures.

Examination of Tables 8, 9, 10, and 11 show a fairly even distribution of intermediate (partial) mortalities between zero percent and one hundred percent mortality, when viewed from a given temperature or given hardness viewpoint.

Parallel lines drawn through the plots in Figure 5 may be closely represented by the following equation (derivation of equations is presented in Appendix A:

$$LC_{50} = 370 \log\left[\left(\frac{H}{504}\right)\left(33.4 - T\right)\right]$$
 Eq. 5-1

or alternately:

 $LC_{50} = 370 \ (\log \in -2.7)$  Eq. 5-2

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# MEDIAN LETHAL CONCENTRATIONS (LC<sub>50</sub>)

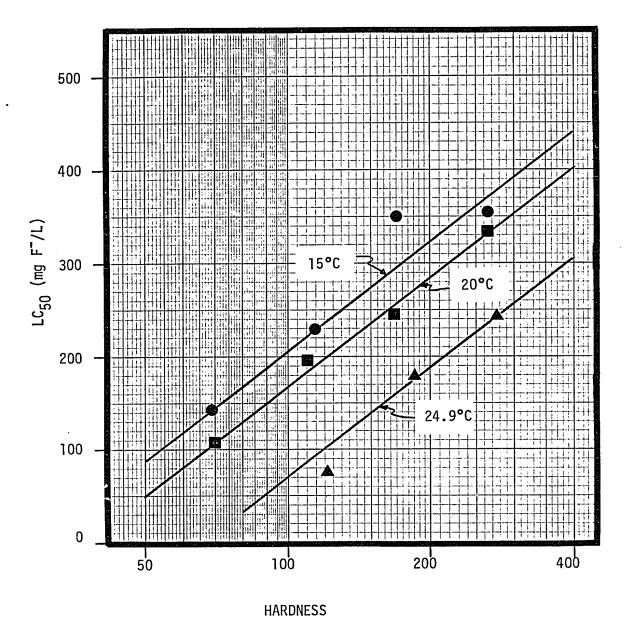
# DAPHNIA magna ACUTE EXPERIMENTS

-	Nomina]	LC <sub>50</sub> (	mg F <sup>-</sup> /L)*
Water T <i>y</i> pe	Temperature ( <sup>°</sup> C)	LCPM **	MAAM ***
	15	359	354 (337 - 369)
Very Hard	20	342	334 (308 - 361)
	25	243	245 (226 - 266)
	15	304	350 (329 - 376)
Hard	20	251	247 (224 - 272)
	25	250	180 (146 - 221)
Moderately	15	223	229 (200 - 268)
Hard	20	187	194 (170 - 224)
	25	78	77 (60 – 98)
	15	161	147 (116 - 178)
 Semi-Soft	20	114	109 (97 - 121)
	25	NA	NA
<ul> <li>* Fluoride concentrations on basis of measurements by ion selective electrode.</li> <li>** Log concentration versus percent mortality method.</li> <li>*** Moving average angle method: LC<sub>50</sub> value followed by 95% C. I. in parentheses.</li> </ul>			

# FIGURE 5

LC<sub>50</sub> VS. HARDNESS AND TEMPERATURE

DAPHNIA magna ACUTE EXPERIMENTS



(mg/L as CaCO<sub>3</sub> - logarithmic scale)

where:

 $LC_{50}$  = median lethal concentration in mg F<sup>-</sup>/L as measured by a selective ion electrode H = water hardness in mg/L as CaCO<sub>3</sub> T = water tempeature in <sup>O</sup>C.  $\in = (H) (33.4^{O}C - T)$ 

The average deviation of the model from the parallel lines of Figure 5 is 4.0% and from the calculated  $LC_{50}$  (per Table 12 - MAAM) is 8.8%.

Pimentel and Bulkley<sup>(76)</sup> have studied the effect of hardness on fluoride toxicity to rainbow trout (<u>Salmo gairdneri</u>) and reported that the fluoride  $LC_{50}$  increased with water hardness. The formula provided by these authors could be used to calculate  $LC_{50}$  for cold water systems and one temperature (12<sup>o</sup>C). Even though the general trend for both species - <u>D. magna</u> and <u>S. gairdneri</u> - is similar, the curve expressing the relationship between fluoride toxicity and hardness for rainbow trout is shallow, indicating a less distinct effect by the modifier on toxicity for this species.

Table 13 compares the sensitivity of <u>S. gairdneri</u> and <u>D. magna</u> to fluorides. The optimum temperature for the daphnids is considered to be  $20^{\circ}$ C, while the <u>S. gairdneri</u> were tested at  $12^{\circ}$ C. Although  $12^{\circ}$ C is outside the range of <u>D. magna</u> tests conducted, it is sufficiently close to the  $15^{\circ}$ C test temperature that an extrapolation to this lower temperature should provide a guide to expected LC<sub>50</sub> values. Table 13

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CALCULATED LC<sub>50</sub> VALUES FOR DAPHNIA magna and SALMO gairdneri

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	LC <sub>50</sub> Values (mg F <sup>-</sup> /L)				
Water Hardness	<u>Salmo gairdneri</u>	Daphnia	a magna		
(mg/L as CaCO <sub>3</sub> )	12 <sup>0</sup> C	20 <sup>0</sup> C	12 <sup>0</sup> C		
50	106	45	121		
100	133	157	233		
200	161	269	344		
300	178	333	409		
NOTES:		I			
LC <sub>50</sub> for <u>D. ma</u>	gna per equation:				
$LC_{50} = 370 \log\left[\left(\frac{H}{504}\right)\left(33.4 - T\right)\right]$					
LC <sub>50</sub> for <u>S. gairdneri</u> per Pimentel and Bulkley (1983)					

shows then the  $LC_{50}$  for trout at the  $12^{\circ}C$  temperature, and the  $LC_{50}$  values for the daphnids at the optimum  $20^{\circ}C$  and at  $12^{\circ}C$ , calculated on the basis of Eq. 5-1.

On the basis of equal temperatures  $(12^{\circ}C)$ , the difference in sensitivity at low hardness is relatively low; e.g., the  $LC_{50}$  is 14% higher for <u>D. magna</u> that for <u>S. gairdneri</u>. At higher hardness levels, the difference in sensitivity becomes more pronounced; e.g., the  $LC_{50}$  is 130% higher for <u>D. magna</u> than for <u>S. gairdneri</u> at a hardness of 300.

At the  $20^{\circ}$ C temperature, where the metabolic rate of the daphnids would be greater, the LC<sub>50</sub> at low hardness is actually lower than that for the trout; however, with increasing hardness, the sensitivity of the daphnids becomes less, so that at a hardness of 300, the <u>D. magna</u> have a LC<sub>50</sub> that is 87% higher than <u>S. gairdneri</u>.

Further discussion of the <u>D. magna</u> acute model follows in Chapter 10.

#### 6.0 DAPHNIA MAGNA CHRONIC EXPERIMENTS

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Chronic (21 day) tests utilizing <u>D. magna</u> were conducted at  $20^{\circ}$ C in very hard, hard, and moderately hard water, at fluoride concentrations ranging from 0 to 158 mg F<sup>-</sup>/L. These tests, involving approximately 190 daphnids, were conducted in accordance with the plan, procedures, and equipment described in Chapters 3 and 4.

#### 6.1 Experimental Data

Preparation and analyses of test solutions proceeded in a manner similar to that for the acute tests. Inasmuch as the chronic test is a renewal test, the daphnids were transferred to new solutions every 48-72 hours according to the following schedule: "Very Hard" on days 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, and both "Hard" and "Moderately Hard" on days 0, 3, 5, 7, 10, 12, 14, 16, 18, 20. Tables 14, 15, and 16 list the pertinent analytical data for the three chronic tests. As in the acute tests, all D.O. measurements were in the 95-100% saturation range. Median temperatures for all chronic experiements were  $20^{\circ}$ C; temperatures were maintianed within  $\pm 1^{\circ}$ C, with a 95-100% C.I. of design temperature  $\pm 1^{\circ}$ C or less.

Variance in measurements of the two primary parameters of fluoride concentration and hardness were generally low as indicated in Table 17. Of the 35 coefficients of variation calculated, only five exceeded the 10% level; these were all in the very hard water where the complexing of

### CHEMICAL ANALYSES OF TEST SOLUTIONS

### D. magna CHRONIC EXPERIMENTS IN VERY HARD WATER

Nominal Fluoride	Ionic Fluoride Note (a, b)	Hardness (mg/L) (Note (b, c)	Alkalinity (mg/L) Note (b, d)	рН	
0	0	283 (271 - 294)	196 (177 - 215)	8.3 (8.0 - 8.6)	
10	8.8	278	194	8.3	
	(6.5 - 11.2)	(264 - 292)	(179 - 210)	(8.0 - 8.6)	
16	15.2	278	194	8.3	
	(11.9 - 18.4)	(261 - 295)	(176 - 213)	(8.1 - 8.6)	
25	24.1	`276	196	8.3	
	(21.1 - 27.1)	(260 – 292)	(178 - 214)	(8.1 - 8.4)	
40	34.0	265	195	8.3	
	(22.7 - 45.2)	(235 - 296)	(179 - 211)	(8.0 - 8.5)	
63	48.1	234	194	8.3	
	(19.4 - 76.7)	(147 - 321)	(174 - 214)	(8.0 - 8.5)	
100	71.0	194	185	8.3	
	(58.5 - 83.4)	(166 - 221)	(167 - 202)	(8.0 - 8.7)	
(	<ul> <li>Notes: (a) Ionic fluoride as measured by selective ion electrode, reported as F<sup>-</sup>.</li> <li>(b) Median value followed by 95% C.I. in parentheses.</li> <li>(c) Hardness in mg/L as CaCO<sub>3</sub></li> <li>(d) Alkalinity in mg/L as CaCO<sub>3</sub>, as titrated with HCl through pH of 4.6.</li> </ul>				

# CHEMICAL ANALYSES OF TEST SOLUTIONS

# D. magna CHRONIC EXPERIMENTS IN HARD WATER

Nominal Fluoride	Ionic Fluoride Note (a, b)	Hardness (mg/L) (Note (b, c)	Alkalinity (mg/L) Note (b, d)	рН
0	0	181 (174 - 189)	126 (113 - 139)	8.2 (7.9 - 8.4)
25	26.1	182	129	8.2
	(25.0 - 27.2)	(164 - 199)	(119 - 139)	(8.1 - 8.3)
40	35.5	159	126	8.2
	(30.4 - 40.7)	(128 - 189)	(117 - 135)	(8.0 - 8.4)
63	49.0	134	126	8.2
	(42.3 - 55.7)	(114 - 154)	(117 - 135)	(7.9 - 8.4)
100	83.8	114	127	8.1
	(75.2 - 92.3)	(99 - 130)	(117 - 137)	(7.8 - 8.5)
158	141.6	102	129	8.2
	(134.4 - 148.8)	(95 - 108)	(122 - 136)	(7.9 - 8.5)
<ul> <li>Notes: (a) Ionic fluoride as measured by selective ion electrode, reported as F.</li> <li>(b) Median value followed by 95% C.I. in parentheses.</li> <li>(c) Hardness in mg/L as CaCO<sub>3</sub></li> <li>(d) Alkalinity in mg/L as CaCO<sub>3</sub>, as titrated with HCl through pH of 4.6.</li> </ul>				

### CHEMICAL ANALYSES OF TEST SOLUTIONS

# D. magna CHRONIC EXPERIMENTS IN MODERATELY HARD WATER

Nominal Fluoride	Ionic Fluoride Note (a, b)	Hardness (mg/L) (Note (b, c)	Alkalinity (mg/L) Note (b, d)	рН
0	0	117 (109 - 126)	80 (74 - 87)	7.9 (7.6 - 8.2)
10	9.9	115	80	8.0
	(8.5 - 11.2)	(104 - 125)	(70 - 90)	(7.8 - 8.2)
16	16.2	115	80	8.0
	(14.7 - 17.7)	(104 - 127)	(73 - 86)	(7.8 - 8.2)
25	25.9	114	80	8.0
	(22.6 - 29.1)	(104 - 124)	(74 - 86)	(7.8 - 8.2)
40	41.2	114	81	8.0
	(36.8 - 45.5)	(105 - 123)	(74 - 88)	(7.8 - 8.2)
63	64.2	111	83	8.0
	(52.5 - 76.0)	(93 - 128)	(75 - 90)	(7.8 - 8.2)
<ul> <li>Notes: (a) Ionic fluoride as measured by selective ion electrode, reported as F<sup>-</sup>.</li> <li>(b) Median value followed by 95% C.I. in parentheses.</li> <li>(c) Hardness in mg/L as CaCO<sub>3</sub></li> <li>(d) Alkalinity in mg/L as CaCO<sub>3</sub>, as titrated with HCl through pH of 4.6.</li> </ul>				

# IONIC FLUORIDE AND HARDNESS MEASUREMENTS

### COEFFICIENTS OF VARIATION

# D. magna CHRONIC EXPERIMENTS

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Water Type	Ionic Fluoride (mg/L)	Coefficient of Variation of Ionic Fluoride (%)	Coefficient of Variation of Hardness (%)
Very Hard	0 8.8 15.2 24.1 34.0 48.1 71.0	- 13.5 10.9 6.3 16.9 30.4 8.9	2.0 2.5 3.1 2.9 5.8 18.9 7.3
Hard	0 26.1 35.5 49.0 83.8 141.6	6.5 7.4 7.0 5.2 2.6	2.1 4.9 9.7 7.6 6.8 3.2
Moderately Hard	0 9.9 16.2 25.9 41.2 64.2	6.8 4.7 6.3 5.4 9.3	3.7 4.7 4.9 4.2 7.7 8.1
	Average:	9.3	5.8

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fluoride anions with hardness cations is strongest. The variances are considered acceptable for this experiment. Only minor variations occurred in the alkalininty and pH measurements.

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Feeding consisted of 0.4 mg of prepared food suspension plus approximately 10 million cells of <u>Selanastrum capricornutum</u> following each water change. No aeration was utilized.

Observations included mortality (lack of antennae or claw movement), time to first brood, and number of "eggs" and neonates produced by the adult daphnids. Neonates were counted by individualy pipetting the organisms out of the test beaker; the number of eggs produced by individual females was established by pipetting all debris from the test beaker, placing this debris on a petri dish, and examining the contents under a dissecting scope at 10X power. The term "eggs" as used in this dissertation includes all self-contained eggs aborted by the adult, eggs contained in the shed carapace, dead embryos and neonates. The term "neonates" refers to live organisms only.

Tables 18, 19, and 20 summarize the reproduction and mortality data for the very hard, hard, and moderately hard experiments, respectively.

Where mortalities were experienced, and number of eggs or neonates are reported on a "per adult" basis, adults are counted on a proportional basis according to length of young-bearing period they survived; for example, if an adult died five days into a 10-day neonate-

### SUMMARY OF REPRODUCTION DATA AND MORTALITY

D. magna CHRONIC EXPERIMENT IN VERY HARD WATER AT 20°C

Nominal Fluoride Concentration (mg/L)	Ionic Fluoride Concentration (mg/L) Note (a)	Average Number of Eggs Per Adult Note (b)	Average Number of Neonates Per Adult Note (b)	Total Number of Mortalities During Test Note (c)	Hatchabil- ity Rate (%) Note (d)
0	0	14.3	8.0	0	55.9
10	8.8	15.2	11.0	0	72.4
16	15.2	29.6	21.2	0	71.6
25	24.1	31.4	22.3	0	71.0
40	34.0	69.9/66.4	33.1/31.4	2	47.3
63	48.1	110.0/89.8	2.0/1.6	2	1.8
100	71.0	101.5	0	0	0
<ul> <li>Notes: (a) Average ionic fluoride concentrations, measured by selective ion electrode, reported as F.</li> <li>(b) Average number calculated on basis of ten adults with/without adjustment for mortalities.</li> <li>(c) Mortalities at 40 mg/L occurred on days 17, 21; at 63 mg/L, on days 11, 13.</li> <li>(d) Hatchability rate is number of neonates divided by number of eggs.</li> </ul>					

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#### SUMMARY OF REPRODUCTION DATA AND MORTALITY

D. magna CHRONIC EXPERIMENT IN HARD WATER AT 20°C

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Nominal Fluoride Concentration (mg/L)	Ionic Fluoride Concentration (mg/L) Note (a)	Average Number of Eggs Per Adult Note (b)	Average Number of Neonates Per Adult Note (b)	Total Number of Mortalities During Test Note (c)	Hatchabil- ity Rate (%) Note (d)
0	0	129.2	122.3	0	94.7
25	26.1	153.5	130.0	0	84.7
40	35.5	143.7	53.6	0	37.3
63	49.0	178.4/131.1	2.2/1.7	3	1.3
100	83.8	174.4	0	0	0
158	141.6	119.0/117.8	0	1	0
Notes: (a) (b) (c) (d)	reported as F <sup>-</sup> .				

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# SUMMARY OF REPRODUCTION DATA AND MORTALITY

D. magna CHRONIC EXPERIMENT IN MODERATELY HARD WATER AT  $20^{\circ}$ C

Nominal Fluoride Concentration (mg/L)	Ionic Fluoride Concentration (mg/L) Note (a)	Average Number of Eggs Per Adult Note (b)	Average Number of Neonates Per Adult Note (b)	Total Number of Mortalities During Test Note (c)	Hatchabil- ity Rate (%) Note (d)
0 10 16 25 40 63	0 9.9 16.2 25.9 41.2 64.2	19.8/18.0 44.3/42.8 73.8/62.7 100.8 153.8 188.5	12.2/11.1 25.6/24.8 44.0/37.4 16.8 0.3 0	1 1 3 0 0 0 0	61.7 57.9 59.6 16.7 0.2 0
Notes: (a) (b) (c) (d)	reported as F <sup>-</sup> . Average number calculated on basis of ten adults with/without adjustment for mortalities. Mortalities at 0 mg/L occurred on days 13; at 10 mg/L, on day 18; at 16 mg/L on 16, 18, 20.				

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bearing period, it was counted as 0.5 adult.

Figures 6, 7, and 8 depict the neonate production in terms of cumulative number of neonates versus day of test for the very hard, hard, and moderately hard experiments, respectively.

#### 6.2 Data Analysis

A review of the chemical analyses yields several conclusions:

- The variances in fluoride analyses and hardness analyses were fairly low throughout the experiments, as evidenced by the low coefficients of variation.
- The hardness levels decreased with increasing fluoride concentrations as expected, due to the complexing of polyvalent cations with the fluoride anion and removal from the system.
- Alkalinity and pH remained essentially constant, thus eliminating these factors as variable parameters in the experiment.

Live neonate production varied widely, as illustrated in Figures 6, 7, and 8.

- Neonate production in hard water, which is the optimum environment (77) for <u>D. magna</u>, was significantly greater than that in

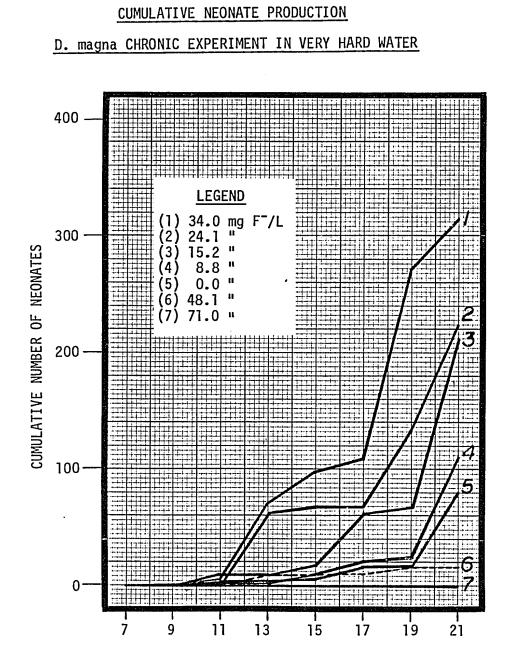
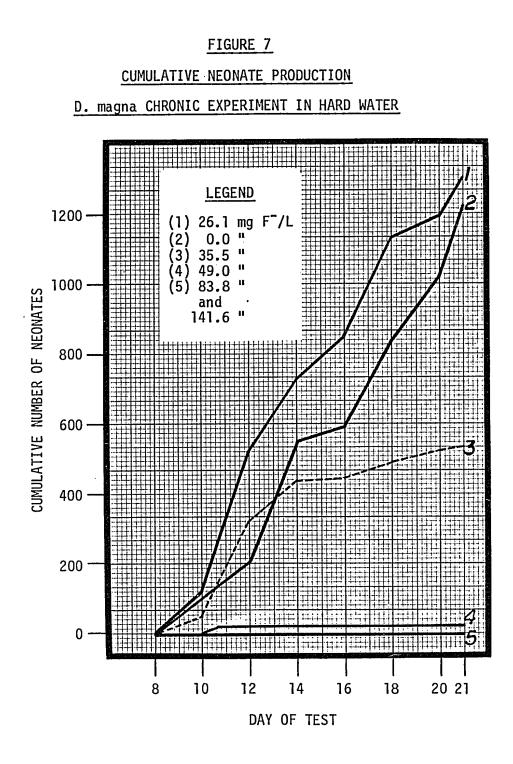
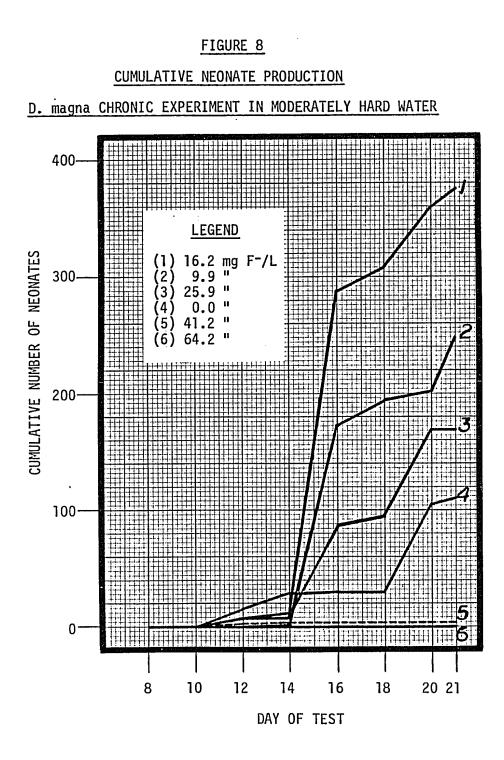


FIGURE 6



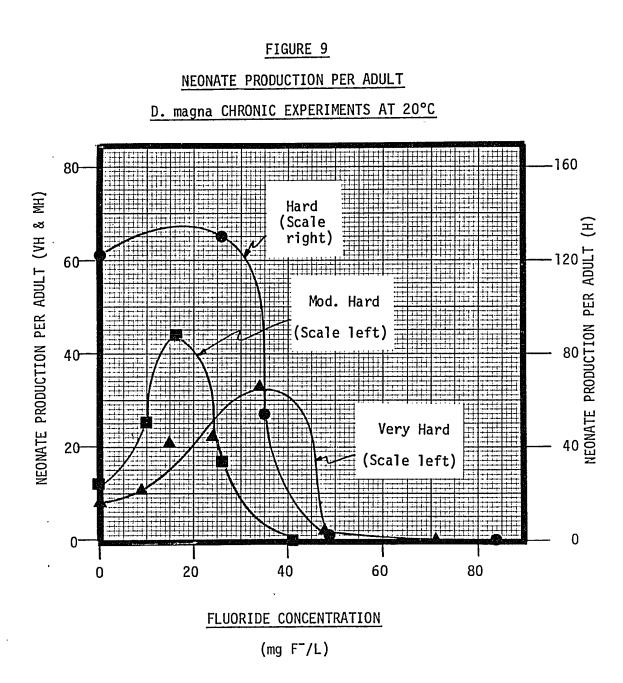


very hard or moderately hard water, by a factor of up to 2:1 at maximum production rates. This is in agreement with the general observation of our stock culture aquariums in which thinning of the daphnids was required much more frequently in the hard water than in other water types.

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- Neonate production in hard water commenced during days 8-10, and at the lower fluoride concentration proceeded at a fairly constant rate until completion of the test. At higher fluoride concentrations, neonate production leveled off early. By comparison, in the very hard water, neonate production was later in starting, had a moderate expansion until approximately day 17, and then a rapid increase until the end of the test. In the moderately hard water, the advent of significant neonate production was later than the other two water types, but then a significant strong growth was experienced during the period of days 14-20.
- In all water types, neonate production in one or more fluoride concentrations exceeded neonate production in the controls, thus indicating a stimulation effect on reproduction.

A summary of neonate production per adult is depicted in Figure 9. Note that maximum neonate production occurs in moderately hard water at approximately 16 mg F<sup>-</sup>/L, in hard water at approximately 18 mg F<sup>-</sup>/L, and in very hard water at approximately 34 mg F<sup>-</sup>/L.



In order to determine the mechanism of increased neonate production at low fluoride concentrations, a count of individual egg production was made. As described earlier, the term "eggs" includes all neonates, partially or fully formed but dead embryos, and undeveloped eggs which were observable either as aborted spheres in the bottom of the test chamber or alternately, contained in the shed carapace.

Figure 10 indicates egg production per adult for the various experimental conditions. Note that egg production peaked in both hard and moderately hard water at approximately 63 mg  $F^/L$  and in very hard water at approximately 55 mg  $F^/L$ .

Observations of egg and neonate production during the experiments indicated several levels of egg and neonate development:

- In the controls or low fluoride concentrations, reproduction was essentially in the form of fully-developed neonates.
- With increasing fluoride concentrations, the quality of reproduction significantly decreased in the following order:
  - Live neonates
  - Dead embryos
  - Partially-formed dead embryos
  - Large, white or colorless eggs
  - Small, white or colorless eggs
  - Small yellow eggs.

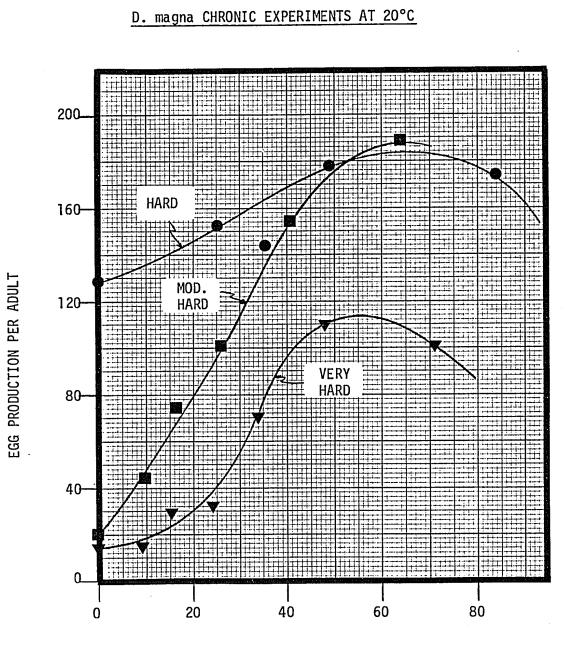


FIGURE 10

EGG PRODUCTION PER ADULT

FLUORIDE CONCENTRATION

(mg F<sup>-</sup>/L)

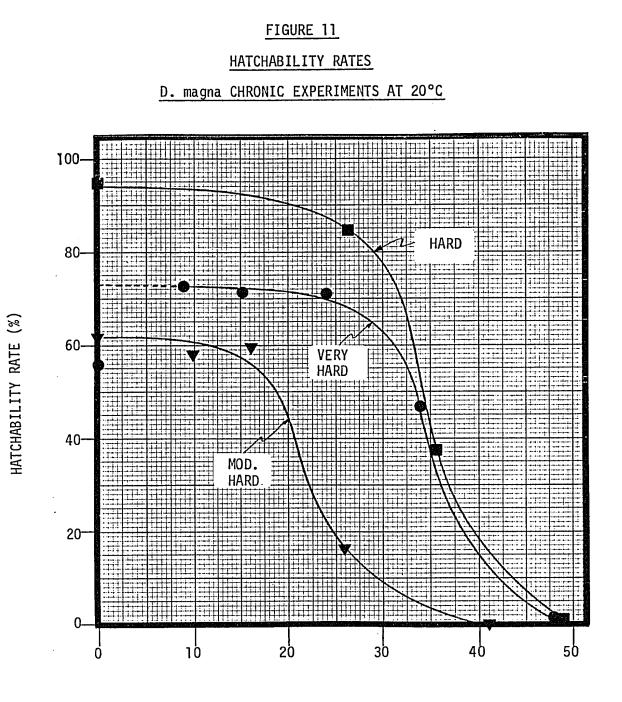
- Generally, at high fluoride levels, the eggs became smaller, but more numerous. A casual observation, and subject to future quantative measurements, is that the total volume of eggs produced (no. eggs x size of eggs) was approximately constant at the various fluoride concentrations.

A relationship between neonate production and egg production may be termed "hatchability", and is defined as follows:

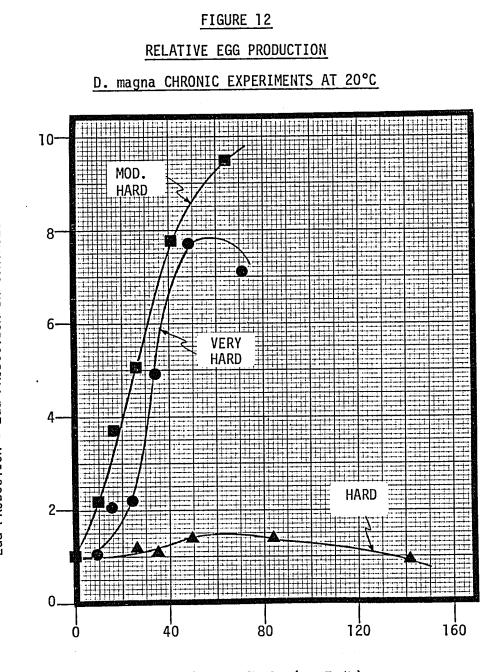
# Hatchability = $\frac{No. neonates}{No. eggs}$

The hatchability rates for the various water types and fluoride concentrations are summarized in Figure 11. Generally, hatchability rates follow a "reverse-S" curve, with maximum hatchability in the hard water, less in the very hard water, and least in the moderately hard water. In the case of the very hard water, the hatchability at 0 mg  $F^{-}/L$ probably should be at approximately 73% as indicated by the dotted line, but is subject to future laboratory verification.

An alternate view of egg and neonate production may be gleaned from Figures 12 and 13, where the ratio of egg and neonate production to that of the controls is plotted against the fluoride concentration. In Figure 13, a "very hard (modified)" plot is shown to indicate the effect of the 73% probable hatchability rate discussed above.

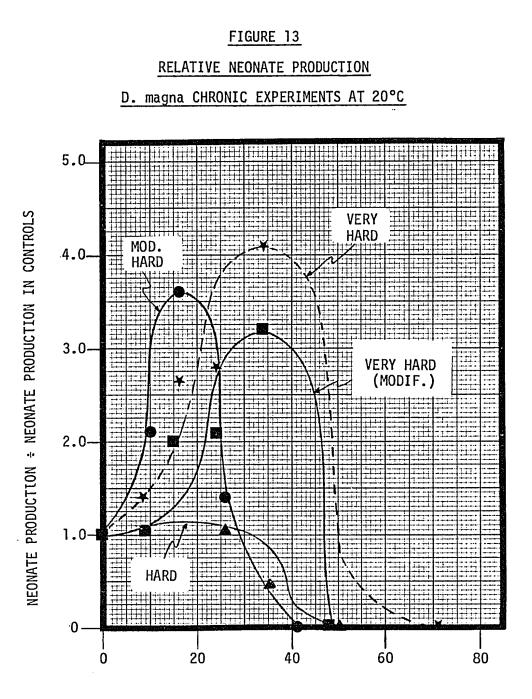


FLUORIDE CONCENTRATION (mg F<sup>-</sup>/L)





EGG PRODUCTION = EGG PRODUCTION IN CONTROLS



FLUORIDE CONCENTRATION (mg F<sup>-</sup>/L)

In these latter two figures, the stimulation effects due to the combined stresses of (1) less than optimum hardness, and (2) increasing fluoride concentrations, can be quantitively observed. In effect, the stresses placed on the organisms have a definite stimulation effect, the net result in terms of live neonate production being the product of the increased egg production and the decreased hatchability rates.

A measure of relative toxicity effects is the measure of concentration wherein neonate production is equivalent to that of the controls. These concentrations, which may be termed "equivalent neonate production (ENP)" levels, can be readily obtained from Figure 13, where the graphs cross the 1.0 level. These values are 28.5, 31.5, and 47.0 mg  $F^/L$  for the moderately hard, hard, and very hard waters, respectively.

Reverting to Tables 6, 7, and 8, hardness levels corresponding to these three ENP fluoride levels were determined by interpolation with the following results:

ENP Fluoride Concentration	Hardness Level	
28.5 mg F <sup>-</sup> /L	. 114 mg/L as CaCO <sub>3</sub>	
31.5 "	170 "	
47.0 "	236 "	

These data follow the equation:

$$\log F_{\rm ENP} = \left[ 0.0028 \left( \frac{\rm H}{100} \right)^{5.1} \right] + \left[ 1.45 \right] \quad Eq. 6-1$$

Where  $F_{ENP}$  = Fluoride concentration (mg F<sup>-</sup>/L) corresponding to equivalent neonate production.

 $H = Water hardness in mg/L as CaCO_3$ 

Thus, a prediction of ENP concentrations may be made at various water hardnesses for <u>D. magna</u> at  $20^{\circ}$ C.

#### 7.0 CERIODAPHNIA AFFINIS/DUBIA ACUTE EXPERIMENTS

Acute (48 hour) experiments utilizing <u>C. affinis/dubia</u> were conducted at 15, 20, and  $25^{\circ}$ C in very hard, hard, moderately hard, and semi-soft water at fluoride concentrations ranging from 0 to 631 mg F<sup>-</sup>/L. These experiments, involving approximately 1,600 daphnids, were conducted in accordance with the plan, procedures, and equipment described in Chapters 3 and 4.

#### 7.1 Experimental Data

The method of preparation and chemical analyses for the <u>C.</u> <u>affinis/dubia</u> acute experiments closely paralleled those for the <u>D. magna</u> acute experiments. Table 21 lists the measured hardness of the control solutions for each temperature; figures are the median values of beginning and ending analyses. As in previous tests, all dissolved oxygen analyses were between 95 and 100% of saturation concentration; all temperatures were maintained within  $\pm 1^{\circ}$ C with a 95% C.I. of design temperature  $\pm 1^{\circ}$ C or less. Median temperatures were 15.2, 20.0, and 24.9°C; for convenience, the "nominal" temperatures of 15, 20, and 25°C are used in the discussion, however, the actual median temperatures are utilized in calculating the models.

Tables 22, 23, 24, and 25 summarize the fluoride concentrations tested and daphnid mortality rate for the experiments in very hard, hard, moderately hard and semi-soft water, respectively. Of the twelve experiments conducted, eleven were judged to be valid on the basis of

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# MEDIAN HARDNESS OF CONTROL SOLUTIONS

# C. affinis/dubia ACUTE EXPERIMENTS

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Water Type	Nominal Test Temperature ( <sup>O</sup> C)	Hardness (mg/L as CaCO <sub>3</sub> )
Very Hard	15 20 25	278 288 290
Hard	15 20 25	174 186 184
Moderately Hard	15 20 . 25	114 117 124
Semi-Soft	15 20 25	80 76 NA

#### SUMMARY OF FLUORIDE CONCENTRATIONS AND DAPHNID MORTALITY

#### C. affinis/dubia ACUTE EXPERIMENTS IN VERY HARD WATER

Nominal Fluoride (mg/L)	Exp	nt at	15 <sup>0</sup> C:	Exp	nt at	20 <sup>0</sup> C:	Experiment at 25 <sup>0</sup> C:					
	Ionic	asure Fluo entra End	ride tion	Percent Mortality	Me Ionic Conce Start	ntrat	ide ion	Percent Mortality	Ionic	easure Fluo entra End	ride tion	Percent Mortality
0	0	0	0	0	0	0	0	0	0	0	0	0
100	-	-	-	-	72	88	80	0	68	80	74	0
158	150	122	136 200	0	122 219	128 202	125 210	0 30	125 218	145 255	135 236	0 10
251 316	210 270	190 265	200	0	219	202	292	70	210	312	230	95
398	360	310	335	20	357	345	351	100	350	430	390	100
447	398	350	374	50	-	-	-	-	-	-	-	-
501	410	430	420	75	-	-	-	-	-	-	-	-
631	560	530	545	100	-	-	-	-	-	-	-	-

All Ionic fluoride concentrations as measured by selective ion electrode, reported as F<sup>-</sup>

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#### SUMMARY OF FLUORIDE CONCENTRATIONS AND DAPHNID MORTALITY

#### C. affinis/dubia ACUTE EXPERIMENTS IN HARD WATER

Nominal Fluoride (mg/L)	Exp	nt at	15 <sup>0</sup> C:	Exp	nt at	20 <sup>0</sup> C:	Experiment at 25 <sup>0</sup> C:					
	Ionic	asure Fluo entra End	ride	Percent Mortality	Me Ionic Conce Start	ntrat	ide ion	Percent Mortality	Ionic	asure Fluo entra End	ride	Percent Mortality
0	0	0	0	0	0	. 0	0	0	0	0	. 0	5
63	49	55	52	, 0	-	-	-	-	-	-	-	-
100	80	88	84	<sup>2</sup> <u>0</u>	83	85	84	0	78	84	81	0
158	135	140	137	5	140	145	142	10	132	136	134	15
251	230	240	235	15	238	247	242	100	225	244	234	80
316	290	290	290	30	305	315	310	100	290	300	295	100
398	355	380	362	50	-	-	-	-	357	400	378	100
447	370	410	390	50	-	-	-	-	-	-		-
501	430	440	435	95	-	-	-	-	-	• 🕳	-	-
631	580	570	575	100	-	-	-	-	-	-	-	-
Notes:												

All Ionic fluoride concentrations as measured by selective ion electrode, reported as F

#### SUMMARY OF FLUORIDE CONCENTRATIONS AND DAPHNID MORTALITY

### C. affinis/dubia ACUTE EXPERIMENTS IN MODERATELY HARD WATER

Nominal Fluoride (mg/L)	Exp	nt at	15 <sup>0</sup> C:	Exp	nt at	20 <sup>0</sup> C:	Experiment at 25 <sup>0</sup> C:					
	Ionic	asure Fluo entra End	ride	Percent Mortality	Me Ionic Conce Start	ntrat	ide ion	Percent Mortality	Ionic	easure Fluo entra End	ride tion	Percent Mortality
0	0	0	0	0	0	0	0	0	0	0	0	0
63	_	-	-	_	66	69	67	Ó	65	73	69	
100	93	88	90	0	103	100	101	10	94	105	99	0 5
158	150	150	150	0	155	164	159	30	151	180	165	65
251	255	251	253	50	256	268	262	100	248	290	269	100
316	330	320	325	25	330	360	345	100	311	365	338	100
398	398	390	394	50	-	-	-	-	-	-	-	-
447	410	430	420	75	-	-	-	-	-	-	-	-
	470	460	465	100	_	-	-	-	_	-	-	-

Notes:

All Ionic fluoride concentrations as measured by selective ion electrode, reported as F<sup>-</sup>

## SUMMARY OF FLUORIDE CONCENTRATIONS AND DAPHNID MORTALITY

# C. affinis/dubia ACUTE EXPERIMENTS IN SEMI-SOFT HARD WATER

	Exp	erime	nt at	15°C:	Ехр	erime	nt at	20°C:	Experiment at 25 <sup>0</sup> C:	
Nominal Fluoride (mg/L)	Ionic	easure Fluo centra End	ride tion	Percent Mortality	Ionic	ntrat	ide ion	Percent Mortality	Measured Ionic Fluoride Concentration Start End Avg.	Percent Mortality
0 25 40 63 100 158 251 316 398 447 501	0 - 38 63 95 150 242 313 381 430 470	0 39 65 100 152 255 320 410 460 475	0 - 38 64 97 151 248 316 395 445 472	0 - 20 0 5 30 30 25 65 100 100	0 24 39 63 95 157 280 - - - -	0 27 44 70 103 179 280 - - - -	0 25 41 67 99 168 280 - -	0 10 5 35 75 100 - - -	Inconsistent obtained at t hardness and temperature	his low

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five percent or less mortality in the controls, ten percent or less mortality at a low fluoride concentration, one hundred percent mortality at a high fluoride concentration, and partial mortality rates at intermediate fluoride concentrations. In the case of the semi-soft experiment at  $25^{\circ}$ C, inconsistent results were obtained, despite the fact that the experiment was repeated twice. As in the case of the <u>D. magna</u> under similar environments, the combination of low hardness coupled with the elevated temperature (thus increasing the organisms' metabolic rate) apparently stressed the daphnids beyond the level where meaningful results could be obtained relative to fluoride toxicity.

#### 7.2 Data Analysis

Median lethal concentrations  $(LC_{50})$  were calculated from the data by the "log concentration versus percent mortality method (LCPM)" and the "moving-average-angle method (MAAM)" for the eleven valid experiments; the results are shown in Table 26.

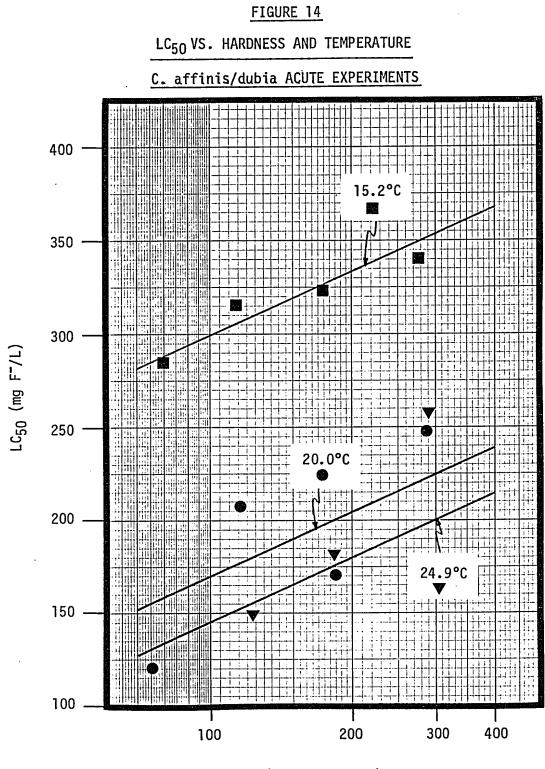
In the MAAM calculations, it should be noted that two adjustments were made in the data: in the MH-15 $^{\rm O}$ C and SS-15 $^{\rm O}$ C tests, the intermediate 0.25 mortality rates were deleted as being judged not meaningful. There is substantial agreement in LC<sub>50</sub> values when calculated by the two methods.

Figure 14 depicts the  $LC_{50}$  values versus hardness at different temperature, calculated by the MAAM. Although the variances are greater

# MEDIAN LETHAL CONCENTRATIONS (LC<sub>50</sub>)

# CERIODAPHNIA affinis/dubia ACUTE EXPERIMENTS

	Nominal	LC <sub>50</sub> (	(mg F <sup>-</sup> /L)*			
Water Type	Temperature (°C)	LCPM **	MAAM ***			
	15	376	340 (304 - 370)			
Very Hard	20	248	248 (226 – 272)			
	25	260	258 (243 - 274)			
	15	329	323 (249 - 405)			
Hard	20	180	171 (146 - 197)			
	25	174	182 (159 - 213)			
Medanataly	15	298	315 (204 - 514)			
Moderately Hard	20	182	208 (194 - 224)			
	25	146	149 (130 - 174)			
	15	265	284 (224 - 352)			
Semi-Soft	20	122	120 (72 - 197)			
	25	NA	NA			
<ul> <li>* Fluoride concentrations on basis of measurements by ion selective electrode.</li> <li>** Log concentration versus percent mortality method.</li> <li>*** Moving average angle method: LC<sub>50</sub> value followed by 95% C. I. in parentheses.</li> </ul>						



HARDNESS (mg/L as  $CaCO_3$ )

than in the <u>D. magna</u> experiments, the <u>C. affinis/dubia</u> tests indicate a similar effect of increasing median lethal concentrations with increasing hardness and decreasing temperature.

Parallel lines drawn through the plots of Figure 14 may be closely represented by the following equations (derivation of equations is presented in Appendix A):

$$LC_{50} = 115 \log \left[ \left( \frac{H}{44.3} \right) \left( 33.4^{\circ}C - T \right) \right]$$
 Eq. 7-1

or alternately:

$$LC_{50} = 115 (log \in -1.646)$$
 Eq. 7-2  
where:

LC<sub>50</sub> = median lethal concentrations in mg F<sup>-</sup>/L as measured by a selective ion electrode.

H = water hardness in mg/L as  $CaCO_3$ 

T = water temperature in Celsius degrees

$$\in$$
 = (H) (33.4 - T)

As developed in Appendix A, the average deviation of the model from the  $20^{\circ}$ C and  $25^{\circ}$ C parallel lines of Figure 14 is 0.4%, and from the calculated LC<sub>50</sub> (per Table 26 - MAAM) is 14.7%. The average deviation of the model from the  $15^{\circ}$ C line of Figure 14 is 35.8%, and from the calculated LC<sub>50</sub> (per Table 26 - MAAM) is 35.5%.

Further discussion of the <u>C. affinis/dubia</u> acute model follows in Chapter 10.

#### 8.0 CERIODAPHNIA affinis/dubia CHRONIC EXPERIMENTS

Chronic (7 day) experiments utilizing <u>C. affinis/dubia</u> were conducted at  $25^{\circ}$ C in very hard, hard, moderately hard, and semi-soft water, at fluoride concentrations ranging from 0 to 63 mg F<sup>-</sup>/L. These tests, involving approximately 250 daphnids, were conducted in accordance with the plan, procedures, and equipment described in Chapters 3 and 4.

#### 8.1 Test Data

Preparation and analyses of test solutions proceeded in a manner similar to that for the acute tests. Tables 27, 28, 29 and 30 list the pertinent analytical data for the four tests. As in the acute tests, all D.O. measurements were in the 95-100% saturation range; temperatures were controlled to within  $\pm 1^{\circ}$ C; median temperature was 24.9°C, with a 95% C.I. of design temperature  $\pm 1^{\circ}$ C or less.

Variance in fluoride and hardness analyses was low as shown in Table 31 where the average coefficient of variation was 5.7% and 5.4%, respectively.

Tables 32, 33, 34, and 35 present the reproduction and mortality data for the very hard, hard, moderately hard, and semi-soft experiments, respectively. Where mortalities were experienced, and number of eggs of neonates are reported on a "per adult" basis, adults are counted on a proportional basis according to length of young-bearing period they

## CHEMICAL ANALYSES OF TEST SOLUTIONS

# C. affinis/dubia CHRONIC EXPERIMENTS IN VERY HARD WATER

Nominal Fluoride	Ionic Fluoride Note (a, b)	Hardness (mg/L) (Note (b, c)	Alkalinity (mg/L) Note (b, d)	рН		
0	0	290 (263 - 316)	213 (187 - 239)	8.3 (8.2 - 8.5)		
10	9.6	292	215	8.3		
	(8.3 - 10.8)	(265 - 318)	(188 - 242)	(8.2 - 8.5)		
16	16.3	292	216	8.3		
	(15.0 - 17.6)	(266 - 318)	(184 - 247)	(8.2 - 8.5)		
25	26.1	292	219	8.3		
	(21.4 - 30.8)	(249 - 335)	(178 - 259)	(8.2 - 8.5)		
40	43.2	291	218	8.3		
	(37.7 - 48.8)	(250 - 333)	(172 - 263)	(8.2 - 8.5)		
63	60.5	271	215	8.3		
-	(45.6 - 75.5)	(254 - 287)	(187 - 243)	(8.2 - 8.5)		
<ul> <li>Notes: (a) Ionic fluoride as measured by selective ion electrode, reported as F.</li> <li>(b) Median value followed by 95% C.I. in parentheses.</li> <li>(c) Hardness in mg/L as CaCO<sub>3</sub>, measured via Calgon-20 titration</li> <li>(d) Alkalinity in mg/L as CaCO<sub>3</sub>, as titrated with HCl through pH of 4.6.</li> </ul>						

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### CHEMICAL ANALYSES OF TEST SOLUTIONS

# C. affinis/dubia CHRONIC EXPERIMENTS IN HARD WATER

Nominal Fluoride	Ionic Fluoride Note (a, b)	Hardness (mg/L) (Note (b, c)	Alkalinity (mg/L) Note (b, d)	рН		
0	0	189 (179 - 199)	132 (124 - 141)	7.8 (7.5 - 8.2)		
6.3	6.2	191	132	7.8		
	(5.8 - 6.5)	(178 - 205)	(112 - 152)	(7.6 - 8.1)		
10	9.7	191	133	7.9		
	(8.7 - 10.8)	(164 - 218)	(115 - 151)	(7.8 - 8.0)		
16	16.2	189	134	7.9		
	(13.8 - 18.6)	(162 - 216)	(99 - 169)	(7.7 - 8.1)		
25	26.2	190	133	7.9		
	(22.1 - 30.2)	(160 - 220)	(118 - 147)	(7.7 - 8.0)		
40	43.3	188	135	8.0		
	(34.5 - 52.2)	(149 - 227)	(113 - 157)	(7.6 - 8.4)		
63	70.7	192	136	8.0		
	(58.6 - 82.8)	(170 - 213)	(119 - 153)	(7.6 - 8.3		
<ul> <li>Notes: (a) Ionic fluoride as measured by selective ion electrode, reported as F.</li> <li>(b) Median value followed by 95% C.I. in parentheses.</li> <li>(c) Hardness in mg/L as CaCO<sub>3</sub>, measured via Calgon-20 titration</li> <li>(d) Alkalinity in mg/L as CaCO<sub>3</sub>, as titrated with HCl through pH of 4.6.</li> </ul>						

## CHEMICAL ANALYSES OF TEST SOLUTIONS

# C. affinis/dubia CHRONIC EXPERIMENTS IN MODERATELY HARD WATER

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Nominal Fluoride	Ionic Fluoride Note (a, b)	Hardness (mg/L) (Note (b, c)	Alkalinity (mg/L) Note (b, d)	рН				
0	0	124 (113 - 135)	93 (81 - 106)	7.7 (7.6 - 7.8)				
6.3	6.0	124	93	7.7				
	(5.0 - 6.4)	(117 - 131)	(81 - 105)	(7.5 - 7.9)				
10	9.5	121	93	7.7				
	(8.6 - 10.3)	(106 - 136)	(82 - 105)	(7.6 - 7.8)				
16	15.5	123	95	7.7				
	(14.5 - 16.5)	(110 - 137)	(84 - 107)	(7.6 - 7.8)				
25	25.4	123	94	7.6				
	(22.8 - 28.1)	(116 - 131)	(86 - 102)	(7.5 - 7.8)				
40	41.2	122	95	7.7				
	(38.0 - 44.5)	(111 - 133)	(88 - 103)	(7.6 - 7.8)				
Notes: (a) Ionic fluoride as measured by selective ion electrode, reported as F <sup>-</sup> . (b) Median value followed by 95% C.I. in parentheses. (c) Hardness in mg/L as CaCO <sub>3</sub> , measured via Calgon-20 titration.								
()	(d) Alkalinity in mg/L as CaCO <sub>3</sub> , as titrated with HC1 through pH of 4.6.							

### CHEMICAL ANALYSES OF TEST SOLUTIONS

## C. affinis/dubia CHRONIC EXPERIMENTS IN SEMI-SOFT WATER

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Nominal Fluoride	Ionic Fluoride Note (a, b)	Hardness (mg/L) (Note (b, c)	Alkalinity (mg/L) Note (b, d)	рН		
0	0	77 (67 - 87)	60 (47 - 73)	7.7 (7.5 - 7.9)		
6.3	6.8 (6.3 - 7.4)	80 (69 - 81)	63 (47 - 79)	7.7 (7.4 - 8.1)		
10	10.4 (10.1 - 10.7)	82 (75 - 89)	64 (48 - 79)	7.7 (7.5 - 7.8)		
16	17.4 (16.7 - 18.1)	81 (71 - 91)	64 (48 - 80)	7.7 (7.4 - 8.0)		
25	28.2 (25.3 - 31.1)	80 (69 - 91)	61 (45 - 77)	7.7 (7.3 - 8.1)		
40	44.8     81     65     7.7       (41.1 - 48.6)     (77 - 85)     (54 - 76)     (7.5 - 7.9)					
<ul> <li>Notes: (a) Ionic fluoride as measured by selective ion electrode, reported as F.</li> <li>(b) Median value followed by 95% C.I. in parentheses.</li> <li>(c) Hardness in mg/L as CaCO<sub>3</sub>, measured via Calgon-20 titration.</li> <li>(d) Alkalinity in mg/L as CaCO<sub>3</sub>, as titrated with HCl through pH of 4.6.</li> </ul>						

# COEFFICIENTS OF VARIATION: FLUORIDE AND HARDNESS

### C. affinis/dubia CHRONIC EXPERIMENT

Water Type	Ionic Fluoride (mg/L)	Coefficient of Variation of Ionic Fluoride (%)	Coefficient of Variation of Hardness (%)
. Very Hard	0 9.6 16.3 26.1 43.2 60.5	6.4 4.0 9.1 6.5 12.5	4.6 4.5 4.6 7.4 7.2 3.0
Hard	0 6.2 9.7 16.2 26.2 43.3 70.7	2.7 5.5 10.7 7.5 10.4 8.7	3.0 3.6 7.1 7.2 7.9 10.5 5.7
Moderately Hard	0 6.0 9.5 15.5 25.4 41.2	3.2 4.4 3.2 5.3 4.0	4.6 2.7 6.2 5.5 3.1 4.6
Semi- Soft	0 6.8 10.4 17.4 28.2 44.8	- 3.6 1.4 1.9 5.2 4.2	6.5 7.1 4.0 6.2 7.1 2.3
	Average:	5.7	5.4

## SUMMARY OF REPRODUCTION DATA AND MORTALITY

C. affinis/dubia CHRONIC EXPERIMENT IN VERY HARD WATER AT  $25^{\circ}$ C

Nominal Fluoride Concentrat (mg/L)		Ionic Fluoride Concentration (mg/L) Note (a)	Average Number of Eggs Per Adult Note (b)	Average Number of Neonates Per Adult Note (b)	Total Number of Mortalities During Test Note (c)	Hatchabil- ity Rate (%) Note (d)		
0		0	12.1/9.7	11.0/8.8	2	90.7		
10		9.6	11.5/10.6	11.5/10.6	1	100.0		
16		16.3	11.4	10.7	0	93.9		
25		26.1	8.0/7.2	7.2/6.5	1	90.3		
40		43.2	10.5/9.7	4.0/3.7	1	38.1		
63		60.5	7.3/6.0	0.1/0.1	3	1.7		
Notes:	(a) (b) (c) (d)	reported as F Average number mortalities. Mortalities at day 3: at 40 m	Average ionic fluoride concentrations, measured by selective ion electrode, reported as F <sup>-</sup> . Average number calculated on basis of ten adults with/without adjustment for mortalities. Mortalities at 0 mg/L occurred on day 4; at 10 mg/L, on day 5; at 25 mg/L on day 3; at 40 mg/L on day 5; at 63 mg/L, two on day 5 and one on day 7. Hatchability rate is number of neonates divided by number of eggs.					

### SUMMARY OF REPRODUCTION DATA AND MORTALITY

C. affinis/dubia CHRONIC EXPERIMENT IN HARD WATER AT  $25^{\circ}$ C

Nominal Fluoride Concentration (mg/L)	Ionic Fluoride Concentration (mg/L) Note (a)	Average Number of Eggs Per Adult Note (b)	Average Number of Neonates Per Adult Note (b)	Total Number of Mortalities During Test Note (c)	Hatchabil- ity Rate (%) Note (d)
0	0	23.7	23.7	0	100.0
6.3	6.2	21.0	21.0	0	100.0
10	9.7	15.2	13.9	0	91.4
16	16.2	22.9	22.6	0	98.7
25	26.2	13.9/12.5	11.4/10.3	1	82.4
40	43.3	15.4/13.9	3.7/3.3	1	23.7
63	70.7	18.6	0	0	0
<ul> <li>NOTES: (a) Average ionic fluoride concentrations, measured by selective ion electrode, reported as F<sup>-</sup>.</li> <li>(b) Average number calculated on basis of ten adults with/without adjustment for mortalities.</li> <li>(c) Mortalities at 25 mg/L occurred on day 3; at 40 mg/L, on day 3.</li> <li>(d) Hatchability rate is number of neonates divided by number of eggs.</li> </ul>					

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## SUMMARY OF REPRODUCTION DATA AND MORTALITY

C. affinis/dubia CHRONIC EXPERIMENT IN MODERATELY HARD WATER AT  $25^{\circ}$ C

Nominal Fluoride Concentration (mg/L)	Ionic Fluoride Concentration (mg/L) Note (a)	Average Number of Eggs Per Adult Note (b)	Average Number of Neonates Per Adult Note (b)	Total Number of Mortalities During Test Note (c)	Hatchabil- ity Rate (%) Note (d)	
0	0	23.1	15.9	0	68.8	
6.3	6.0	12.7	6.6	0	52.0	
10	9.5	12.0	2.9	0	24.2	
16	15.5	14.8	3.6	0	24.3	
25	25.4	11.7	1.7	0	14.5	
40	41.2	18.2/16.4	0	1	· 0	
<ul> <li>Notes: (a) Average ionic fluoride concentrations, measured by selective ion electrode, reported as F.</li> <li>(b) Average number calculated on basis of ten adults with/without adjustment for mortalities.</li> <li>(c) Mortality at 40 mg/L occurred on day 3.</li> <li>(d) Hatchability rate is number of neonates divided by number of eggs.</li> </ul>						

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### SUMMARY OF REPRODUCTION DATA AND MORTALITY

C. affinis/dubia CHRONIC EXPERIMENT IN SEMI-SOFT HARD WATER AT  $25^{\circ}$ C

Nominal Fluoride Concentratio (mg/L)	Ionic Fluoride n Concentration (mg/L) Note (a)	Average Number of Eggs Per Adult Note (b)	Average Number of Neonates Per Adult Note (b)	Total Number of Mortalities During Test Note (c)	Hatchabil- ity Rate (%) Note (d)			
0	0	20.2/18.2	19.3/17.4	2	95.6			
6.3	6.8	14.9/10.4	10.6/7.4	3	71.2			
10	10.4	16.8/14.3	15.5/13.2	3	92.3			
16	17.4	13.4/9.4	4.7/3.3	3	35.1			
25	28.2	15.0/9.0	4.3/2.6	4	28.9			
40	44.8	12.6/11.3	0	1	0			
-	reported as F	reported as F <sup>-</sup> . Average number calculated on basis of ten adults with/without adjustment for						
	) Mortalities at day 4; at 10 m and one on day	Mortalities. Mortalities at 0 mg/L occurred on day 6; at 6.3 mg/L, one on day 3 and two on day 4; at 10 mg/L, one on day 3 and two on day 7; at 16 mg/L, two on day 2 and one on day 4; at 25 mg/L, on day 2; at 40 mg/L on day 2. Hatchability rate is number of neonates divided by number of eggs.						

survived; for example, if an adult died two days into a four day neonate bearing period, it was counted as 0.5 adult.

Figures 15, 16, 17 and 18 depict the live neonate production in terms of cumulative number of neonates versus day of test for the very hard, hard, moderately hard, and semi-soft experiments, respectively.

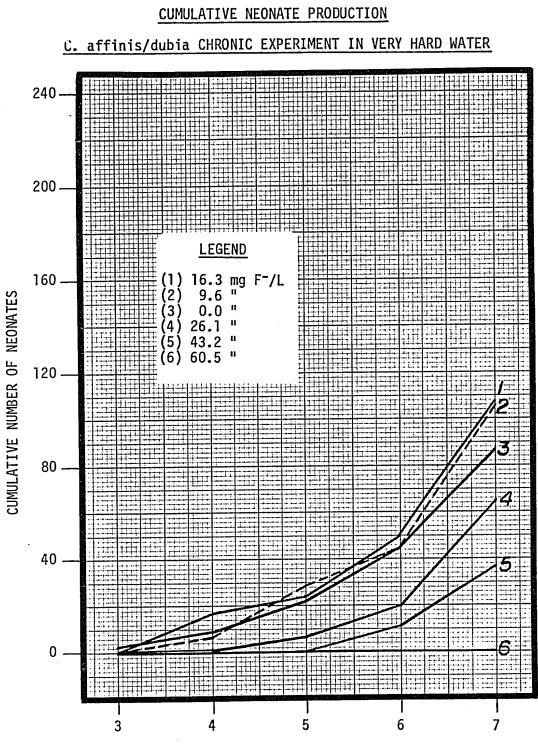
#### 8.2 Data Analysis

A review of the chemical analyses yields several observations:

- The variances in fluoride analyses and hardness analyses were fairly low, as evidenced by the low coefficients of variation.
- The hardness levels decreased with increasing fluoride concentrations as expected, due to the complexing of polyvalent cations with the fluoride anion and removal from the system.

Neonate production varied widely, as indicated in Figures 15, 16, 17, and 18:

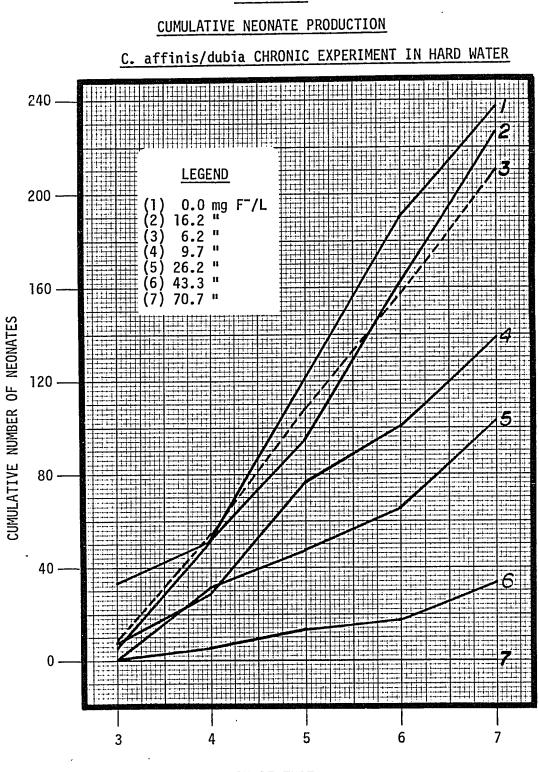
- Neonate production in hard water was significantly greater than that in waters of other hardnesses, by a factor of up to 2:1 at maximum production rates. This is in agreement with the <u>D. magna</u> experiments reported upon in Chapter 6.



### FIGURE 15

DAY OF TEST

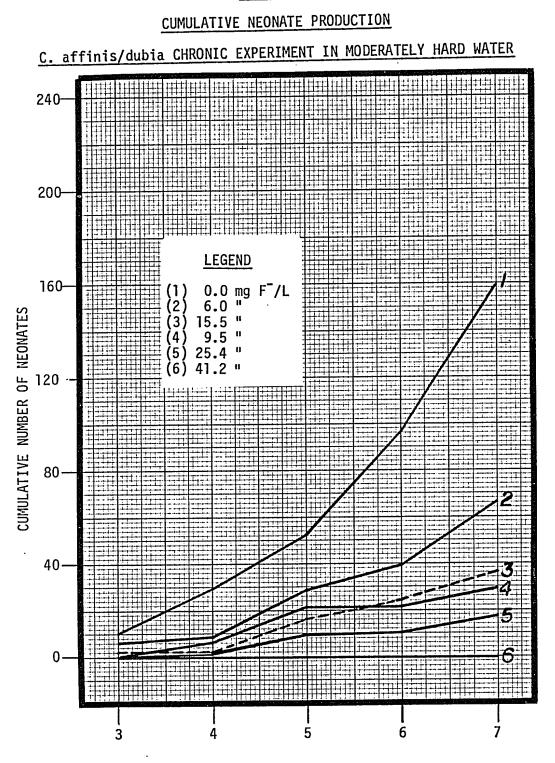
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## FIGURE 16

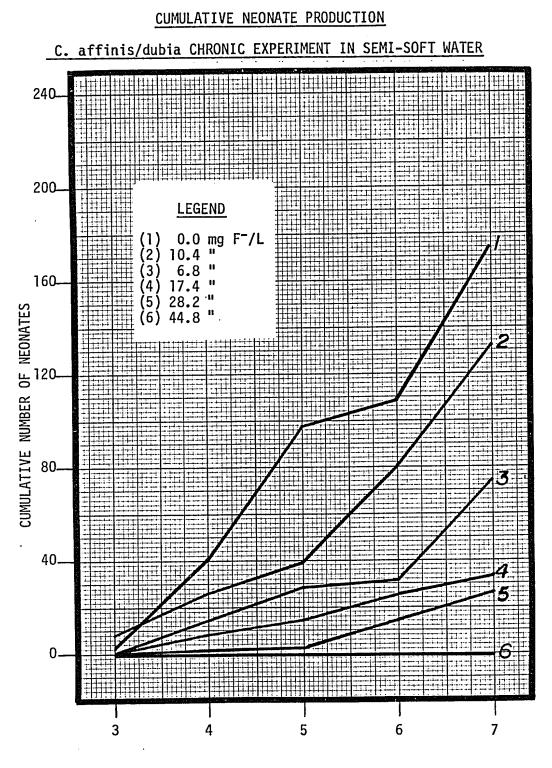
DAY OF TEST





DAY OF TEST

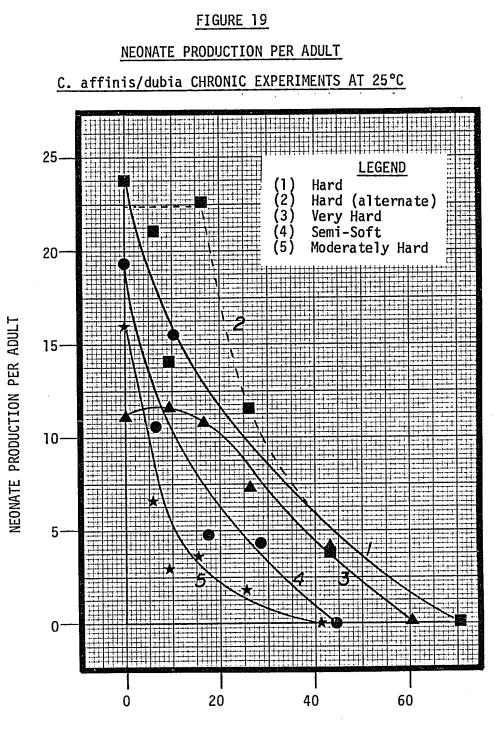




DAY OF TEST

- Significant neonate production was recorded by day 3 in hard water, whereas equivalent production was only reached in the other hardnesses by day 4.
- The rate of neonate production was fairly constant at any given fluoride concentration, as compared to a more variable rate experienced by the <u>D. magna</u> in their chronic experiment. This simply may be due to the fact that the chronic test for the <u>C. affinis/dubia</u> extends over only 7 days rather than 21 days as in the <u>D. magna</u> tests, and averaging of numbers may smooth out the cumulative curve.
- In the very hard water tests, neonate production at 10 mg F<sup>-</sup>/L and 16 mg F<sup>-</sup>/L exceeded that in the controls. In the other hardnesses, maximum neonate production was observed in the controls, unlike the case of the <u>D. magna</u>, where a definite stimulation in neonate production was observed at low fluoride concentrations relative to the controls. This observation of <u>C. affinis/dubia</u> neonate production is the same when measured on a "cumulative total number of neonates" or "cumulative number of neonates per equivalent adult" basis.

Neonates per equivalent adult is depicted in Figure 19. Note that maximum neonate production occurs in very hard water at approximately 8 mg  $F^{-}/L$ , whereas in other water hardnesses, maximum production occurs in the controls and falls off with increasing fluoride concentration. This



FLUORIDE CONCENTRATION (mg F<sup>-</sup>/L)

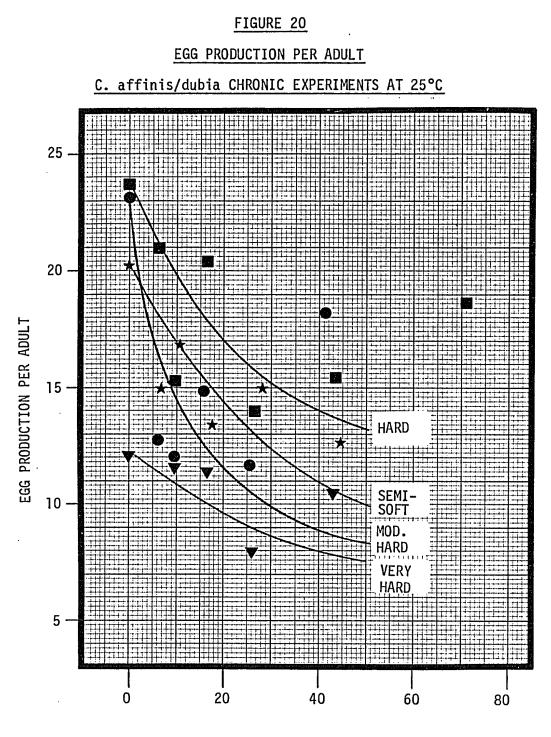
is different from the <u>D. magna</u> chronic tests, wherein low fluorides in all cases provided a stimulation effect and neonate production exceeded that of the controls.

Similarly to the <u>D. magna</u> chronic tests, egg production was counted. As described earlier, the term "eggs" includes all neonates, partially or fully formed but dead embryos, and undeveloped eggs observable either as aborted spheres in the bottom of the test chamber or contained in the shed carapace. Figure 20 indicates egg production per equivalent adult for the various test conditions. The data points are quite variable; the curved lines indicate the general trend of the egg production at the various fluoride concentrations. In all cases, the egg production decreases rapidly from the control level; the stimulation effect observed in the <u>D. magna</u> experiments is not apparent in the case of the <u>C. affinis/dubia</u>.

Observation of the neonate production levels in the controls and low fluoride concentrations (Tables 32, 33, 34, and 35) show values that although different, are not widely separated in many cases.

The question may be posed as to whether a "significant difference" exists between many of these values. In order to answer this question, a statistical analysis of the data was performed using an analysis of variance of the individual neonate production. Specifically, a "t test" based on pooled-variance estimates as described by Prof. Glantz of the University of California<sup>(78)</sup> was used. Values of "t" were calculated

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FLUORIDE CONCENTRATION (mg F<sup>-</sup>/L)

comparing neonate production per equivalent adult in the controls versus that in the low fluoride concentrations; the calculated "t" was then compared to critical values of "t" at a probability level of 1%. Results of the calculations are shown in Table 36.

In effect, the testing for significance leads to the following general observations:

- In very hard water, a statistically significant difference in neonate production may not be discernible until fluoride concentrations increase to between 16.3 and 26.1 mg F<sup>-</sup>/L.
- In hard water, there is a problem in interpretation due to reversal in significance with increasing fluoride concentration. If the data point for 9.7 mg F<sup>-</sup>/L is ignored, then a similar statement to that for hard water could be made; viz., a difference may not be discernible until fluoride concentration increases to between 16.2 and 26.2 mg F<sup>-</sup>/L. The dotted line marked "Hard (alternate)" on Figure 19 shows this phenomena; the 22.4 mg/L is the average concentration of the three pertinent data points. If the 16.2 mg F<sup>-</sup>/L data point is ignored, then a significant difference may occur at the 6.2 to 9.7 mg F<sup>-</sup>/L level.
- In moderately hard water, an immediate significant difference may be discernible at the 6.0 mg F<sup>-</sup>/L level.

### TESTS FOR SIGNIFICANT DIFFERENCES

### IN

# NEONATE PRODUCTION LEVELS

# C. affinis/dubia CHRONIC EXPERIMENTS AT 25°C

Water T <i>y</i> pe	Ionic Fluoride (mg/L)	Comparison Neonates in Control vs. ne- onates in fluor- ide solution	Significance at 1% Probability Level				
Very Hard	9.6 16.3 26.1	11.0 vs. 11.5 11.0 vs. 10.7 11.0 vs. 7.2	Not significant Not significant Significant				
Hard	6.2 9.7 16.2 26.2	23.7 vs. 21.0 23.7 vs. 13.9 23.7 vs. 22.6 23.7 vs. 11.4	Not significant Significant Not significant Significant				
Moderately Hard	6.0	15.9 vs. 6.6	Significant				
Semi- Soft	6.8 10.4 17.4	19.3 vs. 10.6 19.3 vs. 15.5 19.3 vs. 4.7	Significant Not significant* Significant				
* Not signif	* Not significant at 1% level; significant at 5% level.						

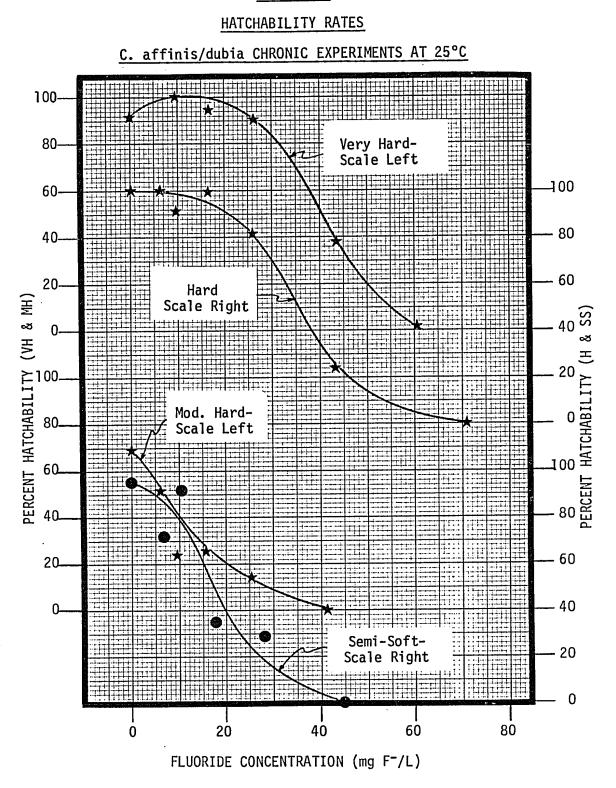
- In semi-soft water, a significant difference may be discernible at the 6.8 mg F<sup>-</sup>/L level. At the 10.4 mg F<sup>-</sup>/L level, the difference may not be significant at the 1% level, but may be significant at the 5% level.

If the 9.7 mg F<sup>-</sup>/L data point in the hard water is ignored, it is interesting to note that for both hard and very hard cases a no-effect level in neonate production may not be reached until somewhere in the  $16.2 - 26.2 \text{ mg F}^{-}/\text{L}$  level. This is similar to the <u>D. magna</u> chronic experiments in which no effect was observed up to 31.5 mg F<sup>-</sup>/L and 47.0 mg F<sup>-</sup>/L level in hard and very hard waters, respectively.

Hatchability, another measure of toxicity, is shown in Figure 21. The curves in this graph generally follow the curves of neonate production (Figure 19):

- In very hard water, the hatchability rate is elevated at low fluoride concentrations, and then decreases at higher concentra-tions.
- In hard water, a generally level rate is experienced at low fluoride concentrations (matches dotted line of Figure 19 more closely than the solid line) and then decreases thereafter.
- In moderately hard and semi-soft waters, the hatchability rate declines continuously in low and high fluoride concentrations.

### FIGURE 21



This discussion will be resumed in Chapter 10 on in context of the other experimental data.

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#### 9.0 PIMEPHALES promelas ACUTE EXPERIMENTS

Acute (96 hour) experiments utilizing fathead minnows (<u>Pimephales</u> <u>promelas</u>) were conducted at  $20^{\circ}$ C in very hard, hard, moderately hard and semi-soft waters, at fluoride concentrations ranging from 0 to 251 mg F<sup>-</sup>/liter. These tests, involving approximately 500 fish, were conducted in accordance with the plan, procedures, and equipment described in Chapters 3 and 4.

#### 9.1 Test Data

The chemical analyses of the test solutions, with reference to fluoride concentration, hardness, alkalinity, and pH, closely paralleled those for <u>D. magna</u> acute tests. Table 37 lists the measured hardness of the control solutions, as an average of the beginning and ending analyses.

#### TABLE 37

### MEDIAN HARDNESS OF CONTROL SOLUTIONS

PIMEPHALES promelas ACUTE EXPERIMENTS

Water Type	Hardness (mg/L as CaCO <sub>3</sub> )
Very Hard	260
Hard	168
Moderately Hard	112
Semi-Soft	72

Fluoride concentrations and mortality rates are indicated in Table 38 for the very hard and hard water experiments, and in Table 39 for the moderately hard and semi-soft water experiments.

Of the four experiments conducted, all were judged to be valid on the basis of no mortality in the controls, no mortality at low fluoride concentration, 100% mortality at a high fluoride concentration, and partial mortality rates at intermediate fluoride concentrations.

For all tests in this group, temperature was maintained at  $20 \pm 1^{\circ}$ C; median temperature was  $20.0^{\circ}$ C with a 95% C. I. of 19.5 -  $20.5^{\circ}$ C. Dissolved oxygen in all samples were within the range of 95% to 100% of saturation concentration.

#### 9.2 Data Analysis

Median lethal concentrations  $(LC_{50})$  were calculated from the data by both the log concentration versus percent mortality (LCPM) method and the moving-average-angle method (MAAM) with results as shown in Table 40.

### FLUORIDE CONCENTRATION AND FISH MORTALITY

IN VERY HARD AND HARD WATER

P. promelas ACUTE EXPERIMENTS AT  $20^{\circ}C$ 

Water Type	Nominal Fluoride Concentration (mg F <sup>-</sup> /L)		oride_Cc (mg_F_/L End	oncentration .) Median	Percent Mortality
Very Hard	0 100 158 251 398 501	0 75 129 242 368 490	0 73 130 240 333 430	0 74 129 241 350 460	0 0 10 75 100 100
Hard	0 63 100 158 251 398 501	0 56 90 154 252 417 530	0 52 96 159 221 380 467	0 54 93 156 236 398 498	0 0 20 25 100 100 100

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### FLUORIDE CONCENTRATION AND FISH MORTALITY

IN MODERATELY HARD & SEMI-SOFT WATER

P. promelas ACUTE EXPERIMENTS AT  $20^{\circ}C$ 

Water Type	Nominal Fluoride Concentration (mg F <sup>-</sup> /L)		oride Co (mg F <sup>-</sup> /L End	ncentration ) Median	Percent Mortality
Moderately Hard	0 40 63 100 158 251	0 39 64 96 152 250	0 43 72 96 165 280	0 41 68 96 158 265	0 0 5 0 75 100
Semi- Soft	0 25 40 63 100 158 251	0 22 37 59 94 148 250	0 22 39 66 100 165 282	0 22 38 62 97 156 266	0 0 5 10 85 100

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#### MEDIAN LETHAL CONCENTRATIONS

#### P. promelas ACUTE EXPERIMENTS

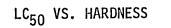
	LC <sub>50</sub> in mg F <sup>-</sup> /L			
Water Type	LCPM	МААМ*		
Very Hard	190	194 (163 - 232)		
Hard	179	230 (205 - 262)		
Moderately Hard	134	142 (122 - 168)		
Semi-Soft	125	124 (107 - 144)		
<pre>* Median value followed by 95% C.I. in par- theses.</pre>				

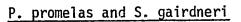
Figure 22 depicts the  $LC_{50}$  versus hardness. The data fit a straight line as shown, especially if the LCPM data point for hard water is used rather than the MAAM data point.

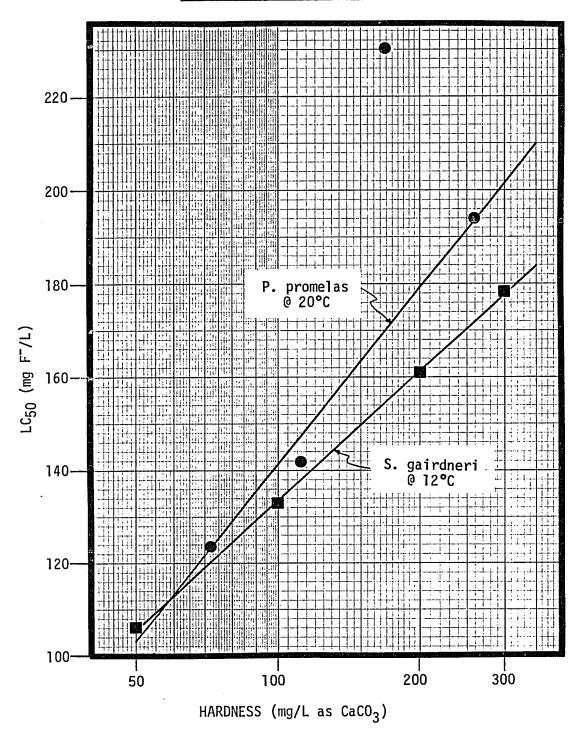
The indicated line follows the following equation:

 $LC_{50} = (126 \log H) - (111)$  Equation 9-1 here H = water hardness in mg/L as CaCO<sub>3</sub> and LC<sub>50</sub> = median lethal concentration in mg F<sup>-</sup>/L as measured by a selective ion electrode.









As indicated in Chapter 5, Pimentel and Bulkley<sup>(45)</sup> studied the effect of hardness of fluoride toxicity to rainbow trout (<u>Salmo gairdneri</u>) at 12  $^{O}$ C; data from their report is plotted on the same scale as for <u>P. promelas</u> as shown in Figure 22. Although the <u>P. promelas</u> curve is more deeply inclined than that for the <u>S. gairdneri</u>, there is exceptional agreement between the curves considering the different species and temperature involved.

This subject will be discussed further in Chapter 10 in the context of other experiments.

#### 10.0 MODELING, RISK AND CRITERIA

Data relative to acute and chronic toxicity of fluoride has been developed for various aquatic organisms. It is the next task, then, to combine these data into a unified statement of toxicity (preferably in the form of a model) that may be used as a standard and/or as a substantive building-block in the establishment of a criterion.

In this chapter, we address the subjects of:

- Standards and criteria: general considerations.
- Risks involved in setting a criterion or standard based on the experiments completed - considerations relative to technical aspects such as quality control and accuracy, but without reference to potential socio-economic factors.
- Overall modeling and establishment of a criterion for fluoride toxicity.

10.1 Standards and Criteria: General Considerations

In general parlance, the term "standard" has been increasingly used to designate both "water quality standards" and "water quality criteria." There is a difference, of course, as indicated by the EPA:<sup>(79)</sup>

"Section 303 of the Clean Water Act provides that water quality standards be developed for all surface waters. A water quality standard consists basically of two parts: (1) a "designated use" for which the water body is to be protected (such as "agricultural", "recreation" or "fish and wildlife") and (2) "criteria," which are numerical pollutant concentration limits or narrative statements necessary to preserve or achieve the designated use. A water quality standard is developed through State or Federal rulemaking proceedings and must be translated into enforceable

effluent limitations in a point source (NPDES) permit or may form the basis of best management practices applicable to nonpoint sources..."

It should be noted that a major difference can exist between an EPA "criteria" and a State "standard". EPA criteria are ". . .based solely on data and scientific judgments on the relationship between pollutant concentrations and environmental and human health effects. Criteria values do not reflect considerations of economic or technological feasibility . . . criteria represent a reasonable estimate of pollutant concentrations consistent with the maintenance of designated water uses . . . States may appropriately modify these values to reflect local conditions. In certain circumstances, the criteria may not accurately reflect the toxicity of a pollutant because of the effect of local water uses . . . "(79)

Our concern in this chapter will be with both "criteria" and "standards"; for convenience, the term "standards" will be used herein for general applications while recognizing that the term "criteria" will be applicable in many instances.

When discussing a standard, we must be specific as to the type of standard under discussion. Standards may fall into three categories or classes:<sup>(80)</sup>

- (A) Effluent standards for point or area sources. These may be in the form of:
  - (1) Concentration at the discharge point (performance standard)
  - (2) Amount per unit time (performance standard)
  - (3) Specification of control methods by use (performance and design standards)
  - (4) Specification and/or restriction of production methods (design standard)
- (B) Ambient standards (water quality)
- (C) Finished drinking water standards for both surface and ground waters. These may be on the basis of:
  - (1) concentration
  - (2) concentration versus exposure.

In this chapter, our primary discussion will be on item B - ambient standards, or alternately, water quality criteria, which can be used as a basis for establishing a standard. It is recognized that an interrelationship exists between these terms and classes; for example, between "ambient" and "drinking water" standards. Drinking water standards may be more severe than standards set for other uses such as "fish and wildlife." However, in water bodies serving multiple uses, the more stringent standard may dictate acceptable levels. Drinking water standards in the USA with reference to fluoride as established by the USPPHS and EPA are shown in Table 41. The standard is set on the basis that in warmer climates, humans and animals drink greater amount of water.

#### TABLE 41

Annual Average Maximum Daily Air Temperature C	Fluoride Concent Recommended	
10.0 - 12.0	1.7	2.4
12.1 - 14.6	1.5	2.2
14.7 - 17.7	1.3	2.0
17.7 - 21.4	1.2	1.8
21.5 - 26.2	1.0	1.6
26.3 - 32.5	0.8	1.4

#### FLUORIDE LIMITATIONS FOR DRINKING WATER

Although these standards are for "finished" drinking water after treatment, the low limits of fluoride concentration, coupled with the non-availability of economical processes for removing fluorides at low concentrations, in effect dictate "ambient" conditions at water intake stations. The water quality criteria for aquatic organisms established herein may then be subject to this overriding consideration depending upon the hydrological conditions (e.g., stream dilution factors) prevailing in the water system being studied.

#### 10.2 Risks

Risk, in the context of aquatic toxicology, has been defined by Rand & Petrocelli<sup>(82)</sup> as ". . .the expected frequency or probability of undesirable effects resulting from a specified exposure to known or potential environmental concentrations of a material. . ." Further, ". . . A material is considered safe if the risks associated with its exposure are judged to be acceptable. Estimates of risk may be expressed in absolute or relative terms. Absolute risk is the excess risk due to exposure. Relative risk is the ratio of the risk in the exposed population to the risk in the unexposed population."

In attempting to provide a criterion or standard for fluorides based on the experiments described herein, it appears that there are several parameters that can significantly influence the risk involved. These may be classified as follows:

A. Experimental parameters

- The aquatic environment in the laboratory to which the test organisms were exposed - how does it compare to the real world?
- 2. Test organisms were they of applicable kind and number of species?
- 3. Laboratory procedures and practices -- are the procedures acceptable? Were the practices employed of sufficient quality and reliability?

B. Data

- Statistical methods were the correct statistical analytical techniques utilized?
- Comparison of data with other research results how does this data compare? Does it make sense?
- C. Modeling and criteria establishment
  - Models how well do they represent the actual relationship?
  - 2. Model use how much interpretation and adjustment from the model is to be made in establishing a criterion or a standard?

In order to answer the indicated questions, a retrospective review of the experimental parameters and observed data follows. The questions on modeling and criteria establishment will be answered in later sections of this chapter.

10.2.1 Experimental Parameters - Aquatic Environment

In previous chapters, test environmental conditions were summarized relative to hardness, pH, alkalinity, and temperature. In order to determine applicability, this data will be compared to conditions on the Ohio River and its major tributaries, which traverse eight states (Pennsylvania, West Virginia, New York, Virginia, Ohio, Kentucky, Indiana, and Illinois). This area of the nation contains many of the steel, aluminum, glass, etc., manufacturing plants which are primary sources of fluoride effluents as indicated in Chapter 1.

The Ohio River Valley Water Sanitation Commission (ORSANCO) periodically publishes data relative to water analyses and conditions. ORSANCO data<sup>(83)</sup> is listed in Table 42 for July-September, 1984; this calendar quarter is the period of "low flow" on the Ohio River, and is generally one of elevated environmental stress to aquatic organisms relative to pollutant concentrations, dissolved oxygen, etc. Data for hardness was derived from 38 sampling locations located along the river, from its tributaries (e.g., starting with Oakmont Station on the Allegheny River and South Pittsburgh Station on the Monongahela River) to Joppa, Illinois, a distance of approximately 1,000 miles. Alkalinity was measured at twelve stations; temperature and dissolved oxygen at 21 stations.

A comparison of the Ohio River and our test analyses (Table 42) indicate good agreement for the hardness concentrations. Temperature comparison is judged to be good, considering that July-September is the highest temperature period for the year. Dissolved oxygen in the laboratory was maintained at 95% or better saturation level; the Ohio River suffered partial oxygen depletion in many instances, down to an average minimum value of 5.0 mg/L. Modern water quality considerations recognize that a reduction in dissolved oxygen below natural levels will have some deleterious effect on organisms <sup>(84,85,86)</sup>. While not influencing criteria considerations, this low value should be comprehended in setting standards.

## TABLE 42

## COMPARATIVE WATER ANALYSES

## CONTROL SOLUTIONS VS. OHIO RIVER

Water Type	Experimental Solutions (Average Concentrations of Controls)	Ohio River (Average Concentrations July - September, 1984)
Hardness (mg/L as CaCO <sub>3</sub> )	VH: 277 H: 179 MH: 117 SS: 74	Highest 25%: 236 Next 25%: 145 Next 25%: 125 Lowest 25%: 83
Alkalinity (mg/L as CaCO <sub>3</sub> )	VH: 189 H: 123 MH: 80 SS: 51	Max.: 52 Avg.: 44 Min.: 36
Temperature ( <sup>O</sup> C)	Max.: 25 Avg.: 20 Min.: 15	Max.: 27.7 Avg.: 25.2 Min.: 22.6
рН	VH: 8.3 H: 8.1 MH: 8.0 SS: 7.7	Max.: 7.9 Avg.: 7.3 Min.: 6.7
Dissolved oxygen (mg/L)	(95 - 100% Sat.) 8.6 - 9.0	Max.: 9.6 Avg.: 6.8 Min.: 5.0

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A difference in alkalinity is observed, with the average concentration more nearly matching that of our semi-soft test solution than that of the higher hardnesses. A corresponding lower level of pH can be observed. A review of the ORSANCO data indicates that the lowest pH and alkalinity values were measured in the upstream reaches of the river (Pittsburgh area); the pH and alkalinity values then generally increase to intermediate values between Pittsburgh and Huntington, West Virginia; with further increases to the Cincinnati area and then a general leveling off. It is most likely that the heavy concentration of industrial facilities and presence of acid mine drainage in the western portions of Pennsylvania and West Virginina contribute and are the primary factor in this phenomena. A further consideration is that the NaF toxicant, unlike some of the metallic toxicants which are significantly affected by relatively small changes in  $pH^{(87)}$ , is stable in the range under consideration.

Thus, it is concluded that the reconstituted waters recommended by the EPA<sup>(88)</sup> and Standard Methods<sup>(89)</sup> and used in our experiments are valid environments when measuring fluoride toxicity against this specific (Ohio River) field location, and no significant increase in risk is introduced.

#### 10.2.2 Experimental Parameters - Test Organisms

Experiments have been completed utilizing the three organisms <u>Daph-</u> <u>nia magna</u>, <u>Ceriodaphnia affinis/dubia</u>, and <u>Pimephales promelas</u>. These three have been widely utilized in previous toxicity testing (Chapter 2)

and are listed in several references (49-52) as preferred test organisms.

The EPA, in their "Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses", in the section on minimum data bases, (90) indicate ". . . To derive a criterion for freshwater aquatic life, the following should be available: Acute tests . . . with freshwater animals in at least eight different families provided that of the eight species:

- at least one is a salmonid fish
- at least one is a non-salmonid fish
- at least one is a planktonic crustacean
- at least one is a benthic crustacean
- at least one is a benthic insect
- at least one of the benthic species is a detritivore".

The EPA lists additional requirements<sup>(90)</sup>, including "Acute-chronic ratios . . . for at least three species of aquatic animals provided that of the three species:

- at least one is a fish
- at least one is an invertebrate
- at least one is a freshwater species (the other two may be saltwater species)".

Further, "At least one test with a freshwater alga or a chronic test with freshwater vascular plant. . .", and "At least one acceptable bioconcentration factor determined with an aquatic animal species, if a maximum permissible tissue concentration is available."<sup>(90)</sup>

The EPA then concludes, "If all the requirements of the minimum data base are met, a criterion can usually be derived, except in special cases..."

Only a limited number of experiments have been heretofore conducted in accordance with modern methodology, as discussed in Chapter 2. The organisms and experiments that have recently been conducted within the framework of acceptable methodology do not fulfill the EPA's minimum data base requirements, and thus are a factor in increasing uncertainty and risk. The experiments conducted in this project will add substantially to the data base, and thus contribute to a reduction in overall risk; however, a risk continues until the full data base requirement has been met.

10.2.3 Experimental Parameters - Laboratory Procedures and Practices

The advantages of using standard test procedures according to Davis<sup>(91)</sup> and listed by Rand & Petrocelli<sup>(92)</sup> include:

- Allows selection of one or more uniform and useful tests by a variety of laboratories.
- Facilitates comparison of data and results and thus increases usefulness of published data.
- Increases accuracy of the data.

- Allows replication of the test.

- Allows the test to be easily initiated and conducted by a variety

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of personnel (if the procedure is well-documented).

- Legal advantage if procedures are accepted by the courts.

- Useful for routine monitoring purposes.

Procedures utilized for the experiments described in this report, were conducted essentially in accordance with the procedures described by the EPA, and in Standard Methods as previously indicated in the text. No significant deviations are known that may increase the level of risk.

With reference to laboratory practices, Rand & Petrocelli<sup>(93)</sup> state: "Important in all phases of aquatic toxicity testing . . . is quality assurance (QA) in the laboratory and adherence to good laboratory practices (GLP) to promote the development of quality test data . . . GLP requirements deal with . . . personnel . . . facilities . . . equipment . . . laboratory operations . . . test chemicals . . . protocols . . . reports . . ." Each of these factors is briefly reviewed below as they apply to our project:

- Personnel: All personnel directly involved in the experiments were trained professionals at the Ph.D, doctoral candidate, or minimum BS level, with experience in the environmental field.
- Facilities: Test animal maintenance, chemical handling, laboratory testing areas, etc., were all located at a University prime area dedictated to research activities and absent of any regular student activities.
- Equipment: All operating equipment such as stills and refrigeration units were maintained for continuous operation; all test

equipment was cleaned, maintained, and calibrated on a regular basis; all glassware was washed with detergent and acid and rinsed with distilled water.

- Operations: Standard operating procedures, methods of animal care, preparation and use of standard reagents and solutions, were in accordance with modern quality laboratory standards.
- Test chemicals: All were of reagent grade as listed in Appendix B.
- Protocols: test methods were in accordance with Standard Methods or EPA publications as previously described; data was recorded directly on a contemporary basis.
- Reports: Reporting procedures were effected in accordance with current University practice.

10.2.4 Data - Statistical Methods

Median lethal concentrations  $(LC_{50})$  were determined by either the "log concentration versus percent mortality method (LCPM)" or the "moving average angle method (MAAM)", as described by the EPA<sup>(74,75)</sup>.

Rand & Petrocelli<sup>(94)</sup> describe a variety of statistical methods available for calculation of  $LC_{50}$ , including the probit method, moving average interpolation, trimmed Spearman-Karber method and others. The authors conclude however, ". . .From a practical point of view, it is not crucial that rigid guidelines be established for selecting among the different available procedures. For most types of data the estimate of the  $LC_{50}$  and its confidence interval will not vary significantly if different methods are used, especially considering the normal biological

and test condition variability that can occur from test to test. Ultimately, all these methods are alternative ways of smoothing the data and then estimating the median effect value. Stephan's <sup>(95)</sup> computations clearly demonstrate that one estimation technique is practically equivalent to another from the point of view of numerical results."

It should be noted that the MAAM is limited to estimating the lethal concentration at the 50% level only, and depends upon relatively equal spacing of concentration level. Both of these factors are applicable in the experiments and calculations described herein.

In comparing "significant versus non-significant" data in the <u>C</u>. <u>affinis-dubia</u> chronic experiments, the "t-test" based on pooled variance estimates as described by  $Glantz^{(78)}$  was utilized. Although other methods are available; (e.g., Bonferroni or Dunnet) for multiple data comparison, the isolated individual data comparisons of Chapter 8 were judged to be susceptible to the t-test method which has been used extensively in the biological field.

In light of the above, it is judged that the statistical methods employed are acceptable, and no unusual risk is associated with their use.

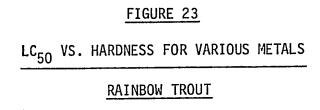
10.2.5 Data - Comparison

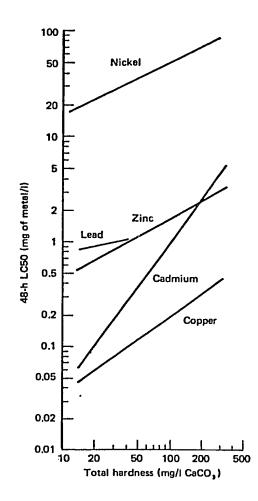
An overall review of the data and comparison to other toxicity tests is in order to determine whether new or exotic relationships are

expressed or whether the indicated relationships fall more in line with previous research. If the former develops, our estimate of risk is elevated; if the latter develops then the risk level is decreased.

Our acute experiments with all three tests organisms indicate that  $LC_{50}$  values increase with increasing hardness, in a logarithmic relationships. According to Rand & Petrocelli<sup>(94)</sup>: "Total hardness of fresh water has little effect on the potentcy of most pollutants (except metals). A few substances change their toxicity up to two or threefold, but the direction of change with hardness varies. ..." The major exception to this general statement is metal toxicity, wherein metals such as lead, zinc, copper, cadmium and nickel become less toxic with increasing hardness. Figure 23 depicts this general relationship as developed by the British Water Pollution Research Laboratory<sup>(95)</sup>. Comparing this figure to Figures 5, 14, and 22 of this report shows that fluoride toxicity acts very similar to that of metals (similar in slope to nickel and zinc, but higher on ordinate scale).

Our acute experiments with <u>D. magna</u> and <u>C. affinis/dubia</u> indicate that  $LC_{50}$  values decrease with increasing temperatures. Although it has been known that temperature can affect toxicity. ". . . there is no general effect of temperature on toxicity. Depending on the species and pollutant, fish in warmer water may be more, less, or equally tolerant. Limited evidence suggests that thresholds of sublethal effect may be about the same at all water temperatures. . ...<sup>(96)</sup> Reports on sodium fluoride toxicity show, for example, <sup>(97)</sup> increasing toxicity with in-





creasing temperature in <u>Salmo gairdneri</u>, but decreasing toxicity with increasing temperature in <u>Escherichia coli</u>. Specific temperature ranges must be specified, of course, for each species.

It is judged that the results relative to hardness and temperature are not unusual, and no element of unusual risk is introduced.

In the chronic tests, we noticed an interesting effect in that increasing amounts of fluoride in some cases (esp. for <u>D. magna</u>) increased the reproduction of the organisms, as measured by the relative number of neonates produced. This effect, while perhaps being unusual, is not unique; stimulation effects of low concentrations of toxicants has been reported upon previously; e.g., Sykora, et al.<sup>(100)</sup> on the effect of ferric hydroxide on brook trout, and McCarthy and Whitmore<sup>(101)</sup> on the effect of phthalates on <u>D. magna</u> and fathead minnows. The effect then is discounted as a factor that might significantly increase risk.

10.2.6 Modeling and Criteria Establishment

Risks associated with these factors will be discussed in section 10.3.

10.3 Modeling and Criterion Establishment

In the first two sections of this chapter, an attempt has been made to "set the stage" for the final compilation of data and an attempt to help define a criterion for fluoride in freshwater.

Definition of some key terms are in order; those presented by Rand and Petrocelli<sup>(102)</sup> are used:

- NOEC: no observed effect concentration the highest concentration of a material in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
- LOEC: lowest observed effect concentration the lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
- LC<sub>50</sub>: median lethal concentration the concentration of material in water to which test organisms are exposed that is estimated to be lethal to 50% of the test organisms.
- EC<sub>50</sub>: median effective concentration the concentration of material in water to which test organisms are exposed that is estimated to be effective in producing some sublethal response in 50% of the test organisms.
- MATC: maximum acceptable toxicant concentration the hypothetical toxic threshold concentration lying in a range bounded at the lower end by the highest tested concentration having no observed effect (NOEC) and at the higher end, by the lowest tested concentration having a significant toxic effect (LOEC) in a life cycle or partial life cycle.
- AF: application factor a numerical, unitless value, calculated as the threshold chronically toxic concentration of a chemical divided by its acutely toxic concentration.

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Mathematically:

NOEC < MATC < LOEC Equation 10-1

$$AF = \frac{MATC}{LC_{50}}$$
 Equation 10-2

or alternately:

MATC = (AF)  $(LC_{50})$ 

An example from our project follows (for  $\underline{D.magna}$  in hard water at 20<sup>o</sup>C):

NOEC*	(neonate	production	basis	)	=	26.1	mg	F <sup>-</sup> /L
LOEC*	("	11	11	)	=	35.5	mg	F <sup>-</sup> /L
LC <sub>50</sub> **	(from acu	ute tests)			= ;	261	mgF	-/L
MATC =	26.1 - 3	35.5 mg F <sup>-</sup> /I	-					
AF =	<u>26.1 - 3</u> 261	<u>35.</u> 5 = 0.2	10 - 0	).1	4			

In the previous chapters, we established models for determining  $LC_{50}$  for water at different temperatures and of different hardnesses; specifically: equations 5-1, 7-1, and 9-1. These can all be represent-

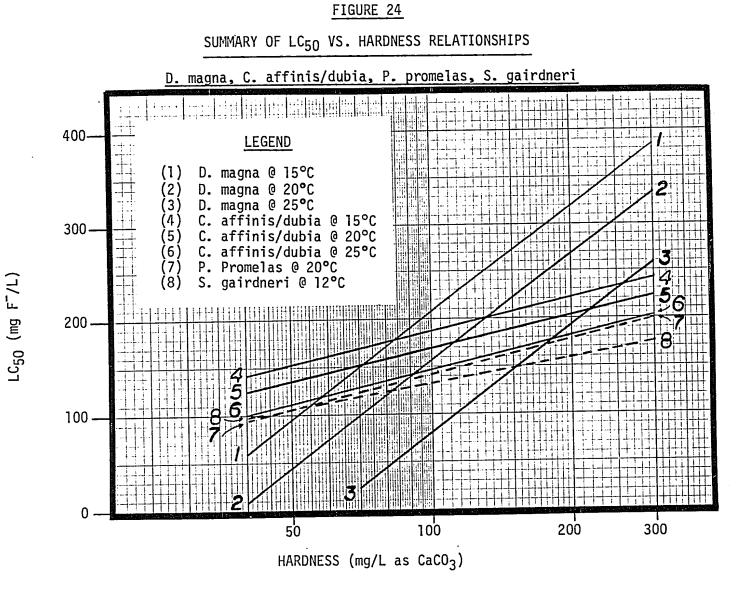
<sup>\*</sup> NOEC and LOEC from Table 19

<sup>\*\*</sup> LC<sub>50</sub> calculated from equation 5-1 for same hardness as that
 of chronic test.

ed by straight lines when plotted on semi-log paper, as presented in Figure 24. Included in this chart are the  $LC_{50}$  values for rainbow trout at  $12^{\circ}C$  as reported by Pimentel and Bulkley.<sup>(45)</sup>

Table 43 lists the MATC,  $LC_{50}$  and AF for the <u>D. magna</u> at 20<sup>o</sup>C experiments and for the <u>C. affinis/dubia</u> at 25<sup>o</sup>C experiments. The MATC values for the <u>D. magna</u> were obtained from Tables 18, 19, and 20, using number of neonates per adult as the measurable effect, and applying equation 10-1. The  $LC_{50}$  were obtained using equations 5-1, 7-1, and 9-1, based on hardnesses equivalent to those of the chronic experiments. AF values were calculated per equation 10-2.

The calculations for Table 43 are straightforward, but a decision was required for the hard and semi-soft experiments for the <u>C. af</u>-Referring to Table 36 for the hard water experiment, the finis/dubia. decision was made to essentially omit the 9.7 mg F<sup>-</sup>/L level and proceed to the 26.2 mg F<sup>-</sup>/L level for the first significant LOEC. Also, referring to Table 36, for the semi-soft water experiment, the LOEC was selected at the 17.4 mg F<sup>-</sup>/L level, which is correct at a 1% probability level but not for a 5% level. These two decisions provide AFs consistent with the previous AFs calculated. These decisions will increase the "risk" factor, however, and must be comprehended in the overall risk The MATC and AF could not be determined for Pimentel and assessment. Bulkely's<sup>(45)</sup> rainbow trout experiments, in that acute tests only were conducted; chronic experiments relative to fluoride toxicity were not conducted.



## TABLE 43

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MATC, LC<sub>50</sub>, AF VALUES D. magna and C. affinis/dubia

Test Organism and Temperature	Water T <i>y</i> pe (mg/L as CaCO <sub>3</sub> )	MATC (mg F <sup>-</sup> /L)	LC <sub>50</sub> (mg F7/L)	Individual AF (%)	Average AF (%)
<u>D. magna</u> 20 <sup>0</sup> C	Very Hard (283) Hard (181) Moderately Hard (117) Semi-Soft	34.0 - 48.1 26.1 - 35.5 25.9 - 41.2 NA	342 267 193 NA	9.9 - 14.1 9.8 - 13.3 13.4 - 21.3 NA	11.0 - 16.2
<u>C. affinis/dubia</u> 25 <sup>0</sup> C	Very Hard (290) Hard (189) Moderately Hard (124) Semi-Soft (77)	16.3 - 26.1 16.2 - 26.2 NA 10.4 - 17.4	197 178 157 133	8.3 - 13.2 9.1 - 14.7 NA 7.8 - 13.1	8.4 - 13.7

Table 43 shows higher MATC values for the <u>D. magna</u> than that for <u>C.</u> <u>affinis/dubia</u>, by an average of approximately 70%. The AF values show relatively good agreement.

A factor to be considered is that the chronic tests for the <u>D. magna</u> <u>na</u> were conducted at  $20^{\circ}$ C, whereas those for the <u>C. affinis/dubia</u> were conducted at  $25^{\circ}$ C. No chronic tests were conducted on the same organism at different temperatures, and the effect on MATC is not known. Rand and Petrocelli<sup>(98)</sup> state (as has been quoted previously): ". . . Limited evidence suggests that thresholds of sublethal effect may be about the same at all water temperatures. . ." It is interesting to observe the potential effect on the <u>D. magna</u> MATC at  $25^{\circ}$ C, holding the AF constant. The results of this calculation are shown in Table 44; the MATC for the <u>D. magna</u> are still substantially above those for the <u>C. affinis dubia</u>. However, not knowing the effect of temperature, and following Rand and Petrocelli's statement of non-effect of temperature, our calculations will be based on the data of <u>D. magna</u> at  $20^{\circ}$ C and <u>C. affinis/dubia</u> at  $25^{\circ}$ C.

TABL	F	44	

CALCULATED MATC FOR D. magna at 25<sup>0</sup>C

Water Type (mg/L as CaCO <sub>3</sub> )	LC <sub>50</sub> (mg F <sup>-</sup> /L)	AF (%)	MATC (mg F <sup>-</sup> /L)
Very Hard (290)	248	9.9 - 14.1	24.5 - 35.0
Hard (189)	172	9.8 - 13.3	16.9 - 22.9
Moderately Hard (124)	100	13.4 - 21.3	13.4 - 21.3
Semi-Soft (77)	NA	NA	NA

In the interests of attempting to narrow the range of the MATC, an analysis was made based on specific data points in the <u>D. magna</u> experiment. For example, from Figure 9, equivalent neonate production was achieved at fluoride levels of 47, 31, and 28.5 mg  $F^{-}/L$  in very hard, hard, and moderately hard waters, respectively; these may be considered to be single-value MATC or "safe concentration" (SC). These would provide corresponding AF values of 0.137, 0.116 and 0.148, respectively. These AF values are 38%, 18% and 10% (average 22%) above the minimum values of the AF in Table 43, and indicate that the low end of the AF are a conservative estimate. If the minimum values of the AF

are multiplied by the 122% factor, resulting values are 0.121, 0.120 and 0.163. It is judged that an overall AF of 12.0% for the <u>D. magna</u> could be utilized without introduction of an unusual large element of risk. Because of the variability of data in the case of the <u>C, affinis/dubia</u> conservatism would dictate that we stay with the average minimum AF value of 8.4% (per Table 43).

Utilizing these AF factors, then, of 12% and 8.4%, safe concentrations (SC) can be calculated as shown in Table 45. These values are plotted in Figure 25.

Figure 25, then, provides us our searched-for criterion. The lowest (safest) values are shown by the starred line. The intersection of the two lines occurs at a hardness of 70 mg/L as  $CaCO_3$ . It is suggested that the minimum value at the low hardness levels correspond to the maximum drinking water standard, or 2.4 mg F<sup>-</sup>/L, which occurs at a hardness of 46 mg/L as  $CaCO_3$ . The suggested criterion, then, is a three-tiered model relative to hardness ranges of 0-46, 47-70, and >70 mg/L as  $CaCO_3$ . Utilizing the equations of the curves of Figure 25, the suggested criterion is:

HARDNESS (H) (mg/L as CaCO <sub>3</sub> )	(mg F <sup>-</sup> /L)	
0 - 46	SC = 2.4	
47 - 70	SC = (48.6 log H) - (78.7)	Eq. 10-3
> 70	SC = (8.56 log H) - (4.6 )	Eq. 10-4

## TABLE 45

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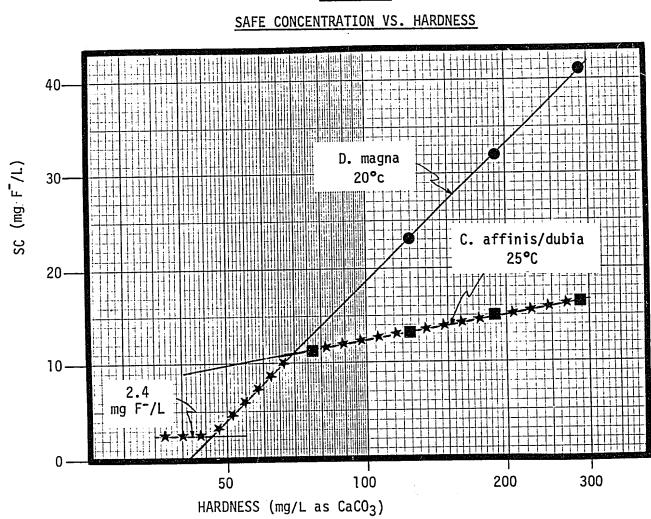
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# SC ON BASIS OF SELECTED AF

# D. magna AND C. affinis/dubia

	D. ma	D. magna at 20 <sup>0</sup> C			C. affinis/dubia at 25 <sup>0</sup> C		
Water Type (mg/L as CaCO <sub>3</sub> )	LC <sub>50</sub> (mg F <sup>-</sup> /L)	AF (%)	SC (mg F <sup>-</sup> /L)	LC <sub>50</sub> (mg F <sup>-</sup> /L)	AF (%)	SC (mg F <sup>-</sup> /L)	
Very Hard (290)	342	12	41.0	197	8.4	16.5	
Hard (189)	267	12	32.0	178	8.4	15.0	
Moderately Hard (124)	193	12	23.2	157	8.4	13.2	
Semi-Soft (77)	NA	NA	NA	133	8.4	11.2	

\*. .



In the interests of simplicity, it is tempting to ignore the <u>D</u>. <u>magna</u> portion of the curve below 70 mg/L hardness level, and establish a single equation criterion; however, the difference in SC values between the two curves is substantial (e.g., 2.4 mg  $F^{-}/L$  vs. 9.4 mg  $F^{-}/L$  at a hardness of 46 mg/L) and conservatism requires the multiple tiers be maintained. Perhaps future research will more clearly define the toxicity effects at the low end of the hardness scale.

An overall MATC based on the values of Table 45 would be 11.2-41.0 mg F<sup>-</sup>/L.

A close approximation to the multiple equation values (Eq. 10-3 and 10-4) can be obtained from the equation:

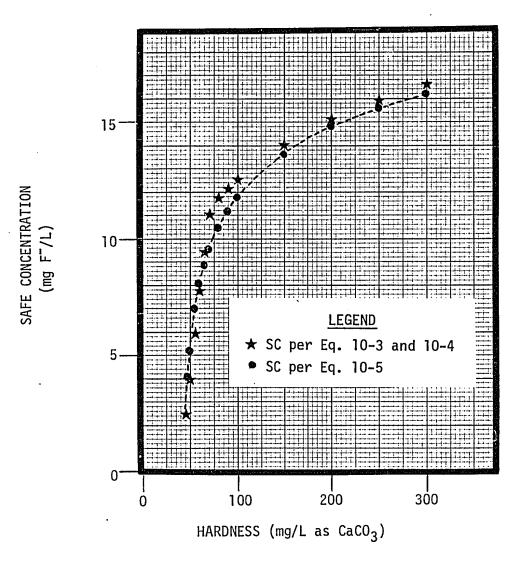
SC =  $3.45 \log (H - 44)^{1.95}$  Eq. 10-5 where:

SC = safe concentration in mg  $F^{-}/L$  (minimum value = 2.4) H = water hardness in mg/L as CaCO<sub>3</sub>

Figure 26 shows a plot of this equation together with a plot of Equation 10-3 and 10-4. The single equation closely approximates the multiple equation values; it slightly over-estimates the SC in the hardness range from 47-62 by an average value of approximately 0.8 mg  $F^{-}/L$ , and slightly underestimates the SC in the hardness range from 63-300 by an average value of approximately 0.6 mg  $F^{-}/L$ . Other equations are possible, of course; however, Eq. 10-5 is suggested because of its close approximations and where deviations do occur, they mainly tend to under-

## FIGURE 26

## SINGLE VS. MULTIPLE EQUATION CRITERION



estimate SC's, including projections out to the 1000 mg/L hardness level. At a hardness of 1000, the difference between the two models is less than 5%.

Having established a criterion, but before leaving this chapter, it is prudent to make a retrospective review of our calculations to determine if we have affected our risk level.

In section 10.2 of this chapter, we judged risk levels at a minimum when viewed relative to the various factors of aquatic environment, test organisms, laboratory procedures and practices, statistical methods, and comparison with other research data.

In establishment of our models and criterion, we have followed the lead of the EPA, Rand and Petrocelli, and others relative to the concepts of NOEC, LOEC, MATC, and AF, which have been fairly well accepted in the field of aquatic toxicology. Items of uncertainty in our calculations occur primarily at three points:

- The handling of data relative to significant and not-significant fluoride levels for two of the <u>C. affinis/dubia</u> data points refer to Table 36 and the discussion in this section on page 105.
- The combination of results for <u>D. magna</u> at 20<sup>o</sup>C and for <u>C. affin-is/dubia</u> at 25<sup>o</sup>C. The discussion is contained in this section on page 141.

- The selection of overall AF factors, as discussed on page 142.

It is believed that a reasoned approach was taken on these factors as discussed in the text, and, although the level of uncertainty has been elevated, it is judged that no unusually large risk factors were introduced.

It is believed that from an overall viewpoint, risks associated with accuracy and quality in this project have been kept to a minimum. A final element of uncertainty, and therefore risk, is associated with the reproducibility of data and testing of the models by other researchers. A recent publication of Grothe and Kimerle $^{(101)}$  describes the results of a nine-laboratory round-robin study of acute toxicity of a spe-Specific test procedures were folcific effluent to Daphnia magna.  $EC_{50}$  ranged from 10.0 to 26.4% effluent and from 3.5 to 9.1% lowed. effluent for 24 hour and 48 hour exposure periods, for an overall factor of 2.6 between the highest and lowest values. Pooled variability of the test data corresponded to a 22% coefficient of variation for the 24 hour tests and 16% for the 48 hour tests. The authors comment favorably on the low variation level, and indicate that the test reproducibility "appears to be as good as, if not better than, commonly accepted analytical methods, as is evident from the results of the ASTM and Chemical Manufacturing Association analytical round-robin studies." Perhaps this is the area of greatest uncertainty in establishing a criterion.

It is interesting to compare our results with the MATC established

by Mr. D. Olson of the Environmental Research Laboratories (46,47) referred to in Chapter 2. Our proposed tentative criterion ranges from 2.4 mg F<sup>-</sup>/L to approximately 16 mg F<sup>-</sup>/L (at a hardness of 300); the MATC established by Olson, based on unknown hardness and temperature, is 6.8 to 13.6 mg/L for fathead minnows, which fits readily into the central portion of our range.

A final word on the criterion - it is suggested that the developed criterion be termed a "tentative criterion", until additional research can be conducted relative to other species, temperatures, and long-term bioconcentration factors; suggestions for additional research are contained in Chapter 12. Although data generated in this project do not fulfill the complete requirements as listed by the EPA for establishing a criterion (see section 10.1 of this chapter), it apparently significantly supplements previous work done on fluoride toxicity and is judged to be sufficient to establish a preliminary or tentative standard in the interests of providing targets and guidance to industry in controlling their effluents.

#### 11.0 CONCLUSIONS

Acute and chronic fluoride toxicity experiments have been completed for the aquatic organisms <u>Daphnia magna</u> and <u>Ceriodaphnia affinis/dubia</u>, and acute tests for <u>Pimephales promelas</u>. These experiments involved a total of approximately 4000 organisms, conductance of more than 3000 chemical and physical tests, and consumed approximately 5500 manhours.

Conclusions from these experiments include:

- A. <u>D. magna</u>, <u>C. affinis/dubia</u>, and <u>P. promelas</u> all exhibit toxic effects when exposed to water solutions of sodium fluoride.
- B. Acute toxicity to <u>D. magna</u> and <u>C. affinis/dubia</u> is modified by the parameters of water hardness and temperature. The relationship between these modifiers and LC<sub>50</sub> may be expressed by the following model:

$$LC_{50} = C_1 (\log \in -C_2)$$

where:	LC <sub>50</sub> = median lethal concentration in mg
	F <sup>-</sup> /L as measured by a selective ion
	electrode
	$\in = (H) (33.4^{\circ}C - T)$
	H = water hardness in mg/L as CaCO <sub>3</sub>
	T = water temperature in <sup>O</sup> C
	C <sub>1</sub> , C <sub>2</sub> = constants for a given species.

Specifically:

For D. magna: 
$$LC_{50} = 370 \ (\log \in -2.7)$$

For <u>C. affinis/dubia</u>:  $LC_{50} = 115$  (log  $\in$  - 1.646)

The above model is based on experiments conducted in the temperature range of  $15-25^{\circ}$ C, and water hardnesses of approximately 70-300 mg/L as CaCO<sub>3</sub>.

C. Acute toxicity to <u>P. promelas</u> is modified by water hardness; the following model expresses this relationship at 20<sup>0</sup>C:

$$LC_{50} = 126 (\log H - 111)$$

where  $LC_{50}$  and H are as defined above. The model is based on experiments at 20<sup>o</sup>C in water hardnesses ranging from 72 - 260 mg/L as CaCO<sub>3</sub>.

- D. The acute toxicity of fluoride to <u>P. promelas</u> at  $20^{\circ}$ C at various hardnesses is similar to that of <u>S. gairdneri</u> at  $12^{\circ}$ C.
- E. Chronic exposure of <u>Daphnia magna</u> to fluorides at 20<sup>o</sup>C over a period of 21 days is accompanied by a stimulation effect in reproduction. Low concentrations of fluoride cause a delayed but large increase in egg production accompanied by a reduced

hatchability rate, with a net result of some increase in neonate production. The concentrations at which this phenomena occurs vary with the hardness of the water. The largest number of neonates produced per adult occurs in hard water which is generally considered to be the optimum environment for D. largest increase proportional to neonate magna. The production in the controls occurs in waters harder and softer With increasing fluoride concentrations, than the optimum. neonate production increases, reaches a maximum, and then declines, ultimately to zero production. The fluoride concentration at which the declining curve reaches an equivalent neonate production (ENP) relative to the controls varies with hardness. In very hard water, the ENP concentration was determined to be 47 mg F<sup>-</sup>/L; in hard water, 31.5 mg F<sup>-</sup>/L, and in moderately hard water, 28.5 mg F<sup>-</sup>/L. These values fit the equation:

$$\log F_{ENP} = \left[0.0028 \left(\frac{H}{100}\right)^{5.1}\right] + \left[1.45\right]$$

This equation is based only on three data points, it is one of many possible models and should be considered as "preliminary" only.

F. Chronic exposure of <u>Ceriodaphnia affinis/dubia</u> to fluorides at  $25^{\circ}$ C over a period of 7 days does not show the stimulation effect such as experienced in the <u>D. magna</u> experiments. No

increase in egg production was effected in any of the water types tested, nor any significant increase in neonate production relative to that in the controls. Earliest and highest egg and neonate production occurs in hard water, with significant reductions in other water types of both higher and lower hardnesses.

- G. Application factors (AF) for <u>D. magna</u> at 20<sup>o</sup>C may range from 9.8% to 21.3% with 12% judged to be a reasonable value. Application factors for <u>C. affinis/dubia</u> at 25<sup>o</sup>C may range from 8.3% to 14.7%, with 8.4% judged to be reasonable.
- H. Maximum acceptable toxicant concentrations (MATC) will vary with hardness. MATC ranges for <u>D. magna</u> are estimated as follows:

Water Hardness (mg/L as CaCO <sub>3</sub> )	M.A.T.C. (mg/L F <sup>-</sup> /L)
Very Hard (283)	34 - 48
Hard (181)	26 - 35
Mod. Hard (117)	26 - 41

MATC ranges for C. affinis/dubia are estimated as follows:

Water Hardness (mg/L as CaCO <sub>3</sub> )	M.A.T.C. (mg/L F <sup>-</sup> /L)
Very Hard (290)	16 - 26
Hard (189)	16 - 26
Mod. Hard (77)	10 - 17

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A tentative criterion can be established based on the following model:

Hardness (H) (mg/L as CaCO <sub>3</sub> )	Safe Concentration (mg F <sup>-</sup> /L)
0 - 46	SC = 2.4 (minimum value)
47 - 70	SC = (48.6 log H) - (78.7)
> 70	SC = (8.56 log H) - (4.6)

All safe concentrations calculated on the basis of this model fall either below or near the minimum values of the MATC ranges listed in paragraph (H) above.

J. An approximation of the criterion concentrations can be calculated from the following model:

SC =  $3.45 \log (H - 44)^{1.95}$ 

with a minimum value of 2.4 mg  $F^{-}/L$ .

K. All of the above models are based on limited data, and subject to validation by additional study in both the laboratory and field. Additional data points for all models would increase validity and allow additional statistical analyses of data. Importantly, bioconcentration factors must be determined as a potential overriding factor relative to the listed models. Chapter 12 lists additional research projects which should be completed before a firm minimum-risk criterion can be established.

## 12.0 FUTURE RESEARCH

Additional research is recommended in order to validate and expand the scope of our models and establish a firm criterion for fluoride toxicity. The following projects are recommended:

- A. Additional acute toxicity experiments with <u>P. promelas</u> at different temperatures to establish a temperature-dependent model.
- B. Chronic experiments with <u>D. magna</u>, <u>C. affinis/dubia</u>, and a vertebrate, at different temperatures, to determine whether chronic toxicity is independent of temperature, or if the toxicity is dependent upon temperature as in the acute case.
- C. Additional acute toxicity experiments to meet EPA's minimum data base; four species have been completed - four additonal sets of experiments will be required, of which one should be with a benthic crustacean, and one should be with a benthic insect. At least one of the benthic organisms should be a detritivore.
- D. Experiments to establish additional acute/chronic ratios; two additional species are recommended, of which one should be a freshwater fish. This could be effected through proper choice of species in (B) and (C) above.

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- E. Chronic experiments on a freshwater alga or vascular plant.
- F. Experimental program to determine bioconcentration factors, maximum permissible tissue concentrations, and final residue values, extended over a complete food chain.
- G. Successive generation survival and reproduction experiments with <u>D. magna</u> to establish the effect of acclimation to fluorides.
- H. Toxicity to selected microorganisms, inasmuch as continued self-purification of rivers and streams is essential.
- I. Additional research on all experiments described in this report so as to obtain additional data points, leading to an increased validity and laboratory verification.
- J. Field verification of laboratory-based models.

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APPENDIX A

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#### APPENDIX A

# DERIVATION OF ACUTE TOXICITY MODELS

## Daphnia magna model

- 1. Given: Figure 5.
- 2. Goal: Establish mathematical model fitting Figure 5 for the parameters of hardness and temperature.
- 3. Examples of calculations are for 15<sup>0</sup>C; calculations for other temperatures follow the same form.
- 4. Obtain slope of curves. For 15<sup>0</sup>C, use (x,y) values of (265, 371) and (28.5, 0).

Slope = (371 - 0)/(log 265 - log 28.5) = 383

5. Form of equation at 15<sup>o</sup>C:

 $LC_{50} = 383 (\log H - \log 28.5)$ 

or in general terms:

 $LC_{50} = 383 \log (H/C)$ where C = intercept on x-axis when y = 0

6. We have:  $LC_{50}$  = function of (H)(1/C)

we need: relationship between 1/C and Temperature (T):

- At  $15^{\circ}C$ :  $1/C_{15} = 1/28.5 = 0.03509$ At  $20^{\circ}C$ :  $1/C_{20} = 1/35.8 = 0.02793$ At  $24.9^{\circ}C$ :  $1/C_{24.9} = 1/64.9 = 0.01541$ Plot these 1/C values against T on linear scales.
- 7. From plot in step 6, obtain slope and intercept, to establish relationship between C and T:

$$1/C = 0.0663 - 0.001985T$$

8. Overall equation, then:

$$LC_{50} = 383 \log [(H) (0.0663 - 0.001985T)]$$

9. Simplify equation so as to eliminate factors <1.0:

$$LC_{50} = 383 \log [(H/504) (33.4 - T)]$$

10. Test model against Figure 5. Adjust parameters incrementally until computer optimizes solution as measured by minimum deviation from Figure 5 and Table 12 - MAAM values:

$$LC_{50} = 370 \log \left[ (H/504) (33.4 - T) \right]$$

11. Place H and T factors together in one term, and factor:

Ceriodaphnia affinis/dubia model

The procedure for establishing this model is analogous to that for the  $\underline{D}$ . magna through step 6. These first six steps provide a general equation:

 $LC_{50} = 115.5 \log (H/C)$ and 1/C values of: At 15.2<sup>o</sup>C: 1/C<sub>15.2</sub> = 3.868 At 20<sup>o</sup>C: 1/C<sub>20</sub> = 0.296 At 24.9<sup>o</sup>C: 1/C<sub>24.9</sub> = 0.183

7. In a plot of 1/C vs. T, the 1/C value at  $15.2^{\circ}$ C is quite removed from the values at 20 and  $24.9^{\circ}$ C. A straight line relationship (such as observed in the <u>D. magna</u> case) cannot be established between the data points. A projection of (x,y) values of (15.2, 3.868) with either (20.0, 0.296) or (24.9, 0.183) yield maximum temperature at y = 0 of 20.4°C and 25.3°C, which are not reasonable. However, a straight line relationship between (20.0, 0.296) and (24.9, 0.183) yields the equation:

1/C = (0.752 - 0.0226T)

Assume this relationship for the purposes of steps 8 and 9.

8. Overall equation:

$$LC_{50} = 115.5 \log [(H) (0.752 - 0.0226T)]$$

9. Simplifying:

$$LC_{50} = 115.5 \log [(H/44.3) (33.3 - T)]$$

10. Note that the term (33.3 - T) very nearly matches the term (33.4 -T) of the <u>D. magna</u> model. It appears, based on the limited data given above, that there may be a basic temperature relationship for the two species. If this is a true relationship, and we use the factor (33.4 - T) as perhaps being more accurate than the (33.3 - T) factor because of the decision made in step 7, then the overall equation becomes:

$$LC_{50} = 115.5 \log [(H/44.3) (33.4 - T)]$$

11. Test the model and adjust for minimum deviation:

$$LC_{50} = 115 \log [(H/44.3)(33.4 - T)]$$

12. Place H and T in one term and factor:

 $LC_{50} = 115 \log ( \in -1.646 )$ where:  $\in = (H)(33.4 - T)$ 

## Common Model

The common model for both species then may be expressed as follows:

LC<sub>50</sub> = C<sub>1</sub> (log 
$$\in$$
 - C<sub>2</sub>)  
where:  $\in$  = (H)(33.4 - T)  
C<sub>1</sub> C<sub>2</sub> = constants for a given species

APPENDIX B

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## APPENDIX B

# CHEMICALS AND MATERIALS

1.	Sodium bicarbonate NaHCO <sub>3</sub> powder, analytical reagent, Mallinckrodt No. 7412.
	Maximum limits of impurities:
	Ammonium (NH <sub>A</sub> )

Ammon1um ( $NH_4$ )
Calcium, magnesium and
R <sub>2</sub> 0 <sub>3</sub> Ppt 0.020%
Chloride (Cl) 0.003%
Heavy metals (as Pb)
Insoluble matter
Iron (Fe) 0.001%
Phosphate (PO <sub>4</sub> ) 0.001%
Potassium (K) 0.005%
Sulfur compounds (as SO <sub>4</sub> ) 0.003%
Assay (NaHCO <sub>3</sub> )

2. Calcium sulfate CaSO<sub>4</sub> <sup>2</sup>H<sub>2</sub>O powder, analytical reagent, Mallinckrodt No. 4300. Maximum limits of impurities:

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Chloride	0.002%
Heavy metals (as Pb)	
Insoluble matter	
Iron (Fe)	
Magnesium and alkali salts	
Nitrate $(NO_3)$	
3	

 Magnesium sulfate, MgSO<sub>4</sub>, anhydrous, certified, Fisher No. M-65. Actual lot analysis:

Ammonia (NH <sub>3</sub> )	0.003%
Arsenic (As)	
Calcium (Ca)	.0.04%
Manganese (Mn)	.0.001%
Nitrate $(NO_3)$	0.005% 1.2%
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4. Potassium chloride, KCl, granular, analytical reagent, Mallinckrodt. Maximum limits of impurities:

> Barium (Ba) . . . . . . . . . . . . . 0.001% Bromide (Br) . . . . . . . . . . . . . . . . 0.01% (approx.) Calcium, magnesium and  $R_2O_3$  Ppt . . . . . . . . . . . . 0.005% Chlorate (ClO<sub>3</sub>) . . . . . . . . . . . 0.001% Heavy metals (as Pb) . . . . . . . . . . . . . 0.005% Insoluble matter . . . . . . . . . . . . . . . . . 0.005% Iodide (I) . . . . . . . . . . . . . . . . . . 0.002% Iron (Fe) . . . . . . . . . . . . . . . . . 0.003% Neutrality . . . . . . . . . . . . . . . 0.003% Nitrogen compounds (as N) . . . . . . . . 0.001% Phosphate (PO<sub>4</sub>) . . . . . . . . . . . . . 0.001%

5. Sodium fluoride, NaF, powder, analytical reagent, Mallinckrodt No. 7636. Maximum limits of impurities:

Chloride (Cl) 0.005%
Free acid (HF)
Free alkali (as Na <sub>2</sub> CO <sub>3</sub> ) 0.10%
Heavy metals (as Pb)
Insoluble matter
Iron (Fe) 0.003 (approx.)
Loss on drying at 150 <sup>0</sup> C 0.3%
Sodium fluosilicate (Na <sub>2</sub> SiF <sub>6</sub> ) 0.10%
Sulfate (SO <sub>4</sub> ) 0.03%
Sulfite (SO <sub>3</sub> ) 0.005%

6. Sodium fluoride, NaF, powder, analytical reagent, Mallinckrodt No. 7636. Certificate of lot analysis:

Chloride (Cl) 0.002%
Heavy metals (as Pb) <0.003%
Insoluble matter <0.02%
Iron (Fe) Passes test
Loss on drying at 150 <sup>0</sup> C 0.03%
Sodium fluosilicate (Na <sub>2</sub> SiF <sub>6</sub> ) 0.01%
Sulfate (SO <sub>4</sub> )
Sulfite (SO <sub>3</sub> ) 0.0005%
Titratable acid (HF) <0.003 meq/gm
Titratable base (as Na <sub>2</sub> CO <sub>3</sub> ) 0.004 meq/gm

7. Sodium carbonate, Na<sub>2</sub>CO<sub>3</sub>, anhydrous powder, analytical reagent, Mallinckrodt No. 7521. Maximum limits of impurities:

Ammonium hydroxide Ppt
Arsenic (As)
Calcium and magnesium Ppt
Chloride (Cl) 0.001%
Heavy metals (as Pb)
Insoluble matter
Iron (Fe) 0.0005%
Loss on heating at 285 <sup>0</sup> C
Nitrogen compounds (as N) 0.001%
Phosphate (PO <sub>4</sub> ) 0.001%
Potassium (K) 0.02%
Silica (SiO <sub>2</sub> ) 0.005%
Sulfur compounds (as $SO_4$ ) 0.003%

- Hydrochloric acid, HCl, reagent A.C.S., Fisher No. A-144, analysis: 8. Assay (HC1) . . . min. 36.5% max. 38.0% Maximum limits of impurities: • Residue after ignition . . . . . . 0.0004% Sulfate (SO<sub>4</sub>) . . . . . . . . . . 0.00008% Sulfite (SO<sub>3</sub>) . . . . . . . . . . 0.00008% Extractable organic substances (not more than about 0.0005%) . . . . passes A.C.S. test Free chloride (Cl) (limit about 0.00004%) . . . . . . . . . . . . . . . . . passes A.C.S. test Iron (Fe) . . . . . . . . . . . . 0.00001%
- 9. Calcium chloride, CaCl<sub>2</sub>, anhydrous, analytical reagent, Mallinckrodt No. 4128. Meets A.C.S. specifications, maximum limits of impurities:

Alkalinity (as Ca(OH) <sub>2</sub> ) 0.020%
Heavy metals (as Pb)
Iron (Fe) 0.001%
Magnesium and Alkali salts (as
sulfates)
Sulfate 0.02%
Assay (CaCl <sub>2</sub> )

Potassium phosphate monobasic (crystals), KH2PO4, analytical reagent, 10. Mallinckrodt No. 7100. Meets A.C.S. specifications, maximum limits of impurities: Chloride (C1) . . . . . . . . . . . . 0.001% Insoluble matter, calcium and Iron (Fe) . . . . . . . . . . . . 0.002% Loss on drying over  $H_2SO_4$  . . . . . 0.20% Nitrogen compounds (as N) . . . . . . 0.001% Sodium (Na) . . . . . . . . . . . . . 0.005% Sodium nitrate, NaNO3, Sigma No. S-5506, lot 68C-0588. 11. Sodium silicate, meta-crystals, Na<sub>2</sub>SiO<sub>3</sub>'9H<sub>2</sub>O, certified, Fisher No. 12. S-408, FW 284.20. Certificate of actual lot analysis: Chloride (Cl) . . . . . . . . . . . . . . 0.01% Sulfate (SO<sub>4</sub>) . . . . . . . . . . . 0.003% Iron (Fe) . . . . . . . . . . . . . . 0.001% 13. Disodium ethylenediamine - tetraacetate, Na<sub>2</sub>C<sub>10</sub>H<sub>14</sub>O<sub>8</sub>N<sub>2</sub>·2H<sub>2</sub>O, certified A.C.S., Fisher No. S-311. Certificate of actual lot analysis: pH of a 5% solution . . . . . . . . . 4.3 Iron (Fe) . . . . . . . . . . . . . 0.003% Nitrilotriacetic acid, (HOCOCH<sub>2</sub>)<sub>3</sub>N . . .0.1%

14. Ferric chloride, FeCl<sub>3</sub><sup>•</sup>6H<sub>2</sub>O, lumps, analytical reagent, Mallinckrodt No. 5029. Meets A.C.S. specifications, maximum limits of impurities:

15. Cupric sulfate, CuSO<sub>4</sub><sup>•</sup>5H<sub>2</sub>O, fine crystals, analytical reagent, Mallinckrodt No. 4844. Meets A.C. S. specifications. Maximum limits of impurities:

Ammonium sulfide metals other than

iron (as Ni)
Chloride
Insoluble matter
Iron (Fe) 0.002%
Nitrogen compounds (as N) 0.001%
Substances not ppted. by $H_2S$ 0.10%

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Cobalt chloride, CoCl<sub>2</sub>.6H<sub>2</sub>O, crystals, analytical reagent, Mallin-16. ckrodt No. 4532. Meets A.C.S. specifications, maximum limits of impurities: Copper (Cu) . . . . . . . . . . . . . 0.002% Iron (Fe) . . . . . . . . . . . . . . 0.001% Lead (Pb) . . . . . . . . . . . . 0.005% Nickel (Ni) . . . . . . . . . . . . . 0.15% Nitrate (NO<sub>3</sub>) . . . . . . . . . . . . 0.01% Substances not ppted. by  $(NH_4)_2S$ (as sulfates) . . . . . . . . . . . 0.25% Sulfate (SO<sub>4</sub>) . . . . . . . . . . . 0.010% Zinc (Zn) . . . . . . . . . . . . . . 0.01% Zinc sulfate, ZnSO4 '7H<sub>2</sub>O, crystals, analytical reagent, Mallinckrodt 17. No. 8880. Meets A.C.S. specifications, maximum limits of impurities: Chloride (Cl) . . . . . . . . . . . . 0.0005% Free acid . . . . . . . . . . . . . to pass test Iron (Fe) . . . . . . . . . . . . . . 0.001% Lead (Pb) . . . . . . . . . . . . 0.003% Nitrate (NO<sub>3</sub>) . . . . . . . . . . . 0.002% Substances not ppted by  $(NH_4)_2S_1$ (as sulfates) . . . . . . . . . . . 0.20%

18. Manganese chloride, MnCl<sub>2</sub>·4H<sub>2</sub>O, crystals, certified, Fisher No. M-87. Certificate of analysis:

Insoluble matter
Sulfate (SO <sub>4</sub> ) 0.001%
Alkalies and earths 0.10%
Heavy metals (as Pb)
Iron and cobalt (as Fe) 0.0008%
Nickel (Ni) 0.001%
Zinc (Zn) 0.02%

19. Sodium molybdate, Na<sub>2</sub>MoO<sub>4</sub> <sup>2</sup>H<sub>2</sub>O, crystals, analytical reagent, Mallinckrodt No. 7882. Maximum limits of impurities:

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Ammonium (NH <sub>4</sub> )
Chloride (C1) 0.005%
Heavy metals (as Pb)
Insoluble matter
Iron (Fe) 0.001%
Phosphate (PO <sub>4</sub> ) 0.0005%
Sulfate $(SO_4)$ 0.015%
Assay

20. Boric acid, H<sub>3</sub>BO<sub>3</sub>, granular, analytical reagent, Mallinckrodt No. 2549. Meets A.C.S. specifications, maximum limits of impurities:

Arsenic (As)
Calcium (Ca)
Chloride (Cl) 0.001%
Heavy metals (as Pb)
Insoluble in methanol 0.005%
Iron (Fe) 0.001%
Nonvolatile with methanol 0.05%
Phosphate (PO <sub>4</sub> ) 0.001%
Sulfate (SO <sub>4</sub> ) <sup>-</sup>

- 21. Total Ionic Strength Adjustment Buffer (TISAB); for use in fluoride determination, Orion Research catalog no. 94-09-09.
- 22. Hardness titrating solution (20), Calgon catalog no. R5011.
- 23. Hardness buffer solution, Calgon catalog no. R5001.
- 24. Hardness indicator powder, Calgon catalog no. R5292.
- 25. Standard (pH): Buffer Solution Concentrate, pH = 7.00 + 0.02, Fisher No. SO-B-109.
- 26. Standard (ph): Buffer Solution Concentrate, pH = 9.00 + 0.02, Fisher No. SO-B-139.
- 27. Standard (fluoride): Fluoride standard, Orion Research No. 940907.
- 28. Standard (hardness): Water hardness standard, Orion Research No. 923206.
- 29. Tetramin Staple Food (flakes); TetraWerke (W. Germany) No. T155-WL-16155; Ingredients: fish meal, torula dried yeast, feeding oat meal, shrimp meal, gluten, cod liver meal, algae meal, casein, chlorophyll, carotene, bixin (from natural sources - leaves and annatto tree seed). Guaranteed analysis:

Min.	crude	protei	n.	•	•	•	•	•	•	•	•	•	•	45%
Min.	crude	fat .		•		•		•	•	•	•	٠	•	5%
Max.	crude	fiber		•	•	•	•	•	•	•	•	•	•	7%
Max.	moist	ure		•			•	•	•	•	•	•	•	.8%
Max.	sodiu	n chlor	ide	•	•	•	•	٠	•	•	•	•	•	3%

APPENDIX C

## APPENDIX C

## EQUIPMENT

## 1. Fluoride Measurement

- A. Meter, Orion Research digital Ionalyzer, Model 601A, Serial A65600
- B. Fluoride electrode, Orion Research, catalog no. 94-09.
- C. Single junction reference electrode, Orion Research, catalog no. 900100.
- D. Magnetic stirrer, Fisher catalog no. 14-511-1V2
- E. Buffer, see Appendix B, item 21.
- F. Standard see Appendix B, item 27.

# 2. pH Measurement

- A. Meter, Corning Model 12.
- B. pH electrode, Orion Research no. 91-04-00.
- C. Magnetic stirrer, Cole-Parmer, Micro-V

## 3. Conductivity Measurement

A. Conductivity Bridge, YSI Model 31 (Yellow Springs Instrument Co, Inc.) Serial 433; Probe: 3403 Cell, K = 1.0

## 4. Refrigeration Unit

A. Refrigerator, Zimney Corp. (Monrovia, California), model 402.

## 5. Dissolved Oxygen Measurement

- A. Oxygen meter, YSF Model 57 (Yellow Springs Instrument Co., Inc.) Serial 9890
- B. Probe no. LN5631

# 6. <u>Scale</u>

A. Balance, Sartorius, Model 2462, Serial 131272.

# 7. <u>Still</u>

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A. Water distillation apparatus, Corning Model AG-2

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# Sensitivity of four New Zealand cladoceran species and *Daphnia magna* to aquatic toxicants

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Abstract Four New Zealand cladoceran species (Daphnia carinata, Simocephalus vetulus, Ceriodaphnia dubia, and Ceriodaphnia cf. pulchella) were compared with Daphnia magna for their acute (short-term) and chronic (long-term) sensitivity to toxicants. In acute tests with 5 reference toxicants and 2 effluent samples (chromium ( $Cr^{6+}$ ), pentachlorophenol, boron, fluoride, biocide (Alfloc 324), kraft bleach effluent pond, and a geothermal discharge), C. dubia was more sensitive than D. magna by up to a factor of 4. In chronic tests on 4 toxicants no differences in sensitivity were observed within 1 order of magnitude. Acute : chronic ratios ranged from 1.3 to 13.5. C. dubia is recommended as a routine test organism because of its good laboratory growth and higher sensitivity than D. magna. D. carinata would also be suitable, but both S. vetulus and C. cf. pulchella showed poor laboratory performance.

**Keywords** aquatic; toxicity; cladocerans; zooplankton; *Daphnia magna* 

## INTRODUCTION

Cladocerans, especially *Daphnia magna*, are extensively used for aquatic toxicity testing, and have been found to adapt well to laboratory conditions. They are often among the most sensitive aquatic organisms used for testing potentially toxic chemicals (Buikema et al. 1980; Mayer & Ellersieck 1986). The functional role of cladocerans in aquatic communities also favours them as test animals. They are among the most important converters of phytoplankton and bacteria in lakes into animal protein that is nutritionally valuable to higher organisms, such as fishes. In addition, they are convenient for laboratory use.

Of the cladocerans, D. magna has been used in most aquatic toxicity testing for a variety of reasons. Familiarity with the organism and the availability of a large data base may have contributed to its popularity with many workers. The relatively large size of D. magna, compared with other cladocerans. is a convenient feature. Standard methods have been developed for acute (short-term) tests for Daphnia toxicity assessment over 24 or 48 h (e.g., UK: HMSO1983; Europe: OECD 1981; Canada; Leonard 1979; US: APHA 1981). Recommendations have also been made for carrying out chronic (long-term) reproduction bioassays with Daphnia over 14 or 21 days (OECD 1981; EPA 1982). Acute tests have been used to provide an extensive database on toxicity levels for a wide range of test substances (e.g., Le Blanc 1980; Mayer & Ellersieck 1986). Long-term chronic tests have been used to estimate maximum acceptable toxicant concentrations (MATC) for pure materials (e.g., Winner & Farrell 1976) and complex effluents (e.g., EPA 1985a).

The disadvantage of *D. magna* is that the species is not native to New Zealand; our present knowledge of the sensitivity of New Zealand cladoceran species to a range of known toxicants is limited to minimal published overseas data for only 2 species (*Ceriodaphnia reticulata* and *Simocephalus vetulus*, Mount & Norberg 1984), although another species (*C. dubia*) has been recommended for whole-effluent toxicity testing (EPA 1985a, 1985b). Additionally, test protocols for *D. magna* require incubation at 20°C to offset mortality in the controls in chronic bioassays (EPA 1982), necessitating relatively long incubations to achieve sufficient reproduction. Performing rapid tests using other cladoceran species, which grow optimally at 25°C (e.g., *C.* 

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*dubia*, EPA 1985b), lowers the unit test cost and increases information output by increasing the throughput of tests.

To date the only cladoceran toxicity tests performed in New Zealand have used imported D. magna (Freeman 1986). Quarantine requirements for this species restrict a more general application for toxicity assessment by regulating authorities. The use of indigenous cladoceran species for ecotoxicity testing is highly desirable.

A species suitable as reference for toxicity tests should be:

- widely distributed;
- sensitive to common pollutants;
- ---- easy to maintain in the laboratory;
- taxonomically distinct and well described; and
- prominent in its ecological community.

Several indigenous cladoceran species potentially meet most of these requirements. They are

Daphnia carinata—the most widely distributed cladoceran inhabiting ponds, lakes, and reservoirs;

Ceriodaphnia dubia-in many lakes and ponds;

- *Ceriodaphnia* cf. *pulchella* (no information on distribution is yet available);
- Simocephalus vetulus—solely littoral or shallow water.

The characteristics of the above species and their wide distribution in New Zealand have been described but the taxonomy of *Ceriodaphnia* needs revision (Chapman & Lewis 1984; Chapman & Green 1987). *C. reticulata*, although present in New Zealand, has been noted in only a few scattered records (Chapman & Lewis 1984). Overseas data for toxicity testing on related genera suggest that we might anticipate each to be suitable for laboratory culture, and sensitive to a range of common pollutants (Mount & Norberg 1984).

Freeman (1986) evaluated *D. magna* using acute tests for a range of toxicants, but the setting of "safe" environmental levels requires additional knowledge of the chronic sensitivity to toxicants. Chronic test methodologies have been developed (e.g., Mount &Norberg 1984) and applied to both pure chemical (e.g., Winner & Farrell 1976) and industrial wastewater discharges (e.g., EPA 1985a), but are expensive to perform routinely. Commonly in the assessment of hazard to aquatic environments, only acute tests are performed and "safe" levels calculated with the help of previously determined "application factors" (acute : chronic ratios). No data on application factors for New Zealand species are available. This study firstly compares the relative sensitivity of several New Zealand cladoceran species and *D. magna* to a range of toxicants; secondly determines the range of application factors (acute : chronic ratios for calculating "safe" environmental levels from acute measurements); and thirdly facilitates comparisons with the international literature for chemical toxicity when assessing the impact of toxicants on local species.

### MATERIALS AND METHODS

A stock culture of *Daphnia magna* was obtained from Mrs D. M. M. Adema, Division of Technology for Society (TNO), Delft, The Netherlands. Stock cultures of Ceriodaphnia dubia, Ceriodaphnia cf. pulchella, and Simocephalus vetulus were isolated from Lake Rotoroa, Hamilton, New Zealand. Daphnia carinata was isolated from a drinking trough at a local showgrounds. Species identification followed the key of Chapman & Lewis (1984). Stock cultures were bred in 8 litres of medium composed of equal volumes of upper Waihou River water and standard dilution water (British Standard 1983) in glass tanks at  $20\pm2^{\circ}$ C. Organisms were fed on alternate days with 2 ml of nutrient solution (0.75 g beef extract and 0.75 g glucose per 100 ml stored at 4°C), and daily with the alga Selenastrum capricornutum. The volume of feed was adjusted for different species and organism densities so that a clearing of turbidity occurred in 24-48 h.

The standard dilution water (HMSO 1983; British Standard 1983) was prepared in ultrapure water (Nanopure System, Barnstead) which had been stored for at least 1 week in glass carboys. Final pH was 7.9  $\pm$  0.2, and hardness 250  $\pm$  25 g m<sup>-3</sup> (as CaCO<sub>2</sub>) with a Ca : Mg ratio of c. 4 : 1.

Before tests, adult cladocerans were sorted by size and isolated in separate 60 ml beakers containing 15 ml of media, so that released young less than 24 h old were available for tests the following day. For acute tests, 10 neonates were placed in 50 ml of test solution in a 60 ml disposable polystyrene beaker for 24 h. All controls and test concentrations were duplicated. For chronic tests, 10 individual neonates were each placed in 15 ml (*Ceriodaphnia*) or 40 ml of test solution (other species) in polystyrene beakers for 14 days. During the test the cladocerans were fed daily with a digested trout pellet-yeast diet (pellets incubated for 1 week in aerated river water, blended, settled (24 h, 4°C)) with supernatant used as feed modified by omission of "Cerophyll" from

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EPA 1985b) at a rate of 0.1 ml of food suspension per 15 ml of test solution for Ceriodaphnia and 0.15 ml per 40 ml for other cladoceran species. Test organisms were transferred to a fresh test solution every 48 h. Chemical analysis of water concentrations of toxicants were not tested. All tests were performed at 20  $\pm$  1°C in the dark. The 20°C incubation temperature was chosen for these experiments so that direct comparisons of toxic sensitivity could be made between species, and because unacceptable mortality occurs for D. magna controls in chronic bioassays at higher temperatures (EPA 1982). A serial dilution factor of 0.5 was used to prepare at least 5 dilutions encompasing the anticipated  $EC_{50}$ (effective concentration resulting in death of 50% of the test organisms) or LOEC (lowest observed effect concentration). The "anticipated" value was estimated from range-finding tests using a wide range of concentrations.

All chronic tests using C. cf. *pulchella* were abandoned because of high mortality in both control and test samples. Not all the combinations of organisms and toxicants used in the acute test could be tested because of equipment and personnel shortages.

For the calculation of the acute 24 h  $EC_{50}$  the number of mobile cladocerans was counted in each container. Those which did not move within 15 s following gentle agitation of the container were considered dead. The probit analysis procedure (Finney 1971) was used to obtain  $EC_{50}$  and  $EC_{10}$ values (g m<sup>-3</sup> for chemicals; % v/v dilution for effluents) (SAS 1985). For chronic tests the total number of young produced per adult female was measured for each test concentration and control. An analysis of variance (ANOVA) with Tukey's significance test (one-tailed, P = 0.05) was used to compare each toxicant concentration mean with the control mean to determine the NOEC (no observable effect concentration), and the LOEC. The MATC (maximum acceptable toxicant concentration) was calculated as the geometric mean of the NOEC and LOEC.

Five reference toxicants and 2 effluent samples were used in tests: Chromium (Cr<sup>6+</sup>), pentachlorophenol (PCP), boron, fluoride, biocide (Alfloc 324), kraft bleach effluent pond, and a geothermal discharge. Hexavalent chromium (Cr<sup>6+</sup>, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, AR grade) has been proposed as a reference toxicant (Lee 1980; HMSO 1983). Pentachlorophenol (PCP, C<sub>6</sub>Cl<sub>5</sub>OH, analytical grade) has been proposed as an organic reference toxicant (Lee 1980) as has the water-soluble sodium salt (EPA 1985b). Only PCP was available in New Zealand and a stock solution of 100 g 1<sup>-1</sup> was prepared in 95% ethanol. Solvent controls performed at the highest test concentration were never significantly different from the nonsolvent controls. Boron (H<sub>3</sub>BO<sub>3</sub>, AR grade) and fluoride (NaF, AR grade) were investigated because of concern about the toxicity of these elements derived from geothermal waters. A biocide, Alfloc 324 (standard grade, Catoleum (NZ) Ltd, Auckland) has industrial applications which could result in environmental discharge. Alfloc 324 is an acidic alcoholic solution of a non-ionic dispersant and substituted isothiazolinones. Geothermal effluent was obtained from the main drain of the Wairakei geothermal field, and kraft bleach effluent from an aerated lagoon of a pulp and paper manufacturer.

#### **RESULTS AND DISCUSSION**

The acute toxicity (24 h EC<sub>50</sub> and EC<sub>10</sub>) values obtained for the 5 cladoceran species exposed to 2 effluent samples and 5 reference toxicants are shown in Table 1. The two *Daphnia* species showed similar sensitivities. *Simocephalus* and *Ceriodaphnia* were similar and generally more sensitive than the *Daphnia* species (except for the biocide).

Interspecific differences in EC<sub>50</sub> values were generally no more than a factor of 3, with a maximum 8-fold difference (between *C. dubia* (most sensitive) and *D. carinata* for  $Cr^{6+}$ ). Of the *Ceriodaphnia* species, *C.* cf. *pulchella* was generally less sensitive than *C. dubia*. *C.* cf. *pulchella* had high mortality (causing abandonment of several tests), and is therefore discussed only where adequate data exist.

Sensitivity differences were greater for EC<sub>10</sub> values with a maximum of 50-fold (between *S. vetulus* (most sensitive) and *C.* cf. *pulchella* for biocide), 7-fold (between *C. dubia* (most sensitive) and *D. carinata* for Cr<sup>6+</sup>), and 6-fold (between *S. vetulus* (most sensitive) and *D. magna* for boron) differences observed. The interspecies differences for calculated EC<sub>50</sub> and EC<sub>10</sub> values suggest that toxicant concentration (or dose) response relationships may vary significantly between species.

Calculation of  $EC_{50}$ :  $EC_{10}$  ratios can be used to indicate differences in concentration response between species. This relationship between the routinely reported  $EC_{50}$  acute response and the threshold  $EC_{10}$  value may be used to compare the sensitivity to toxicants of a wider range of species than the ACR (acute : chronic ratio) value which necessitates using more costly chronic tests. In effect the  $EC_{10}$  value is used as an indicator of the MATC value.  $EC_{50}$  :  $EC_{10}$  ratios were calculated from the data presented in Table 1 where 95% confidence limits had been estimated. For a given toxicant the  $EC_{50}$ :  $EC_{10}$  ratios for different species were generally similar with a median mean value of  $\sim 1.7$  (range 1.3-5.4; coefficients of variation 10-94%). Biocide data showed high variability of ratios. For a given cladoceran species, the  $EC_{50}$ :  $EC_{10}$  ratios for the range of toxicants tested gave a median mean value of 2.1 (range 1.8-4.0; coefficients of variation 27–104%). S. vetulus data showed the widest range of ratios. However, no significant differences were found between species which suggests that no one species was consistently more sensitive to threshold levels of toxicants. Rather the threshold response levels varied with the nature of the chemical toxicant and the test species. Thus, for acute tests using different cladoceran species, a 2fold (and up to 5-fold) difference in their threshold concentration sensitivity should be anticipated given comparable  $EC_{50}$  values.

The chronic toxicity (14-day NOEC and LOEC) values obtained for the 5 cladoceran species for 1 effluent sample and 3 reference toxicants are shown in Table 2. The 4 species successfully tested appeared to have similar sensitivities: for a given toxicant the MATC values for different species were within 1 order of magnitude.

Uncertainty associated with a calculated MATC value is indicated by the LOEC and NOEC values. The "true" no effect concentration could fall anywhere in the interval NOEC  $\pm$  (NOEC-LOEC). The choice of the serial dilution factor for a test determines the width of the NOEC-LOEC interval. EPA (1985b) report that for a serial dilution factor of 0.3 the NOEC could have a relative variability as high as  $\pm$  300%; and for a serial dilution factor of 0.5 the relative variability could be  $\pm$  100%. A factor of 0.5 was used for these experiments. Other factors which can affect test precision include test organism age, condition, sensitivity, temperature control, and feeding (EPA 1985b). For these tests the coefficient of variation (CV) of the number of neonates born in

Table 1 Comparison of acute toxicity measurements (mean value and 95% confidence limits, more accurately called Fiducial limits) for 5 cladoceran species and 7 toxicants. All values in g m<sup>-3</sup> except where indicated by  $\dagger$ , where % v/v dilution.

Sample		D. magna	D. carinata	S. vetulus	C. dubia	C. cf. pulchella
Kraft bleach	EC <sub>50</sub> †	76.5	91.9	60.5	66.6	38.7
effluent pond	201	(62.0–92.5)	(80.9–110.6)	(25.0–100)	(55.6–86.7)	(0-75.0)
-	EC10 <sup>†</sup>	52.6	75.0	28.6	50.0	14.6
	10	(25.4-64.0)	(44.1-84.0)	()	(23.1–58.7)	()
Geothermal	EC50†	~100	~100	26.8	25.7	43.2
discharge	50	(96.5–100)	(91.3–100)	(16.3–43.4)	(6.55–66.3)	(14.5–51.1)
-	EC10 <sup>†</sup>	71.5	86.7	19.0	10.0	33.2
		<u>(</u> 50.0–100)	(50.0-96.1)	(0.47–23.8)	(0.11420.7)	(1.75–41.6)
Biocide	EC <sub>50</sub>	6.57	10.1	1,80	11.1	14.1
(Alfloc 324)	50	(2.07–12.6)	(6.1–50.0)	(0.34-5.70)	(7.65–35.0)	(9.9–50.0)
	EC <sub>10</sub>	2.28	6.84	0.16	5.28	8.23
		(0.117–4.77)	(1.0-8.7)	(0.0006–0.63)	(0.94–7.66)	(-5.0-13.8)
Chromium (Cr <sup>6+</sup> )	EC <sub>50</sub>	0.224	0.423	0.154	0.053	0.196
		(0.143-0.353)	(0.317-0.570)	(0.005–0.5)	(0.039-0.079)	
	EC <sub>10</sub>	0.069	0.260	0.016	0.037	0.036
		(0.028–0.112)	(0.119–0.340)	(0.005–0.077)	(0.0086-0.046	
PCP	EC <sub>50</sub>	0.343	0.570	0.206	0.202	1.79
		(0.101-0.501)	(0.171–0.959)	(0.1410.294)	(0.097–0.259)	(0-2.7)
	$EC_{10}$	0.129	0.130	0.097	0.138	1.02
		(0.005–0.249)	(0.0033-0.310)	(0.040-0.142)	(0.019–0.189)	
Boron	EC <sub>50</sub>	319.8	267.7	123.4	180.6	101.2
		(264.3–496.7)	(191.4–376.1)	(33.6–196.5)	(101.1–232.2)	
	EC <sub>10</sub>	250.0	138,8	38.1	130.4	48.8
		(162.7–302.9)	(64.7–193.6)	(1.01–81.9)	(37.4–173.4)	(19.5–69.7)
Fluoride	EC <sub>50</sub>	353.6	353.6	201.5	157.9	83.2
	50	(250.9–498.2)	(250.9-498.2)	(142.6–279.4)	(110.0-225.1)	
	EC <sub>10</sub>	292.1	292.1	109.6	75.1	27.9
	10	(157.0-373.9)	(157.0–373.9)	(49.5–152.2)	(33.1–180.3)	()

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control tests ranged from 23 to 64% for *Daphnia* species and *C. dubia*. Control values were much higher for *S. vetulus* (213% CV for Cr<sup>6+</sup>; 117% CV for PCP) and *C.* cf. *pulchella* (224% CV for PCP), indicating that the laboratory test conditions were less suitable for these species. High CV values for the number of neonates born to control organisms greatly reduce the test precision for detecting the NOEC and hence the suitability of an organism for routine toxicity assessment.

Application factors may be derived from acute : chronic ratios (ACR) and applied to acute test results to determine "safe" environmental concentrations. Acute  $EC_{50}$  and  $EC_{10}$  values, together with chronic MATC and ACR values, are shown in Table 3. For the 4 cladoceran species (*C*. cf. *pulchella* excluded) and 5 toxicants tested the ACR values based on  $EC_{50}$ results ranged from 1.3 to 13.5 (median 4.5). When ACR values have not been measured and it is necessary to estimate MATC values from acute test

Table 2Comparison of chronic toxicity measurements for 5 cladoceran species and 5 toxicants.LOEC, lowest observable effect concentration; NOEC, no observable effect concentration; MATC,maximum acceptable toxicant concentration (geometric mean of NOEC and LOEC); -, not determined;TA, test abandoned. All values in g m<sup>-3</sup> except where indicated by †, where % v/v dilution.

Sample		D. magna	D. carinata	S. vetulus	C. dubia	C. cf. pulchella
Kraft effluent†	LOEC NOEC	75 50	75 50		•	-
Chromium (Cr <sup>6+</sup> )	MATC LOEC NOEC MATC	$\begin{array}{c} 61.2 \\ 0.100 \\ 0.025 \\ 0.050 \end{array}$	$\begin{array}{c} 61.2 \\ 0.100 \\ 0.050 \\ 0.071 \end{array}$	>0.100	- 0.010 Control 0.005	TA
РСР	LOEC NOEC MATC	$\begin{array}{c} 0.100 \\ 0.050 \\ 0.071 \end{array}$	0.500 0.250 0.354	0.100 0.050 0.071	$0.250 \\ 0.100 \\ 0.158$	ТА
Boron	LOEC NOEC MATC	32.0 18.0 24.0			18.0 10.0 13.4	-
Fluoride	LOEC NOEC MATC	>50.0	>50.0	-		-

Table 3 Comparison of acute/chronic ratios (ACR) calculated for toxicity tests on 4 cladoceran species. All values (taken from Table 1 and 2) in g m<sup>-3</sup> except where indicated by  $\ddagger$ , where % v/v dilution.

		Acute		MATC value		
		EC <sub>50</sub>	EC <sub>10</sub>	(NOEC*LOEC) <sup>1/2</sup>	ACR <sub>50</sub>	ACR <sub>10</sub>
D. magna	Kraft effluent†	76.5	52.6	61.2	1.3	0.86
	Chromium (Cr <sup>6+</sup> )	0.224	0.069	0.050	4.5	1.4
	PCP	0.343	0.129	0.071	4.8	1.8
	Boron	319.8	250.0	24.0	13.3	10.4
	Fluoride	353.6	292.1	>50	<7.1	<5.8
D. carinata	Kraft effluent†	91.9	75.0	61.2	1.5	1.2
	Chromium (Cr <sup>6+</sup> )	0.423	0.260	0.071	1.6	3.7
	PCP	0.570	0.130	0.354	6.0	0.37
	Fluoride	353.6	292.1	>50	<7.1	<5.8
S. vetulus	Chromium (Cr <sup>6+</sup> ) PCP	0.154 0.206	$0.016 \\ 0.097$	>0.10 0.071	<1.5 2.9	<0.16 1.4
C. dubia	Chromium(Cr <sup>6+</sup> )	0.053	0.037	0.005	10.6	7.4
	Boron	180.6	130.4	13.4	13.5	9.7
	PCP	0.202	0.138	0.158	1.3	0.87

results, then an ACR value of 10 is recommended (EPA 1985a). These results would appear to support the EPA value which will provide a conservative estimate.

Environmentally acceptable threshold toxicity levels may be better estimated from measured threshold values (such as an acute  $EC_{10}$  value) so as to minimise toxic concentration response effects. ACR values were calculated from  $EC_{10}$  values and ranged from < 0.16 to 10.4 (median 1.8) (Table 3), indicating that acute  $EC_{10}$  values are more comparable with chronic MATC values, and have a lower maximum ACR uncertainty than  $EC_{50}$  values. The results therefore suggest that  $EC_{10}$  values provide a better measure of threshold effects and should be reported in addition to  $EC_{50}$  values for acute tests. Relatively high levels of uncertainty associated with  $EC_{10}$  estimates in standard acute tests (see Table 1) may be reduced by either increasing the numbers of test organisms, or the test dilution factor in order to improve precision. For definitive toxicity tests, chronic tests must be undertaken since these assess both direct lethal and reproductive toxic effects.

The overall suitability of the individual species for toxicity testing showed some distinct differences between the organisms tested. Both S. vetulus and C. cf. pulchella showed poor survival characteristics and highly variable neonate production under the laboratory test conditions. This resulted in the abandonment of several tests with these organisms. Such results do not favour use of these organisms for performing laboratory toxicity tests. Of the New Zealand species tested, D. carinata and C. dubia appeared to be the most suitable for routine laboratory testing and of these C. dubia appeared to be more sensitive to toxicants (an effect which may be related to the smaller size of these organisms). The use of C. dubia for 7-day chronic tests is in fact recommended for whole-effluent toxicity testing (EPA 1985a, 1985b), though their major disadvantage is the very small size of the 2-4 hour-old neonates which creates handling difficulties. D. carinata has the advantages of a relatively large size (similar to D. magna) and good neonate production making this species particularly easy to handle, and potentially suitable for use for chronic toxicity testing.

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### Response of spinach (Spinacea oleracea) to the added fluoride in an alkaline soil

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### ABSTRACT

The influence of soil contamination by inorganic fluoride (NaF) on the uptake and accumulation of fluoride in the shoot and root of spinach (*Spinacea oleracea*) was investigated in pot experiment under controlled conditions. The soluble fluoride in soil varied between 2.57 mg kg<sup>-1</sup> soil and 16.44 mg kg<sup>-1</sup> soil in the treatment range of 0 800 mg NaF kg<sup>-1</sup> dry wt. [control] and 219.8 mg kg<sup>-1</sup> dry wt. (at 800 mg NaF kg<sup>-1</sup> or). The fluoride concentration in shoot and root showed a linear trend. At the added fluoride concentration beyond 200 mg NaF kg<sup>-1</sup> of soil, the spinach root retained more fluoride than shoot. In the treatment range 0 800 mg NaF kg<sup>-1</sup> of soil, the spinach root retained more fluoride than shoot. In the treatment range 0 800 mg NaF kg<sup>-1</sup> soil, the water labile fluoride in the juice varied from 0.32 to 0.78 ppm in shoot and 1.03 to 2.79 ppm in the root. No visible symptom of phyto toxicity was noticed with the treatment from 0 to 800 mg NaF kg<sup>-1</sup> soil. It was inferred from this study that spinach (*S. oleracea*) accumulates fluoride at tissues level and has a distinct mechanism of partitioning of water labile fluoride and total fluoride in the tissues.

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#### 1. Introduction

Water acts as predominant source of fluoride to cause fluorosis in the endemic areas, although some food materials also contribute significantly to the total intake of fluoride (Gulati et al., 1993; Singh et al., 1993). Atmospheric pollution by fluoride is considered a high phyto toxicity risk as this form of fluoride is often readily absorbed by the plants and is toxic to many plant species at relatively low concentration (WHO, 1984). Stevens et al., 1997, 1998 found that the ionic species of fluoride in solution also had a marked influence on the uptake of fluoride by plants root with complexed species being more readily taken up by the roots than the free fluoride ion. The greater solubility of fluoride under acidic conditions was explained by the formation of AIF complexes, whereas under alka line conditions by the desorption of free fluoride as a result of repul sion by the negatively charged surfaces (Wenzel and Blum, 1992). Fluoride is usually readily bound to soil surfaces at neutral pH and is not available to plants. In soil solutions of neutral to alkaline pH, fluoride exists predominantly as the free fluoride ion. (Barrow and Ellis, 1986; Wenzel and Blum, 1992).

Plants do not require fluoride, and tissues concentration from uncontaminated soils rarely exceed 30 mg kg<sup>-1</sup>, dry mass (Kabata Pendias, 2001).The fluoride content of both leafy and root vegetables usually do not differ appreciably from those of cereals with a exception of spinach which is usually enriched in fluoride

\* Corresponding author. Tel./fax: +91 522 2464664. E-mail address: jhask 01@yahoo.com (S.K. Jha). (Madhavan and Subraminian, 2006) and it is known as good accu mulator of fluoride (Sheldrake et al., 1978). The objective of this study was to characterize the fluoride uptake and partitioning water labile and total fluoride in spinach (*Spinacea oleracea*) in an alkaline soil of Indo Gangetic plain.

### 2. Material and methods

Samples were collected from the upper part of soil (0-15 cm) classified as Typic Natraustalf, from Central Soil Salinity Research Institute research farm, located at central Indo Gangetic plains. The soils was mixed, dried, grinded and sieved through 2.0 mm sieve. The initial characteristics of the initial soil such as pH, EC, sand, silt, clay, organic carbon, total fluoride, CaCl<sub>2</sub> extractable fluoride were determined which is given in Table 1. Thoroughly mixed 8 kg soil was filled into earthen pots lined with polythene sheet. A mixture of nutrients was added to each pot; 300 mg N as urea and 150 mg P as  $KH_2PO_4 \times H_2O$ . The soils were contaminated with graded concentration i.e. 0, 50, 100, 200, 400, 600 and 800 mg NaF kg<sup>-1</sup> by adding Sodium Fluoride (NaF) to the pots and thoroughly mixed. Each treatment was replicated four times. Fifteen seeds of spinach (S. oleracea) cv. "Palak All Green" were sown. Six plants were maintained in each pot. Another 300 mg N as urea was applied at 20 days after sowing as top dress. The irrigation was applied with de-ionised water. All the plants were harvested at 35 days. Half of the plant sample was used for determination of the water labile fluoride in the juices of shoots and roots immediately, whereas the other half was segregated into shoots and roots and, dried, weighed, milled to pass through 0.2 mm sieve and kept for the total fluoride determination in the shoots and roots. Similarly, soil samples collected from each pot after the harvest, were subjected to the analysis of pH, CaCl<sub>2</sub> extractable fluoride and total fluoride.

### 2.1. Soil analysis

pH (1:2) and EC (1:2) of the initial soil was determined by using ORION ion Analyser (5-Star series). Textural analysis (Sand, Silt, and Clay) of the soil was carried out by International Pipette Method (Klute, 2002), organic carbon by Walkley Black

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 Table 1

 Analysis of initial soil used in the experiment

Parameters	Value
Sand (%)	49.1
Silt (%)	18.5
Clay (%	32.5
Organic carbon (%)	0.41
pH (1:2)	8.42
EC (1:2) (dS m <sup>-1</sup> )	0.65
$CaCl_2$ extractable fluoride (mg kg <sup>-1</sup> )	6.01
Total fluoride (mg kg <sup>-1</sup> )	311
Al <sub>2</sub> O <sub>3</sub> (%)	1.25
Fe <sub>2</sub> O <sub>3</sub> (%)	4.56

Method,  $Fe_2O_3$  and  $Al_2O_3$  (Jackson, 1979). Soluble fluoride (0.01 M CaCl<sub>2</sub> extractable) by the method adopted by Larsen and Widdowson, 1971, the detection limit of the method (LOD) was 0.02 mg l<sup>-1</sup>. The total fluoride in the soil was determined by Alkali fusion method using ion selective electrode technique (McQuaker and Gurney, 1977), the LOD value was 0.05 mg l<sup>-1</sup>.

### 2.2. Water labile fluoride in the juice

The fresh shoots and roots of the spinach were thoroughly washed with distilled water and the excess water was drained out, and remaining was wiped out with the filter paper. Shoots were cut into small pieces and the juice was extracted by using USHA LEXUS grinder cum juicer. Similarly the roots were grinded in mortal and pestle and the juice were extracted by using a masoline cloth, as the quantity of the roots was not sufficient to be fed into the juicer.

Five milliliters of the juice, each of shoots and roots were taken separately and mixed with TISAB-IV (4 g CDTA + 58 g NaCl and 57 ml glacial acetic acid in 1 litre of distilled water adjusted to pH 5.0–5.5 by 6 N NaOH) in 1:1 ratio and the fluoride concentration was measured directly by using fluoride ion selective electrode (Njenga et al., 2005) with the help of ORION ion analyzer 5 star series. The recovery value of fluoride by this method was 96–102%.

#### 2.3. Total fluoride in shoots and roots

The total fluoride in the shoots and roots were determined by extracting the grinded and sieved samples with 0.1 N perchloric acid (Villa, 1979). The average recoveries based on the spiked samples at two different levels of fluoride were  $96 \pm 8\%$ .

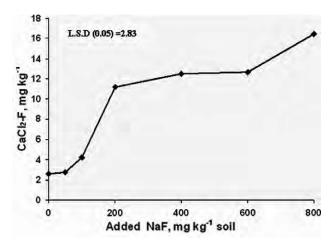
### 2.4. Statistical analysis

The comparison of the treatment means were done by ANOVA and the level of significance were determined at p = 0.05 and considered as significant. The data of the CaCl<sub>2</sub> extractable fluoride in soil and the added fluoride were subjected to the moment correlation coefficient. The comparison of distribution of fluoride in the root and shoot were done by chi-square test.

### 3. Results

### 3.1. Soluble fluoride in soil

The variation of soluble fluoride (CaCl<sub>2</sub> extractable) in the soil with the added fluoride was presented (Fig. 1). The product moment correlation coefficient showed a positive relationship (r + 0.92)between the concentration of added fluoride and the CaCl<sub>2</sub> extract able fluoride in the soil. The correlation was significant at probabil ity level p = 0.05. The soluble fluoride varied between 2.57 mg kg<sup>-1</sup> and 16.44 mg kg<sup>-1</sup> in the treatment range of 0 800 mg NaF kg<sup>-1</sup> soil. The data of soluble fluoride content of the soil under different added fluoride was subjected to one way ANOVA. The difference of the soluble fluoride content in soil were statistically not signifi cant up to the added fluoride of 50 mg NaF kg<sup>-1</sup> soil, followed by a statistically significant increase in the soluble fluoride with the added fluoride up to 200 mg NaF kg<sup>-1</sup> soil. In the added fluoride range of 200  $600 \text{ mg NaF kg}^{-1}$  soil, the soluble fluoride content in the soil was statistically much the same which was followed by further significant increase at 800 mg NaF kg<sup>-1</sup> soil. The pH of the soil showed an increasing trend with the added fluoride



**Fig. 1.** Effect of added NAF on the Cacl<sub>2</sub> extractable – F concentration in soil. LSD (0.05): Least significance difference between the means at the 0.05 probability level.

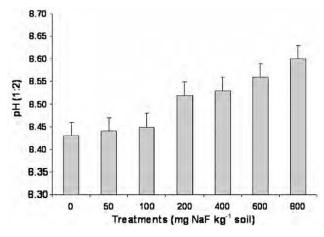


Fig. 2. Effect of added NaF on the changes of soil pH. The bar indicates the least significance difference between the means at the 0.05 probability level.

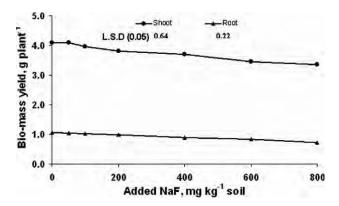
>200 mg NaF kg<sup>-1</sup> soil. The pH varied from 8.43 to 8.6 in the added fluoride range of 200 800 mg NaF kg<sup>-1</sup> soil (Fig. 2).

### 3.2. Bio mass yield

No visible symptom of phyto toxicity was noticed in the *S. oler acea* with the added fluoride range of 0 800 mg NaF kg<sup>-1</sup> soil. However the root and shoot dry weight bio mass per plant was found to decrease significantly at 600 mg NaF kg<sup>-1</sup> soil and 800 mg NaF kg<sup>-1</sup> soil respectively over the control (Fig. 3).

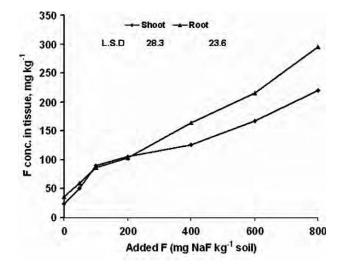
#### 3.3. Fluoride concentration in shoot and root tissues

The fluoride concentration in the shoot and root (Fig. 4) showed a linear trend with the added fluoride in the soil. The fluoride con centration in the root and the shoot were increased statistically with added fluoride. The concentration of fluoride in the shoots varied between 23.5 mg kg<sup>-1</sup> dry wt. (control) and 219.8 mg kg<sup>-1</sup> dry wt. (800 mg NaF kg<sup>-1</sup> soil). The fluoride concentration in root and shoot were subjected to chi square test. The fluoride concen tration of the root and shoot were statistically on par up to the added fluoride of 200 mg NaF kg<sup>-1</sup> soil, at the higher level of added fluoride, the difference in fluoride concentration was statistically significant. However the *S. oleracea* showed a significant difference in the fluoride uptake between the root and shoot. At the added

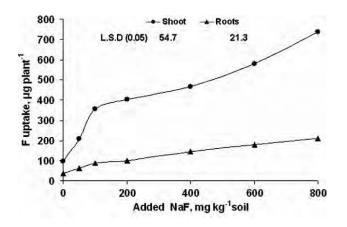


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Fig. 3. Effect of NaF on the bio-mass yield of *Spinacea oleracea*. LSD (0.05): Least significance difference between the means at the 0.05 probability level.

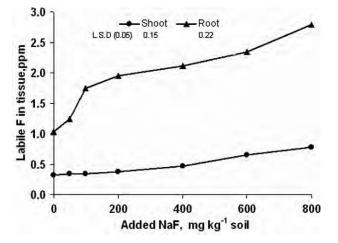


**Fig. 4.** Effect of NaF on the F concentration in shoot and root of *Spinacea Oleracea* LSD (0.05): Least significance difference between the means at the 0.05 probability level.



**Fig. 5.** Effect of NaF on the F uptake of *Spinacea oleracea*. LSD (0.05): Least significance difference between the means at the 0.05 probability level.

range of 0 800 mg NaF kg<sup>-1</sup> soil, the uptake of fluoride in the root followed a linear response (Fig. 5). In this range of fluoride concen tration, the uptake in shoot followed a sigmoid response.



**Fig. 6.** Effect of added NaF on the labile – F concentration in shoot and root of *Spinacea oleracea*. LSD (0.05): Least significance difference between the means at the 0.05 probability level.

### 3.4. Water labile fluoride in juice

It was found that the water labile fluoride in the juices of shoots did show any regular pattern, water labile fluoride in the juice re mained statistically on par up to the added fluoride level of 400 mg NaF kg<sup>-1</sup> soil followed by an increase at the added fluoride of 600 mg NaF kg<sup>-1</sup> soil which was again followed by a statistically similar value at 800 mg NaF kg<sup>-1</sup> soil added fluoride (Fig. 6). In case of root, there was a sharp increase of the water labile fluoride in juice up to the level of 100 mg NaF kg<sup>-1</sup> soil, beyond this, there was a gradual increase in the water labile fluoride. The difference in water labile fluoride in juice extract of root with the added fluoride was statistically significant. The water labile content varied between 0.32 0.78 ppm in shoots and 1.03 2.79 ppm in roots.

### 4. Discussion

The total fluoride concentration in the soil generally do not cor relate well with the uptake of fluoride by roots presumably be cause it is the only fluoride in solution or easily desorbable fluoride which is taken up by the plants (Brewer, 1965; Cooke et al., 1976; Gisiger, 1968). Therefore for all the soils, it is the soluble fluoride content that is biologically important to plants and ani mals (Davison, 1983). Hence soluble fluoride (CaCl<sub>2</sub> extractable) was determined in the soils of different treatments. At such low concentrations, adsorption by soil primarily occurs through ex change with  $OH^-$  of  $Al(OH)_3$ , which is very low in this soil, rather than with the crystal lattice OH<sup>-</sup> of clay minerals. Hence the solu ble fluoride in soil did not increase up to the added fluoride of 50 mg NaF  $\mathrm{kg}^{-1}$  soil. The sudden increase in the soluble fluoride beyond this concentration of added fluoride may be due to the lack of adsorption sites for fluoride in the soil. When the concentration of the added fluoride increased from 200 to 600 mg NaF kg<sup>-1</sup> soil, the crystal lattice OH<sup>-</sup> of clay minerals, which happens to be at higher level due to alkaline soil, also appeared to be replaced by F<sup>-</sup>. As a result there was no significant increase in the soluble fluo ride concentration due to maximum adsorption but with subse quent addition of 800 mg NaF  $kg^{-1}$  soil, there was further a sharp increase in soluble fluoride which may be mainly due to the release of OH<sup>-</sup> during the adsorption process (Bower and Hatcher, 1967). This release of OH<sup>-</sup> in turn increased the pH (Fig. 2) and hence more fluoride was leached out in the soil solution due to high alka linity which is in agreement with earlier findings (Stevens et al., 1998).

The increased concentration of fluoride in the plant shoot be yond a certain added level of fluoride may be attributed to the fact that when a high concentration of the fluoride is added to the soil or soil solution, pH becomes more alkaline, fluoride could increase in the soil solution and more fluoride would be potentially avail able for uptake by the plant root (Stevens et al., 1998). Garber, 1968 reported that larger doses of the order of 30 60 mg F% (300 600 ppm) added to the soil increased the fraction of fluoride in spinach by 190 675%.

Fluoride uptake in the *S. oleracea* followed a linear and sigmoid response in the root and shoot respectively. Hewitt and smith (1974) attributed sigmoidal uptake pattern to substrate co opera tion (the affinity of an enzyme for its substrate increasing with substrate concentration). At the added fluoride concentration be yond 200 mg NaF kg<sup>-1</sup> soil, the spinach root retained more fluoride than shoot. This may be partly due to dilution of fluoride in shoot due to increased shoot biomass and partly due to restricted trans location of fluoride from the root to shoot. This suggests *S. oleracea* has a tolerance mechanism for fluoride at tissue level. Higher con centration of fluoride in roots than in shoot, probably due to low permeability through the endodermis (Keller, 1980; Takmaz Nis aneiouglu and Davison, 1988).

S. oleracea have ability to tolerate high level of added fluoride in the soil, either by excluding fluoride at the root or detoxifying fluo ride at cellular level in plant. Critical concentrations are very much dependent on plant species. Visible symptom of fluoride toxicity does not appear even at a higher level of added fluoride. Fluoride toxicity in plants is normally manifested by marginal necrosis (tip burn, scorching, or lesions) on foliage, which begins on the margin or tips of the leaves and moves inward (Brewer, 1966). All the vegetables do not accumulate fluoride to the same extent and variations among vegetables are high (Khandare and Rao, 2006). It is reported that spinach is a good accumulator of fluoride (Sheldrake et al., 1978). The water soluble fluoride in soil recorded in this experiment is also encountered in the fluoride polluted areas such as brick kiln (Supharungsun and Wainwright, 1982) and aluminum smelter industries (Milhaud et al., 1990). The spin ach grown in those areas may have a lot of health consequences in long run. Singh et al. (1995) has also reported that leafy and roots vegetables accumulate more fluoride.

Consumption of vegetable juices as healing therapy has become popular (Njenga et al., 2005) and has concern for the fluoride con centration in the extracted juices. Most of the water labile fluoride present in the root than shoot. Venkateswarlu et al. (1965) re ported that most of the fluoride adsorbed by roots of *Hordeum vulgare* was desorbed in water, indicating that most of it retained in the apoplast. The water labile fluoride and total fluoride in leaves and roots varied differently with the added fluoride. This might be due to assimilation of fluoride as complex form in the shoot of spinach and not available as labile fluoride. This indicated that spinach plant has distinct mechanism for partitioning both the water labile fluoride and total fluoride at tissue level.

### 5. Conclusions

The results of the pot experiment indicate that spinach accumu lates much of fluoride in its tissues. It was also found that the plant has a partitioning mechanism for water labile and total fluoride in its tissues. It restricts translocation of fluoride from root to the shoot, sparing the plant part mostly used for consumption. High uptake capacity of the spinach for fluoride from soil, necessitate its monitoring from time to time. When the F content in the plant is high it may have several health consequences as it is a popular vegetable. Therefore it is desirable to that this type of vegetable be cultivated away from the source of fluoride accumulation in soil.

### **Conflict of interest statement**

The authors declare that there are no conflicts of interest.

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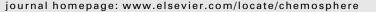
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### Fluoride toxicity effects in onion (Allium cepa L.) grown in contaminated soils

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### ABSTRACT

A pot experiment was carried out under controlled condition to investigate the accumulation, uptake and toxicity effects of fluoride (F) in onion (*Allium cepa L.*) grown on the soil contaminated by inorganic fluo ride (NaF). Six different levels of soil contamination were used by adding 0, 100, 200, 400, 600 and 800 mg NaF kg<sup>-1</sup> to the soil. The F concentration in shoot, bulb and root varied between 16.3 and 109.1 mg F kg<sup>-1</sup>, 15.8 and 54.3 mg F kg<sup>-1</sup> and 18.6 and 151.6 mg F kg<sup>-1</sup>, respectively. The visible symptoms of F toxicity in terms of tip burning and death of the plant was noticed in highly contaminated soils (>400 mg NaF kg<sup>-1</sup> soil). The phyto toxic threshold limit ( $LC_{50}$ ) in onion shoot was found to be 55 mg F kg<sup>-1</sup>, beyond which the biomass yield decreased by 50%. It was also inferred from the study that there is a partitioning of F in onion, with more accumulation in roots and shoots than in bulbs. The order of retention of fluoride in onion found to be roots > shoot > bulb.

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### 1. Introduction

Fluorine is widespread in the environment, occurs in soil, water, air and vegetations. The principal source of fluoride (F) that causes fluorosis in humans is believed to be the sources of drinking waters. However, some food materials also contribute considerable amount to the total intake of fluoride (Singer and Ophauge, 1979; Singh et al., 1993). F is not an essential element for plant but is essential for animals and humans. The continuous ingestion by animals in excessive amounts of F may lead to fluorosis and the sub optimal levels in the diet can have an equally damaging effect (Kabata Pendias, 1989; Stevens et al., 1995). The F level in air, water and soil needs to be at an appropriate level for living organ ism (Khoshoo, 1985). The world Health Organisation (WHO, 1984) and Bureau of Indian Standards (BIS, 2003) have laid down the maximum permissible limits of F in drinking water as  $1.5 \text{ mg L}^{-1}$ and 1.0 mg L<sup>-1</sup>, respectively, but there is no stringent threshold limits of F in soil and plants above which the ingestion may be det rimental to human health. The gaseous F emitted from the indus trial source is known to have phyto toxic effects on plants. Plants can also incorporate F from contaminated soils (Arnesen, 1997). The F thus absorbed is translocated to the shoots, causing physio logical, biochemical and structural damage and even cell death, depending on the concentration in the cell sap (Miller, 1993). Some plants accumulate F and even at higher concentration (up to 4000  $\mu$ g F g<sup>-1</sup> do not show sign of toxicity (Jacobson et al., 1966; Sheldrake et al., 1978). Most other plants show sign of toxicity at

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much lower concentration (Brewer, 1966), while some species are extremely sensitive to concentration  $<20 \ \mu g \ g^{-1}$  (Jacobson et al., 1966; Istas and Alaerts, 1974).

For all soils, it is the soluble F content that is biologically impor tant to plants and animals (Davison, 1982). The solubility of F in soil is controlled mainly through F adsorption by inorganic constit uents of the soil and soil pH (McLaughlin et al., 2001; Loganathan et al., 2003). In normal soil, the fluorine is strongly adsorbed to the soil (Barrow, 1986) and hence plant uptake of F is generally mini mal (Singh, 1990; Mclaughlin et al., 1997). The greater solubility of F under acidic conditions was explained by the formation of AlF<sub>x</sub> complexes, whereas under alkaline conditions by desorption of free F as a result of repulsion by the negatively charged surfaces (Wenzel and Blum, 1992).

Onion, is one of the most important commercially valuable crop in India and is consumed by the population as salad, vegetable and spice. Onion was therefore chosen to study F accumulation, uptake and toxicity when grown on contaminated soil.

### 2. Materials and methods

Soil samples were collected from the upper part of soil (0 15 cm) classified as Typic Natrustalfs, from the research farm of Central Soil Salinity Research Institute, Regional Research Sta tion, Lucknow. The soil was thoroughly mixed, dried, ground and sieved through 2.0 mm sieve. The basic characteristics of the initial soil such as pH, EC, sand, silt, clay, organic carbon, total fluoride, and CaCl<sub>2</sub> extractable fluoride were determined and are given in Table 1. Thoroughly mixed 8 kg soil was filled into earthen pots lined with polythene sheet. A mixture of nutrients was added to

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<sup>0045-6535/\$ -</sup> see front matter  $\odot$  2009 Elsevier Ltd. All rights reserved. doi:10.1016/j.chemosphere.2009.03.044

Parameters	Value
Sand (%)	49.1
Silt (%)	18.5
Clay (%)	32.5
Organic carbon (%)	0.41
pH (1:2)	8.42
EC (1:2) (dS m <sup>-1</sup> )	0.65
$CaCl_2$ extractable fluoride (mg kg <sup>-1</sup> )	6.01
Total fluoride (mg kg <sup>-1</sup> )	311
Al <sub>2</sub> O <sub>3</sub> (%)	1.25
Fe <sub>2</sub> O <sub>3</sub> (%)	4.56

each pot, 1.16 g N as urea and 890 mg P as  $KH_2PO_4 \times H_2O$  and 340 mg K as murette of potash. The N was applied in two splits, half as basal and other half at 60 d after plantation. The soils of each pot were contaminated with graded concentration i.e. 0, 100, 200, 400, 600 and 800 mg NaF kg<sup>-1</sup> by adding sodium fluoride (NaF) and thoroughly mixed. Each treatment was replicated four times. 8 Nos. of 20 d onion (Allium cepa L.) seedling cv. "Pusa Red" were transplanted. Five plants were maintained in each pot. The irrigation was applied with de ionised water. All the plants were harvested at 90 d after transplanting. The plants were air dried for 2 d and segregated into shoots, roots and bulb. It was then oven dried at 70 °C, weighed till a constant weight was achieved. It was then milled to pass through 0.2 mm sieve and kept for the total F determination in the shoots, roots and bulb. Similarly, soil samples collected from each pot after the harvest, were subjected to the analysis of pH, soluble F (CaCl<sub>2</sub> extractable).

### 2.1. Soil analysis in the experimental soil

pH and EC of the initial soil was determined by using ORION ion Analyser (5 Star series). Textural analysis (Sand, Silt and Clay) of the soil was carried out by International Pipette method (Klute, 2002), organic carbon by Walkley Black method,  $Fe_2O_3$  and  $Al_2O_3$ (Jackson, 1979), CaCl<sub>2</sub> (0.01 M) extractable F by the method adopted by Larsen and Widdowson (1971). The detection limit of the method (LOD) was 0.02 mg L<sup>-1</sup>. The total F in the soil was determined by Alkali fusion method using ion selective electrode technique (McQuaker and Gurney, 1977), the LOD value was 0.05 mg L<sup>-1</sup>.

#### 2.2. Total F determination in shoots, roots and bulb

The Total F in the shoots, roots and bulb were determined by extracting the grinded and sieved samples with 0.1 N perchloric acid (Villa, 1979). Accurately weighed 1 g of powdered samples was stirred magnetically with 25 mL of 0.1 N perchloric acid for 25 min. Subsequently 25 mL of 0.1 N perchloric acid was added be fore measurement. The average recoveries based on the spiked samples at two different levels of F were 96 ± 8%.

### 2.3. Statistical analysis

The comparison of the treatment means were done by ANOVA and the level of significance were determined at p = 0.05 and con sidered as significant

### 3. Results and discussion

#### 3.1. Soluble F in soil

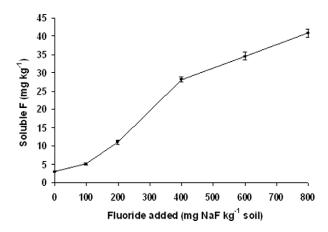
The total F concentration in the soil generally do not correlate well with the uptake of F by roots presumably because it is the only F in solution or easily desorbable F which is taken up by the plants (Brewer, 1965; Cooke et al., 1976; Gisiger, 1968). Therefore for all the soils, it is the soluble F content that is biologically important to plants and animals (Davison, 1983). Hence soluble F (CaCl<sub>2</sub> extract able) was determined in the soils of different treatments. The var iation of soluble F in the soil with the added F was presented (Fig. 1). It was found that the soluble F varied between 2.93 mg  $F kg^{-1}$  and 30.86 mg  $F kg^{-1}$  in the treatment range of 0 800 mg NaF kg<sup>-1</sup> soil. Up to the added 100 mg NaF kg<sup>-1</sup> soil, the soluble F was at par with the control. There was a sharp increase of the sol uble F with the added F up to 200 mg NaF kg<sup>-1</sup> soil and then in creased steadily with the increase of the added F which may be mainly due to the release of OH<sup>-</sup> during the adsorption process (Bower and Hatcher, 1967). This release of OH<sup>-</sup> in turn might have increased the pH (Fig. 2) and hence more F leached out in the soil solution due to high alkalinity, which is in agreement with earlier findings (Stevens et al., 1998).

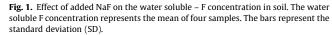
### 3.2. Biomass yield

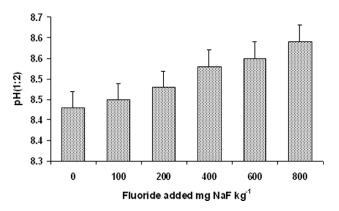
Visible symptom of F toxicity was noticed in *Allium cepa L*. from the added dose of 400 mg NaF kg<sup>-1</sup> soil onwards. The toxicity symptoms were in terms of tip burning and even plant death. There was no significant decrease in shoot, root and bulb biomass in soil treated with low NaF concentrations (up to 200 mg NaF kg<sup>-1</sup> soil) (Fig. 3). A decrease of 20%, 59% and 70% biomass was observed at 400, 600 and 800 mg NaF kg<sup>-1</sup> in roots, shoots and bulbs, respec tively. This suggested that the plant is unable to tolerate addition of F higher than 400 mg NaF kg<sup>-1</sup> soil. Some species like Gladiolus and Freesia are extremely sensitive to even lower concentration of F (Jacobson et al., 1966; Istas and Alaerts, 1974).

### 3.3. F uptake by onion tissues

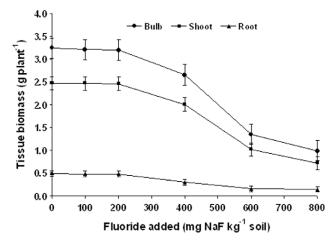
The concentration of F in the shoot, onion bulb and root showed almost a linear trend with the added fluoride in the soil (Fig. 4). The concentration of F in shoot, bulb and roots varied between 16.3 and 109.1 mg F kg<sup>-1</sup>, 15.8 and 54.3 mg F kg<sup>-1</sup> and 18.6 and 151.6 mg F kg<sup>-1</sup>, respectively, in the treatment range of 0 800 mg NaF kg<sup>-1</sup> soil. The F concentration in the shoot and root showed a steady in crease with the increase in added NaF. However, the concentration of F in bulb up to 200 mg NaF kg<sup>-1</sup> soil, was statistically at par (p < 0.05) with control. The onion bulb consumed as spice and sal ad in India, is comparatively less contaminated than shoots which



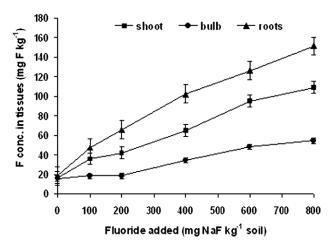




**Fig. 2.** Effect of added NaF on the changes of soil pH. The bar indicates the least significance difference (LSD) between the means at the 0.05 probability level.

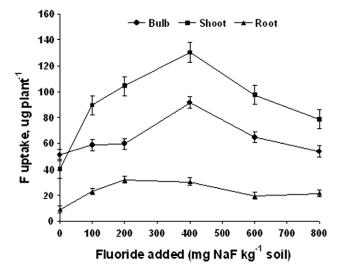


**Fig. 3.** Effect of NaF on the biomass yield of onion (*Allium cepa L*.): the biomass yield represents the mean of four samples. The bars represent least significance difference (LSD) between the means at the 0.05 probability level.



**Fig. 4.** Effect of NaF on the F concentration in shoot, bulb and root of onion (*Allium cepa L.*): the F concentration represents the mean of four samples. The bars represent least significance difference (LSD) between the means at the 0.05 probability level.

is used as leafy vegetable. It was also found that there is a parti tioning of F with more accumulation in roots and shoots than in

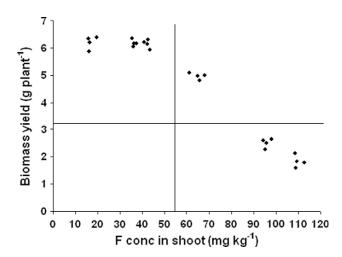


**Fig. 5.** Effect of NaF on the F uptake of onion (*Allium cepa L*.): the uptake value represents the mean of four samples. The bars represent least significance difference (LSD) between the means at the 0.05 probability level.

bulbs. The higher biomass of bulb may have dilution effect leading to lower concentration. The order of retention of F in onion found to be roots > shoot > bulb. The higher concentration of F in roots than in shoot is probably due to low permeability through the endodermis (Keller, 1980; Takmaz Nisaneiouglu and Davison, 1988). Despite having higher biomass of the bulb, the total uptake of bulb is less than that of shoot (Fig. 5), which is due to higher con centration of fluoride in shoot. The low uptake of root was because of lower biomass of the roots.

### 3.4. Shoot F and phyto toxic threshold limit

The scattered diagram (Fig. 6) shows that the biomass yield de creased with increase in the F concentration in shoot (r –0.96, p < 0.05). The phyto toxic threshold limit for F is defined here as mean F concentration in shoot beyond which the biomass yield de creased by 50%. The phyto toxic threshold limit (LC<sub>50</sub>) in onion shoot was found to be 55 mg F kg<sup>-1</sup>. The information on the phy to toxic threshold limits of F on plants is quite limited especially when the ionic species of F are taken up by the plant roots from



**Fig. 6.** Cate and Nelson diagram showing  $LC_{50}$  of F in shoot tissue for onion (*Allium cepa L.*).

the soil. However, Arnesen (1997) found the visible toxicity symp toms due to F in white clover only at 200 mg  $F kg^{-1}$  of soil and at larger than 400 mg F kg<sup>-1</sup>soil, showed toxic effects in all the plants. A solution culture study on tomato (Lycopersicon esculentum L.) re vealed that the activities of F in solution significantly limited dry weight of tomato shoot between 1476 and 2412  $\mu$ M F (Stevens et al., 1998). The linear regression equation for the shoot F concen tration and biomass yield showed that shoot F concentration ac counted 92.34% variability in biomass yield. LC50 determined in this study may vary depending upon the types of soil and the vari ations in sensitivities of onion cultivars to F. The variability be tween plant species in sensitivity to gaseous F is well documented (Weinstein, 1977; Ivinskis and Murray, 1984) whereas further research is needed to know the variability be tween cultivars sensitive to soil F, which was beyond the scope of the present study.

### 4. Conclusions

The results of the present study indicated that among the edible parts of onion, F is accumulated more in the shoot than in the onion bulb. However, the roots are the main accumulation site. This indicated that onion plant has a partitioning mechanism for F in its tissues. Both the shoot and the bulb of onion are consumed in India as vegetable and spice, respectively. Even onion bulb is consumed as salad along with the food. The F accumulation pattern in the onion tissues in this study suggests that there should be a constant monitoring of uptake in the onion and should not be grown in the F contaminated areas.

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### Environmental Contamination and Toxicology

## Toxicity of Fluoride to the Endangered Unionid Mussel, *Alasmidonta raveneliana*, and Surrogate Species

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The Appalachian elktoe, Alasmidonta raveneliana, is a federally-listed endangered unionid mussel whose range once included the Cumberland and Tennessee River drainages, but is now limited to the Tennessee River and its tributaries, Nolichucky River system, Pigeon River system, Mills River, and Little River (Burch 1975, USFWS 1996, Bogan 2002). The decline in abundance of this species and other unionids is believed to have resulted from habitat destruction, competition with nonindigenous species, the loss of host fish species that are necessary for larval transformation to the juvenile stage, and contamination (Williams et al. 1993, USFWS 1994, The Nature Conservancy 1992). One of the healthiest remaining populations of the Appalachian elktoe mussel is found in North Carolina's North Toe River that now receives discharges from feldspar mining operations containing significant concentrations of fluoride. Fluoride is known to be toxic to fish, zooplankton, aquatic insects and some adult unionid mussels (Smith et al. 1985, Feiser 1986, Camargo and Tarazona 1990, Muley 1990). However, no fluoride toxicity data were available for early life stages of the endangered A. raveneliana. The present study was conducted primarily as a means of evaluating fluoride as a possible limiting factor in the recovery of this mussel species. A second goal was to test several fish species as potential hosts for the mussel larvae during transformation.

### MATERIALS AND METHODS

Adult A. raveneliana, Actinonais pectorosa, Utterbackia imbecillis and Lampsilis fasciola mussels were collected from the Little Tennessee River, Franklin, North Carolina (A. raveneliana); Lake Chapman, Athens, Georgia (U. imbecillis); and the Clinch River, Virginia (L. fasciola, A. pectorosa), with the use of glassbottomed buckets or by SCUBA diving (U. imbecillis). The mussels were transported to the USEPA Region 4 laboratory in Athens, GA in wet burlap bags held in an insulated container. Upon arrival at the laboratory, the mussels—except U. imbecilis, were placed in a recirculating water bath at 16°C, the same as water from which they were collected. U. imbecillis were held at 20°C, similar to the temperature of Lake Chapman. Collection and transport followed appropriate permit requirements.

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Unionid mussels have a parasitic stage during which their larvae (glochidia) encyst on gills or fins of host fish and transform into juvenile mussels. This process can take a week to several weeks to complete depending on the mussel species and temperature. To produce juvenile mussels in the laboratory, we collect glochidia from female mussels and place them on the gills of fish hosts, then maintain the fish until the juveniles drop off. They are ~0.25-0.35 mm in length at that time.

Glochidia for fish infections and toxicity tests were collected from adult *A*. *raveneliana* by puncturing marsupia (egg-filled gills) with a scalpel and washing larvae out with water from a squirt bottle. This mode of collection appeared to be non-lethal to the adult females that were returned to their point of collection within a week of glochidia harvest. Tagged elktoe mussels from which glochidia were removed were recollected in a healthy condition a year later. Glochidia from the other more common mussel species were more completely harvested, generally resulting in the death of the adult.

The banded sculpin, *Cottus carolinae*, a known host for *A. raveneliana* glochidia, (Gordon and Moorman 2001) were collected in north Georgia. The mottled sculpin, *Cottus bairdii*, were collected in South Carolina for testing as a host because its range is currently sympatric with *A. raveneliana* while *C. carolinae*'s is not (USFWS 1996). Largemouth bass, *Micropterus salmoides* and smallmouth bass, *M. dolomieu* were also infected with Appalachian elktoe glochidia because they were readily available and are widely distributed fish.

Sculpins were infected by collecting glochidia from the female mussels, placing them in a beaker containing well water with a bubbling airstone for 30 minutes to allow the glochidia to attach to gills. The bass species were large enough that glochidia could be directly pipetted onto their gills. Sculpin were then held under low-light conditions in aquaria with recirculating water at 16°C for approximately six weeks until juvenile mussels excysted and dropped to the bottom of the tank. They were then siphoned from the tank and used in toxicity tests. Juvenile U. *imbecillis* mussels were produced on Lepomis macrochirus (bluegill), A. *pectorosa* on M. dolomieu, and L. fasciola on M. salmoides in five gal aquaria containing flow-through water at 20°C.

Test solutions were prepared by dilution of a stock of NaF dissolved in dilution water, measured with the fluoride probe to determine concentrations, and distributed to six-well plates that were used as test chambers. Once collected, glochidia were rinsed with clean test dilution water, soft or moderately hard (USEPA 1994), tested for viability, and randomly distributed into triplicate chambers per test concentration in six-well plates containing one of five concentrations of the fluoride solution (NaF; Fisher Scientific, 99.3% pure), or the dilution water control (Table 1). Lethality was assessed at the end of 24 hr and 48 hr using separate sets of glochidia (Keller and Ruessler 1997).

	Glochidia tests	96-hr tests	9-d tests
Renewal	None	None	Daily
Feeding regime	None	None	Algae
Lighting regime	16L:8D	16L:8D	16L:8D
Test temperature	25 <u>+</u> 1℃	25 <u>+</u> 1°C	25 <u>+</u> 1°C
Light intensity	500-1000 Lux	500-1000 Lux	500-1000 Lux
Test chamber	6-well plates (Polyethylene)	60x15 mm glass petri dish	60x15 mm glass petri dishes
Test solution volume	5 ml	10 ml	10 ml
Test	31, 62, 125, 250	31, 62, 125, 250	31, 62, 125, 250
concentrations	and 500 mg F/L	and 500 mg F/L	and 500 mg F/L
Aeration	None	None	None
Number of replicates	3	3	3
Number of mussels per replicate	100	10	15
Test water	EPA moderately	EPA moderately	EPA moderately
	hard reconstituted	hard reconstituted	hard reconstituted
	water	water	water

Table 1.	Test conditions for 96-hr and 9-d toxicity tests with glochidia and
juvenile	e mussels.

Acute toxicity tests with the juveniles were conducted as described in Keller and Ruessler (1997). Test solutions were prepared by dilution of a stock, measured with the fluoride probe to determine concentrations, and distributed to 60x15 mm diameter glass petri dishes that were used as test chambers. The dishes were then covered with their glass lids. Dilution water consisted of USEPA (1994) moderately hard reconstituted freshwater at a hardness of 84 mg/L as CaCO<sub>3</sub>. For some toxicity tests, dilution water hardness was reduced to 28-68 mg/L as CaCO<sub>3</sub> by addition of deionized water to better match conditions in streams containing the Appalachian elktoe. Tests were conducted for 96 hr, with daily determinations of juvenile survival, in static conditions with no feeding. LC<sub>50</sub>s were calculated for all toxicity tests using Trimmed Spearman-Karber.

Nine-day (216 hr) tests were conducted using the same methods as the acute tests, except that the mussels were fed algae to promote growth. At the end of nine days, remaining live *A. raveneliana* mussels were placed in vials containing 90% ethanol preservative until their shell lengths could be measured. Shell length (anterior to posterior) was measured to the nearest hundredth millimeter using a microscope and ocular micrometer at 100X. These data were then analyzed via ANOVA and Duncan's test to determine if there were significant differences between the control and test concentrations. Fluoride concentrations were measured with an Orion ion selective probe #940900 having a detection limit of 0.02 mg/L at pH 5-7, and calibrated against a fluoride standard. Initial

measurements were also validated by separate analysis using USEPA Method 300, an ion chromatography method. Data are presented as the concentration of the F ion (mg F/L).

### **RESULTS AND DISCUSSION**

Both species of sculpin produced juvenile A. raveneliana. This is significant given the fact that C. bairdii was not previously documented as a fish host. The A. raveneliana recovery plan (USFWS 1996) indicated that there was a need to identify additional host fish species. Our documentation of C. bairdii as a host, a fish that is sympatric with A. raveneliana, helps fulfill that stated need.

After four weeks of incubation, eighteen juvenile A. raveneliana were collected from the tanks containing approximately ten C. carolinae at 16°C. The tanks were siphoned for juveniles weekly thereafter, however no more juveniles were found for another month, when 186 were collected from the tank containing ten C. bairdii. Gordon and Moorman (2001) collected juveniles after 19 days' incubation. Since they did not report their culture temperature, it is possible that Gordon and Moorman (2001) held their fish at a higher temperature (>16°C), which decreased the incubation period. A similar incubation period (~6 wk) was required to produce juveniles cultured in our first attempt. At that time, 100 juvenile A. raveneliana were collected from the tank containing C. carolinae five weeks after glochidia were placed on the sculpin. The C. bairdii died before any juveniles were produced. Micropterus salmoides and M. dolomieu were also infected with A. raveneliana glochidia, but they did not produce any juveniles.

Twenty-four hr glochidia toxicity tests performed with *A. raveneliana* and *U. imbecillis* mussels at 25°C produced LC<sub>50</sub>s of 288 mg/L F and 351 mg/L F, respectively (Table 2). The NOEC for *A. raveneliana* glochidia was 250 mg/L F based on ANOVA and Dunnetts test. Forty-eight hr results were not reliable because of high control mortality (>25%) in both mussel species. Four species of mussels were used in juvenile toxicity tests--*L. fasciola*, *A. raveneliana*, *A. pectorosa*, and *U. imbecillis*. Ninety-six hr LC<sub>50</sub>s for fluoride ranged from 172 mg/L F for *L. fasciola* to 347 mg/L F for *A. pectorosa*, in water with hardness ranging from 28 to 84 mg/L as CaCO<sub>3</sub> (Table 2). The NOEC for juvenile *A. raveneliana* mussels was 250 mg/L F, based on ANOVA and Dunnetts test. These values are similar to LC<sub>50</sub>s (96- to 240-hr) for several species of fish, e.g., 124 mg/L F for brown trout, *Salmo trutta* (Wright 1977) and 315 mg/L F for fathead minnow, *Pimephales promelas* (Smith et al. 1985), and for adult *Indonaia caeruleus* mussels in 96-hr tests, LC<sub>50</sub>=247 mg/L F (Muley 1990).

Two juvenile tests were conducted for nine days (216 hr) resulting in little decrease in LC<sub>50</sub>s, i.e., *A. raveneliana*, LC<sub>50</sub> of 223 mg/L F and *L. fasciola*, LC<sub>50</sub> of 177 mg/L F, compared to those determined at 96 hr (Table 1). However, based on an ANOVA and Duncan's test, there were significant differences ( $p \le 0.05$ ) in growth (based on shell lengths) between controls and juvenile mussels exposed to fluoride (Table 3). Control mussels had a mean shell length ( $\pm$  s.d.) of 0.362 $\pm$ 

Mussel Species	Life Stage <sup>a</sup>	Test Duration (h)	Hardness (mg/L as CaCO <sub>3</sub> )	LC <sub>50</sub> (mg F/L)	Lower 95% Confidence Limit (mg F/L)	Upper 95% Confidence Limit (mg F/L)
Alasmidonta raveneliana	j	96	28	303	287	320
A. raveneliana	j	168	28	240	213	271
A. raveneliana	j	216	28	223	195	255
A. raveneliana	g	24	30	288	278	299
Actinonaias pectorosa	j	96	84	298	266	334
A. pectorosa	j	96	68	347	n/c <sup>b</sup>	n/c
A. pectorosa	j	96	30	178	n/c	n/c
A. pectorosa	j	96 ·	28	259	233	288
Utterbackia imbecillis	j	96	34	234	206	265
U. imbecillis	g	24	30	351	349	354
Lampsilis fasciola	j	96	32	172	164	180
L. fasciola	j	168	32	172	163	181
L. fasciola	j	216	32	177	n/c	n/c

 Table 2. Twenty-four hour to nine-day toxicity of fluoride to glochidia and juvenile mussels of several species at different water hardnesses.

<sup>a</sup>g=glochidia, j=juvenile <sup>b</sup>n/c=not calculable

0.019 mm, while mussels exposed to fluoride had mean lengths of  $0.348\pm0.027$  mm,  $0.333\pm0.022$  mm and  $0.318\pm0.021$  mm, for 31, 62 and 124 mg/L F, respectively. Using these results, the acute to chronic ratio (96 hr LC<sub>50</sub> divided by the geometric mean of the chronic No Observed Adverse Effect Concentration [NOAEC] and Lowest Observed Adverse Effect Concentration [LOAEC], USEPA (1985) for F in these mussels is at least 10. Note that no NOAEC other than zero was defined.

These results suggest that glochidia and juvenile mussels are similarly sensitive to acute exposures to fluoride and that hardness has little or no effect on its toxicity. Acute toxicity occurred at concentrations that are two orders of magnitude greater than the North Carolina water quality standard of 1.8 mg F/L (NCDENR 2003). The North Toe River receives five point sources of mining waste containing fluoride. The maximum concentration of fluoride reported in these undiluted effluents is 70 mg F/L, less than half the 96 hr to 216 h LC<sub>50</sub> determined for unionid mussels in the present study. Median instream concentrations of

Fluoride Concentration			Mean (±stdev.) Shell Length by Replicate	Mean (±stdev.) Shell Length by Treatment
(mg F/L)	Replicate	n	(mm)	(mm)
0	1	15	0.3663 ±0.0209	0.362 ±0.019
	2	15	0.3613 ±0.0187	
	3	14	0.3560 ±0.0150	
31	1	12	0.3429 ±0.0253	0.348 ±0.027*
	2	15	0.3522 ±0.0250	
	3	14	0.3558 ±0.0117	
62	1	14	$0.3360 \pm 0.0230$	0.333 ±0.022*
	2	10	0.3299 ±0.0249	
	3	12	0.3329 ±0.0214	
124	2	15	0.3251 ±0.0205	0.318 ±0.021*
	3	15	0.3118 ±0.0914	

**Table 3.** Shell lengths (anterior to posterior) for juvenile *Alasmidonta raveneliana* exposed to fluoride in soft water for nine days.

\*denotes a treatment that is significantly different from the control ( $p \le 0.05$ )

fluoride (after mixing with receiving waters) downstream of the discharge pipes of these five facilities are typically less than the State standard. While maximum concentrations ranged from about 1.5 to 8.0 mg F/L over the past two years, exceeding the standards periodically, acute fluoride toxicity to mussels is unlikely.

Sublethal effects occurred at concentrations as low as one-tenth of the 96 hr  $LC_{50S}$ , and one-fifth of the 9-d  $LC_{50S}$ . Few studies have evaluated growth impacts in juvenile unionids resulting from short-term exposure to pollutants. The success of the current study is therefore of particular value. Results of the 9-day tests indicated that subchronic exposures of juvenile mussels to fluoride can impair their growth at 31 mg F/L, but this concentration is about 17 times that permitted by the State of North Carolina. Facilities are generally meeting this standard; the ambient concentration of fluoride in the North Toe River at Penland, NC (which is within a mile downstream of four of the five mine discharges) ranged from <0.1 to 3.0 mg/L F with a median of 0.8 mg F/L over the past decade. These concentrations are significantly higher than those from the North Toe River ambient monitoring station near Ingalls, NC (upstream of all five facilities) which ranged from <0.1 to 2.0 mg/L F with a median of 0.2 mg F/L over the past decade, but they are still well below the sublethal effects concentrations determined in this study. Because growth impacts were measurable after such a short time in this study and mussels live for decades, it would be worthwhile to perform more lengthy exposures at lower concentrations. Smaller adults are

known to suffer greater predation effects and reduced reproductive success compared to those of normal size and this could have a negative impact on populations in the long term.

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### INFLUENCE OF LOW-CHITIN KRILL MEAL ON REPRODUCTION OF *CLETHRIONOMYS GLAREOLUS* (SCHREBER, 1780)

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Abstract—1. Clethrionomys glareolus fed on a diet containing krill meal assimilated excessive amounts of fluoride.

2. Excess of fluoride caused disturbances in the reproduction of these animals: reduction of the number of ltters, higher mortality of offspring and degenerative changes in seminiferous tubules.

3. The disturbances in the reproduction occurred still more distinctly in the successive generation. 4. Among the bank vole offspring which received the diet with the greater amount of krill meal, both

in the parent generation and in the generation  $F_1$ , a changed sex ratio was found, which was evidenced by a significantly higher number of males.

### INTRODUCTION

Research on the utilization of krill meal concentrated mainly on the question of whether krill meal can constitute full value source of protein in animal nutrition. Results of most of these studies indicate that it can be a partial or full substitute for fish meal. However, in cases where rats and laboratory mice were given diets with krill meal for a longer period of time or when amounts of krill meal in the diet were great, it was observed that krill meal influenced the organism negatively (Pastuszewska, 1979; Heinz et al., 1981; Seidler et al., 1982; Pastuszewska et al., 1983b). These animals exhibited changes in the reproduction cycle, anatomicopathologic changes of alimentary tract and distinct overgrowth and discolouration of incisors. The aforementioned authors do not determine univocally the toxic agent responsible for originating these disturbances although disfigurement of incisors indicates the excess of fluoride ions in the organism, and it is commonly regarded as the typical symptom of toxicity of this element.

Euphausia superba Dana contains great amounts of fluoride which is mainly situated in the shell (Soevik and Braekkan, 1979; Boone and Manthey, 1983; Buchholz, 1983; Adelung et al., 1987). After the death of krill fluoride passes from the shell into muscles. Intensity of this migration depends on the freezing temperature, on the length of time it remains in the frozen state and on the method of preparation (Christians and Leineman, 1983). Moreover, the average content of fluoride is about 50% higher in krill larvae than compared with the adults (Hempel and Manthey, 1981). That is why the amount of fluoride in various parts of krill meal is different. Thus, it seems that divergences in the opinions concerning the possibility of using krill meal as food additive may be explained mainly by different content of fluoride in the diets containing krill meal as well as by different and most often too short duration of experiments. Krill meal also contains quite substantial amounts of chitin, which according to Seidler *et al.* (1982) can cause anatomicopathologic changes of alimentary tract in mice. In order to eliminate possible harmful influence of chitin *C. glareolus* was used (in this study) whose natural food contains certain amounts of this component.

The aim of the present study was to investigate the effect of low-chitin krill meal on the reproduction of bank vole. Up till now, research on the effect of krill meal on reproduction of animals has been limited only up to the time of obtaining generation  $F_1$ . This period of time is too short to obtain objective assessment of the effect of krill meal in the diet on the reproduction of animals especially, if the diet does not contain much krill meal. Toxicity of fluoride depends not only on the dose quantity but also on the length of time during which this element is received. This is why it was decided in this study that breeding observations should be carried out on successive generations.

#### MATERIALS AND METHODS

Assessment of the influence of low-chitin krill meal on the reproduction of C. glareolus was carried out on 284 animals. The bank voles came from the Mammals Research Institute, Polish Academy of Sciences in Białowieża.

Experimental diets were made on the basis of data taken from literature concerning bank vole's food maintaining the same percentage of components as in the natural food, i.e. seeds, green feed and animal food. The control diet (A) included seeds (wheat), 65%; dried green feed (clover, alfalfa, grasses), 22% as well as meat and bone meal, 13%. Two experimental diets were made in which protein from meat and bone meal was substituted in 50% and 100% respectively by protein from the low-chitin krill meal which was provided by Sea Fishery Institute in Gdynia. As a result, the amount of krill meal in these diets was in percentages as follows: in diet B, 6.2% in diet C, 12.4%. Other components were left in the same amounts as in the control diet. All diets were enriched by vitamins, Polfamix (1 gram/kilogram diet). In order to prevent the animals from selective eating of

Table 1 Chemical composition of experimental diets. A-control diet, B and C-experimental diets

Diet	A	В	С
Ash			
0 o	6 81	7 11	7 25
Ether extract			
0/0	7 04	6.83	6 78
Crude fibre			
0/0	5 74	6 38	6.81
N ° °	3 29	2 22	
Na	5 29	3 22	3 32
°'0	0.49	0 48	0 51
ĸ	0.47	040	0.51
0	0 56	0.55	0 56
Ca			
°,0	112	1 00	0 90
Mn			
ppm	20.00	22.50	25 00
Zn			10.00
ppm	17.50	17 00	19 00
Cd	0 25	0 25	0.35
ppm Pb	0 25	0 23	0.55
ppm	4 00	4 50	5 00
Cu		150	5.00
ppm	3 00	3 50	4 50
F			
ppm	18 37	46 60	96 96

particular components, the diets were ground and mixed thoroughly. Food and water were provided *ad libitum* once daily, in the morning. Chemical composition of the diets is presented in Table 1.

Two groups of bank voles were taken into account during the breeding observations. The first group constituted of 3–4 month old animals which were given experimental diets for six weeks after which they were combined in pairs and observed for seven months being still fed on the same diets. The second group constituted bank voles from generation  $F_1$ which were constantly fed on the experimental diets and which were taken from the parents fed on the same diets for half a year. At the age of 3–4 months they were combined in pairs and observed for half a year. In both groups the control constituted bank voles tested in parallel which were given diet with meat and bone meal.

During the breeding observations of the two groups the following data were registered: number of litters and their size, mortality of offspring in the nest period, period of time from mating to producing the first litter, period of time between successive litters and changes concerning incisors of the animals under study

Testes were taken for histological examinations from males of both groups receiving diets A and C. The testes were fixed in Bouin solution, embedded in paraffin and sectioned at  $10 \,\mu$  The sections were stained with haematoxylin and eosin.

The content of fluoride in femur and in blood, was examined in the bank voles receiving experimental diets for five months and in 8–9 month old animals from generation  $F_1$ . Bank voles were etherized. The removed femurs were carefully cleaned and dried Samples of blood were taken from vein sinus. Flouride extraction by microdiffusion was followed by colorimetric analysis. The detailed analytical method was described in Bäumler and Glinz (1964) and Culik (1986).

The received results were analyzed statistically with calculation of standard deviation and with examination of significance of differences between average values by Student's t-test. The statistical significance of deviations of sex ratio from the norm was assessed by means of chi-square test.

### RESULTS

The number of litters per one fertile female was lower in the bank voles fed the experimental diets (B and C) while in the animals receiving diet C it was significantly lower (P < 0.01) compared with bank voles receiving control diet (Table 2). Regardless of the diet, the litter size in all bank voles was similar. The number of days between mating and producing the first litter was higher in the bank voles receiving food with krill meal. The C. glareolus fed on all diets produced successive litters every 41-48 days on average and the differences between them were statistically insignificant (Table 2). In bank voles receiving the food with krill meal the mortality of offspring in the nest period was higher than in the control bank voles (Table 3). There were greater numbers of males (P < 0.05) among the offspring born by the bank voles receiving diet C than among the offspring born by bank voles receiving diet B and diet A (Table 3). In order to inspect whether increase of mortality of bank voles receiving diet C during nest period can affect females to a higher degree, the sex ratio of the young was determined immediately after birth. The received results indicate that the significant (P < 0.05) deviations from the expected sex ratio 1:1 in favour of males occur already at the moment of birth.

In the generation  $F_1$  the bank voles receiving diets B and C exhibited fewer number of litters compared with the control animals, whereas in bank voles receiving diet C the number of litters per one fertile female was significantly lower (P < 0.01, Table 2).

	Group	>	I			II	
	Die	t A	В	С	Α	В	С
Number of	Mated Dead	29 2	29 3	30 5	14	15 3	15 5
pairs	Fertile	23	20	21	12	10	8
Number of li one fertile fe		2 57 ± 0.69	2.10 ± 0 91	$1.86 \pm 0.87^{a}$	$2.58 \pm 0.86$	1 90 ± 0 70	1 37 ± 0 49°
Litter size		$3.00 \pm 1.11$	$353 \pm 095$	$2.97 \pm 1.02$	2 74 <u>+</u> 0 76	$2.84\pm0.74$	3 54 ± 0 98ª
	lays from mating the first litter	56 70 ± 28.90	64.80 ± 36 70	68 00 ± 30 30	64 90 ± 16 20	72 00 ± 14 10	$84\ 70\pm 8\ 70^{\circ}$
Number of d successive ht		41 20 ± 16.30	46.70 ± 28 50	47 70 ± 27 20	31 60 ± 18.20	32.70 ± 11 50	34 40 ± 12 60

Table 2. The influence of food with low-chilin krill meal on the reproduction in *Clethrionomys glareolus* (mean values  $\pm$  SE)

I-the first experimental group (parent generation).

II—the second experimental group (generation  $F_1$ ).

A-control diet, B and C-experimental diets.

a - P < 0.01 as compared with control diet

Table 3 Mortality among young voles in the nest period and sex ratio

	Group		I			II	
	Diet	Α	В	С	A	В	C
Number of fertile pair	'S	23	20	21	12	10	8
Number Born of young Weaned	33 '+'-	167 57 52	153 48 44	116 41 25	85 29 30	54 16 9	39 6 4
Mortality of young in nest period in %		34 33	39.87	43 11	30.59	53 70	74 36
Sex ratio		1 10 1	1 09.1	1 64.14	1 02·1 <sup>b</sup>	1 35 1 <sup>b</sup>	1 78 1ªb

1-the first experimental group (parent generation)

II—the second experimental group (generation  $F_1$ )

A-control diet, B and C-experimental diets

a-P < 0.05 as compared with control diet

b-sex ratio at birth

The average litter size in C. glareolus fed on diet B was similar to the litter size in the control group. Statistically greater litters (P < 0.01) were observed in bank voles receiving diet C (Table 2). The number of days from mating to producing the first litter was higher in the animals receiving experimental diets than it was in the animals fed on the control diet, whereas in the bank voles eating more krill meal (diet C) this period was significantly longer (P < 0.01, Table 2). Also significantly longer (P < 0.01) was the period between mating and producing the first litter in the bank voles from the generation  $F_1$  fed on diet C compared with the animals from parent generation (the first experimental group) fed on the same diet (Table 2). Hence also the number of litters in animals from generation  $F_1$  is lower than in the bank voles from parent generation, but this difference is not significant. The period of time between producing successive litters in C. glareolus in the second experimental group was the same at the administration of three different diets and did not differ from the time between successive litters produced by the bank voles from the parent generation (Table 2). In the animals

from generation  $F_1$  receiving control diet the mortality of offspring in the nest period was similar to the data received for bank voles from parent generation fed on the same diet. In animals fed on the experimental diets, the mortality of offspring in the nest was higher (Table 3). Significant (P < 0.05) change of sex ratio in favour of males (Table 3) was observed among the offspring of bank voles from generation  $F_1$ receiving diet C as well as in the animals from parent generation fed on the same diet.

The histological picture of testes from the second experimental group (generation  $F_1$ ) receiving diet C indicates that a large number of seminiferous tubules underwent degenerative changes (Fig. 1). These tubules have a diminished surface of germinal epithelium or do not have it at all. The particular sex cells do not form concentrically arranged layers but they are, so to say, mixed. Some of the sex cells are of a very large size usually containing pyknotic nuclei (Fig. 2). Empty spaces resembling vacuoles can be seen between the cells. They might presumably have come into being after the disintegration of the cells of germinal epithelium of changed appearance the



Fig. 1. Microscopic picture of transverse section of testis of male from the second experimental group (generation  $F_1$ ) receiving diet C (magnified  $240 \times$ ) group of tubules with distinct retroactive changes Amount of germinal epithelium is reduced (1) or there is hardly any at all (2).

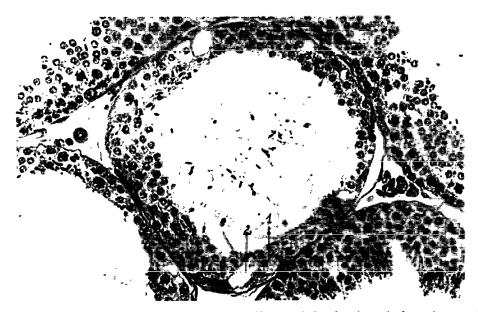


Fig 2. Microscopic picture of section across seminiferous tubule of male testis from the second experimental group (generation  $F_1$ ) receiving diet C (magnified  $360 \times$ ) diminished amount of cells of germinal epithelium, presence of distinctly enlarged cells containing vacuolated cytoplasm and pyknotic nucleus (1); there are empty spaces between cells of germinal epithelium resembling vacuoles (2), resulting probably after decomposition of cells of changed view.

more so as in some changed sex cells a bright cytoplasm with marked slight vacuoles was observed. Beside the epithelia which underwent degenerative changes, there are epithelia having normal histological structure. The epithelia of control males had normal histological structure (Fig. 3). No markedly changed epithelia were observed in testes of *C. glareohus* from the first experimental group receiving diet C. Growth of incisors was found in 50% of bank voles

fed on diet C and in about 30% of bank voles

receiving diet B during the breeding observations in the first experimental group. Changes of appearance of incisors of the animal fed on diet C was observed at about the 5th month of being fed on this diet and in the bank voles receiving diet B they were observed at about the 6th month. Incisors elongated gradually and distorted (Fig. 4). No changes in the shape of incisors were observed in the control bank voles (Fig. 5). Changes in the shape of incisors in the second experimental group were observed earlier, at

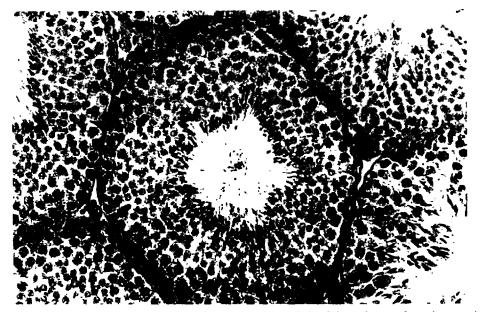


Fig. 3. Microscopic picture of the section across seminiferous tubule of the male testis from the second experimental group receiving diet A (control diet) (magnified  $360 \times$ )

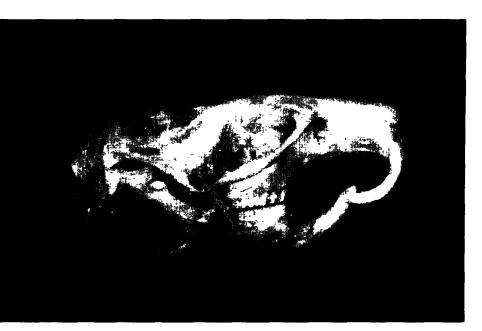


Fig. 4 Picture of incisors of bank voles fed on low-chitin krill meal diet for a longer period of time.

about the 3rd month of life in the animals fed on diet C and at about the 4th month of life in the bank voles receiving diet B. In the final period of observation the overgrowth of incisors was found in bank voles from generation  $F_1$  in 90% of animals fed on diet C, and in about 60% of bank voles fed on diet B.

In bank voles fed on experimental diets for five months the amount of fluoride accumulated in their femur was significantly higher (P < 0.001) compared with the amount of fluoride accumulated in femur of animals receiving control diet (Table 4). In males and females the amount of fluoride in bone tissue was similar (Table 4). In 8–9 month old *C. glareolus* from generation  $F_1$  receiving experimental diets the amount of fluoride in the femur was also significantly higher (P < 0.001) compared with control animals (Table 4) and this amount was the same in males and females. The amount of fluoride in bone of 8–9 month old bank voles from generation  $F_1$  was significantly higher (P < 0.001) in all examined animals compared with the amount of this element deposited in the femur of bank voles from parent generation receiving the same diets for five months. This is the evidence for accumulation of fluoride in the bone tissue of the examined animals.

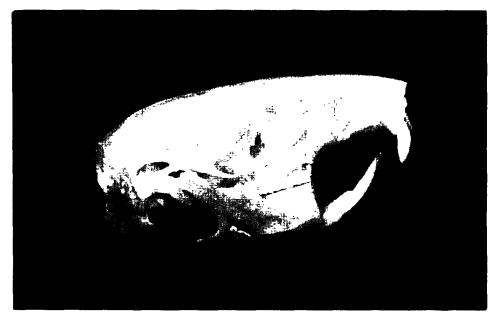


Fig. 5. Picture of incisors of bank voles not receiving low-chitin krill meal in their food.

	Group					II	
Tissue	Diet	Y	B	C	A	8	С
Bone	रीर २५ Total	$\begin{array}{c} (19) \ 485 \ 00 \ \pm \ 71.00 \\ (18) \ 480 \ 00 \ \pm \ 66 \ 00 \\ (37) \ 480 \ 00 \ \pm \ 69.00 \end{array}$	$\begin{array}{c} (22) \ 1006 \ 00 \pm 220 \ 00^4 \\ (14) \ 956 \ 00 \pm 133 \ 00^4 \\ (36) \ 986 \ 00 \pm 190 \ 00^4 \end{array}$	$(15) 2818.00 \pm 191.00^{4}$ $(17) 2803 00 \pm 223.00^{3}$ $(32) 2810 00 \pm 205 00^{3}$	$\begin{array}{c} (12) \ 1692.00 \pm 91.00 \\ (14) \ 1086 \ 00 \pm 83.00 \\ (26) \ 1089 \ 00 \pm 85 \ 00 \end{array}$	$\begin{array}{l} (14) \ 2619 \ 00 \ \pm \ 157.00^{\circ} \\ (15) \ 2623.00 \ \pm \ 154.00^{\circ} \\ (29) \ 2621 \ 00 \ \pm \ 153.00^{\circ} \end{array}$	$\begin{array}{l} (10) \ 5734.00 \pm 254 \ 00^{\circ} \\ (14) \ 5762.00 \pm 338.00^{\circ} \\ (24) \ 5750.00 \pm 299.00^{\circ} \end{array}$
Blood	చేచే ఛం Total	$\begin{array}{c} (6) \ 0.19 \pm 0.04 \\ (6) \ 0.19 \pm 0.04 \\ (12) \ 0.19 \pm 0.04 \end{array}$	$\begin{array}{l} (6) \ 0.39 \pm 0 \ 0.4^{4} \\ (6) \ 0.38 \pm 0 \ 0.3^{4} \\ (12) \ 0.39 \pm 0 \ 03^{4} \end{array}$	(6) $0 84 \pm 0.09^{4}$ (6) $0.83 \pm 0.06^{4}$ (12) $0.84 \pm 0.07^{4}$	$\begin{array}{c} (6) \ 0 \ 22 \pm 0.04 \\ (6) \ 0 \ 21 \pm 0.06 \\ (12) \ 0 \ 22 \pm 0.05 \end{array}$	(6) $0.46 \pm 0.06^{b}$ (6) $0.45 \pm 0.07^{b}$ (12) $0.45 \pm 0.07^{b}$	(6) $1.03 \pm 0.16^{a}$ (6) $1.05 \pm 0.14^{a}$ (12) $1.04 \pm 0.14^{a}$
I-the II-the Acon	I-the first experimental group (paren II-the second group (generation $F_1$ ) Acontrol diet, B and C-experimer	I—the first experimental group (parent generation) II—the second group (generation $F_1$ ) A—control diet, B and C—experimental diets	ation) s				

Bank voles receiving experimental diets for five months exhibited significantly higher contents of fluoride in blood (P < 0.001) compared with animals fed on control diet (Table 4). In 8-9 month old C. glareolus from generation F<sub>1</sub> receiving experimental diets the content of fluoride was also significantly higher (for diet B P < 0.01 and for diet C P < 0.001, Table 4). The content of fluoride in the blood of bank voles from generation  $F_1$  fed on diet B and C was significantly greater than in the blood of bank voles receiving the same diets for five months (for diet B P < 0.01 and for diet C P < 0.001). The concentration of fluoride in blood was the same in males and females receiving experimental diets for five months and in 8-9 month old animals from generation  $F_1$ (Table 4).

### DISCUSSION

The abnormal shape of incisors and changes in their pigmentation are the most visible symptoms of excessive amounts of fluoride in the body of rodents (McClure and Mitchell, 1931: Newman and Markey, 1976). Elongation of incisors in C. glareolus in this study depended on the concentration of fluoride in the diet and on the length of time during which the food was provided to the animals. Deformation of incisors in the bank voles appeared earlier when animals were fed on the food containing greater amount of fluoride (diet C) and in animals from generation F<sub>1</sub> compared with parent generation. The earlier elongation of incisors of bank voles from generation F<sub>1</sub> resulted mainly from the fact that these animals were exposed to harmful effect of fluoride during adolescence This is so because the growing animals exhibit greater ability to accumulate fluoride in the bone than the adult animals (Savchuck and Armstrong. 1951). Moreover, the bank voles from generation F<sub>1</sub> received fluoride during prenatal development, because this element is transferred through placental barrier to skeletal system of the fetuses (Fleming and Greenfield, 1954; Brzeziński et al., 1961; Katz and Stookey, 1973). The confirmation of the excess of fluoride in the body of C. glareolus fed on the diet containing low-chitin krill meal is its presence in the blood and bone of these animals. The amount of deposited fluoride in the femur of bank voles depended on the content of this element in the diet (Table 4). Moreover, greater content of fluoride in the femur of the bank voles from generation  $F_1$ (Table 4) resulted mainly from much longer time of receiving the experimental diets. These results agree with the reports of other authors according to which the amount of accumulated fluoride in the bone is positively correlated with the content of fluoride in food or in drinking water and with the time of administering (Weber and Reid, 1969; Newman and Markey, 1976; Ekstrand et al., 1981; Khalawan, 1981; Boros et al., 1984). Simon and Sutie (1968), Patz (1973) and Boros et al. (1984) found that the amount of fluoride in blood plasma depends also on the amount of fluoride in the food. It seems that slightly higher content of fluoride in the blood of the bank voles from generation F<sub>1</sub> receiving food with krill meal in comparison with the animals from parent generation was the result of lower capacity of

A—control diet, B and C—experimental diets Number of animals appears in parentheses a-P < 0.001, b-P < 0.01 as compared with control diet

The excess of fluoride in the body of the C. glareolus was undoubtedly the cause of the observed disturbances in their reproduction. The animals from the first experimental group receiving food with krill meal gave birth to a smaller number of litters than the control bank voles and the longevity of their offspring in the nest period was lower (Tables 2 and 3). The reduction of the number of litters and higher mortality of offspring occurred even more distinctly in the bank voles from the second experimental group (generation  $F_1$ , Tables 2 and 3). Since the number of days between successively produced litters was similar in the experimental bank voles and in the control group ones, the smaller number of litters receiving food with krill meal resulted from the lengthening of time between the mating and giving birth to the first litter (Table 2). Pastuszewska et al. (1983b) providing food with krill shell meal to growing rats found the retarded development of seminiferous epithelium in the testes of these animals. It may be supposed that similar phenomenon could take place in C. glareolus fed on diets with krill meal. However, it was not the only cause of decrease in the number of litters in these animals, because histological picture of male testes from the second experimental group fed on diet C indicates that a large part of seminiferous epithelia underwent degenerative changes. In the first experimental group so clearly changed seminiferous epithelia were not observed in the testes of males receiving the same food. It may be, however, considered that in spite of the lack of distinct changes in the histological picture, the normal functioning of these males' testes had already been disturbed and could be the cause of a slight delay in giving birth to the first litter by this group of animals. The degenerative changes of the germinal epithelium were caused by the toxic effect of fluoride. The conclusion is confirmed by the studies of Kour and Singh (1980). These authors, administering fluoride to the males of mice in drinking water at the amount of 500 and 1000 ppm, observed similar changes in the seminiferous epithelia; these changes clearly intensified alongside the lengthening of time of fluoride intake by these animals. As in these investigations bank voles were receiving much smaller amounts of fluoride than the mice in the study by the aforementioned authors, and moreover the fluoride from krill meal is assimilated to a lower degree than the fluoride from sodium fluoride (Pastuszewska et al., 1983a) clear degenerative changes were not observed in bank voles until the second generation. Kanwar and Singh (1981) and Singh (1982) report that administering fluoride in drinking water to laboratory mice causes significant decrease of manganese and zinc in liver and kidneys of these animals. Decrease of these elements in soft tissues may have a harmful influence on their normal functioning. Among other things, both of these elements condition normal development of testes and normal spermatogenesis (Underwood, 1962). Deficiency of these elements leads to degenerative changes of seminiferous tubules and their atrophy (Boyer *et al.*, 1942; Swenerton and Hurley, 1968; Diamond *et al.*, 1971). Thus, it is likely that administering to bank voles food with greater amount of krill meal and hence with greater amount of fluoride could decrease the level of zinc and manganese in the soft tissues of these animals and consequently it could result in the degenerative changes of seminiferous tubules. This hypothesis will be confirmed in further studies.

Addition of low-chitin krill meal to the food of C. glareolus also increased the mortality of the young in their nest period. The increase of the mortality of the young obtained from the bank voles of parent generation (the first experimental group) was admittedly not great, but the increase of the mortality of the offspring of animals from the second experimental group was so great that carrying out further breeding observations became impossible (Table 3). The mortality of the young in the nest period in bank voles not receiving krill meal was approximate to the data by Drożdż (1963) and Buchalczyk (1970). The observed increase of mortality of the offspring of bank voles fed on food with krill meal was thus probably caused by the toxic influence of fluoride. The size of litter can influence the longevity of offspring (Gustaffson et al., 1980). However, the litter size in this study was similar in the control of animals and in the experimental ones. Therefore, it can be said that mortality of the offspring of the bank voles fed on food with krill meal depends on the amount of fluoride in the diet, as well as on time of feeding. When the amount of fluoride in food or drinking water is great, high mortality is observed already in the first generation (Messer et al., 1973; Marks et al., 1984).

In the offspring of bank voles receiving more krill meal in food (diet C) a changed sex ratio was found in both generations, evidence manifested by the greater number of males (Table 3). Investigations by Buchalczyk (1969) showed that sex ratio in young bank voles at the time of weaning amounted 1:1. Similar data were obtained by Mazak (1962), Zejda (1968) and Gustaffson *et al.* (1980).

Since sex ratio among the offspring of bank voles receiving the control diet was also 1:1 in this study, it may be suggested that sex ratio disturbances in the animals fed on diet C were a result of a great amount of fluoride in this food.

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### TOXICITY OF AQUEOUS AND SEDIMENT-ASSOCIATED FLUORIDE TO FRESHWATER ORGANISMS

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**Abstract**—Inorganic fluorides were declared toxic under the Canadian Environmental Protection Act in 1993 based on their potential to cause long-term harmful effects in aquatic and terrestrial ecosystems, but information on the toxicity of sediment-associated fluoride to freshwater benthic organisms was considered incomplete. The purpose of this study was to determine the toxicity of aqueous and sediment-associated fluoride to several species of freshwater organisms and to determine if toxic effects could be expected under environmentally realistic exposures. Toxicity of fluoride (as NaF) in short-term (48–96-h) lethality tests was greatest for the amphipod *Hyalella azteca* (median lethal concentration [LCS0] = 14.6 mg F<sup>-</sup>/L), followed by the mayfly *Hexagenia limbata* (32.3), the midge *Chironomus tentans* (124.1), the fathead minnow *Pimephales promelas* (262.4), and the cladoceran *Daphnia magna* (282.8). Relative toxicity in long-term (10–28-d) growth and survival tests in spiked sediment was similar. *Hyalella azteca* was the most sensitive species for growth (25% inhibitory concentration [IC25] = 290.2  $\mu$ g F<sup>-</sup>/g), followed by *C. tentans* (661.4), *H. limbata* (1,221.3), and *P. promelas* (>5,600); *H. azteca* was also the most sensitive species for survival (LC50 = 1,114.6  $\mu$ g F<sup>-</sup>/g), followed by *H. limbata* (1,652.2) and *P. promelas* and *C. tentans* (>5,600 for both). Concentrations of fluoride measured in sediments near some industrial point sources exceed some of these toxicity thresholds. Fluoride is highly mobile in aquatic systems and could potentially reach toxic levels in the water column during dredging to remove fluoride-contaminated sediment.

Keywords-Fluoride Toxicity Freshwater organisms Sediment

### INTRODUCTION

Inorganic fluorides were declared toxic under paragraph 11(a) of the Canadian Environmental Protection Act in 1993 based on the conclusion of the Priority Substances List Assessment Report that "inorganic fluorides are entering the Canadian environment from anthropogenic sources in quantities resulting in concentrations in some Canadian waters, plants, and air, that may cause long-term harmful effects to biota in aquatic and terrestrial ecosystems" [1]. The report recommended the acquisition and evaluation of additional data in several areas to permit a more complete assessment of fluorides, and one of these areas was "the relationship between the levels of fluoride in sediment and toxicity to benthic organisms (in areas of Canada where high levels of inorganic fluorides in sediments are known or expected to occur)."

The major anthropogenic sources of inorganic fluorides in Canada are phosphate fertilizer production, chemical production, and aluminum smelting. These three sources collectively account for over 75% of the estimated 23,500 tons of inorganic fluorides released to the Canadian environment annually. More than 13,500 tons of this material are released in effluents [2]. The average concentration of inorganic fluoride in freshwaters across Canada is 0.05 mg F<sup>-</sup>/L, with concentrations in the vicinity of industrial activities reaching levels as high as 11.0 mg F<sup>-</sup>/L [2]. The toxicity of fluoride in water has been determined for a variety of freshwater organisms, including several species of salmonids, sticklebacks, cyprinids, caddisfly

larvae, bivalves, cladocerans, and algae (see [2] for a review). The most sensitive freshwater species tested to date is the fingernail clam, *Musculium transversum*, based on an eightweek LC50 of 2.8 mg F<sup>-</sup>/L. By "dividing the lowest-observed-effect-levels by a factor of 10 to account for differences in inter-species sensitivity and to extrapolate laboratory findings to the field," Environment Canada and Health Canada [1] have estimated the effects threshold for freshwater species to be 0.28 mg F<sup>-</sup>/L.

Data on levels of inorganic fluorides in sediments near known discharges to the Canadian environment are limited to one location in the marine environment. Fluoride levels in sediments located within 150 m of an aluminum smelter outfall in British Columbia averaged 1,370 (range 149–3,460)  $\mu$ g F<sup>-</sup>/g dry weight, with concentrations of 290, 220, and 140  $\mu$ g F<sup>-</sup>/g observed at distances of 150, 400, and 800 m, respectively, from the plant [3,4]. To our knowledge, no published data exist on the toxicity of sediment-associated fluoride to aquatic organisms.

The purpose of this study was to determine the toxicity of aqueous and sediment-associated fluoride to several species of freshwater organisms and to determine if toxic effects could be expected under environmentally realistic exposures. This study was part of a larger study to assess the toxicity of sediments near the Reynolds Metals Company aluminum production plant in Massena (NY, USA), which discharges its effluent into the international section of the St. Lawrence River near Cornwall (ON, Canada) [5,6], as well as the potential impact on the aquatic ecosystem of a proposed dredging operation to remove sediments contaminated with polychlori-

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nated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), fluoride, cyanide, aluminum, and dibenzofurans from the river bottom [7].

### METHODS

Preliminary tests to determine the toxicity of aqueous and sediment-associated fluoride to fathead minnows (*Pimephales promelas*) were conducted in 1993, and tests on a broader range of organisms were conducted in 1994.

### Toxicity of aqueous fluoride to freshwater organisms

Preliminary 7-d growth and survival tests were conducted with larval fathead minnows exposed to reagent-grade sodium fluoride in solution. Sodium fluoride is the compound most frequently used to assess the toxicity of fluoride to aquatic organisms [2]. Environment Canada's test protocols [8] were followed. Briefly, four replicates of 10 larvae (<24 h posthatch) each were exposed to concentrations of 250.0, 125.0, 62.5, 31.0, 15.5, and 7.8 mg F<sup>-</sup>/L plus a control (~0.2 mg/L) under static renewal conditions at 25°C. Larvae were fed and examined daily for mortality and swimming behavior, and mean dry weight was determined at the end of the test. As fluoride is known to be more toxic in soft water [1], testing was conducted at two hardnesses: 280 mg/L as CaCO<sub>3</sub> (well water) and 160 mg/L (~ hardness of St. Lawrence River water). The no-observed-effect concentration (NOEC), lowestobserved-effect concentration (LOEC), and IC25 were determined for both endpoints. The ICp is defined as "the inhibiting concentration for a (specified) percentage effect. It represents a point estimate of the concentration of test material that causes a designated percentage impairment in a quantitative biological function such as growth of fish" [8]. In this study, IC25 refers to the concentration of fluoride estimated to cause a 25% inhibition of growth or survival of P. promelas relative to the control. Inhibition concentration values were calculated using linear interpolation.

A series of acute lethality tests was used to determine the toxicity of fluoride to *P. promelas* and four species of invertebrates, namely, the mayfly *Hexagenia limbata*, the midge *Chironomus tentans*, the amphipod *Hyalella azteca*, and the cladoceran *Daphnia magna*. As in the preliminary tests, appropriate amounts of reagent-grade NaF were added to laboratory dilution water to obtain the desired exposure concentrations. Hardness was adjusted to 140 to 150 mg/L (similar to concentrations observed in the St. Lawrence River during the course of the larger study), pH was  $7.8 \pm 0.2$ , and temperature was 20°C. Each test species was exposed to a geometric series of five concentrations of fluoride; concentrations varied with the test species and ranged from 6 to 5,600 mg F<sup>-</sup>/L. Test conditions for each test organism are described briefly here.

Tests on juvenile (10-d-old) *P. promelas* followed U.S. Environmental Protection Agency protocols [9]. Two replicates of 10 fish each, plus one control, were exposed for 96 h under static renewal conditions. Fish were fed 0.2 ml brine shrimp nauplii following renewal of the solution at 48 h. Tests on *H. limbata* followed methods used by the Ontario Ministry of the Environment (D. Bedard, Toronto, ON, Canada, personal communication). Two replicates of five mayfly larvae (three to four months old) each, plus one control, were exposed for 96 h under static conditions. Larvae were fed 0.2 ml of *Hexagenia* diet. The diet was prepared by blending 0.9 g (dry wt) of finely crushed Tetra conditioning food (Tetra Werke, Melle,

Germany) and 100 ml of laboratory dilution water into a fine slurry. Tests on C. tentans also followed the methods of the Ontario Ministry of the Environment. Two replicates of 10 larvae (10-d-old) each, plus one control, were exposed for 96 h under static conditions. Two grams of silica sand were added to each test container, and the larvae were fed 0.005 g Tetra fish food on the first day of the test. Tests on H. azteca followed procedures recently developed at the National Water Research Institute (K.E. Day, National Water Research Institute, Burlington, ON, Canada, personal communication). Two replicates of five amphipods (1-7-d-old) each, plus one control, were exposed for 48 h under static conditions, without feeding. Tests on D. magna followed Environment Canada's protocols [10]. Four replicates of three animals each were exposed for 48 h under static conditions without feeding. The LC50 for fluoride was calculated for each test species.

### Toxicity of sediment-associated fluoride to freshwater organisms

Preliminary 21-d growth and survival tests were conducted with P. promelas exposed to sediment from Long Point, Lake Erie, spiked with NaF. Long Point sediment is routinely used as a control sediment in toxicity testing at the National Water Research Institute [11]. For each treatment, an appropriate amount of NaF was weighed out, dissolved in 10 ml of dilution water, and thoroughly blended with 325 ml of wet control sediment. A 1,300-ml volume of dilution water (water:sediment ratio = 4:1, v/v) was gently poured into the test container, which was then covered to prevent evaporation and allowed to equilibrate overnight before beginning the test. Test protocols of the Ontario Ministry of the Environment were followed [12]. Briefly, four replicates of 10 three- to four-monthold (250-400 mg wet wt) fish each were exposed to nominal concentrations of 1,100, 500, and 110  $\mu$ g F<sup>-</sup>/g dry weight plus a control (~5  $\mu$ g F<sup>-</sup>/g) under static conditions at 20°C. The highest concentration tested was similar to the concentration observed in a sediment sample collected within 50 m of the main plant outfall at Reynolds Metals on November 17, 1993 [5]. Fish were fed 1% of mean body weight per day. Hardness of the dilution water was adjusted to 160 mg/L to approximate the hardness of St. Lawrence River water. Growth was measured as the difference between the average final weight and average initial weight of all fish in each replicate.

A series of growth and survival tests was conducted with P. promelas, H. limbata, C. tentans, and H. azteca. Tests on P. promelas followed the same protocols as the preliminary tests (described previously), except that three replicates of 10 fish each were exposed to each treatment and the test fish were smaller (80-110 mg). Tests on H. limbata and C. tentans also followed the previously described protocols [12]. Both tests were conducted under static conditions at 20°C in glass jars containing 1,300 ml water and 325 ml sediment. Three replicates of 10 mayfly larvae or 15 midge larvae were exposed to each treatment. Tests on mayfly larvae were 21 d in duration, and tests on midge larvae were 10 d in duration. Mayfly larvae were three to four months old (5-25 mg) and were fed 1 ml Hexagenia diet weekly. Midge larvae were 10 to 12 d old and were fed 1 ml of the same diet per chamber. Tests on H. azteca followed procedures recently developed at the National Water Research Institute (K.E. Day, personal communication). Three replicates of 10 amphipods (1-7-d-old) each were exposed for 28 d under static conditions at 20°C in jars containing 200 ml water and 50 ml sediment; the animals were fed weekly. Each

Table 1. Toxicity of aqueous fluoride (as NaF) to larval fathead minnows, *Pimephales promelas*, in 7-d growth and survival tests at 25°C

	Concn. of fluoride (mg F <sup>-</sup> /L)			
	Hardness 280 mg/L as CaCO <sub>3</sub>		Hardness 160 mg/L as CaCO <sub>3</sub>	
	Survival	Growth	Survival	Growth
NOEC <sup>a</sup> LOEC <sup>b</sup> IC25 <sup>c</sup>	125 250 145	63 125 94	63 125 132	63 125 72

<sup>a</sup> NOEC = no-observed-effect concentration.

 $^{b}$  LOEC = lowest-observed-effect concentration.

 $^{\circ}$  IC25 = 25% inhibitory concentration.

test species was exposed to a geometric series of four concentrations of fluoride in sediment. Concentrations varied with the test species and ranged from 175 to 5,600  $\mu$ g F<sup>-</sup>/g dry weight. Two endpoints were used, namely, the LC50 for survival and the IC25 for growth. Growth was measured as the difference between the average final weight and average initial weight of all test organisms in each replicate.

#### Analysis of water and sediment for fluoride

A 100-g (wet wt) sample of the Long Point control sediment was analyzed for fluoride. The sediment was placed in a plastic jar and frozen, then freeze-dried using a Labconco Lyph-Lock 6<sup>®</sup> freeze dryer fitted with a Model 77560 Lyph-Lock Stoppering Tray Dryer® (Kansas City, MO, USA) for precise temperature control. After drying, the sample was homogenized using a mortar and pestle. Samples of overlying water (250 ml) from one replicate of each of the 110- and  $1,100-\mu g-F^{-}/g$ preliminary spiked sediment tests with P. promelas were collected at the beginning and end of the tests and analyzed for fluoride. Similarly, samples of overlying water were taken on days 0, 12, and 21 from one replicate of each treatment of the spiked sediment tests with mayfly larvae. Laboratory dilution water was also analyzed for fluoride. All analyses were performed by the Wastewater Technology Centre (Burlington, ON, Canada) using the ion-selective electrode method [13].

### RESULTS

### Toxicity of aqueous fluoride

Fluoride was found to be more toxic to larval *P. promelas* in water with a hardness of 160 mg/L than in water with a hardness of 280 mg/L in 7-d growth and survival tests (Table 1). Hardness had a greater effect on survival than growth of fish.

Results of short-term lethality tests on five freshwater organisms (Table 2) showed that *H. azteca* was the most sensitive species (LC50 = 14.6 mg  $F^{-}/L$ ), followed by *H. limbata* (LC50 = 32.3 mg  $F^{-}/L$ ) and *C. tentans* (LC50 = 124.1 mg  $F^{-}/L$ ). *Pimephales promelas* and *D. magna* were considerably less sensitive to fluoride, with LC50s of 262.4 and 282.8 mg  $F^{-}/L$ , respectively.

### Toxicity of sediment-associated fluoride

No significant difference was observed in survival or growth of juvenile *P. promelas* exposed for 21 d to nominal concentrations of 1,100, 500, and 110  $\mu$ g F<sup>-</sup>/g dry weight in sediment relative to the Long Point, Lake Erie, control sediment (Table 3). Only two of the 40 control minnows and one

Table 2. Toxicity of aqueous fluoride (as NaF) to *Pimephales* promelas, Daphnia magna, Hyalella azteca, Hexagenia limbata, and *Chironomus tentans* in short-term lethality tests at 20°C

Test species	Test duration (h)	LC50 <sup>a</sup> as mg/L (95% confidence limits)
P. promelas (juvenile)	96	262.4 (200-400)
D. magna	48	282.8 (200-400)
H. azteca	48	14.6 (12.5–25)
H. limbata	96	32.3 (10.3-51.6)
C. tentans	96	124.1 (91.6–152.9)

<sup>a</sup> LC50 = median lethal concentration.

minnow in the 500- $\mu$ g-F<sup>-</sup>/g exposure died during the test. Furthermore, no significant difference was observed in survival or growth of juvenile *P. promelas* exposed for 21 d to nominal concentrations ranging from 700 to 5,600  $\mu$ g F<sup>-</sup>/g relative to the control sediment. Only one of the 30 control minnows and three minnows in the 700- $\mu$ g-F<sup>-</sup>/g exposure died during the tests.

Results of long-term (10–28-d) growth and survival tests on four species of freshwater organisms showed that *H. azteca* was the most sensitive species for the growth endpoint (IC25 = 290.2  $\mu$ g F<sup>-</sup>/g), followed by *C. tentans* (661.4  $\mu$ g F<sup>-</sup>/g), *H. limbata* (1,221.3  $\mu$ g F<sup>-</sup>/g), and *P. promelas* (see previous discussion); *H. azteca* was also the most sensitive species for the survival endpoint (LC50 = 1,114.6  $\mu$ g F<sup>-</sup>/g), followed by *H. limbata* (1,652.2  $\mu$ g F<sup>-</sup>/g). Survival of fathead minnows and chironomids were unaffected by the highest concentration tested (5,600  $\mu$ g F<sup>-</sup>/g).

The concentration of fluoride in laboratory dilution water was 0.16 mg F<sup>-</sup>/L, with a standard deviation (SD) of 0.10. Concentrations measured in overlying water from the spiked sediment tests with *P. promelas* in 1993 and *H. limbata* in 1994 increased over the duration of the tests (Table 4). In all tests at nominal exposures of 1,100 µg F<sup>-</sup>/g or greater, the concentration of fluoride in overlying water on day 21 was almost exactly one one-hundredth of the original concentration spiked into the sediment.

### DISCUSSION

### Toxicity of aqueous and sediment-associated fluoride

A recent review of the literature on the behavior of fluoride in aquatic systems concluded that fluoride ion is not very toxic to aquatic organisms, especially in hard, cold waters [14]. Results of the present study generally support this view. Fluoride was found to be more toxic to larval fathead minnows (*P. promelas*) under the moderately hard water conditions characteristic of the St. Lawrence River (160 mg/L) than at the hardness of well water (280 mg/L). Fathead minnows and

Table 3. Toxicity of sediment-associated fluoride to juvenile *Pimephales promelas* (250–400 mg wet wt) in 21-d growth and survival tests at 20°C

Nominal concn. in sediment	Endpoint and response		
$(\mu g F^{-}/g dry wt)$	Survival	Weight change	
1,100	100%	+0.5% (+1.63 mg)	
500	98%	+0.7% (+1.85 mg)	
110	100%	-1.3% (-4.15 mg)	
5ª	95%	+1.4% (+3.75 mg)	

<sup>a</sup> Measured concentration was 4.62 µg F<sup>-</sup>/g dry weight.

Table 4. Concentrations of fluoride (as NaF) in overlying water during 21-d growth and survival tests on spiked sediment with *Pimephales* promelas and *Hexagenia limbata* 

Nominal concn.	Concn. measured in overlying water (mg/L or ppm)			Conversion factor
( $\mu g F^{-}/g dry wt or ppm$ )	Day 0	Day 12	Day 21	- (water to sediment) <sup>a</sup>
Control (4.62; <i>H. limbata</i> )	0.169 <sup>b</sup>	0.247	< 0.03	$19 \times$
110 (P. promelas)	0.55		3.18	$35 \times$
700 ( <i>H. limbata</i> )	3.10	11.3	10.4	$67 \times$
1,100 (P. promelas)	1.87		10.1	$109 \times$
1,400 (H. limbata)	3.26	13.7	14.0	$100 \times$
2,800 (H. limbata)	7.89	24.9	26.8	$104 \times$
5,600 (H. limbata)	17.7	56.8	60.9	$92 \times$

<sup>a</sup> Concentration in water on day 21 used for all exposures except the control, where data from day 12 were used.

<sup>b</sup> Concentration in laboratory dilution water = 0.16 mg/L (standard deviation = 0.1).

D. magna were less sensitive than H. azteca, H. limbata, and C. tentans in aqueous exposures to fluoride, with LC50s of 262.4 mg/L (96 h) and 282.8 mg/L (48 h), respectively, at  $20^{\circ}$ C and a hardness of 140 to 150 mg/L CaCO<sub>3</sub>. To our knowledge, the latter three species have never been tested before; however, other studies reported comparable results for fathead minnows and cladocerans. One study reported 96-h LC50s of 205 and 180 mg F<sup>-</sup>/L for fathead minnows at 20°C and hardnesses of 256 and 92 mg/L CaCO<sub>3</sub>, respectively [15], and another study [16] reported 96-h LC50s of 124 to 194 mg F<sup>-</sup>/L for fatheads at 15°C and hardnesses ranging from 69 to 292 CaCO<sub>3</sub>. The 48-h LC50 for D. magna was estimated to be 247 mg F<sup>-</sup>/L at 20°C [17]. Four species of cladocerans (D. magna, Daphnia carinata, Simocephalus vetulus, and Ceriodaphnia dubia) were found to be relatively tolerant of NaF, with 24-h LC50s ranging from 353.6 mg F<sup>-</sup>/L (D. magna) to 157.9 mg F<sup>-</sup>/L (*C. dubia*) at 20°C [18]. The LC50 for *C.* cf. pulchella was only 83.2 mg F<sup>-</sup>/L, but the author noted that this species had poor survival in laboratory settings.

Rainbow trout (Oncorhynchus mykiss) and fathead minnows were found to be more sensitive to fluoride than sticklebacks (Gasterosteus aculeatus), although the differences between species were not great: 96-h LC50s ranged from 180 to 460 mg F<sup>-</sup>/L over a range of pH, temperature, and hardness [15]. In addition to the present study, several studies have shown that some aquatic invertebrates are more sensitive to fluoride than fish. For example, net-spinning caddisfly larvae (Family hydropsychidae) were more sensitive to fluoride than either brown trout (Salmo trutta fario) or rainbow trout [19]. For comparison, 120-h LC50s were 135.6 mg F<sup>-</sup>/L for brown trout, 92.4 mg F<sup>-</sup>/L for rainbow trout, and 13.4 to 31.9 mg  $F^{-}/L$  for three species of hydropsychids at hardnesses of 22 to 42 mg CaCO<sub>3</sub>/L. Rainbow trout and the fingernail clam, Musculium transversum, are among the most sensitive freshwater species tested to date; a 20-d LC50 of 3.7 mg F<sup>-</sup>/L has been reported for trout at 13°C and a hardness of 3 mg CaCO $_3/$ L, and an eight-week LC50 of 2.8 mg F<sup>-</sup>/L has been reported for *M. transversum* [1].

Concentrations of fluoride in river water collected from 11 sites in the vicinity of the Reynolds Metals plant (Massena, NY, USA) in 1990 ranged from 0.10 to 0.21 mg F<sup>-</sup>/L, except for a concentration of 1.40 mg F<sup>-</sup>/L measured immediately adjacent to the main plant outfall [20]. These concentrations are well below levels that were acutely toxic to the five or-

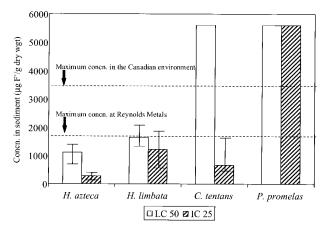


Fig. 1. Toxic thresholds (median lethal concentration [LC50] for survival and 25% inhibitory concentration [IC25] for growth, with 95% confidence interval [CIs]) for four species of aquatic organisms exposed for 10 to 28 d (see text) to fluoride in spiked sediment texts relative to the highest concentrations reported from the Reynolds Metals study area [5] and in the Canadian environment [1].

ganisms tested in this study (14.6–282.8 mg  $F^{-}/L$ ). The highest concentration reported to date from Canadian surface waters is 11.0 mg  $F^{-}/L$  downstream of an open pit tin mine tailings pond in Nova Scotia [2].

Relative sensitivity of the test organisms to fluoride in longterm (10–28-d) growth and survival tests in sediment was similar to that in short-term (48–96-h) lethality tests in water. In aqueous tests, *H. azteca* was the most sensitive species, followed by *H. limbata*, *C. tentans*, and *P. promelas*. Survival followed the same pattern in the spiked sediment tests, except that fathead minnows and chironomid larvae were equally tolerant. Chironomid larvae were found to be more sensitive than mayfly larvae for the growth endpoint.

Fluoride may have significant chronic effects on freshwater organisms. At sublethal levels, fluoride toxicity to trout is characterized by a reduced respiratory rate [21]. It has been suggested that fluoride may inhibit enzyme activity in fish [22], and oxygen consumption and acetylcholinesterase activity were reduced in the freshwater field crab (Barytelphusa guerini) after 15 d exposure to fluoride [23]. Fluoride may also cause avoidance behavior in exposed animals. For example, the migration of Pacific salmon (Oncorhynchus sp.) was inhibited by fluoride levels in the Columbia River downstream of an aluminum plant [24]. Avoidance behavior of salmon was demonstrated at concentrations as low as 0.5 mg F-/L. Mayflies (H. limbata) and fathead minnows were observed to avoid the sediment surface during toxicity tests with contaminated sediments from the Reynolds Metals study area; mayflies refused to burrow and fatheads refused to forage in sediment containing 891 to 1,680 µg F<sup>-</sup>/g dry weight [5,6]. As these sediments were contaminated with many other substances, avoidance behavior cannot be directly linked to fluoride.

Toxic thresholds (LC50 and IC25) for four species of test organisms exposed to fluoride-spiked sediment are shown in Figure 1, where they are compared with maximum concentrations occurring in the environment. The highest concentration detected in sediment from the Reynolds Metals study area was 1,680  $\mu$ g F<sup>-</sup>/g dry weight in a sample collected 50 m from the main plant outfall in 1993 [5]. The highest concentration observed to date in Canada is 3,460  $\mu$ g F<sup>-</sup>/g dry weight in sediment near an aluminum smelter on the Kitimat River in

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British Columbia [1]. Results of sediment toxicity tests suggest that growth and survival of fathead minnows and survival of chironomids would be unaffected by even the highest concentrations likely to be encountered in the Canadian environment. However, it is possible that growth and survival of H. limbata and survival of H. azteca could be affected by the highest concentration measured in sediment from the Reynolds Metals study area, and it is likely that growth of H. azteca and C. tentans would be affected. The average concentration of fluoride in sediment within 50 m of the Reynolds Metals plant outfall was 1,244 µg F<sup>-</sup>/g based on five samples collected between 1990 and 1994 [5,6,20], and the average concentration in sediment from within 150 m of the smelter in Kitimat Arm, British Columbia, was 1,370 µg F-/g based on nine samples collected in 1990 [1]. These concentrations exceed the threshold for survival of H. azteca in the present study and the threshold for growth of all test species except fathead minnows, suggesting that concentrations of sediment-associated fluoride in localized areas in Canadian waterways may be potentially toxic to freshwater organisms.

### Potential impact of proposed sediment dredging operations

Fluoride is highly mobile in aquatic systems. Elutriate testing was conducted in 1990 on sediment from a number of sites near the Reynolds Metals plant to determine the potential for fluoride and other contaminants to desorb from sediments into the water column during dredging [20]. River water was analyzed, mixed with sediment, filtered, and reanalyzed (volumes of water and sediment not reported). The concentration of fluoride increased 360-fold, from 0.100 mg/L in river water to 36.0 mg/L in elutriate prepared from the most contaminated sediment tested (323  $\mu$ g F<sup>-</sup>/g dry wt).

Results of spiked sediment tests in the present study also show that fluoride is highly mobile. Fluoride began leaching from the sediment into the water within 24 h of the test solutions being prepared with a minimum of disturbance and before the test organisms were added (day 0; Table 4). Data from the mayfly tests show that equilibrium was reached by day 12 of these tests. Using data from both the fathead minnow and the mayfly tests, concentrations of fluoride in sediment and water were found to be highly correlated at and above exposures of 700  $\mu$ g F<sup>-</sup>/g dry weight in sediment ([F<sup>-</sup>] as ppm in water = -0.37 + 0.01 [F<sup>-</sup>] as ppm in sediment; r = 0.99; n = 5).

The behavior of a contaminant in spiked sediment tests does not necessarily represent the behavior of the same contaminant under field conditions, where other pollutants are usually present and environmental conditions may vary. Concentrations of 17.9 and 23.3 mg F<sup>-</sup>/L were measured in elutriates prepared from sediment samples collected from the Reynolds Metals study area that contained 1,190 and 1,160  $\mu$ g F<sup>-</sup>/g dry weight, respectively [5,6]. These elutriates were prepared by combining clean water with sediment in a 4:1 (v/v) ratio (as in the present study), mixing it vigorously for 1 h, then allowing the mixture to settle for 48 h prior to analysis. Neither filtration nor adjusting the pH between 3.0 and 11.0 affected the amount of fluoride entering the elutriate. Using the relationship derived from the spiked sediment tests (see the previous discussion), concentrations approaching 11 mg F-/L would have been expected in these elutriates. Differences between predicted and actual concentrations could be due to differences in test conditions; for example, sediment in the elutriate tests was disturbed vigorously over a short period of time, while sediment

in the spiking tests was disturbed over several weeks by the test organisms. However, fluoride was also measured in overlying water at the end of 21-d fathead minnow and mayfly toxicity tests on the sediment containing 1,160  $\mu$ g F<sup>-</sup>/g dry weight, and concentrations were again higher than predicted (39.0–41.5 vs 11.2 mg F<sup>-</sup>/L) [6]. These results suggest that fluoride may desorb from the sediment into the water column more readily under certain field conditions, for example, when significantly disturbed during dredging activities, than in static tests in the laboratory. It follows that the toxic thresholds reported in this study for various organisms may be somewhat high and should be regarded with caution when extrapolating to field situations.

It can be concluded from this study that sediment-associated fluoride, at levels found in the vicinity of industrial point sources in the Canadian environment, has the potential to harm some species of freshwater organisms.

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# Evaluating Risks to Terrestrial Wildlife from Environmental Fluoride

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### ABSTRACT

Information and approaches to evaluating health risks to terrestrial wildlife from fluoride contamination in the environment are few in the literature. We use environmental field data from a phosphate ore processing site, toxicity reference values (TRVs), and bioaccumulation factors relative to site conditions to develop risk-based concentrations (RBCs) for total fluoride in terrestrial biota and soil. RBCs were derived specifically for forage that are protective of terrestrial mammalian and avian ecological receptors through multiple exposure pathways, and which can be used to evaluate site remediation or as environmental monitoring action levels. Following review of the literature, we recommend fluoride TRVs for mammalian and avian wildlife, and bioavailability factors for estimating exposures related to aerial deposition of fluoride and fluoride gases. For large ungulates, information on fluoride bioavailability from feed and soil, and related effects thresholds, are summarized from studies on grazing livestock. The resultant RBCs for fluoride in forage range from 14 to 63 mg/kg dry weight, based on no-effect and low-effect concentrations, respectively. These concentrations bracket most state, provincial, and international regulatory standards of vegetation levels for protection of livestock and wildlife.

Key Words: fluoride, wildlife risk, ecological risk assessment, toxicity reference value.

### INTRODUCTION

Reports in the scientific literature of predictive risk analyses for wildlife exposures to fluoride are few (Newman 1984; Purucker *et al.* 2007; Salatas *et al.* 2009). Nonetheless, reports of numerous animal toxicity studies are available, having been stimulated by the need to understand the potential effects of fluoride on grazing

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livestock due to phosphate fertilizer use (Cronin *et al.* 2000), and as a result of finding fluorosis in livestock and wildlife exposed to emissions from aluminum smelters and phosphate ore processing facilities (Weinstein and Davison 2004). Based on reviews of available risk assessments and the scientific literature on fluoride toxicity and bioavailability, we recommend toxicity reference values (TRVs) and bioavailability factors for ecological risk assessment (ERA), and use concentration data from a fluoride-contaminated semi-arid habitat to derive risk-based concentrations (RBCs) for total fluoride in various environmental media that are protective of terrestrial wildlife through multiple routes of exposure. Fluoride RBCs were derived for both biotic and abiotic environmental media to demonstrate their utility for setting levels of concern or action levels for monitoring fluoride in the environment.

The typical approach to characterizing risk to chemically exposed wildlife receptors at a hazardous waste site is to compare estimates of site-specific dietary exposures to dose-based TRVs (USEPA 1998). Dietary dose routes may include inhalation, drinking surface water, and ingestion of contaminated food items and soil. The risk estimates, more properly termed "hazard quotients" since probabilities of effects are not quantified (Hope 2012), are the ratio of the estimated exposure to the TRV. Although increasing emphasis is being placed on incorporating full dose-response information in the toxicological component of wildlife risk assessments (Allard et al. 2010), typically two levels of TRVs are used in an ERA: the no-observed-adverse-effects level (NOAEL) to represent exposures below which no unacceptable levels of impacts are expected and which are intended to be protective of all individuals in a population, and the lowest-observed-adverse-effects level (LOAEL) that corresponds to the lowest exposures that are associated with statistically significant impacts in experimental test groups. The true threshold for toxicity is generally between the NOAEL and LOAEL doses, and the use of both TRVs can provide context in evaluating the potential for ecological risk. For this reason, we select both NOAEL and LOAEL TRVs to develop a range of RBCs for total fluoride for each wildlife receptor.

Ecological RBCs are those concentrations in environmental media that are associated with a defined level of acceptable risk or hazard for ecological receptors. Since they can be calculated for wildlife based on generic exposure parameters, such as bodyweight and ingestion rates found in U.S. Environmental Protection Agency (USEPA 1993) guidance, they are not necessarily dependent on site-specific data if site-use factors (*i.e.*, relationship of forage or home range of a receptor to the size of the site) or bioavailability factors are assumed. However, a fluoride RBC for a single medium that is considered protective of wildlife exposures to all other media at a particular site may be useful for setting action levels for monitoring purposes. In this article, we develop fluoride RBCs for forage vegetation that account for wildlife exposures to other media and are dependent on the relationships among site-specific fluoride concentrations in those media.

### **METHODS**

### Site Characteristics and Contaminant Data

The fluoride RBCs were developed using data on total fluoride concentrations collected in 2009 from areas outside the property boundaries of two phosphate ore

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processing facilities at the Eastern Michaud Flats (EMF) site in southeastern Idaho (USA). Fluoride contamination at the EMF site resulted from air emissions from a former phosphorus plant and from an operational phosphoric acid plant, both of which began operating in the 1940s. The facilities used fluorapatite (Ca5(PO4)3F) ore from southeastern Idaho as the primary raw material. Fluoride releases from various heat processes at the facilities likely occurred as gaseous hydrogen fluoride and silicon tetrafluoride, as well as particulates (ATSDR 2003). At facilities, baghouses and scrubbers controlled air emissions from furnace off-gases; nonetheless, although concentrations have been decreasing over time, elevated fluoride in vegetation and soil located within 3–5 miles of the facilities have been found over decades of monitoring.

Fluoride concentration data are reported in Formation Environmental (2010) and were collected to support an ERA specifically for fluoride at the EMF site; methods for sample collection and analysis were based on a work plan and quality assurance measures that followed procedures of, and were approved by the USEPA. The data are from a single sampling effort consisting of 5 to 16 samples, depending on medium, with each sample a composite of up to 30 specimens collected over a 0.3 acre sampling unit.

### **Ecological Receptors**

The wildlife receptors selected for RBC development are characteristic of the sagebrush steppe found in the semi-arid, temperate climate of southeastern Idaho where the EMF site is located. The sagebrush steppe communities of Michaud Flats are dominated by sagebrush (*Artemisia* spp.), with other shrubs and perennial grasses, dominated by wheatgrass species. Wildlife receptors of concern are the deer mouse (*Peromyscus maniculatus*) as a representative small mammal, coyote (*Canis latrans*) as a representative mammalian carnivore, red-tailed hawk (*Buteo jamaicensis*) as a representative avian carnivore, and horned lark (*Eremophila alpestris*) as a representative passerine or songbird (Formation Environmental 2010). Other wildlife such as mule deer, a large herbivore, and sage grouse, a gallinaceous game bird, had been shown to be at lower risks at the site due to lower dietary exposures, and RBCs are not developed for them. RBCs were developed for applicable exposure media for the receptors of concern: unwashed forage vegetation, unwashed browse vegetation, small mammals, terrestrial invertebrates (insects), and soil.

### **Toxicity Reference Values**

Studies containing critical data for developing mammalian and avian TRVs for fluoride were identified through literature searches, published summaries of ecotoxicity data, and recent reviews compiled in ERAs for fluoride-contaminated sites (Formation Environmental 2009; Salatas *et al.* 2009). In addition to the literature search, toxicity data for mammals and birds were found in the Oak Ridge National Laboratory (ORNL) compilation (Sample *et al.* 1996) and the EcoTox database (USEPA 2012), and data for mammals are available in ATSDR (2003), Environment Canada (1996), and NRC (1993). Based on the literature searches and reviews, studies selected for identifying fluoride TRVs for mammals are summarized in Table 1; critical studies for both avian and mammalian receptors are discussed further in this

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Source	Species	Endpoint	Exposure conditions and outcome
Aulerich <i>et al.</i> 1987	Mink (Mustela visons)	Reproduction, kit mortality	Mated pairs of adult mink fed NaF-supplemented diets for 382 days, total fluoride at 68, 95, 143, 229, and 385 mg/kg; weaned offspring kits fed the same diet. No significant effects on body weight, reproduction (percentage of fertilization, mean gestation time, mean litter, lactation). Decreased survivability of kits on highest concentration (14% at 3 weeks, compared with 86% for the control). Dietary no-effect concentration of 229 mg/kg and NOAEL of 31.37 mg/kg-d; LOAEL of 52.75 mg/kg-d, based on kit survival.
Shupe <i>et al.</i> 1987a	Mink	Bone structure	Dietary concentrations of NaF for 7–8 mos. NOAEL of 4.75 mg/kg-d (dietary no-effect concentration of 125 mg/kg dw), LOAEL of 11.93 mg/kg-d (dietary LOEC of 307 mg/kg dw); osteofluorosis in kits and adults.
Araibi <i>et al.</i> 1989	Rat	Reproduction	Dietary NaF for 60 days. NOAEL of 11.2 mg/kg-d; LOAEL dose of 22.4 mg/kg-d resulted in a 50% reduction in fertility (pregnancy rate and total number of offspring), and decreased percentage of seminiferous tubules containing spermatozoa and decreased testosterone levels in exposed male adult rats.
Krasowska 1989	Bank vole (Clethriono- mys glareolus)	Reproduction	Dietary study over two generations with fluoride-containing chitin; fluoride dietary concentrations of 18.37, 46.6, and 96.96 mg/kg dw. No statistically significant mortality for any dose; elevated mortality observed for the highest dose. Reduced number of litters per fertile female in the highest dose, and reduced litter size, number of days from mating to producing the first litter, and number of days between successive litters. A significant reduction in the latter three endpoints was noted in the second generation of test animals. NOAEL calculated at 5.3 mg/kg-d, LOAEL at 10.98 mg/kg-d.

 Table 1.
 Summary of mammalian toxicity studies for fluoride.

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Source	Species	Endpoint	Exposure conditions and outcome
NTP 1990	Місе	Growth, mortality	Dose of NaF at 200 mg/L in drinking water for 6 months. resulted in no effects; reduced weight gain and mortality at 300 mg/L. NOAEL and LOAEL calculated by Salatas <i>et al.</i> (2009) at 24.5 and 42.1 mg/kg-d, respectively.
Marks <i>et al.</i> 1984	Rats	None	Dietary NaF for 3 mos. No effect dose of 23 mg/kg-d.
Maurer <i>et al.</i> 1990	Rats	Skeletal abnormalities	Dietary doses of 4.5 or 11.3 mg/kg-d of fluoride for 95–99 weeks resulted in reduced body weight, hyperostosis of skull, hyperkeratosis, and acanthosis in the stomach versus controls (0.1 mg/kg-d).
Chinoy et al. 1995, Chinoy and Sequeira 1992	Rats, mice	Decreased sperm motility and count	Dietary sodium fluoride for 30–50 days. Adverse effects at 4.5 mg/kg-d of fluoride.
Tao and Suttie 1976	Mice	None	Dietary NaF for 3 generations. No effect dose of 13 mg/kg-d.
Eckerlin <i>et al.</i> 1986	Red foxes	Survival	Dietary concentrations of 98–137 mg/kg dw resulted in decreased milk production and decreased survival of kits.
Mosekilde <i>et al.</i> 1987, Kragstrup <i>et al.</i> 1989	Pigs	Skeletal abnormalities	Dose of 2 mg/kg-d for 6 months. resulted in alterations in bone remodeling.
Purohit <i>et al.</i> 1999	Rabbit	Congestion, edema and desquamation of respiratory epithelium in lungs	Dietary NaF for 6 mos. Adverse effects at 4.5 mg/kg-d of fluoride.
Shupe <i>et al.</i> 1963	Dairy cows	Dental fluorosis and skeletal abnormalities	Dietary concentration of 27 mg fluoride/kg feed was associated with minor dental fluorosis and slight skeletal changes; 49 mg fluoride/kg feed was associated with adverse osteofluorosis and dental fluorosis.
Shupe <i>et al.</i> 1992	Cattle	Dental fluorosis and skeletal abnormalities	Dietary concentrations of 60–93 mg/kg in vegetation resulted in severe dental and osteofluorosis. Concentrations of 25–60 mg/kg in vegetation resulted in mild to moderate dental and osteofluorosis. Less than 25 mg/kg of fluoride in vegetation resulted in minimal dental changes.

 Table 1.
 Summary of mammalian toxicity studies for fluoride. (Continued)

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Source	Species	Endpoint	Exposure conditions and outcome
Snow and Anderson 1986	Dogs	Skeletal abnormalities	Dietary dose of 0.32 mg/kg-d of fluoride (dw) for 6 mos. resulted in alterations in bone remodeling
Suttie <i>et al.</i> 1985	White tailed deer	Dental fluorosis and skeletal abnormalities	Dietary concentrations of 10, 35, or 60 mg/kg for 2 yrs. At 35 mg/kg, mottling of incisors characteristic of dental fluorosis was noted; at 60 mg/kg, increased wear of molars and hyperostoses of the long bones of the leg were observed.

 Table 1.
 Summary of mammalian toxicity studies for fluoride. (Continued)

Sources: ATSDR (2003), Formation Environmental (2009, 2010), Salatas et al. (2009), and USEPA Region 10.

mg/kg-d = milligrams fluoride per kilogram bodyweight per day.

article. The preferred endpoints on which TRVs are based are those that may affect a population of organisms, and include sublethal measures associated with growth, reproduction, and survival, particularly survival to reproductive age.

The procedure for selecting fluoride mammalian TRVs from the studies in Table 1 followed USEPA (2007) guidelines for developing ecological soil screening levels (Eco-SSLs). Three studies were identified as sufficient for developing mammalian TRVs (discussed further below): Aulerich *et al.* (1987), Araibi *et al.* (1989), and Krasowska (1989), consistent with a recent review in Formation Environmental (2010). Rather than using the geometric mean of NOAELs from the three studies as the TRV, which was found to be greater than the lowest bounded LOAEL from the Krasowska (1989) study, the NOAEL and LOAEL values from Krasowska (1989) were selected as the fluoride TRVs for mammals. According to the Eco-SSL guidelines, when the geometric mean NOAEL for a chemical is greater than the lowest LOAEL for any of the three endpoints, then the lowest bounded NOAEL (*i.e.*, a NOAEL with a paired LOAEL) should be selected for the NOAEL-based TRV. The use of a geometric mean NOAEL greater than the lowest bounded LOAEL might not be protective of wildlife exposures.

Avian TRVs were taken from a study of reproductive effects (adult fertility and hatching success) in Eastern screech owls (*Otus asio*) fed a NaF-supplemented diet (Pattee *et al.* 1988). Avian NOAEL and LOAEL TRVs are identified as 8.02 and 32.9 mg/kg-d, respectively.

### **Bioavailability Factors**

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The Krasowska (1989) feeding study, from which the mammalian TRVs were derived, used chitin rather than a more typical NaF-supplemented diet for the exposures of test organisms. Because the bioavailability of fluoride from chitin is considered to be lower than from a NaF-supplemented diet (Pastuszewska *et al.* 1983; Krasowska 1989), we assumed that fluoride bioavailability to mammals from the TRV study food source and from food items at the EMF site were not substantially

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different from each other, and thus no bioavailability factors were used to model mammalian exposures from food ingestion.

For soil, because of complexion with calcium in the calcareous soils of the EMF site, fluoride was assumed to be less bioavailable following soil ingestion than from the chitin feed used in the Krasowska (1989) study. For that reason and based on the recommendation in Cronin *et al.* (2000), fluoride bioavailability for mammalian ingestion of soil was assumed to be 65%, consistent with USEPA (1995) and Formation Environmental (2010). Similarly, fluoride was assumed to be less bioavailable to avian receptors at the EMF site due to the calcareous soils than from the NaF-supplemented feed used in the avian toxicity study (Pattee *et al.* 1988). Avian bioavailability was assumed to be 75% for plant and invertebrate food sources, 70% for small mammal food sources for avian carnivores, and 65% for ingested soil, consistent with Formation Environmental (2010).

#### **Risk-Based Concentrations**

For each receptor of concern, an RBC was calculated for unwashed forage so that the associated concentrations of fluoride in all other consumed media would result in a total hazard quotient no greater than 1 for that receptor. Thus, for each receptor a protective concentration of fluoride in unwashed forage (the RBC) was developed that relates to protective concentrations in each of the other environmental media that the receptor may consume. The protective concentrations are calculated for all media for each receptor regardless of whether the receptor actually consumes the media. Using coyote as an example, an RBC in forage was calculated that relates to protective concentrations of fluoride in small mammals and soil, which are the two media to which the coyote is actually exposed.

The approach used to develop forage RBCs for fluoride is consistent with the method of USEPA (2005) for developing ecological soil screening levels (Eco-SSLs), except that the focus of RBC development in this case is on forage rather than soil. The risk basis of the threshold is the hazard quotient (HQ), which relates dose to toxicity. The  $Dose_{Total}$  term is derived in Eq. (1) for multiple sources of dietary intake plus soil ingestion.

$$Dose_{Total} = \frac{(C_{forage} \times IR_{forage}) + (C_{browse} \times IR_{browse}) + (C_{prey} \times IR_{prey}) + (C_{soil} \times IR_{soil})}{BW}$$
(1)

where  $C_{forage}$ ,  $C_{brouse}$ ,  $C_{prey}$ ,  $C_{soil}$ , = concentrations of fluoride in unwashed forage, unwashed browse, prey, and soil (mg/kg dw except mg/kg ww for small mammals), respectively; IR = ingestion rate (kg/day, units consistent with concentration term); and BW = bodyweight of receptor species (kg). Relative bioavailability was accounted for by incorporating bioavailability factors into the  $Dose_{Total}$  terms for each receptor and medium; bioavailability factors consisted of numerical equivalents of the percent-based values identified above. The  $Dose_{Total}$  term conservatively assumes that the receptor acquires 100% of its dietary intake from the site (*i.e.*, the site use factor (SUF) is set at 1). The RBC for a single dietary item is derived using Eq. (2) while setting the HQ to 1.

$$RBC_{dietitem} = (HQ = 1) \times TRV \times \frac{BW}{IR_{dietitem}}$$
(2)

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To derive an RBC for fluoride in forage, the terms  $C_{browse}$ ,  $C_{prey}$ , and  $C_{soil}$  in Eq. (1) are replaced with functions that relate those concentrations to  $C_{forage}$  (*i.e.*, to the concentration in unwashed forage). The functions were developed using data on total fluoride collected from the EMF site and followed the preferred hierarchy in USEPA (2005), with preference for regression equations to characterize association functions, followed by association factors as simple ratios when data are limited or regressions are not significant. The term association function is used since the regressions do not describe causative relationships such as uptake or transfer factors.

With  $C_{browse}$  and  $C_{prey}$  replaced by association functions and  $C_{soil}$  by an association factor (see Results), dietary ingestion rates and bodyweights were used in Eqs. (1) and (2) to solve for  $C_{image}$  that result in a total HQ of 1 for each wildlife receptor. Total ingestion rates are from Nagy (2001), and dietary components as percentages of the ingestion rate are from USEPA (1993), except Martin et al. (1951) for the horned lark. Soil ingestion percentages of total diet are from Beyer et al. (1994). Body weights are from Godin (1977) for coyote, USEPA (1993) for red-tailed hawk and deer mouse, and Dunning (1993) for horned lark. The coyote diet was assumed to consist of 100% small mammals, at a dietary ingestion rate of 0.429 kg/day dw (1.684 kg/day ww), with soil ingestion at 2.7% of the diet and body weight at 13.6 kg; the deer mouse diet was assumed to consist of 21.5% browse (shrubs), 42.8% forage (forbs), and 35.7% terrestrial invertebrates (insects), with an ingestion rate of 0.0035 kg/day dw, soil ingestion assumed at 2.0% of the diet, and body weight at 0.021 kg; the horned lark diet was assumed to consist of 80% forage and 20% insects. with an ingestion rate of 0.00658 kg/day dw, soil ingestion assumed at 2.3% of the diet, and body weight at 0.031 kg; the red-tailed hawk diet was assumed to consist of 100% small mammals, with an ingestion rate of 0.0858 kg/day dw (0.215 kg/day ww), soil ingestion assumed at 2.0% of the diet, and body weight at 1.056 kg. Home ranges can be found in USEPA (1993) for the red-tailed hawk and deer mouse, and in DeGraaf and Rudis (1986) for horned lark and coyote.

## RESULTS

The relationships between total fluoride concentrations in unwashed forage and those in other environmental media at the EMF site are depicted for each sample in Figure 1, with other environmental media consisting of browse vegetation, soil, and tissues of terrestrial invertebrates and small mammals. The regressed data were fit to linear or logarithmic equations, and resultant best fit regression equations and correlation coefficients are displayed in the legend to Figure 1.

The correlation coefficients for small mammals and invertebrates tend to be dependent on single high concentrations (Figure 1); the dependency on a small number of high concentration values is a major source of uncertainty for each association. The slope of approximately unity for the regression relationship between total fluoride in unwashed forage and in washed forage  $(r^2 = 0.96)$  suggests that most of the fluoride in forage was incorporated into the tissue or strongly adsorbed. For unwashed forage and soil, regressions showed weak to no correlations, and regression coefficients are not tabulated. Instead, following USEPA (2005) guidance,

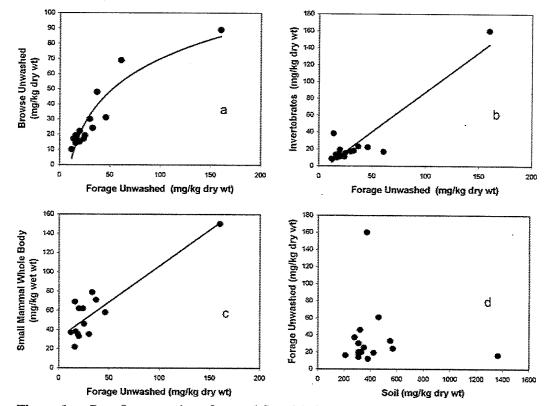


Figure 1. Best fit regressions for total fluoride in environmental media at the EMF site. (A) Browse-unwashed vs. forage-unwashed, n = 16, logarithmic, y = 31.138 Ln(x) - 73.332,  $r^2 = 0.86$ ; (B) terrestrial invertebrates vs. forage-unwashed, n = 16, linear, y = 0.9424x - 6.8714,  $r^2 = 0.86$ ; (C) small mammal vs. forage-unwashed, n = 14, linear, y = 0.7479x + 31.55,  $r^2 = 0.78$ ; (D) forage-unwashed vs. soil, n = 14, non-significant. Units are in dry weight, except small mammals are in wet weight.

the relationship between unwashed forage and soil fluoride was characterized by an association factor of 0.095, calculated as the 95% upper confidence limit on the mean (0.08) of 14 ratios of unwashed forage to soil fluoride. Reasons for the weak relationships between fluoride concentrations in soil and those in biotic media at the site are unknown, but the weak relationships are consistent with direct uptake from air as the primary source of vegetation fluoride at the EMF site (*e.g.*, see review in TCEQ 2009).

The resultant RBCs for each receptor and each medium are shown in Table 2. The lowest RBCs for fluoride in unwashed forage were calculated at 14 and 63 mg/kg dw for the NOAEL- and LOAEL-based TRVs for protection of coyote and deer mouse, respectively. These concentrations are identified as risk-based threshold concentrations for fluoride in unwashed forage in that they serve as thresholds to indicate the potential for risks to multiple receptors.

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		Risk-based concentrations						
Receptor of concern	TRV type	Forage (mg/kg dw)	Browse (mg/kg dw)	Small Mammal (mg/kg ww)	Invertebrates (mg/kg dw)	Soil (mg/kg dw)		
Coyote	NOAEL	14		42		149		
	LOAEL	72		85		758		
Deer mouse	NOAEL	30	33		22	318		
	LOAEL	63	55		52	659		
Horned lark	NOAEL	43	<u> </u>		· 34	456		
	LOAEL	175		-	158	1837		
Red-tailed hawk	NOAEL	30		54		315		
	LOAEL	241		212		2537		

 Table 2.
 Summary of fluoride risk-based concentrations to protect ecological receptors at the EMF site.

*Bold and italics* = Lowest RBCs in each environmental medium for NOAEL and LOAEL toxicity reference values. dw = dry weight; ww = wet weight.

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#### DISCUSSION

#### Mammalian Toxicology and TRVs

Numerous reviews have described the effects of chronic exposures of mammals to fluoride, including dental and skeletal lesions, lameness, appetite impairment, reproductive effects, and behavioral changes (NAS 1971; Newman 1979; Environment Canada 1996; ATSDR 2003; Doley *et al.* 2004; OMOE 2004; TCEQ 2009). The sensitive life stage for fluoride effects on animals is during bone growth and tooth development, and fluoride deposition in bone occurs mainly in regions undergoing active ossification or calcification. Osteofluorosis lesions are mainly located on the bone surface and are seen as a general thickening of the bones or as excessive surface formations (exostosis), with proliferation of deeper endosteal cells in severe cases. Mineralization of tendons at the point of attachment to the bone is also commonly observed (Shupe *et al.* 1963). Studies with grazing livestock noted that dental fluorosis is readily observable as an early indication of potential fluoride effects on bone (NAS 1971; Shupe and Olson 1983; Shupe *et al.* 1987b).

Following dietary exposure in mammals, the low pH of the stomach strongly favors formation of hydrogen fluoride, which is passively absorbed in the stomach and intestine. At physiological pH, hydrogen fluoride dissociates into fluoride ion ( $F^-$ ) and hydrogen ion ( $H^+$ ). The fluoride ion is believed to replace the hydroxyl ion ( $OH^-$ ), and possibly the bicarbonate ion ( $HCO_3^-$ ), associated with hydroxyapatite of calcified tissues (Kaminsky *et al.* 1992), a mineral phase during the formation of teeth and bone.

Although most of the published toxicity studies have been performed with small mammals or livestock, field studies have noted similar dental fluorotic lesions and osteofluorosis in wildlife exposed to mining sources or industrial discharges of fluorides, including the roe deer (Capreolus capreolus), mule deer (Odocoileus hemionus hemionus), white-tailed deer (O. virginianus), blacktailed deer (O. hemionus columbianus), wood mouse (Apodemus sylvaticus), common shrew (Sorex araneus), short-tailed field vole (Microtus agrestis), bank vole (Clethrionomys glareolus), and cotton rats (Sigmodon hispidus) (Kierdorf and Kierdorf 1989; Kierdorf et al. 1993; Kay et al. 1975; Karstad 1967; Suttie et al. 1987; Cooke et al. 1990; Vikøren and Stuve 1996; Boulton et al. 1994a,b; Schultz et al. 1998; Doley et al. 2004; Richter et al. 2010; Paranjpe et al. 1994). Although doses have been difficult to quantify in field studies, dose-effects were quantified in a controlled captive study of white-tailed deer exposed to dietary NaF, where a 2-year exposure found dental fluorosis at a 35 mg/kg diet, and increased wear of the molars and mild hyperostoses of the long bones of the leg at 60 mg/kg diet (Suttie et al. 1985). The effects observed in the captured deer were reported to be similar to those observed in affected wild deer near sources of industrial releases and in cattle at similar exposure levels (Karstad 1967; Kay et al. 1975; Newman and Yu 1976). A finding of fluorosis effects in bison exposed to fluoride in geyser areas of Yellowstone National Park (USA) support the likelihood that the tolerance of wild ungulates, including deer and bison, to fluoride is similar to that for domestic ungulates (Shupe et al. 1984).

As mentioned, summaries of mammalian and avian ecotoxicity data were found in the ORNL compilation (Sample *et al.* 1996) and in the EcoTox database (USEPA 2012), and additional data for mammals are available in summaries used for evaluating human health risks (ATSDR 2003; Environment Canada 1996; NRC 1993). However, neither ecotoxicity profiles nor TRVs for fluoride are available in the Eco-SSL database (USEPA 2007), in the compilation for performing ERAs for combustion releases (USEPA 1999), or in a recent U.S. Department of Energy compilation of screening values for ecological receptors (Friday 2005). The ORNL publication of Sample et al. (1996), one of the most widely used ecotoxicity compilations, recommends fluoride toxicity benchmarks for mammals that are based on the Aulerich et al. (1987) study of minks (Table 1). The Aulerich et al. (1987) data have also been used to develop mammalian TRVs in several ERAs, including at the U.S. Department of Energy-Idaho National Laboratory and Savannah River (USA) facilities, mine sites in the western United States (Sampson et al. 1996), and more recently for exposures of pronghorn to a fluoride-contaminated sagebrush habitat in the western United States (Purucker et al. 2007) and to evaluate several small mammal species near a proposed aluminum smelter (SENES 2007). The state of Oregon (USA) (ODEQ 2001) and the LANL (2012) ECORISK database use the ONRL benchmarks to develop protective soil levels for fluoride exposures of mammals.

The Aulerich et al. (1987) study has been widely used because it included chronic exposures to a wildlife species during a critical life stage (reproduction), several dose levels that yielded both NOAEL and LOAEL effects, and measures of physiology, growth, reproduction, and mortality endpoints. In addition to kit mortality on which the TRVs are based, musculoskeletal deformation of the sagittal crests was noted in half of the male adults at the LOAEL daily dose per bodyweight of 52.75 mg/kg-d. The Shupe et al. (1987a) feeding study (Table 1) noted the same sagittal crest deformation, as well as other indications of osteofluorosis, in exposed minks at a much lower dose of 11.93 mg/kg-d, with severe effects at 31.4 mg/kg-d. USEPA (1995) used the NOAEL of 4.75 mg/kg-d from the Shupe et al. (1987a) study as the TRV in an ERA for the EMF site. The Shupe et al. (1987a) study was of shorter duration than the Aulerich et al. (1987) study, and did not cover reproductive endpoints, and was subsequently not selected as suitably confident for TRV derivation during a review of fluoride toxicity studies for a more recent ERA at the EMF site (Formation Environmental 2010). Note that allometric scaling originally advocated in Sample et al. (1996) and used in the USEPA (1995) ERA is no longer recommended because the scaling data were based on acute exposures primarily for anti-cancer drugs (Allard et al. 2010; USEPA 2007).

The reviews in Formation Environmental (2009, 2010) identified Araibi *et al.* (1989) and Krasowska (1989) in addition to Aulerich *et al.* (1987) as of sufficient quality for derivation of mammalian TRVs for fluoride (Table 1). The TRVs derived from the Araibi *et al.* (1989) study, with a LOAEL of 22.4 mg/kg-d, were considered to have moderate to high confidence since the study was over a chronic timeframe, at a sensitive life stage (reproduction), used adequate controls, and included appropriate statistical procedures. The LOAEL TRV for reproductive and mortality effects in the Krasowska (1989) study of bank voles, at 10.98 mg/kg-d, was considered to have moderate to high confidence, with some uncertainty due to the use of a krill-based diet with uncertain fluoride bioavailability from the chitin matrix and uncertain interactions with other constituents of krill (note that the LOAEL

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of 10.98 mg/kg-d is similar to the Shupe *et al.* [1987a] value for mink). Krasowska (1989) states that, based on Pastuszewska *et al.* (1983), the bioavailability of fluoride from chitin is lower than from NaF, and the absorbed doses of fluoride would have been lower than estimated in the study. In the Krasowska (1989) study, the high percentage of test organisms exhibiting effects (*e.g.*, 60–90% for incisor effects in the second generation) suggests that risks to small mammals from fluoride ingestion at or greater than the LOAEL could be significant.

A recent ERA for an aluminum smelter site in Iceland (Salatas *et al.* 2009) identified a study of mice conducted by the U.S. National Toxicology Program (NTP 1990) to derive TRVs specifically for fluoride exposures of the wood mouse as the critical mammalian receptor for the site. The NOAEL and LOAEL TRVs are higher than those derived from the Araibi *et al.* (1989), Krasowska (1989), and Shupe *et al.* (1987a) studies, but are similar to those of Aulerich *et al.* (1987) (Table 1).

The studies identified in Table 1 tend to be limited to small- and medium-sized mammals exposed under laboratory conditions. Toxicity data on wild ungulates or large grazers have not been of sufficient quality for developing fluoride TRVs in published ERAs. For example, risks to mule deer in the USEPA (1995) and Formation Environmental (2010) ERAs at the EMF site were assessed using TRVs developed from mink and bank vole studies summarized above. Whether the sensitivity of large ungulates to fluoride differs from that of smaller animals is uncertain. The findings of dental fluorosis in captive white-tailed deer at a dietary concentration of 35 mg/kg (Suttie *et al.* 1985) and in cattle at 27 mg/kg (Shupe *et al.* 1963) suggest that large grazers may show similar or even greater sensitivity to fluoride as small mammals, for which fluorosis effects were observed in bank voles at 46.6 mg/kg with no effects at 18.37 mg/kg (Krasowska 1989), and in mink at 229 mg/kg but not at 143 mg/kg (Aulerich *et al.* 1987).

Because livestock sensitivity to fluoride may differ from smaller mammals used in toxicity tests, livestock-specific dietary thresholds can be used to evaluate risks in lieu of dose-based TRVs extrapolated from laboratory studies. The NAS (1974) assessment of fluoride toxicity and the review in Shupe and Olson (1983) of cattle exposed to fluoride over multiple years confirmed an earlier compilation of dietary thresholds for dental fluorosis and adverse health effects in livestock (Suttie *et al.* 1958): 30 mg/kg dry weight (dw) diet as a threshold for slight exostosis and cartilage calcification, 40 mg/kg dw diet for moderate dental fluorosis and other effects, and 50 mg/kg dw diet for severe effects. Because these diet-based thresholds are comparable with the 35 mg/kg dietary concentration that resulted in dental fluorosis in the white-tailed deer study (Suttie *et al.* 1985), they may be useful as diet-based adjuncts to dose-based TRVs for evaluating risks to large grazing mammals.

Bone levels of fluoride are sometimes measured during toxicity tests and field exposures, from which effects thresholds can be estimated. Dietary no-observed-effects concentrations (NOECs) have been associated with average femur concentrations ranging from 960 to 2485 mg/kg dw for bank voles and mink (Krasowska 1989; Shupe *et al.* 1987a), with dietary low-observed-effects concentrations (LOECs) at femur concentrations of 2800 to 5110 mg/kg dw. In white-tailed deer, dietary LOECs for fluorosis and mild hyperostoses were associated with mandible concentrations at 4550 and 6600 mg/kg, respectively, compared with a NOEC of 1700 mg/kg for

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control deer (Suttie *et al.* 1985). Consistent with these studies, USEPA (1995) recommended 2000 mg/kg dw as a femur concentration threshold for potentially toxic exposure in mammalian wildlife.

#### **Avian Toxicology**

Fluoride toxicity data are not as well developed for birds as they are for mammals largely because of the lack of teeth that serve as indicators of fluorosis in mammals. Toxic effects are reported as changes in egg quality and shell thickness, bone breaking strength, and lower reproductive success (Guenter and Hahn 1986; Carrière *et al.* 1987; Bird and Massari 1983; Hoffman *et al.* 1985; Fleming *et al.* 1987).

The Pattee *et al.* (1988) study, used herein to develop avian RBCs, is considered to have high confidence for use in TRV development because of the use of dietary exposures, development of both NOAEL and LOAEL effects for chronic exposure, and the evaluation of multiple endpoints. The study observed reproductive effects in Eastern screech owls (*Otus asio*) fed a NaF-supplemented diet. The TRVs have been used in ERAs at Department of Energy (USA) sites and for an aluminum smelter (SENES 2007), and to develop soil screening levels for birds in the ECORISK (LANL 2012), ORNL (Sample *et al.* 1996), and Oregon (ODEQ 2001) compilations.

An alternative avian toxicity study has been used in an ERA for an aluminum smelter in Iceland (Salatas *et al.* 2009). Based on the similarity of dietary preferences between the wild turkey and the rock ptarmigan, which was the key avian receptor at the site, the ERA selected a study of fluoride exposures of turkeys (Nahorniak *et al.* 1983) as the critical avian toxicity study. Newly hatched turkeys were exposed to NaF in their diet for up to 18 weeks, and NOAEL and LOAEL TRVs for reduced bodyweight were identified as 13.2 and 26.5 mg/kg-d, respectively.

#### Fluoride Bioavailability in the Terrestrial Environment

Bioavailability is incorporated into modeling exposures to wildlife receptors when the fraction of fluoride absorbed from a contaminated source differs from the fraction absorbed from the exposure medium in the test used to derive the TRV; this difference is termed "relative bioavailability." Essentially, when the TRV is based on an administered dose determined from a feeding study, the TRV accounts for limitations in desorption from ingested items and absorption kinetics from the gastrointestinal tract. If bioavailability is assumed to be the same in both the TRV feeding study and for the receptor at the contaminated site, then no bioavailability factor is incorporated into the exposure modeling. Any differences in bioavailability between the test study and the modeled exposures would need to be accounted for by use of a bioavailability factor.

We evaluated the use of dietary bioavailability factors to model mammalian exposures to fluoride based on the review in Cronin *et al.* (2000), who recommended that dietary absorption of fluoride be assumed at 75%, based on studies that reported fluoride bioavailability at 75% for dairy cattle fed either a NaF-supplemented diet or atmospherically contaminated hay (Shupe *et al.* 1962), 75% for goats fed a NaFsupplemented diet (Clay and Suttie 1985), and 70 and 79% for rats fed a dietary solution of NaF or the solid form of calcium fluoride (CaF<sub>2</sub>) (Harkins *et al.* 1963).

Boulton *et al.* (1994a) demonstrated that the bioavailability of fluoride from contaminated vegetation to field voles can differ depending on the source of contamination, suggesting differences in biotic chemical speciation.

The consideration of bioavailability in modeling wildlife exposures to dietary fluoride in ERAs has been inconsistent. The USEPA (1995) ERA for the EMF site assumed 70% bioavailability of fluoride from small mammals as dietary items to mammalian predators, based on the assumption that 90% of fluoride is incorporated into prey bones, that the bioavailability of fluoride from bone during ingestion by predators is less than 50%, and that fluoride is typically 75% bioavailable from feed sources, based on Cronin *et al.* (2000). In contrast, fluoride bioavailability was not considered in exposure modeling for mammalian or avian receptors in the recent ERA for a smelter site (Salatas *et al.* 2009). Because knowledge of the relative bioavailability of fluoride for wildlife has been incomplete, the World Health Organization (WHO 2002) recommended more research on fluoride bioavailability to animals.

In contrast to food sources, estimating wildlife exposure to soil fluoride typically includes bioavailability factors. During development of livestock tolerance levels for New Zealand soils, the Cronin *et al.* (2000) review recommended ingested fluoride absorption from soil at 20 to 38% for mammals. Deposited atmospheric fluoride is strongly retained by soil, forming complexes with aluminum, iron, and calcium, thereby limiting bioavailability (ATSDR 2003; Weinstein and Davison 2004). The degree of fluoride adsorption to soil particles is greatest in acidic non-calcareous soils containing aluminum hydroxides, where fluorides occur predominantly as aluminum fluorosilicate complexes. In alkaline soils with sufficient calcium carbonate (CaCO<sub>3</sub>), typical of many western U.S. soils, soluble fluoride compounds are almost completely fixed as  $CaF_2$  (Brewer 1966). Fluoride bioavailability is generally considered to be higher from  $CaF_2$  than from aluminum complexes. An assumption of reduced fluoride bioavailability is appropriate for acidic soils or in the presence of aluminum hydroxides or calcium carbonates, which is characteristic of soils at the EMF site from which the present RBCs were derived.

Modeling the uptake of fluoride into plants as food sources for wildlife is problematic in that the bioavailability of soil fluoride for plant uptake is extremely variable, with uptake seemingly unrelated to fluoride content of the soil, such that uptake factors are considered unreliable (Longanathan *et al.* 2001; ATSDR 2003). In contrast, fluorides taken up into plants from atmospheric sources are related to air concentrations (TCEQ 2009), although the mechanisms are unexplained (Weinstein and Davison 2004).

#### SUMMARY AND CONCLUSIONS

The NOAEL- and LOAEL-based threshold concentrations for fluoride in unwashed forage at the EMF site were calculated at 14 and 63 mg/kg dw, respectively. These threshold concentrations can be identified as levels of concern or action levels that are protective of the terrestrial mammalian and avian receptors of concern under the exposure conditions of the sagebrush steppe environment from which they were developed. The lowest RBCs for fluoride in each of the other environmental

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media in Table 2 are also considered thresholds that are protective of all receptors. The range of forage threshold concentrations bracket most state, provincial, and international regulatory standards of vegetation threshold levels for protection of livestock and wildlife, which range from 30 to 80 mg/kg dw (IDAPA 1998; OMOE 2004; Doley *et al.* 2004; TCEQ 2009; WDEQ 2009). For example, the state of Idaho where the EMF site is located, has promulgated forage levels that support air permits for atmospheric fluoride releases, with the annual average limit set at 40 mg/kg dw and a never-to-exceed limit set at 80 mg/kg dw (IDAPA 1998). The Idaho forage standards are based on the recommended dietary thresholds for fluorosis in livestock presented in Shupe and Olson (1983). The adjacent state of Wyoming (USA), with semi-arid habitats and soils similar to Idaho, has promulgated a lower annual average forage limit at 30 mg/kg dw (WDEQ 2009).

Soil RBCs calculated herein are less certain than for forage because of the lack of significant relationships between soil fluoride concentrations and those in biotic media at the EMF site. This uncertainty limits comparisons with other fluoride ecological soil screening values. The lowest soil NOAEL and LOAEL RBCs of 149 mg/kg and 659 mg/kg in Table 2 bracket the low end, but are below the high end, of the soil threshold range developed for New Zealand livestock (326–1400 mg/kg), with the range based on soil ingestion rates and fluoride bioavailability (Cronin *et al.* 2000). The LANL (2012) ECORISK database provides ranges of NOAEL-based soil ecological screening levels (ESLs) for birds and mammals depending on dietary compositions. The ESLs for birds (54–1000 mg/kg) and for mammals (120–4600 mg/kg) bracket the soil NOAEL RBCs derived for birds and mammals in Table 2.

The fluoride RBCs in Table 2 are specific to terrestrial animals typical of the sagebrush steppe environment of the western United States. By conservatively setting site use factors for all receptors at 1, they are sufficiently protective of mammalian and avian wildlife regardless of home range size. The RBCs entail some uncertainty because of the limited ranges of concentration data used to develop the media associations, and because the data were collected during a single sampling event. The use of TRVs derived from exposures of small mammals and birds under laboratory test conditions and extrapolated to wildlife exposures entails additional uncertainty. Nonetheless, available wildlife exposure studies support an assumption of reasonably similar sensitivities to laboratory test organisms. The RBCs are also specific to environmental contamination related to air releases of fluoride; associations among media related to phosphate fertilizer application to agricultural fields might be dominated by fluoride in soil and may not be similar to those described herein. With these uncertainties in mind, the finding of significant associations between concentrations in forage and other media support the RBC development as a reasonable approach in deriving levels of concern for terrestrial wildlife exposures to fluoride.

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# Effects of Dietary Fluoride on Reproduction in Eastern Screech-Owls

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Abstract. Sixty-six eastern screech-owls (Otus asio) were paired and randomly assigned to dietarv treatment groups of 0, 40, or 200 ppm (mg/kg) fluoride (as sodium fluoride) in November 1981. Hatching success was adversely affected at the 200 ppm (mg/kg) level, suggesting potential detrimental impacts to wild populations exposed to fluoride pollution. Eggshell thickness was unaffected. Although fluoride concentrations were elevated in bone and eggshells, large variations among individuals were observed as well as a trend for eggshell residues to increase with sequence of laying. Females had higher residues of fluoride in bone than males. Although fluoride levels in bone and eggshells are useful indicators of exposure, the variability in residues among individuals makes residue data from field collections of limited usefulness in assessing hazards in wild birds.

Fluoride is ubiquitous in the environment; it is estimated to constitute between 0.06% and 0.09% by weight of the upper layers of the lithosphere (Koritnig 1951; Leech 1956). Fluoride is also an environmental pollutant, originating from such anthropogenic sources as steel, aluminum, ceramics, and phosphate fertilizer production as well as coal combustion (Smith and Hodge 1979). The best known examples of fluoride pollution are associated with aluminum production (NRC 1971). Problems associated with fluoride pollution occur in many localities and may become more wide-spread in the future if not stringently controlled.

Little is known about the effects of fluoride on wild birds. Chronic fluoride toxicosis has been frequently observed in cattle and deer (Shupe et al. 1963; Krook and Maylin 1979); fluorotic lesions in permanent dentition are the most obvious signs. Structural bone changes also occur in response to ingestion of elevated levels for prolonged periods (Shupe and Alther 1966; Shupe et al. 1963). Yu and Driver (1978) suggested that poultry tolerate higher levels of fluoride exposure than sheep and cattle and found 150 ppm to cause detectable physiological and biochemical changes. Guenter (1979) found that 200 ppm enhanced egg production and feed efficiency in chickens. Merkley and Sexton (1982) reported no deleterious effects when 100 ppm was added to the drinking water of chickens, whereas van Toledo and Combs (1984) found decreases in feed consumption, egg production, and eggshell characteristics (breaking strength, weight, thickness) at 900–1,200 ppm of fluoride.

In wild birds, Newman (1977) suggested that house martin (*Delichon urbica*) nesting densities were lower adjacent to an aluminum smelter in Czechoslovakia due to fluoride emissions. Van Toledo (1978) reported that a crippled tawny owl (*Strix aluco*) with elevated fluoride levels and the discovery of two unsuccessful tawny owl nests with indented eggs were associated with aluminum smelter emissions. Since insectivorous and carnivorous birds have apparently been affected by fluorides, eastern screech-owls, who eat a variety of small prey including mice and insects, were selected to test the effects of dietary fluoride on residue accumulation and reproduction. Hoffman *et al.* (1985) reported potential teratogenic effects,

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growth, and blood chemistry in 1- and 7-day-old owls from this study.

#### Materials and Methods

Sixty-six eastern screech-owls from a captive colony at the Patuxent Wildlife Research Center, Laurel, Maryland were paired and randomly assigned to outdoor pens on 25 November 1981 (Pattee 1984). Each pair of owls was fed 100 g/day of a commercial bird of prey diet; the diet was supplemented with Vionate<sup>2</sup> (1%), calcium phosphate (0.5%), and the antibiotic, nf 180® (0.0055%:N-[5-nitro-2-furfurylidene]-3-aminol-2-oxazolidone). Diets were mixed in a Hobart® vertical cutter mixer and three treatment levels were prepared by the incorporation of sodium fluoride: control (no fluoride added); 40 ppm (mg/kg) fluoride; 200 ppm (mg/kg) fluoride. Eleven pairs were randomly assigned to each treatment level; treated diets were started on 30 November 1981. Uneaten food was removed daily and the amount uneaten estimated. Birds were maintained on the treated diets for 5-6 months until each clutch hatched or failed. Adults and young were either sacrificed immediately after hatch or were placed on untreated food and sacrificed when the youngest nestling in each brood was 7 days old (Hoffman et al. 1985).

Birds were weighed at the start of the experiment (November 30), on 1 February, and at the time of sacrifice. Nest boxes were monitored until egg laying commenced. Once laying started, boxes were checked daily at 0900 and eggs numbered sequentially. Five to 7 days after the last egg was laid, the third egg was removed and stored at 5°C until processed. An estimated hatch date was determined based on when the last egg laid would hatch and birds were left undisturbed until 2–3 days prior to this date. At this time, boxes were again checked daily and the status of each egg determined. Unhatched eggs were removed when it became apparent that they would not hatch. Eggs were opened, their contents examined for evidence of embryonic development, and the shells preserved (Pattee 1984) for thickness measurements (Ratcliffe 1967) and determination of fluoride content.

The shells from all third eggs were analyzed for fluorides as well as those of an additional 19 unhatched eggs from clutches in which at least two eggs failed to hatch. Due to their medullary bone content, the femurs from 15 randomly selected pairs (five per treatment) were also analyzed for fluorides. Food samples collected during the study were pooled by treatment and analyzed for fluoride, then pooled into a single sample and analyzed for nutrient content.

Eggshells and bone were analyzed for fluoride as outlined by Singer and Armstrong (1968) with the following modifications. Fifty mg of ash were dissolved with 2 ml of 3N nitric acid; the pH was adjusted to 1.5 using 3N sodium hydroxide, then to a final pH of 5.4 with a 15% sodium acetate solution. Sample volume was adjusted to 5 ml, then 5 ml of TISAB buffer solution (Orion instrument manual 1977—EDTA substituted for CDTA) was added. Fluoride measurements were made with an Orion Model 96-09 combination electrode in conjunction with an Orion 901 research ionanalyzer. Recovery data from spiked chicken eggshells averaged 93% with a lower limit of reportable fluoride of 0.1 ppm (mgd/kg). Feed samples were analyzed for fluoride content and nutrient content by Hazleton Laboratories America, Inc. (3301 Kinsman Blvd, Madison, WI 53707). Analyses ac-

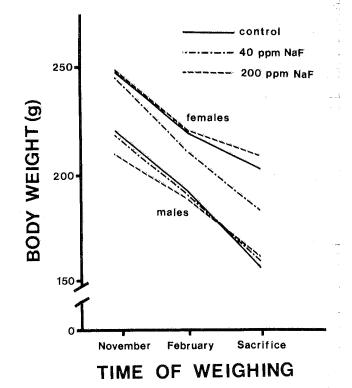


Fig. 1. Body weights of male and female eastern screech-owls fed fluoride between November 1981 and May 1982

cording to the Association of Offical Analytical Chemists (1980) were as follows: fluorine, methods 25.049–25.055; moisture, method 16.233; fat, method 7.058; ash, method 14.006; crude fiber, method 7.066; protein, method 2.057; calcium, methods 2.109–.113 and 7.091–.095; phosphorus, methods 2.019–.025, 7.120–.123, 11.032–.034. Other nutrients analyzed included carbohydrates (Watt and Merril 1963; pp 164) and calories (Watt and Merril 1963; pp 159–160).

Data concerning food consumption, body weights, clutch size, number of fertile eggs, number of eggs hatched, residues in eggshells, shell thickness, and thickness index were compared by one-way ANOVA ( $P \le 0.05$ ). The percentage of fertile eggs laid and the percentage of eggs incubated that hatched were arcsin transformed to equalize variance before one-way ANOVA was conducted. Retransformed means are reported herein. Fluoride residues in bone were compared by two-way ANOVA (treatment and sex). Residue values of fluoride in eggshells were converted to common logarithms to correct for the skewed distribution of values before comparison by one-way ANOVA. When significant treatment differences were detected, Duncans's Multiple Range Test ( $P \le 0.05$ ) was used to separate means. Correlation coefficients were used to examine relationships among residue levels in bone, eggshells, and number of fertile eggs.

#### Results

Food consumption of any given pair varied throughout the study period but monthly means were similar for different treatment groups. Pairs

<sup>&</sup>lt;sup>2</sup> Reference to trade names does not imply endorsement by the U.S. Fish and Wildlife Service

			Fertility		Hatching success <sup>a</sup>		
Treatment	N	Eggs/Pair (±SE)	Number/Clutch (±SE)	%ь	Young/Clutch (±SE)	%	
Control	11	$5.6 \pm 0.24 A^{\circ}$	$5.5 \pm 0.46A$	99.9A	$4.2 \pm 0.42 A$	96.4A	
40 ppm	10	$5.2 \pm 0.26A$	$4.4 \pm 0.48 AB$	91.5A	$3.2 \pm 0.44 \text{AB}$	79.9A	
200 ppm	11	$5.1 \pm 0.24 \mathrm{A}$	$3.9~\pm~0.46B$	82.0A	$2.6~\pm~0.42B$	64.6A	

Table 1. Reproductive success of eastern screech-owls fed fluoride

<sup>a</sup> One egg removed from each clutch; these eggs excluded from hatching success data

b Percentages retransformed from arcsine transformed data analyses

 $_{\circ}$  Means with different letters significantly different (P < 0.05)

consumed 1,300-1,700 g/month, about 50% of the food provided. Males were lighter than females and body weights of both sexes declined throughout the study (Figure 1), a normal occurrence for this species (Wiemeyer In Press). Within sex, there were no differences between treatment groups except for the females fed 40 ppm who were significantly lighter at the end of the study than the females from the control or 200 ppm groups.

Of the 33 pairs of birds, all females laid eggs except one in the 40 ppm treatment group where the male died 11 March. Nesting chronology with respect to initiation of egg laving, initiation of incubation, hatching date, or intervals between any of these events was similar among treatment groups. No significant differences occurred among treatments with regard to clutch size, percent fertility, or percentage of eggs hatched of those incubated (Table 1). Reproductive success in the 200 ppm NaF group, as measured by the number of young produced per clutch (Table 1), was significantly lower than controls. Pairs receiving 200 ppm NaF produced significantly fewer fertile eggs than controls although no significant differences (P = 0.072) occurred between controls and F-treatments in percentage of eggs that were fertile.

Eggshell thickness and the thickness indices were not significantly different among treatments, whereas eggshell fluoride concentrations increased with increasing dietary fluoride (Table 2). Results from a number of haphazardly collected eggshells (Table 3) suggest a trend for residues to increase with egg order within the clutch. At the 200 ppm dietary level, about a 3-fold increase occurred within clutches. Femur fluoride concentrations also increased with increasing dietary fluoride (Table 4) in both sexes. However, there were no significant differences between treatment groups. Females exhibited significantly higher levels than males within the 40 ppm and 200 ppm treatment groups. Fluoride concentrations in femurs of females were significantly correlated (r = 0.51, N = 15) with those in  

 Table 2. Mean shell thickness, thickness index, and geometric

 mean fluoride concentrations (dry weight) of eastern screechowl eggshells from birds fed fluoride

Treatment	N	Shell thickness (mm ± SD)	Thickness index (±SD)	ppm Fluoride
Control	11	$0.250 \pm 0.017$	$1.277 \pm 0.079$	6.4A <sup>a</sup>
40 ppm	10	$0.230 \pm 0.018$	$1.186 \pm 0.087$	53.3B
200 ppm	11	$0.240 \pm 0.022$	$1.212 \pm 0.122$	87.2B

<sup>a</sup> Means with different letters were significantly different (P < 0.05); all others were not different

the shell of the third egg but were not correlated with number of fertile eggs laid (r = -0.50, N =15). Fluoride concentrations in the shell of the third egg were not significantly correlated with the number of fertile eggs in that clutch (r = -0.34, N = 31) nor was the fluoride concentration in the femures of the males correlated with the number of fertile eggs in their clutch (r = -0.08, N = 15).

Wet weight concentrations of fluorides in the diet were 56.5 ppm and 232 ppm versus the predicted 40 ppm and 200 ppm, 10-20% higher than expected due to fluoride in the control diet (27.2 ppm). Nutrient content (Table 5) data are provided to confirm the adequacy of the diet and may be compared to those reported by Pattee (1984). The calcium/phosphorus ratio was 1.7/1.0.

#### Discussion

The addition of 200 ppm fluoride to the diet of eastern screech-owls significantly reduced reproductive success as indicated by the number of young produced. Success intermediate between controls and the 200 ppm group was noted at 40 ppm. The reduction in number of young produced in the 200 ppm group appeared attributable to a combination of factors which, by themselves, were

	Pen number and treatment											
Egg Laying Sequence	664 (Control)	655A (40 ppm)	674 (40 ppm)	635 (200 ppm)	661 (200 ppm)	672 (200 ppm)	679 (200 ppm)					
1	7.7	26	12	a	72	30	74					
2	8.6	32	13		140	42	74					
3	6.6	97	17	30	140	72	200					
4		88		74	_	86	220					
5		_		86	210	130						

Table 3. Fluoride content (ppm, dry weight) of eggshells of eastern screech-owls from clutches in which two or more eggs failed to hatch

<sup>a</sup> Dash indicates that egg hatched and shell was unavailable

Table 4. Fluoride levels (ppm, dry weight) in femurs of eastern screech-owls fed fluoride

	NI	Sex	ppm ± SD
Treatment	N	363	ppin ± 5D
Control	5	Male	964 ± 565
Control	5	Female	$1,040 \pm 536$
40 ppm	5	Male	724 ± 122
40 ppm	5	Female	$1,600 \pm 552$
200 ppm	5	Male	1,134 ± 259
200 ppm	5	Female	$1,720 \pm 286$

Table 5. Nutrient content of the diet given eastern screech-owls November 1981 to June 1982

Moisture (%)	56.2
Protein (%)	20,5
Fat (%)	9.7
Ash (%)	6.0
Crude fiber (%)	3.4
Carbohydrates (%)	4.2
Calories (per 100 g)	186.0
Calcium (%)	1,74
Phosphorus (%)	1.05

not significant. For example, 65-68% of the reduction could be attributed to a decrease in egg fertility. Our findings coincide with the results of van Toledo and Combs (1984), who found differences in egg production of chickens fed fluoride. However, our results conflict with those of Bird and Massari (1983), who found an increase in the fertility of American kestrels (Falco sparverius) fed fluoride. Their kestrel data appear suspect because their control birds exhibited fertility rates of only 35-41% compared to 85-95% in our study. Van Toledo and Combs (1984) found major differences between two strains of white leghorn hens and there may be even greater interspecific differences. A reduction of 40% in the number of young produced, as found in the 200 ppm group of our study, could have long-term impacts on the success and survival of wild bird populations. This reduction in reproductive success might explain the extirpation of wild house martins reported by Newman (1977). Certainly differences in sensitivity to fluorides exist.

Wright et al. (1978) reported fluoride levels (dry weight) in small mammals from a fluoride polluted environment ranging from 12.6 ppm (muscle) to 4.387 ppm (femur). Andrews et al. (1982) found in a fluoride-contaminated grassland fluoride levels (dry weight) in invertebrates ranging from 321-3,204 ppm and in small mammals from 27.6 ppm (muscle) to 1,283 ppm (femur) with whole body levels of 332 ppm in Microtis agrestis and 1,063 ppm in Sorex araneus. Although the availability of fluoride stored in bone, muscle, and other tissues is unknown, the levels of fluoride utilized in this study appear to fall within the range of environmental samples from polluted areas.

The thin eggshells found in wild tawny owl nests by van Toledo (1978) and attributed to fluoride could not be confirmed by this study, even though our eggshell residues approached or exceeded those found in tawny owl eggshells. Nor could we confirm the trend towards thicker eggshells reported by Bird and Massari (1983). Again, their kestrel data must be qualified, because the treated birds had significantly thicker shells than the controls before treatment; shell thickness of birds on the fluoride diet increased only 0.004-0.006 mm.

Fluoride concentrations in tissues and shells were variable, although a trend towards higher residues at higher treatments was apparent. We also noted the same trend reported by Bird and Massari (1983) for fluoride levels in eggshells to increase in successively laid eggs. Eggshell fluoride levels for controls were similar to those from uncontaminated locations (van Toledo 1978; Seel 1983), whereas eggshell concentrations from our 200 ppm treat-

ment were comparable to those found in Great tits (Parus major) and tawny owls from contaminated environments (van Toledo 1978). The data suggest that eggshell fluoride residues have limited usefulness in interpreting impacts on reproduction. Not only does egg order affect residue concentration in eggshells, the variability between individuals serves to further confound treatment differences. Some birds from high and low treatment levels had similar residues. This is also evident in the bone fluoride levels and their relationship to productivity. This variability suggests different uptake, excretion, and/or deposition rates among individual birds; part of this may be tied to calcium uptake as evidenced by the differences between males and females on the same diets. This is especially noteworthy because females dumped fluoride into their eggshells yet still had higher bone fluoride levels than males. Whether this is also tied to bone volume, as reported by van Toledo and Combs (1984), was not investigated.

Bone fluoride levels are of limited usefulness because of the variability in residue levels. However, they are of comparative value. Bird and Massari (1983) reported lower bone levels than this study at comparable or higher dosages; this appears to be related to their shorter treatment time and their analysis of only the diaphyses. Seel and Thomson (1984) determined fluoride concentrations in femurs of predatory birds in the British Isles and found mean levels greater than 1,500 ppm (dry weight) in 4 of 12 species and levels greater than 500 ppm in 8 of 12 species. It should be noted that the controls in our study had femur fluoride levels exceeding 900 ppm, possibly reflecting the 27 ppm (wet weight) fluoride in the diet. The value of bone fluoride concentrations is rather like that of bone lead concentrations (Pattee 1984)-useful as an indicator of exposure but not specific enough to be useful in assessing hazard.

The information presented in this paper and by Hoffman *et al.* (1985) indicates that fluoride ingestion may have a detrimental effect on reproductive success of eastern screech-owls. The levels of sodium fluoride used in our study yielded bone and eggshell residues which are comparable to those found in wild populations of birds. However, the data also suggest that individual variability seriously hampers the usefulness of fluoride residue data in evaluating field situations. The available literature also suggests major species differences in fluoride accumulation and, perhaps, their response to elevated levels. Based on the results of the present study, a closer scrutiny of the problem in the field in concert with additional laboratory work appears justified, since fluoride ingestion at environmentally realistic levels may induce moderate to severe impacts on recruitment in some avian populations.

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# PREDICTING CADMIUM, LEAD AND FLUORIDE LEVELS IN SMALL MAMMALS FROM SOIL RESIDUES AND BY SPECIES-SPECIES EXTRAPOLATION

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#### Abstract

The effects of heavy metals on wild mammals are often assessed by analysing residues in body organs. This paper reviews published studies to determine whether cadmium (Cd), lead (Pb) and fluoride (F) residues in small mammals can be predicted directly from residues in soil or, when this is not possible, from residues in other species. It was found that residues in soil could be used to predict Cd and Pb concentrations in small mammals. There were significant (P < 0.05) relationships between Cd residues in soil and in the liver and kidneys of wood mice Apodemus sylvaticus and common shrews Sorex araneus; similar relationships occurred in field voles Microtus agrestis (0.05 < P < 0.10). There were also significant relationships between Pb residues in soil and body organs for wood mice and field voles. Insufficient data were available to relate either Pb levels in soil to those in shrews or F levels in soil to residues in any species. However, both Cd and F residues in any one of the three small mammal species examined could be predicted from the corresponding residues in the other species, there being significant relationships between species for residues in the liver and kidneys (Cd) or bone (F). Too few data were available to determine species-species relationships for Pb.

Keywords: Cadmium, lead, fluoride, soil, small mammals, extrapolation.

# INTRODUCTION

Small mammals are often used as bio-indicators of pollution, residues being determined in either the whole body or in specific organs (Martin & Coughtrey, 1982; Talmage & Walton, 1991; Wren, 1986). Such analyses demonstrate that intake and accumulation of the pollutant by mammals occurs. For elements such as cadmium (Cd), lead (Pb) and fluoride (F), it is now well established that food-chain transfer and uptake by small mammals does occur on contaminated sites (Andrews *et al.*, 1989*a,b*; Hunter *et al.*, 1987*a,b,c*) and it can be argued that further studies to demonstrate this are unnecessary. Quantification of pollutant residues in the body organs of wild mammals remains important, however. This is because residue data are largely used to assess whether sufficient xenobiotic has been accumulated to cause death or, when exposure is less severe, sub-lethal effects (Ma *et al.*, 1991; Shore & Douben, 1994*a*; Stickel *et al.*, 1969, 1979).

The measurement of pollutant residues in mammals raises practical and ethical difficulties. Direct sampling of body organs requires an extensive trapping programme and involves killing the animals. Such terminal sampling may also pose scientific problems in that it may compromise studies on population dynamics. However, it may be possible, for certain heavy metals at least, to estimate the magnitude of residues in small mammals using soil residue data rather than by analysing body organs directly. Examination of residue data for Cd and Pb for a number of contaminated sites indicated that there was a positive association between residues in soil and in the organs of wood mice Apodemus sylvaticus (Talmage & Walton, 1991). A positive correlation between Cd residues in soil and in the liver of rock squirrels Spermophilus varigatus has also been demonstrated (Sharma & Shupe, 1977). The possibility of using soil data to predict accurately the magnitude of metal residues in various small mammal species has not previously been examined in detail.

The prime objective of the present study was to use data from published studies to investigate the relationships between residues of Cd and Pb in the soil and in the body organs of small mammals; there were insufficient data to investigate the relationship between residues in soil and in tissues for F. A second objective was to investigate the relationships between different small mammal species in the amounts of pollutant accumulated in body organs. This was done to assess the possibility of predicting residue levels in absent or rare species from residues in more common species which can be captured in sufficient numbers to allow adequate replication. Such extrapolation would be of value when soil data are not available and when considering pollutants for which there is no significant relationship between concentrations in soil and in the body organs of small mammals. Sufficient data were available to examine species-species relationships for Cd, F and, to a limited extent, Pb.

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#### METHOD

Data were collated from published studies which reported Cd, Pb and F levels in small mammals and, for Cd and Pb, in the soil of the habitat in which the animals were captured. Cd, Pb and F levels in small mammals were most often reported as total body concentrations or as residues in the liver, kidney and, for Pb and F, bone, these being the tissues which accumulate the highest concentrations of these elements. In the present study, data for total body concentrations were excluded because such measures have little toxicological relevance and can vary markedly with factors such as body fat. Data for Cd, Pb and F concentrations in body organs were most numerous for the wood mouse, field vole *Microtus agrestis* and common shrew *Sorex araneus* and analyses in the present study were restricted to these species.

In all the studies examined, soil samples were taken from the top 5 or 10 cm soil layer. Residues were for total Cd and Pb in soil, and all were reported on a dry weight basis although the drying procedures varied slightly from study to study. Pb and F concentrations in bone in small mammals were also all reported on a dry weight basis. However, liver and kidney residues were occasionally given as wet concentrations in the original studies. These values were converted to dry weight concentrations either using data given in the study for the water content of the organs or, where such data were not available, by multiplying the wet weight concentration by 3.5 (Talmage & Walton, 1991).

The residue data for soil and small mammals in each of the studies examined were presented as mean values. Therefore, in the present review, it is the mean residue level which is given for soil and animals for each site. In those studies where residues in body organs were reported for small mammals captured in different seasons on the same site (Andrews *et al.*, 1989*a*; Ma *et al.*, 1991), the data were pooled and the mean residue level for all the animals calculated.

Residue data for soil and small mammals were most numerous for Cd. Soil Cd levels could be related to residues in small mammals for between 8 and 12 sites, depending upon which species was examined (Table 1). Two sites, the first at a copper/cadmium refinery and the other 1 km away from the refinery (sites 3 and 4 respectively, Table 1), were sampled in two separate studies but the reported soil and small mammal Cd residues were higher in the second study than in the first (Table 1). This may have been a result of sampling in different areas within the same general region in the two studies or reflected an increase in habitat contamination in the interval between sampling due to continued input of Cd. Because of the differences in residue levels, the data from these studies were treated independently and not combined.

Measures of the levels of Pb in soil and small mammal species were only available for between 5 and 8 sites (Table 2). Soil data for road verges were excluded because Pb residues decline sharply with increasing distance from the road (Welch & Dick, 1975) and may vary by three orders of magnitude within the home range of individual animals; thus, it would be impossible to relate Pb levels in soil to those in the organs of road-side small mammals. However, residues in the organs of small mammals from road verges were included in the data set used to compare accumulation of Pb by different species.

Residues of F in bone, the main site of accumulation in mammals (Walton, 1988), have been measured in wood mice, field voles and common shrews on a number of sites (Table 3) and residues in different species were compared. The F residues reported were measured

Site	a.h			Cadmium	concentra	tion (mg kg	<sup>1</sup> DW) in		Reference
		Soil	A. sy	lvaticus	М. а	grestis	S. ai	raneus	
			Liver	Kidney	Liver	Kidney	Liver	Kidney	
1	mine	23-9	0.71	1-78	1-80	5-27	219	149	Andrews & Cooke, 1984; Cooke <i>et al.</i> , 1990
2	reference	0.52	0.26	1.28	1.1	1.7	2.9	4-1	Andrews & Cooke, 1984; Cooke et al., 1990
3a	refinery	8.5	1.46	7.4	7.71	23.3	280	193	Hunter & Johnson, 1982
3b	refinery	15.4	18.2	41-7	22.7	88.8	578	253	Hunter et al., 1987a, 1989
4a	Nr. refinery	3.1	1.38	5.51	1.42	4.06	237-3	139.4	Hunter & Johnson, 1982
4b	Nr. refinery	6.9	1.8	8.5	8.7	23.9	245	156	Hunter et al., 1987a, 1989
5	reference	0.75	0.51	1.46	0.66	1.3	25.4	25.7	Hunter & Johnson, 1982
6	reference	0.8	0.4	2.0	0.7	1.7	13.6	20.5	Hunter et al., 1987a, 1989
7	Budel	5-5			0.34	1.85	185	144	Ma et al., 1991
8	Arnhem	1.2			0.12	0.17	21.0	25.3	Ma et al., 1991
9	Y Fan mine	11-2	2.49	10.3	1.06	8.91		(1997) (1997) (1997)	Johnson et al., 1978
10	reference	1.55	0.86	1.67	0.27	0.82	-	-	Johnson et al., 1978
11	S'sea waste	45-9	4.36	18.0	_	—		_	Johnson et al., 1978
12	reference	1.1	0.66	2.19		-	-		Johnson et al., 1978
13	Minera mine	92.2	9-84	39.7	-	-		-	Johnson et al., 1978
14	reference	1.44	0.48	1.68		-	<u></u>	_	Johnson et al., 1978

Table 1. Cadmium concentrations in soil and small mammals from polluted and uncontaminated (reference) sites

<sup>a</sup>Route of contamination is predominantly from aerial deposition apart from mine and waste sites. <sup>b</sup>Sites 3 and 4 were sampled twice (see text for details).

Site <sup>a,b</sup>		Lead concentration (mg kg <sup>-1</sup> DW) in						Reference			
	Soil	A	. sylvatio	cus	Л	A. agres	tis	2	S. aranei	IS	
		Liver	Kidney	Femur	Liver	Kidney	Femur	Liver	Kidney	Femur	
I mine	4234	_	_	_	12.8	21.1	129	22.3	89.6	610	Andrews et al., 1989a
2 reference	113	_	-	-	6-1	5.9	20.1	7.1	21.3	55-3	Andrews et al., 1989a
3 Budel	130	-	-	-	1.2	4.2	-	2.7	31.9	-	Ma et al., 1991
4 Arnhem	177	-	-		1.1	3.1		4.5	47.5	-	Ma et al., 1991
5 M'ra mine	8430	11+7	46.6	352	_	-	-	-		-	Johnson et al., 1978
6 reference	96-3	7.85	12.7	11.5	-	_	_	-	-	_	Johnson et al., 1978
7 YF mine	14010	13.0	39.2	189	13-7	60-4	448	-		-	Johnson et al., 1978
8 reference	78.0	5.37	9.4	21.1	4-67	8.26	10.2	-		-	Johnson et al., 1978
9 S'sea waste	4030	12.1	65-2	672		-			_	_	Johnson et al., 1978
10 reference	76-1	6-63	14-1	34-2	-	-	_	_		-	Johnson et al., 1978
11 reference	0.03	0.5	0.8	2.0	_			2.2	18.2	57.3	Ma, 1989
12 reference	90	9	5	_	5	9					Williamson & Evans, 1972
13 reference	_	3.5	$0.4^{h}$	25	-	-	-	$0.4^{b}$	8.6	41	Chmiel & Harrison 1981
14 road verge	-	8.5	9.8	67		-	-	17.2	45 7	193	Chmiel & Harrison, 1981
15 road verge	-	12	-	-	10.5	9.5	-	14	27		Williamson & Evans, 1972
16 road verge	-	9.5	6.5	-	5	5	-	11	17.5	_	Williamson & Evans, 1972
17 road verge	-	9.0		-	13-6	-				-	Jefferies & French, 1972
18 road verge	_	8.4		-	9.0	-	-	-	-	-	Jefferies & French, 1972

Table 2. Lead concentrations in soil and small mammals from polluted and uncontaminated (reference) sites

"Route of contamination is predominantly from aerial deposition apart from the mine and waste sites.

"Value quoted as '< 0.4'.

in the femur, apart from animals taken from Anglesey in which various other parts of the skeleton (not specified) or the whole skeleton was used.

The relationships between residues in soil and small mammals and between the residues in small mammals of different species were examined by linear regression analysis using untransformed or log transformed concentration data. Semi-logarithmic relationships between residues in soil and body organs gave the best fit; such relationships were also found between intake and organ concentration for Cd and Pb and may reflect regulation or saturation in body organs when metal intake is high (Shore & Douben, 1994a,b). Examination of the residuals from the regression analysis did not reveal any obvious deficiencies in the models. Relationships between species were best represented as log-log plots. For these data,

linear regression analysis was not carried out because definition of the line was highly dependent on single, outlying points of datum. The significance of the associations between species was assessed from the Spearman Rank correlation coefficients  $(r_s)$ ; a probability of P < 0.05 was taken as statistically significant using a two-tailed test.

#### RESULTS

# Relationships between metal residues in soil and small mammals

There were clear positive relationships between the levels of Cd in the soil and the liver and kidneys of small mammals (Fig. 1). Common shrews accumulated the largest Cd residues and the relationship between soil

Site	Bor	Bone fluoride (mg kg <sup>+</sup> DW)					
	Apodemus sylvaticus	Microtus agresis	Sorex araneus				
1 mine tailings	344	551	1069	Cooke et al., 1990			
2 Northumb. ref.	160	75.5	346	Cooke et al., 1990			
3 Anglesey site A	1437	588	2093	Walton, 1985, 1986			
4 Anglesey site B	357	215	1003	Walton, 1985, 1986			
5 Anglesey site C	392	333	1489	Walton, 1985, 1986			
6 Anglesey site D	281	288	1323	Walton, 1985, 1986			
7 Anglesey site E	226	167	770	Walton, 1985, 1986			
8 Anglesey site F	377	436	1705	Walton, 1985, 1986			
9 Anglesey site G	336	374	1296	Walton, 1985, 1986			
10 Anglesey site H	250	295	1205	Walton, 1985, 1986			
11 Derbys, ref. <sup>a</sup>	189	117		Wright et al., 1978			
12 Derbys. ref. <sup>b</sup>	158.7	<u> </u>	216	Wright et al., 1978			

Table 3. Bone fluoride concentrations in small animals from eleven UK sites

"Animals trapped in May.

<sup>b</sup>Animals trapped in October.

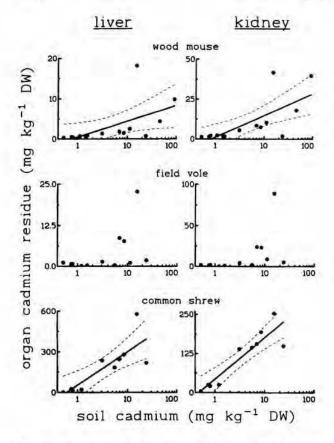


Fig. 1. The relationships between cadmium residues in soil and in the liver and kidneys of wood mice *Apodemus sylvati*cus, field voles *Microtus agresis* and common shrews *Sorex araneus*. Regression lines for y on x (solid line) and 95% confidence intervals (dashed lines) are shown. Data are taken from Table 1 and the significance of the regressions and the regression equations are given in Table 4.

and body organ residues was significant for both the liver and the kidney (Table 4). The corresponding relationships for wood mice were also significant (Fig. 1 and Table 4). Therefore, soil Cd residues can be used to predict Cd levels in the body organs of both species. There was also a positive association between Cd residues in the soil and in the body organs of field voles but these just failed to reach statistical significance (Table 4).

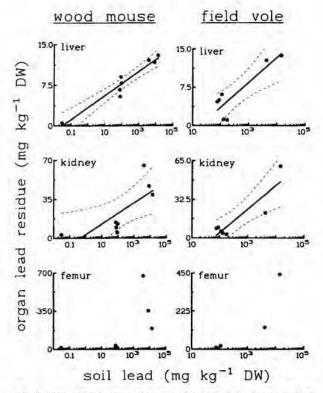


Fig. 2. The relationships between lead residues in soil and in the liver and kidneys of wood mice *Apodemus sylvaticus*, field voles *Microtus agrestis* and common shrews *Sorex araneus*. Regression lines for y on x (solid line) and 95% confidence intervals (dashed lines) are shown. Data are taken from Table 2 and the significance of the regressions and the regression equations are given in Table 4.

There were statistically significant relationships between the levels of Pb in the soil and in the body organs of small mammals (Fig. 2 and Table 4). The liver and kidney Pb concentrations in wood mice and field voles could be predicted from the levels of Pb in the soil (Fig. 2). There were relatively few data for femur Pb (Table 2) but residues tended to be positively correlated with soil Pb levels for both wood mice ( $r_s = 0.714$ , n = 7, 0.05 < P < 0.10) and field voles ( $r_s = 1.000$ , n = 4,  $P \le 0.10$ ). There were insufficient data to define the relationship between Pb residues in the soil and in common shrews.

Table 4. Regression equations and model summary for the relationships between metal residues in soil and in the liver and kidneys of small mammals. Data are in Tables 1 and 2

Species	Metal	Organ	Regression equaton	<b>R</b> <sup>2</sup>	F	Significance
wood mouse	Cd	liver	$liver[Cd] = 0.47 + 3.92 \log_{10}soil[Cd]$	0-314	$F_{1,12} = 5.49$	<i>P</i> < 0.05
wood mouse	Cd	kidney	kidney[Cd] = 1.11 + 13.6 log <sub>10</sub> soil[Cd]	0-505	$F_{1,12} = 12.3$	<i>P</i> < 0.005
field vole field vole	Cd Cd	liver kidney	Ξ	0·257 0·264	$\begin{array}{l} F_{1,10} = 3.45 \\ F_{1,10} = 3.59 \end{array}$	0.05 < P < 0.01 0.05 < P < 0.01
common shrew	Cd	liver	$liver[Cd] = 53.8 + 247 log_{10}soil[Cd]$	0-671	$F_{1.8} = 16.3$	<i>P</i> < 0.005
common shrew	Cd	kidney	kidney[Cd] = 42.8 + 133 log_{10}soil[Cd]	0.831	$F_{1.8} = 39.3$	<i>P</i> < 0.001
wood mouse	Pb	liver	liver[Pb] = $3.32 + 2.22 \log_{10}$ soil[Pb]	0-930	$\begin{array}{l} F_{1,6} = 80.0 \\ F_{1,6} = 8.96 \end{array}$	<i>P</i> < 0.001
wood mouse	Pb	kidney	kidney[Pb] = $1.93 + 9.94 \log_{10}$ soil[Pb]	0-599		<i>P</i> < 0.05
field vole	Pb	liver	liver[Pb] = $-6.23 + 4.89 \log_{10}$ soil[Pb]	0·777	$\begin{array}{l} F_{1.5} = 17.4 \\ F_{1.5} = 18.6 \end{array}$	P < 0.01
field vole	Pb	kidney	kidney[Pb] = $-35.2 + 19.9 \log_{10}$ soil[Pb]	0·788		P < 0.01

definition of the regression equation being highly dependent upon a single outlying point. However, soil Pb was significantly correlated with Pb residues in the kidney ( $r_s = 1.00$ , n = 5, P < 0.05) in this species.

# Relationships between organ metal concentrations in different species

There were significant positive relationships between wood mice, field voles and common shrews for Cd concentrations in both the liver and the kidney (Fig. 3). It therefore appears possible to predict the magnitude of Cd residues in the body organs of one species by analysing residues in other species. However, the exact relationships between the Cd concentrations in the body organs of different small mammals requires better definition using a larger data set; for example, it is not clear in the present study whether the relationships between species such as the common shrew and the wood mouse are curvilinear or linear (Fig. 3) although no significant departure from linearity was detected (Runs test: P > 0.05 in all comparisons between species).

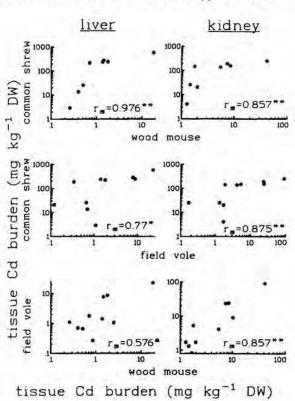
Comparison of Pb residues in different small mammal species was hampered by lack of data from common origin. However, Pb levels in the liver of common shrews were significantly correlated with Pb levels in the liver and kidney of field voles (Table 5). In contrast, kidney Pb concentrations in shrews did not appear to be correTable 5. Spearman rank correlation coefficients for relationships between the lead content in the liver and/or kidney of wood mice, field voles and common shrews. Data are given in Table 2

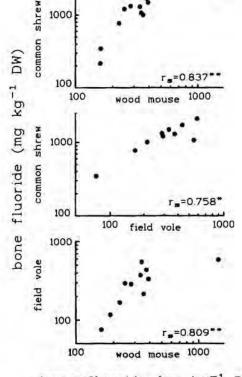
	wood mouse	fie vo	common shrew	
	liver	liver	kidney	liver
field vole: liver	0.664			
field vole: kidney	0.700	0.895**		
common shrew: liver	0.600	0-886*	0.886*	
common shrew: kidney	0.300	0.086	0.086	0.600

\*P<0.05, \*\*P<0.01.

lated with liver or kidney Pb in field voles (Table 5). Similarly, liver Pb concentrations in wood mice were not significantly correlated with Pb levels in the liver and kidneys of either field voles or common shrews (Table 5). There were insufficient data to compare kidney Pb concentrations in wood mice with those in other species.

As with Cd and Pb residues in the liver and kidney, F concentrations in bone were highest in common shrews and lower but similar in field voles and wood mice. There were significant relationships between the three species in the amount of F accumulated in bone (Fig. 4). This demonstrates that, as for Cd, it should be





bone fluoride (mg  $kg^{-1}$  DW)

Fig. 3. Scatterplot of the relationships between small mammals for the cadmium residues accumulated in the liver and the kidneys. The data points represent the mean residues in the small mammal populations on different sites. Data are taken from Table 1 and the significance of the relationships, assessed by Spearman Rank Correlation Coefficients  $(r_s)$  are represented as: "0.05 < P < 0.1, "P < 0.05, "\*P < 0.01.

Fig. 4. The relationships between different small mammals for the fluoride residues accumulated in bone. The data points represent the mean residues in the small mammal populations on different sites. Data are taken from Table 3 and the significance of relationships, assessed by Spearman Rank Correlation Coefficients ( $r_s$ ) are represented as: \*P < 0.05, \*\*P < 0.01.

possible to estimate F residue magnitude by species to species extrapolation.

# DISCUSSION

The present study has demonstrated that there are significant relationships between residues of Cd and Pb in soil and in the liver and kidneys of certain small mammal species. These results support those of earlier work on Cd (Sharma & Shupe, 1977; Talmage & Walton, 1991) which indicated a positive association between Cd residues in soil and in the body organs of terrestrial mammals. However, the significant relationship between soil and body organ Pb residues demonstrated in the present study contrasts with the lack of any such correlation found in rock squirrels (Sharma & Shupe, 1977). This apparent anomaly may reflect that the range of soil Pb contamination examined was much greater in the present study and relationships between soil and organ Pb concentrations may be difficult to detect when relatively narrow ranges of contamination are examined.

The results of the present study are important because they demonstrate the feasibility of using soil residue data to predict mean Cd and Pb concentrations in the body organs of small mammals. Although such prediction ignores variation in metal accumulation between individuals arising from differences in age (Hunter et al., 1989) or other factors, this does not invalidate the usefulness of this approach. This is because the potential effects of Cd and Pb to wild mammals are usually assessed on the basis of the average residue level in the population (Scheuhammer, 1991; Shore & Douben, 1994a,b) and it is this value which is predicted from the soil concentration data. It is also important to note that the relationships between Cd and Pb residues in soil and in small mammals appear to be robust. The data used in the present review were derived from several independent studies and included sites contaminated from a variety of sources. Despite this, the relationships between Cd and Pb residues in soil and in the body organs of rodents and shrews were mostly highly significant. This suggests that these relationships accommodate, to some extent, differences in Cd and Pb bio-availability which may occur because of inter-site variation in contaminant source or form. Such robustness is a prerequisite if soil residue data are to be used widely to assess metal concentrations in mammals.

Although the regression models for the relationship between residues in soil and small mammals are defined in the present review, these models require improvement; the aim was to demonstrate the feasibility of using soil data to predict residues in small mammals as the lack of available data makes accurate definition of the relationships impossible. Data are required for more sites so that factors which have a major influence on metal accumulation by small mammals can be identified and incorporated into the models, thereby improving their robustness. It is also necessary to ascertain whether the semi-logarithmic relationship between residues in soil and in free-living small mammals (Figs 1 and 2) is due to metal regulation/saturation in body organs at high intakes or because animals which accumulate very high residues die and so are not sampled during trapping programmes. Such information is necessary for accurate estimation of hazard to small mammals.

The use of soil data to predict residue magnitude in small mammals may be possible for metals other than Cd and Pb and for non-metallic compounds which bioaccumulate. It has already been demonstrated that antimony, selenium, mercury, arsenic, chromium and PCB residues in small mammal tissues are elevated above background levels on contaminated sites (Ainsworth et al., 1990; Batty et al., 1990; Bull et al., 1977; Clark, 1987; Martin & Coughtrey, 1982). Whether extrapolation of residues from soil to small mammal organs is also possible for essential trace elements, which can be toxic if accumulated in excess amounts, is unknown. Homeostatic mechanisms exist in mammals to regulate the levels of these elements (Underwood, 1977) but significant correlations were observed between copper (Cu) and zinc (Zn) levels in soil and in the livers of rock squirrels (Martin & Coughtrey, 1982). Whether this correlation was a result of high intakes or disruption of Cu and Zn metabolism by intake of co-contaminants such as Cd is uncertain, however.

Soil data may not be good predictors of residue levels in mammals for elements where bio-availability or route of exposure varies markedly between areas; it has been suggested that this may account for apparent differences in the amounts of F accumulated by small mammals from two regions with similar levels of contamination (Walton, 1987). Extreme heterogeneity in the distribution of the pollutant in the soil, as occurs with Pb on road verges, also precludes using soil data to predict residue levels in small mammals. In these circumstances, an ability to predict accurately the mean concentration of the pollutant in the organs of one species from the average residue level in another species may be valuable. Such extrapolation could be used to estimate residue levels in species which are absent or rare on sites where they would normally be common. If the organ concentration associated with lethality or impaired reproduction is known, it would be possible to diagnose whether contamination was the likely cause for the absence or rarity of species on contaminated sites. The feasibility of such an approach for Cd and F in small mammals has been clearly demonstrated in the present study.

Whether there are significant inter-species relationships for the accumulation of Pb is less certain. In the present study, identification of correlations between species for Pb was hampered by lack of data and inclusion of data for animals captured on road verges which may have added a confounding factor; the availability of Pb derived from exhaust emissions may vary between species for a variety of reasons but such factors may not necessarily apply where Pb is derived from other sources. A more comprehensive data set is required to elucidate whether the magnitude of residues of Pb, and also of other pollutants, can be predicted by species to species extrapolation.

In general, inter-species extrapolation of residue levels would be valuable if it could be done across a range of taxa. This would allow estimation of residue levels in a wide range of species from measurements made on one or several indicator species, perhaps invertebrate; good relationships between metal residues in soil or leaf litter and invertebrates have been demonstrated (Hopkin, 1993). Such extrapolative methods are likely to be limited by heterogeneity in the distribution of the contaminant and inter-species differences in home range size relative to the spread of contamination. However, these methods offer the advantage that variation between sites in bio-availability may affect many of the organisms of interest and would be incorporated within the inter-species comparisons to some extent. Identification and quantification of inter-species relationships in contaminant accumulation merits further study.

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# STUDIES ON THE IMPACT OF FLUORIDE TOXICITY ON GROWTH PARAMETERS OF *Raphanus sativus* L.

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#### ABSTRACT

A study was concluded during 2011 2013 on the effect of various concentrations 50, 100, 200 and 400 mg NaF/kg soil on different growth parameters in *Raphanus sativus* L. Different concentrations of sodium fluoride inhibited seedling germination percentage, length of root, length of shoot, plant height, number of leaves, size of leaf, number of flowers per plant, fruit-set percentage and seed-set percentage. The plants growth in soil supplemented with 400 mg NaF/kg soil shows maximum reduction in their growth parameters as compared to control plants.

#### KEYWORDS: Growth Parameter, Fluoride, Raphanus sativus L.

Fluoride a toxic substance is present in air, water and soil. Industrial growth as well as human activities are responsible for increasing environmental pollution. Several workers have reported adverse effect of fluoride on root, shoot and leaf elongation (Wang et al., 1995 and Nagoor, 1997). Even at lower ambient fluoride concentrations, a number of physiological changes may be initiated in plant without the appearance of visible injury symptoms. Some of these changes may have important consequences such as reduction in growth or yield. Thus, the effect of fluoride on growth may be complex, varying from positive to negative effects (Davison, 1982).

Moreover, Gupta et al., (1999) have reported the high fluoride concentration in ground water at Agra district, Hence a study was conducted on the effect of fluoride on the growth parameters of *Raphanus sativus* L. at Agra.

# **MATERIALSAND METHODS**

A field experiment was conducted during 2011 2013 with *Raphanus sativus* L. seeds (collected from the Division of genetics, I.A.R.I., New Delhi) at Botanical garden, Agra College, Agra in microplots of 1.5m x 1.5m containing loam soil (pH 7.8, ECF 1.5 ds/m, available N 150 kg/ha, P 15 kg/ha, K 225 kg/ha, organic carbon 0.15%, soluble cations 15.01 me/1, soluble anions 15.01 me/1, CaCO3 0.8%). Each microplots was separated by polythene line upto 60 cm depth. Between two microplots a bund of 0.5 m was left. The treatments were replicated thrice. The seeds were sterilized and then soaked for a period of 24

hours in distilled water. Sodium fluoride (NaF) was added @ 50, 100, 200 and 400 mg/kg soil (dry wt) in different microplots. For control, NaF was not added in alternate microplots between the ones supplemented with NaF. The seeds were sown in these microplots in respective years. The crop was raised to maturity by irrigation with distilled water. Observations of different growth parameters were recorded and statistically analysed.

#### **RESULTS AND DISCUSSION**

Data of the table 1 clearly indicate that *Raphanus sativus* L. plants grown in soil supplemented with various levels of Sodium Fluoride (NaF) exhibited a marked reduction in growth parameters i.e. seedling germination percentage, length of root, length of shoot, plant height, number of leaves, size of leaf, number of flower per plant, fruit-set percentage and seed-set percentage as compared to control plants. Also, the reduction in above growth parameters increased with the increase in the level of NaF in soil. In addition to this, the plants grown in soil supplemented with 400 mg NaF/kg soil showed maximum reduction in their growth parameters as compared to control plants.

Several workers have reported adverse effects of fluoride on plants (Yang and Miller, 1963; Posthumus, 1983; Rathore and Agarwal, 1989; Fornasiero, 2001, 2003; Elloumi et al., 2005; Reddy and Kaur, 2008). Our findings are in conformity with those of Wang et al., (1995) and Nagoor, (1997). They have reported the inhibition of root,

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S.	Parameters		Ν	NaF (Mg/Kg) so	il	
No.	rarameters	Control	50	100	200	400
1	Seedling germination %	$95.1\pm2.48$	$92.0\pm1.02$	$90.16 \pm 1.68$	$88.54 \pm 1.60$	$85.32\pm0.82$
2	Length of root after 45 days (cm)	21.52±2.35	18.06±1.60	15.36±1.85	11.42±1.24	9.12±1.63
3	Length of shoot after 45 days (cm)	27.18±2.25	25.02±1.70	19.26±1.72	15.47±2.32	10.26±1.09
4	Plant height after 45 days of germination	48.70±3.14	45.16±2.30	39.58±1.15	34.60±1.18	28.02±1.35
5	Number of leaves	$16.20\pm0.84$	$15.10 \pm 1.72$	14.07±1.24	12.19±1.68	8.96±1.20
6	Size of the leaf (cm)	$18.20\pm1.62$	$21.54 \pm 1.72$	$20.78 \pm 1.85$	$19.35\pm1.91$	$15.95\pm1.99$
7	Number of flower/plant	685	660	560	530	412
8	Fruit set %	85.1±1.94	78.3±1.78	65.5±2.18	60.2±1.84	40.8±1.44
9	Seed set %	92.2±1.52	84.26±1.77	73.6±1.68	64.37±1.14	51.50±2.12

Table 1 : Morphological Parameters in Raphanus sativus L. Plants Treated With Different Concentration of NaF

Mean value  $\pm$  SD

shoot and leaf elongation by sodium fluoride treatments.

Present findings are also supported by Singh et al. (1978a and b), Pant (1997), Reddy and Kaur (2008), Gupta et al., (2009). Chang, (1966) has observed fluoride prevented the dephosphorylation of phylin compound in the plant tissue and retarted the rate of seedling root growth during germination. Shaddad et al., (1989) have also supported adverse effect of NaF supplied in various concentrations on seed germination, seedling growth, transpiration rate and growth criteria of Zea mays L., Helianthus annus and Vicia faba L. According to them, the germination of the treated seeds significantly dropped as the concentration of NaF increased. However, low doses of the applied inhibitors stimulated the germination of maize grains. The results are also supported by the view that fluoride induce alternations in metabolism resulting in the reduction in crop field (Weinsten, 1977). The reduction in yield of plants grown in soil with higher concentration of Sodium Fluoride can also be attributed to the fact that fluoride causes pollen sterility (Schulzbach and Pack, 1972).

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STUDIES ON THE ACUTE TOXICITY OF FLUORIDE ION TO STICKLEBACK, FATHEAD MINNOW, AND RAINBOW TROUT

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<u>Abstract:</u> We have studied the acute toxicity of fluoride ion to <u>Gasterosteus</u> <u>aculeatus</u>, <u>Pimephales</u> <u>promelas</u>, and juvenile <u>Salmo</u> <u>gairdneri</u>. LC50 values varied with species and (due to precipitation) initial water hardness. Exposure to elevated fluoride levels in water resulted in increased blood fluoride levels in <u>Salmo</u> <u>gairdneri</u>.

#### Introduction

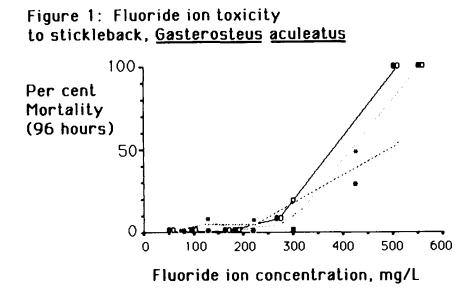
Interest in environmental fluoride ion and fluoride salts has long been spurred by observance of differing effects of fluoride, depending on exposure level. While the toxicity of high levels and the benefits of trace levels appear well established (Underwood, 1971), the question of what level is safely tolerable in the environment remains less clearly delineated (U.S. Environmental Protection Agency, 1980b). As with other potential pollutants, fluoride's effects in aqueous systems and on aquatic life have been of particular concern. Our laboratory has carried out a number of static bioassay studies intended to define the acute toxicity of fluoride ion to sticklebacks (Gasterosteus aculeatus), fathead minnows (Pimephales promelas), and juvenile rainbow trout (Salmo gairdneri) (Holsen et al., 1985). As will be discussed below, our results suggest that fluoride may not be as acutely toxic to fish as certain earlier studies concluded. There are indications of a threshold toxicity effect in all three species. Our results also support the suggestions of others (Herbert and Shurben, 1964; Vallin, 1968; Pimentel and Bulkley, 1983) that the observed protective effect of high water hardness may be due to the precipitation of insoluble calcium fluoride from hard water. Finally, measurements of blood fluoride levels in rainbow trout exposed to fluoride indicate a modest increase in blood fluoride at sublethal levels, but markedly higher concentrations in the blood of fish exposed to fluoride levels near the LC50. Following a summary of our results, we will discuss our findings in the context of data previously reported by other researchers, and of regulatory concerns.

#### Static Bioassays

In Table I, we summarize our static bioassay conditions, and the 96-hour LC50 values derived. All fish used were obtained from Alex Fish Co., San Rafael, CA. Sticklebacks and fathead minnows used were typically less than one gram in size; trout were generally less than three grams. Bioassays were run in duplicate, using ten individuals per ten liter tank. Except as indicated, initial fluoride levels were not replenished during the course of an experiment. Bioassay water was dechlorinated San Francisco tap water, which generally has a hardness below 50 mg/L (as CaCO<sub>3</sub>), and a fluoride ion level of <u>ca</u>. 0.5 mg/L. Fluoride

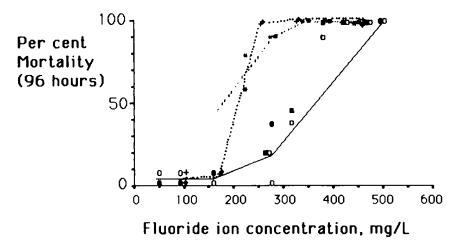
ion concentrations were adjusted with reagent grade sodium fluoride, and hardness was adjusted using reagent grade calcium sulfate and magnesium sulfate. Fluoride ion concentration was monitored by means of an Orion fluoride electrode, and hardness was measured by EDTA titration. Combinations of high fluoride and moderate to high hardness caused rapid precipitation of finely divided solid, which spectrographic analysis indicated to consist of calcium and magnesium salts. In two of the fathead minnow experiments, fluoride levels were restored after precipitate formation. Because of the incompatibility of high fluoride and high hardness, there was no attempt to restore hardness levels after precipitate formation.

LC50 values were determined graphically; data was plotted on log-probit paper, with test concentrations entered on the log scale and per cent mortality on the probit scale (American Public Health Association, 1981). From our results, any protective effect of water hardness appears slight, and is probably due to loss of fluoride ion to precipitation. Figures 1, 2 and 3, graphing mortality directly against fluoride level, appear to show a threshold toxicity effect as exposure concentrations approach the LC50 value for each species. While trout and fathead minnows appear more sensitive to fluoride ion than do sticklebacks, the overall range of 96-hour LC50 values observed in our studies (180 to 460 mg/L, depending on species and conditions) varied by a factor of only 2.55.



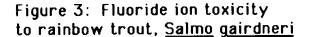
Notes on test conditions:

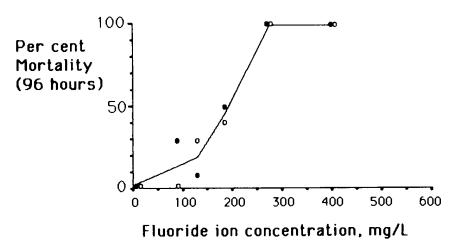
```
Temperature 20° C, hardness 78 mg/L--e,o
Temperature 20° C, hardness 146 mg/L--e,o
Temperature 20° C, hardness 300 mg/L-- *, *
```



Notes on test conditions:

Temperature 16-20° C, hardness 20-48 mg/Le,o
Temperature 15-19° C, hardness 10-44 mg/L
Temperature 20° C, hardness 92 mg/L+,+
Temperature 20° C, hardness 256 mg/L+,+





Notes on test conditions:

Temperature 15° C, hardness 23-62 mg/L -- e,o

#### Blood Fluoride

For measurements of blood fluoride in trout, somewhat larger individuals (3.4-5.1 grams) were used than in the 96-hour bioassays, to facilitate collection of sufficient blood for fluoride measurements. Tanks were set up at several fluoride concentrations, with the LC50 chosen as the highest concentration. At intervals, living fish were removed from the test tanks, wiped dry, and their tails were amputated with a scalpel. Microhematocrit tubes were used to collect  $10-20 \ \mu$ L of blood from the vein paralleling the backbone. A microtechnique, in which the fluoride electrode was placed flat against a l-cm filter paper disk moistened with sample, was used to measure fluoride levels in 10  $\mu$ L of fish blood after mixing with 10  $\mu$ L of ionic strength adjustment buffer ("TISAB").

Our blood fluoride results are summarized in Table 2. High mortality prevented measurements beyond one day at the 200 ppm exposure, but extended survival at lower concentrations permitted measurements over a 10-day period. While the data are limited, they indicate a leveling-off of blood fluoride levels within a few days. These results supplement earlier studies by others, which found that prolonged exposure of fish to fluoride results in accumulation of fluoride both in bone and in soft tissue (Neuhold and Sigler, 1960; Wright and Davison, 1975; Wright, 1977; Milhaud, El Bahri, and Dridi, 1981). One study has suggested that although trout may be relatively sensitive to fluoride as compared with other fish, adaptation may also be possible; a case of wild trout successfully adapted to 14 mg/L of fluoride was cited (Sigler and Neuhold, 1982). In some, but not all, marine organisms, prolonged exposure to moderate fluoride levels appears to be tolerable (Hemens and Warwick, 1972; Hemens, Warwick, and Oliff, 1975; Milhaud, El Bahri, and Dridi, 1981).

#### Discussion

A range of widely divergent LC50 values has been reported for fluoride in rainbow trout and other species of fish. While the reported variations may predominantly reflect variables such as exposure time, precipitation due to water hardness, fish size, differences in strains of fish tested, and test temperature, it may also be that the conclusions of certain early studies cited below cannot be confirmed. In 1960, Neuhold and Sigler determined a 24-day LC50 for fluoride of 2.7-4.7 mg/L in rainbow trout; in 1961, Angelovic <u>et al</u>. measured a 10-day LC50 of 5.9-7.5 mg/L for the same species. Soon thereafter, a 21-day rainbow trout LC50 value of 8.5 mg/L (in soft water) was reported (Herbert and Shurben, 1964). However, in 1968, Vallin reported that rainbow trout in hard (320 mg/L, as CaCO<sub>3</sub>) water survived 100 mg/L of fluoride for 21 days; formation of a precipitate of calcium fluoride was also mentioned. Much more recently, a study of the effect of water hardness on fluoride toxicity in rainbow trout (Pimental and Bulkley, 1983) found 96-hour static LC50 values ranging from 51 mg/L to 193 mg/L, depending on hardness. Our own 96-hour static LC50 value of 200 mg/L for rainbow trout, measured at an intermediate initial hardness level, corresponds roughly to conditions under which Pimental and Bulkley obtained an LC50 value of 128 mg/L. It is of interest to note that although the earliest studies indicate much higher toxicity for fluoride than we found, some studies also suggest threshold toxicity effects for fluoride ion (Herbert and Shurben, 1964; Wright, 1977).

Discussions of fluoride toxicity are complicated by the status of fluoride both as a beneficial trace element (Underwood, 1971; McKee and Wolf, 1977; National Academy of Sciences (U.S.A.), 1972; U.S. Environmental Protection Agency, 1980a) and as a potential toxin in larger doses (Windholz <u>et al.</u>, 1983; McKee and Wolf, 1971; California Department of Health Services, 1984; U.S. Environmental Protection Agency, 1980b). Imputed levels of hazard, such as the California "STLC" (soluble threshold limit concentration) of 180 mg/L for leachable fluoride, were set at least partly on the basis of the earliest studies, which indicated a higher fish toxicity level for fluoride than more recent experiments. Drinking water standards, which are relatively low (<u>ca</u>. 1 mg/L), may also have tended to influence regulatory views of potential hazards due to elevated fluoride ion concentrations in water. The available data suggest that a uniform consensus about the maximum safe level of fluoride ion for fish in natural waters of varying hardness has not yet been achieved.

Species	96-hr LC50, mg/L	Replenished to maintain <u>level?</u>	Initial hardness (mg_CaCO3/L)	Initial PH	Final PH	Тетр., 
Stickleback	340	no	78	7.4	7.7-7.9	20°
Stickleback	380	no	146	7.4	7.5-7.9	20°
Stickleback	460 (1)	no	300	7.4	7.6-7.9	20 °
Rainbow trout	200	no	23-62	7.4	7.7-8.0	15°
Fathead minnor	ar 315	no	20-48	8.0-8.2	7.9-8.0	16-20°
Fathead minnor	w 315	no	10-44	7.5	7.7-8.0	15-19°
Fathead minnor	a 180 (1)	yes	92 (2)	7.4	7.7-7.8	<b>2</b> 0°
Fathead minnor	a 205	yes	256 (3)	7.5-7.6	7.6-7.7	20°

Fluoride

#### Table 1: Summary of Fish Bioassay Results

(1) by extrapolation

- (2) Due to rapid precipitation, this hardness was maintainable only in the control tank. Hardness dropped to 10 in test tanks within a few hours, staying high only in the control tank.
- (3) Within a few hours, actual hardness dropped to 12 in the 400 ppm fluoride tank, and to 75 in the 100 ppm fluoride tank.

Exposure <u>Time</u> 4 hr	Blood level in <u>Control fish</u> <2 (4)	Blood level, fish in 75 ppm tank	Blood level, fish in 150 ppm tank -	Blood level, fish <u>in 200 ppm tank</u> <u>3.0 ± 1.1 (4)</u>
l day	<0.5 (4)	6.0 ± 4.6 (5)	7.8 ± 4.4 (5)	17.4 ± 9.6 (5)
2 days	<0.5 (1)	4.7 ± 4.5 (5)	9.0 ± 7.7 (4)	-
3 days	<0.5 (4)	6.7 ± 2.3 (5)	$3.0 \pm 0.9$ (5)	-
6 days	<0.5 (3)	4.1 ± 2.3 (5)	$2.2 \pm 0.5$ (4)	-
8 days	0.5 (3)	3.8 ± 1.1 (5)	-	-
10 days	<0.5 (3)	3.4 ± 1.5 (5)	-	-

Table 2: Rainbow trout (Salmo gairdneri) blood fluoride levels

Source of fish: Alex Fish Co., San Rafael, CA

Weight range: 3.4-5.1g

Length: 6.8-8.4 cm

Numbers in parentheses indicate the number of individuals sampled. Indicated uncertainties are standard deviations.

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(Received in UK 28 May 1985)

# Attachment 1 – Exhibit U

Final Report, Acute and Chronic Toxicity of Boron, Fluoride, and Manganese to Freshwater Organisms, by David J. Soucek and Amy Dickinson, Illinois Natural History Survey, dated October 14, 2010

# --FINAL REPORT--

## Acute and Chronic Toxicity of Boron, Fluoride, and Manganese to Freshwater Organisms

by

David J. Soucek and Amy Dickinson Illinois Natural History Survey Institute of Natural Resource Sustainability University of Illinois, Urbana-Champaign 1816 S. Oak St. Champaign, IL 61820

Submitted to: Brian Koch and Robert Mosher Illinois Environmental Protection Agency 1021 North Grand Avenue East Springfield, Illinois 62794-9276

October 14, 2010

## A. BORON

## PURPOSE

This study was designed to generate further data on acute and chronic boron toxicity in support of an effort by Illinois Environmental Protection Agency (IL EPA) to update their State general-use standard for boron. First, we conducted acute toxicity tests with boron on a variety of freshwater species, including a fingernail clam and a stonefly, as well as several commonly used standardized test organisms. Next, we sought to further clarify whether hardness or pH affect boron toxicity by conducting tests at three hardnesses and three pHs with two different test organisms, *C. dubia* and the amphipod *Hyalella azteca*. Finally, we conducted chronic boron toxicity tests with two species (*H. azteca* and *P. promelas*) in an effort to generate acute to chronic ratios (ACRs) for use in a chronic boron standard.

#### MATERIALS AND METHODS

## Culture and holding of test organisms

Five species (four invertebrates and one vertebrate) were selected to generate acute toxicity data for boron based on data gaps in the literature, and the need for acute to chronic ratios (ACR) for use in chronic standard development. Useful data are available from the literature for a number of fish species, but we included fathead minnow, *Pimephales promelas*, because of the need to generate an ACR. There are relatively fewer data available on toxicity of boron to invertebrates. No published data exist for mollusks so we included a native fingernail clam, *Sphaerium simile*. The only insect data point available in the literature is for *Chironomus* (Maier and Knight, 1991), which is the least sensitive species tested, so we chose a winter stonefly, *Allocapnia vivipara*. Finally we tested the crustaceans *Ceriodaphnia dubia* and *Hyalella azteca* because of their greater availability and usefulness in testing under a variety of water quality conditions.

The cladoceran, *C. dubia*, and the amphipod *H. azteca* were cultured in-house (Soucek laboratory, Illinois Natural History Survey) according to U.S. EPA methods (USEPA 2000, 2002). *C. dubia* were cultured in moderately hard reconstituted water (USEPA 2002), which will also be referred to as our "hard 100a" water (Table 1), at 25 °C and a 16:8 (L:D) photoperiod. *C. dubia* were fed approximately 0.3 ml of a YTC/*Pseudokirchneriella subcapitata* (3.0 X 10<sup>7</sup> cells/ml) mixture (1:1, v.v) daily. Amphipods, *H. azteca*, were cultured in a "reformulated moderately hard reconstituted water, RMHRW" (Smith et al. 1997), which will be referred to as "hard 100b" (Table 1), at 22 °C and a 16:8 (L:D) photoperiod. *H. azteca* were fed *Pseudokirchneriella subcapitata* (3.0 X 10<sup>7</sup> cells/ml) and TetraMin® (TetraWerke, Melle, Germany) flake food. Other details of crustacean culturing followed recommendations of USEPA (2000, 2002). For use in tests with different hardnesses and pHs, *C. dubia* were cultured in test water for at least two generations prior to use in testing. *H. azteca* were cultured in test water for the different hardnesses, but for the different pH tests, organisms were acclimated to test water for three to four days prior to testing.

*Pimephales promelas* for use in both acute and chronic testing were obtained as embryos from Aquatic Bio Systems, Fort Collins, CO, and upon receipt, were transferred to aquaria containing our "hard 100a" water. Embryos were received <24 h after fertilization and chronic bioassays (see below) were initiated upon receipt. A separate cohort for acute testing was maintained in aquaria at 25 °C and a 16:8 (L:D) photoperiod, and upon hatching, larvae were fed brine shrimp (Brine Shrimp Direct, Ogden, UT) twice daily. Other details of fathead minnow holding followed recommendations of American Society of Testing and Materials (ASTM) method E 1241-05 (2005).

Sphaerium simile were field-collected from Spring Creek, near Loda, IL, in Iroquois County. Clams collected from this site were previously identified to species by Dr. Gerald Mackie of the University of Guelph, Department of Zoology, Guelph, Ontario, Canada. Clams were collected as adults, returned to the laboratory (at INHS, Champaign, IL) in site water, and they subsequently released juveniles from their brood chambers in the laboratory. Juveniles were used for testing. The juvenile clams were gradually acclimated to laboratory conditions for approximately two weeks. Twenty percent of the water was changed daily until holding water was 100% "hard 100a" water; afterward, 50% of the water was changed daily. The temperature of the clam holding water was gradually adjusted (1 °C/day) from the water temperature at the time of collection to a test temperature of  $22 \pm 1$  °C. The clams were held in aquaria containing 6 L with a photoperiod of 16:8 (L:D). Prior to testing, clams were fed daily a suspension of the green alga (*Ankistrodesmus falcatus*) at a rate of 1.25 mg (d.w.) per gram of clam (w.w.). Other details of clam holding conditions followed recommendations of ASTM E729 (2002).

Allocapnia vivipara were field-collected from Stoney Creek, near Muncie, IL, in Vermilion County, as later instar nymphs at 4 °C. Stoneflies were returned to the laboratory in site water, and were gradually acclimated to laboratory conditions for approximately two weeks; temperature was gradually adjusted (1 °C/day) to a test temperature of  $12 \pm 1$  °C, and 50% of the water was changed every third day until holding water was 100% "hard 100a" water. The stoneflies were held in 6 L aquaria with a photoperiod of 16:8 (L:D). Prior to testing, stoneflies were fed maple leaves that were collected from Stoney Creek and rinsed with deionized water. Other details of stonefly holding conditions followed recommendations of ASTM E729 (2002).

## Test chemicals and dilution waters

The boron source for both acute and chronic toxicity tests was a combination of sodium tetraborate decahydrate or borax (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>•10H<sub>2</sub>O, 99.5+%, CAS # 1303-96-4) and boric acid (H<sub>3</sub>BO<sub>3</sub>, reagent grade, CAS# 10043-35-3). Previous studies investigating boron toxicity to invertebrates have used both boric acid and borax. In two studies that used boric acid as the boron source, pH of various treatments ranged from 6.7 to 8.1 (Gersich 1984), and 7.1 to 8.7 (Lewis and Valentine 1981). Maier and Knight (1991) used borax as their boron source, and the pH of their treatments was 9.1, while the pH of their controls ranged from 7.3 to 8.6. Because it was our intention to study the effect of pH on boron toxicity, having a range of pHs in treatments within a given test was undesirable.

Both boric acid and borax readily dissolve in water to form undissociated boric acid  $(H_3BO_3)$  and borate anion  $(B(OH)_4)$ , and different proportions of these two species are present depending on pH (Power and Woods 1997). Therefore, we decided to use boric acid and borax as a buffer system in which a given combination of the two salts would be used to match the desired pH of the dilution water, thereby allowing for a relatively constant pH for all treatments within a given test. In most cases 82% of the boron in solution was as boric acid and 18% was as borax, allowing for a test pH of ~8.0. Tests with different target pHs had different ratios of boric acid to borax (detailed in Table 1). We also conducted one acute test with *C. dubia* using only boric acid to determine if the boron source used affected its toxicity.

We used a variety of dilution waters depending on the species tested, the desired hardness, and the desired pH. Waters were formulated by adding a combination of four to five salts to distilled/deionized water (Table A.1). All tests with P. promelas, S. simile, and A. vivipara, were conducted using our "hard 100a" water, which is called Moderately Hard Reconstituted Water (MHRW) in U.S. EPA (2002). Tests with C. dubia and H. azteca were conducted at three different hardnesses (~100, 300, and 500 mg/L as CaCO<sub>3</sub>) and three different pHs (6.5, 7.5, and 8.5), but different recipes were used for the two species to achieve these water quality formulations because the formulations for H. azteca were based on a water recipe developed by Smith et al. (1997), and Borgmann (1996), which were both specifically developed for use with Hyalella. Different hardnesses were achieved by adding MgSO<sub>4</sub>, CaSO<sub>4</sub>, and in the case of H. azteca, CaCl<sub>2</sub> in the same ratios as found in the corresponding hardness = 100 recipe. All toxicity tests were conducted as static, non-renewal tests; therefore, pH could not be varied by the addition of acid because the alkalinity of the dilution water would change the pH too much by 48 hours after the start of the test (DJS personal observation). Instead we added different amounts of NaHCO<sub>3</sub> depending on the desired test pH (Table A.1). This resulted in relatively stable pH readings for the duration of the 96-h acute tests, and between changeovers in the chronic bioassays.

#### Acute test procedures

For *P. promelas, C. dubia, H. azteca, S. simile,* and *A. vivipara*, static, non-renewal, acute toxicity tests were conducted according to guidelines detailed in ASTM E729-96 (2002)., Treatments were comprised of a 50% dilution series. Five to six concentrations were tested using various dilution waters (as described above (Table A.1)) as both the diluent and control with four replicates tested per concentration. Tests with *C. dubia* were conducted for 48 h with a 16:8 (L:D) photoperiod with all others being 96 h in duration. Further details on test conditions for each species are provided in Table A.2. For *H. azteca* and *A. vivipara*, nitex mesh was added to each test chamber to provide substrate for these benthic invertebrates. Percent survival in each replicate was recorded every 24 h and at the end of the exposure period. A dissecting microscope was used to assess survival of all species. At the end of 96 h tests, fingernail clams were transferred to boron free dilution water with food for evaluation of survival. Individuals with undetectable foot movement or ciliary motion were considered dead.

Standard water chemistry parameters were measured at both the beginning and the end of each exposure period, including temperature, pH, conductivity, dissolved oxygen, alkalinity and hardness. The pH measurements were made using an Accumet<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet® gelfilled combination electrode (accuracy  $\leq \pm 0.05$  pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments (RDP, Dayton, OH, USA) model 55 meter. Conductivity measurements were made using a Mettler Toledo<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model MC226 conductivity/TDS meter. Alkalinity, and hardness were measured by titration as described in American Public Health Association (APHA) et al. (2005). At both the beginning and end of acute tests, water samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of boron concentrations by inductively couple plasma - atomic emission spectrometry according to U.S. EPA method 200.7 (Martin et al. 1994). To address the potential need to account for total versus dissolved boron, samples from the acute toxicity test with S. simile (selected at random), were analyzed for both total and dissolved boron at the beginning and at the end of the test. For measurement of dissolved boron, samples were filtered using 0.45 µm cellulose nitrate filters (Whatman<sup>®</sup>), Maidstone, England). Total boron was determined with unfiltered samples.

## Chronic test procedures

Hyalella azteca -- A 42-d, water only, static-renewal, chronic reproduction bioassay was conducted with H. azteca using recommendations detailed in the U.S. EPA sediment toxicity testing guidelines (USEPA 2000), but with modifications. Treatments included five nominal boron concentrations (3.125, 6.25, 12.5, 25, and 50 mg B/L) and a control with no boron added. The control and dilution water was our "hard 100b" recipe (Table A.1). Test chambers were 300-ml, high form beakers and 200 ml of test solution was used per test chamber. Organisms were 7- to 14-d old at the beginning of the test, and we loaded 10 into each of four replicate chambers per treatment. A 1.2- by 2.5-cm conditioned maple leaf strip was added to each test chamber for food and substrate, and 200 µl of a 5 g/L Tetramin® suspension (in deionized water) was added each time test solutions were changed. Test solutions were not aerated. Every three to four days, complete water renewals were conducted, with test organisms being transferred to new beakers containing fresh test solutions. Survival was evaluated with every changeover. After the first appearance of mating pairs (day 25), the number of pairs per test chamber was recorded daily, and discarded tests solutions (after changeovers) were carefully searched for young. Young began to appear on day 35, and the number produced was recorded until the end of the test (day 42). At the end of the test, adult amphipods were sexed and then dried in an oven (60 to 70 °C) for at least 48 h before they were weighed to the nearest 0.001 mg. Endpoints calculated included % survival, mean dry weight (per individual), number of mating pairs, # of young per female.

*Pimephales promelas* -- A 32-d, water only, static-renewal, chronic early life-stage toxicity test bioassay was conducted with *P. promelas* using guidelines detailed in ASTM E 241-05 (2005), but with modifications. The primary modification was that the test was conducted as a static-renewal test rather than a flow-through test. Treatments included

five nominal boron concentrations (2.75, 5.5, 11, 22, and 44 mg B/L) and a control with no boron added. The control and dilution water was our "hard 100a" recipe (Table 1). The test was initiated with embryos  $\sim 14$  h post fertilization; 60 embryos were placed into each of six 1 L beakers containing a test solution (described above). Beakers were aerated vigorously to prevent accumulation of fungus. On day two, percent survival of embryos was assessed and then the number of organisms was thinned to 40 per treatment, and 10 embryos were placed into each of four replicate 600-ml beakers per treatment. Embryos began to hatch on day two, and by day four, hatching was completed. Only two embryos failed to hatch, both in the 5.5 mg/L treatment. Test solutions were not aerated. Fish were fed brine shrimp (Artemia sp.) following ASTM (2005) guidelines. Approximately every three days, complete water renewals were conducted, with test organisms being transferred to new beakers containing fresh test solutions. Survival was evaluated daily until the end of the test (day 32). At the end of the test, fish were dried in an oven (60 to 70 °C) for at least 48 h before they were weighed to the nearest 0.001 mg. Endpoints calculated included % survival of embryos before thinning, % survival after 32 d, total survival (= [% embryo survival before thinning)]/100 \* % survival at the end of 32 d), and mean dry weight per fish.

Fish test water chemistry – Temperature and dissolved oxygen were measured daily in each test replicate for the fish test. Care was taken to minimally disturb the fish during this process. Other standard water chemistry parameters were measured at the beginning of the test and in the "in" and "out" water from every changeover for both species; these included pH, conductivity, alkalinity and hardness. In addition, total ammonia was measured frequently during the fish test. The pH, dissolved oxygen, conductivity, alkalinity and hardness measured as described above. Ammonia was measured using a Thermo® Orion 4-Star ion selective electrode meter with a Thermo® Orion ammonia probe (model # 9512). Renewal "in" water and discarded "out" water samples from each treatment were collected at each changeover and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of boron concentrations by inductively couple plasma - atomic emission spectrometry according to U.S. EPA method 200.7 (Martin et al. 1994).

## Statistical analysis

All LC50 values were calculated using the trimmed Spearman-Karber method (USEPA 2002). For chronic toxicity tests, we followed guidelines detailed in U.S. EPA (2002). Briefly, data for survival, and sub-lethal endpoints (amphipod dry weight, # females, # young per female, fathead minnow dry weight) were tested for normality using the Shapiro-Wilk's Test, and homogeneity of variance using Bartlett's test. Data that passed both of these tests were analyzed for differences among means using Dunnett's test. For the *Hyalella* chronic test, one replicate beaker was lost resulting in unequal numbers of replicates so Bonferroni's test was used to analyze weight and reproduction data, while Fisher's exact test was used to analyze survival data. Those that did not pass normality or homogeneity of variance tests were analyzed using Steel's Many-One test. The No Observable Adverse Effects Concentration (NOAEC) was the highest concentration whose mean for a given endpoint was not significantly different from that of the control,

and the Least Observable Adverse Effect Concentration (LOAEC) was the lowest concentration whose mean was significantly different from the control. We also calculated Maximum Acceptable Toxicant Concentrations (MATC) as the geometric mean of the LOAEC and the NOAEC, and Acute to Chronic Ratios (ACR) as the LC50 divided by the MATC. For the ACR, we used the LC50 that was generated for a given species in the same dilution water as was used in the chronic test.

## RESULTS

#### Acute toxicity

For the 96-h boron toxicity tests with fish, clams, and the stonefly, mean water temperatures remained within 1 °C of targets, mean pH values ranged from 7.9 to 8.0 with low variability within tests, and hardnesses ranged from 91 to 102 mg/L as CaCO<sub>3</sub>, again with low variability within tests (Table A.3). For the fingernail clam test, both total and dissolved boron was measured, and with the exception of one spurious value (day zero, 100 mg/L treatment), total and dissolved boron measurements were similar for the day zero samples, with a mean ratio of dissolved to total B of 1.009 (Table A.4). On day four, more variability was observed, with some ratios being greater than one, and some being less. Ratios of dissolved to total boron did not appear to be related to concentration, and the day four geometric mean was 0.981, with the overall geometric mean ratio being 0.994.

The 96-h LC50 values based on measured boron concentrations ranged from 79.7 mg B/L (fathead minnow) to >447 mg B/L for *S. simile* (Table A.3). For the *S. simile* test, no clams died in any test concentration, and therefore an LC50 could not be calculated.

For the 48- or 96-h boron toxicity tests with the crustaceans *C. dubia* and *H. azteca*, mean water temperatures remained within 1 °C of targets. Mean pH values and hardness were variable due to the experimental design, but within given tests, pH values were stable from day zero to day four and had low variability (Table A.5). The one exception to this was the *C. dubia* test using only boric acid as the boron source. In this test, pH values ranged from 6.8 in the highest test concentration on day zero to 7.8 in the control. The geometric mean of all measured pH values in this test was 7.4.

The 48-h boron LC50s for *C. dubia* ranged from 91 for the pH 6.5 test to 165 mg B/L for the first hard 100a test (Table A.5). Investigating the effects of pH on boron toxicity, we included all tests conducted at various pH values with hardness of ~90 mg/L (n = 6) and conducted regression analysis of pH versus log LC50. The resulting line was positively sloped, but the regression was not statistically significant at the  $\alpha = 0.05$  level (R<sup>2</sup> = 0.5708, p = 0.0823). Likewise, we investigated the influence of hardness on boron toxicity to *C. dubia* by including all tests conducted at various hardness levels but with pH of ~8.0 (n = 4). Conducting a log hardness versus log LC50 regression resulted in a negatively sloped (the higher the hardness the lower the LC50), but statistically insignificant line (R<sup>2</sup> = 0.5329, p = 0.2700).

We conducted similar analyses of the LC50s for *H. azteca*. The 96-h boron LC50s for this species ranged from 64 for the pH 8.5 test to 269 mg B/L for the hard 100c test (Table A.5). Comparing pH versus log LC50 for the tests with hardness values of ~100 mg/L (n = 4) resulted in a plot that was best fit by an upside down, U-shaped line. While the R<sup>2</sup> value was high (0.9311), the p-value was insignificant (0.2624), due to low sample size. For the hardness solutions based on Smith et al (1997) water (the "b" series), increasing hardness decreased boron toxicity in a marginally significant manner (R<sup>2</sup> = 0.9933, p = 0.0522). However, the 300 and 500 mg/L hardness test solutions also had higher chloride concentrations than the 100 mg/L hardness solution (Table A.1), thus presenting a potential confounding factor. Using Borgmann (1996) water as a base, thus keeping chloride concentration constant (the "c" series), increasing hardness in the "b" series resulted in lower LC50s (Table A.5), suggesting the reduced toxicity at higher hardness in the "b" series tests was actually due to increased chloride.

#### Chronic toxicity

<u>Fathead minnows</u> - Basic water quality parameters in the 32-d chronic static renewal bioassay with *Pimephales promelas* (Table A.6) met the basic acceptability requirements as outlined in ASTM E241-05 (2005). Temperature variability was within acceptable limits, and dissolved oxygen did not drop below 5 mg/L (Table A.6). Unionized ammonia concentrations never reached 0.05 mg/L. Measured boron concentrations were generally similar to nominal concentrations (Table A.7), with no major differences between "in" water and "out" water samples. The overall geometric mean percent difference between nominal and measured concentrations was 2.7%.

Percent survival of embryos before thinning was high, with no treatment having a percent survival lower than 93% (Table A.8). Most larvae emerged on day three with no substantial differences among treatments in average day of hatch, and hatching rates were high with all eggs hatching in every treatment except for two individuals in the 11.2 mg/L treatment (Table A.8). After thinning, survival was relatively high in all treatments until ~day 17, when survival in the 44.5 mg/L treatment began to drop (Fig. A.1). At the end of the 32-d test, three treatments (control, 2.8, and 11.2 mg/L) had greater than 90% survival and 87.5% of the fish had survived in the 5.7 mg/L treatment. Two treatments had significantly lower survival than the control: 23 mg/L (80%) and 44.5 mg/L (15%). Because embryo % survival before thinning was high for all treatments, total survival values were similar to % survival values of thinned fish at the end of the test (Table 8, Fig. A.1). Dry weights of individual fish in controls met acceptability requirements of 0.25 mg, but after excluding treatments for which survival was significantly lower, no significant differences among treatments were observed in mean dry weight per fish (Fig. A.2).

<u>Amphipods</u> - Basic water quality parameters in the 42-d chronic static renewal bioassay with *Hyalella azteca* were similar to those observed in the fish test, but with slightly higher hardness because of the different dilution water used (Table A.6). Temperature variability was within acceptable limits, and dissolved oxygen did not drop below 6.6 mg/L (Table A.6). As with the fathead minnow test, measured boron concentrations were generally similar to nominal concentrations (Table A.9), with no major differences between "in" water and "out" water samples. The overall geometric mean percent difference between nominal and measured concentrations was 3.5%.

At the end of 42 d, % survival of the controls was 90%, and although survival in the four lowest boron treatments (3.2, 6.6, 13.0, and 25.9) ranged from 72.5 to 87.5%, only the highest concentration (51.1 mg/L, 37.5%) had significantly lower survival than the control (Fig. A.3). After excluding the highest treatment (51.1 mg/L) from further analysis because of its lower survival rate, there were no differences among treatments in the number of females present (Fig. A.4) or dry weight of individual amphipods (Fig. A.5). However, there were significant differences from the control in # offspring produced per female, with both the 13.0 and the 25.9 mg/L having significantly lower means (Fig. A.4).

<u>Chronic values</u> – Because there were no significant differences among treatments in fathead minnow dry weight, the NOAEC (23.0 mg/L) and LOAEC (11.2 mg/L) values for *P. promelas* were derived from survival data. The resulting MATC from these values was 16.0 mg/L, and using the 96-h LC50 of 79.7 mg/L produced an ACR of 5.0. For *H. azteca*, the NOAEC (13.0 mg/L) and LOAEC (6.6 mg/L) values were derived from the number of offspring produced per female. This resulted in an MATC of 9.3 mg/L, and with the 96-h LC50 of 107 mg/L, the ACR was 11.5.

dilution waters	<sup>a, o, c, a</sup> used fo	<u>or definitive b</u>	<u>oron toxicity</u>	<u>testing with f</u>	<u>reshwater s</u>	pecies.
Water name	KC1	NaHCO3	MgSO <sub>4</sub> (an)	CaSO <sub>4</sub> (an)	CaCl <sub>2</sub>	B ratio <sup>*</sup>
hard 100a	4	96	60	60	0	82 / 18
hard 100b	4	96	30	50	50	82 / 18
hard 100c	4	84	30	0	111	82 / 18
hard 300a	4	96	192	192	0	82 / 18
hard 300b	4	96	90	150	150	82 / 18
hard 300c	4	84	30	190	111	82 / 18
hard 500a	4	96	320	320	0	82 / 18
hard 500b	4	96	150	250	250	82 / 18
hard 500c	4	84	30	408	111	82 / 18
рН 6.5а	4	4	60	60	0	99.1 / 0.9
pH 6.5b	4	4	30	50	50	99.1 / 0.9
рН 7.5а	4	40	60	60	0	93.2 / 6.8
pH 7.5b	4	40	30	50	50	93.2 / 6.8
pH 8.5a	4	400	60	60	0	75.7 / 24.3
pH 8.5b	4	400	30	50	50	75.7 / 24.3
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Table A.1. Salt concentrations (mg/L) added to deionized water for generation of dilution waters<sup>a,b,c,d</sup> used for definitive boron toxicity testing with freshwater species

<sup>\*</sup>B ratio = ratio of % boron added to highest test concentration as boric acid / borax.

<sup>a</sup> hard 100a was used for tests with *P. promelas, S. simile, A. vivipara,* and *C. dubia.* For *C. dubia,* and additional acute test was conducted in this water using boric acid only.

<sup>b</sup> hard 300a, hard 500a, pH 6.5a, pH 7.5a, pH 8.5a were used for tests with C. dubia.

<sup>c</sup> hard 100b & c, hard 300b & c, hard 500b & c, pH 6.5b, pH 7.5b, pH 8.5b were used for tests with *H. azteca*.

<sup>d</sup> hard 100c, 300c, and 500c also had 1 mg/L NaBr.

organisms.					
		Organism			
Parameter	P. promelas	C. dubia	H. azteca	A. vivipara	S. simile
1. Temperature (°C)	$25 \pm 1$	$25 \pm 1$	$22 \pm 1$	$12 \pm 1$	$22 \pm 1$
2. Test chamber size	250 ml	50 ml	50 ml	250 ml	150 ml
3. Test solution vol.	200 ml	40 ml	40 ml	200 ml	120 ml
4. Age of organisms	<7 <b>-</b> d	<24-h	7-14 d	nymphs	juveniles
5. # org./chamber	10	5	5	5	5
6. # chambers/trt.	4	4	4	4	4
7. Feeding	none	none	none	none	none
8. Aeration	none	none	none	none	none
9. Test duration	96-h	48-h	96-h	96-h	96-h
10. Endpoints	survival	survival	survival	survival	survival
11. Control % Surv.	$\geq 90$	$\geq 90$	$\geq 90$	$\geq$ 90	≥ 90

Table A.2. Test conditions for acute toxicity bioassays with various freshwater organisms.

with three neshwater s	pecies.			
Species	temp. (s.d)	pH (s.d)	hardness (s.d.)	LC50 (95% C.I.)
	°C	S.U.	mg/L as CaCO3	mg B/L
Pimephales promelas	24.7 (0.3)	8.0 (0.1)	91 (1)	79.7 (72 – 88)
Sphaerium simile	21.1 (0.1)	7.9 (0.1)	102 (3)	>447 (n.a.)
Allocapnia vivipara	11.2 (0.1)	7.9 (0.1)	98 (3)	476 (401-566)

Table A.3. 96-h boron LC50s and measured water quality conditions<sup>\*</sup> for toxicity tests with three freshwater species.

\*water quality values are geometric means of measurements taken in all test concentrations throughout the duration of the test.

Table A.4. Nominal and measured boron concentrations (mg B/L) for unfiltered (total B) and filtered<sup>a</sup> (dissolved B) samples from the 96-h acute toxicity test with the fingernail clam (*Sphaerium simile*).

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Nominal	total B	dissolved B	ratio <sup>b</sup>	total B	dissolved B	ratio
concentration	day 0	day 0	day 0	day 4	day 4	day 4
Control	< 0.2	< 0.2	na	< 0.2	0.51	na
25	26	26	1.000	32	30	0.938
50	54	56	1.037	54	56	1.037
100	110	170°	na	120	110	0.917
200	220	220	1.000	230	240	1.043
400	440	440	1.000	460	450	0.978
<u> </u>	£ 1. 0	1		4 1	0.001 0	11

Geometric mean of day 0 values = 1.009, and day 4 values = 0.981. Overall geometric mean = 0.994

<sup>a</sup> samples were filtered with 0.45 µm pore sized cellulose nitrate filters.

<sup>b</sup> ratio = dissolved B divided by total B.

<sup>c</sup> measurement for this sample was extreme and because the day 4 sample was similar to the nominal concentration, the ratio for day 0 at this concentration was not calculated.

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	Ceriodaph	<i>inia dubia</i> 48-h tes	ts							
Test water	temp. (s.d)	pH (s.d)	hardness (s.d.)	LC50 (95% C.I.)						
	°C	S.U.	mg/L as CaCO <sub>3</sub>	mg B/L						
hard 100a (boric acid)	24.0 (0.1)	7.4 (0.3)	90 (4)	102 (82 – 126)						
hard 100a (first)	24.3 (0.1)	8.0 (0.2)	91 (3)	165 (137 – 198)						
hard 100a (second)	25.0 (0.0)	8.1 (0.1)	89 (2)	109 (93 – 128)						
hard 300a	25.0 (0.0)	8.1 (0.1)	282 (3)	104 (87 – 123)						
hard 500a	25.0 (0.0)	8.1 (0.1)	469 (1)	93 (77 – 114)						
рН 6.5а	25.0 (0.2)	6.7 (0.1)	85 (1)	91 (79 – 106)						
pH 7.5a	24.9 (0.1)	7.6 (0.0)	87 (1)	115 (108 – 122)						
<u>pH 8.5a</u>	25.0 (0.0)	8.4 (0.1)	84 (1)	<u>142 (130 – 155)</u>						
	<u>Hyalella</u>	a azteca 96-h tests								
Test water	temp. (s.d)	pH (s.d)	hardness (s.d.)	LC50 (95% C.I.)						
	°C	S.U.	mg/L as CaCO <sub>3</sub>	mg B/L						
hard 100b	22.2 (0.4)	8.1 (0.0)	106 (4)	107 (70 – 163)						
hard 300b	21.5 (0.1)	8.1 (0.1)	302 (4)	151 (110 – 207)						
hard 500b	22.2 (0.4)	8.1 (0.1)	507 (9)	170 (121 – 239)						
hard 100c	22.0 (0.2)	8.1 (0.1)	111(1)	269 (223 - 326)						
hard 300c	22.1 (0.1)	8.1 (0.1)	291 (3)	203 (170 - 232)						
hard 500c	22.1 (0.1)	8.1 (0.1)	475 (4)	188 (154 – 230)						
pH = 6.5	21.0 (0.0)	6.6 (0.1)	102 (1)	104 (78 – 140)						
pH = 7.5	21.0 (0.0)	7.6 (0.0)	102 (1)	127 (90 – 178)						
<u>pH = 8.5</u>	21.0 (0.0)	8.4 (0.1)	103 (1)	64 (41 - 101)						
*	°C         S.U.         mg/L as C           ard 100a (boric acid) 24.0 (0.1)         7.4 (0.3)         90 (4           ard 100a (first)         24.3 (0.1)         8.0 (0.2)         91 (3           ard 100a (second)         25.0 (0.0)         8.1 (0.1)         89 (2           ard 300a         25.0 (0.0)         8.1 (0.1)         282 (3           ard 500a         25.0 (0.2)         6.7 (0.1)         85 (1           4 7.5a         24.9 (0.1)         7.6 (0.0)         87 (1           4 8.5a         25.0 (0.0)         8.4 (0.1)         84 (1           Hyalella azteca 96-h tests           est water         temp. (s.d)         pH (s.d)         hardness           °C         S.U.         mg/L as C           rd 100b         22.2 (0.4)         8.1 (0.0)         106 (4           rd 300b         21.5 (0.1)         8.1 (0.1)         507 (9           rd 500b         22.2 (0.4)         8.1 (0.1)         111 (1           rd 300c         22.1 (0									

Table A.5. Mean boron LC50s for *Ceriodaphnia dubia* and *Hyalella azteca* at various levels of water hardness and pH<sup>\*</sup>.

water quality values are geometric means of measurements taken in all test concentrations throughout the duration of the test.

<u>Ilyutettu uztecu</u>	•					
	Pim	ephales prome	elas 32-d chror	nic test		
Parameter	mean*	5 <sup>th</sup> %ile	95 <sup>th</sup> %ile)	min	max	
Temperature (°C	C) 24.7	24.4	25.0	23.6	25.5	
D.O. (mg/L)	6.50	5.75	7.12	5.13	7.50	
pH	8.0	7.6	8.2	7.5	8.2	
Hardness (mg/L	.) 89	87	92	84	94	
Alkalinity (mg/	L) 67	60	80	58	86	
	Ŀ		42-d chronic	test		
Parameter	mean*	5 <sup>th</sup> %ile	95 <sup>th</sup> %ile	min	max	
Temperature (°C	C) 22.5	22.2	23.3	22.1	23.8	
D.O. (mg/L)	7.3	6.8	7.6	6.6	8.0	
pH	7.9	7.6	8.1	7.5	8.1	
Hardness (mg/L	.) 105	102	108	102	110	
Alkalinity (mg/]	L) 69	62	84	60	86	

Table A.6. Water quality data for chronic bioassays with Pimephales promelas and Hvalella azteca.

Table A.7. Boron measurement data from samples collected on 19 occasions throughout the 32-d chronic bioassays with *Pimenhales promelas*.

the 52-a cm	Unic bloas	says with I	internutes p	omeius.			
Nominal	overall	in water	out water	$5^{th}$	95 <sup>th</sup>	min	max
Conc.	mean <sup>a</sup>	mean	mean	%ile	%ile		
Control <sup>b</sup>	0.03	0.02	0.03	0.0	0.1	< 0.02	0.14
2.75 mg/L	2.8	2.7	2.9	2.7	3.1	2.6	3.1
5.5 mg/L	5.7	5.6	5.8	5.4	6.2	5.2	6.3
11 mg/L	11.2	11.0	11.5	10.0	12.0	10	12
22 mg/L	23.0	22.4	23.6	21.9	24.3	21	27
44 mg/L	44.5	43.5	45.8	41.8	47.0	40	47

<sup>a</sup> All means are geometric means. <sup>b</sup> Means shown for controls are for samples that had measureable boron. Nine of 19 control samples had boron less than detection limit of 0.02 mg/L.

Uluassays with	in r internates prometas.			
Treatment	embryo % survival	mean (s.d) day <sup>a</sup>	% hatch	total <sup>b</sup>
	before thinning	of hatch	after thinning	survival
Control	93.3	3.0 (0.5)	100	88.6
2.8 mg/L	98.3	3.1 (0.5)	100	90.9
5.7 mg/L	100	3.3 (0.4)	95	87.5
11.2 mg/L	95	2.8 (0.6)	100	90.3
23.0 mg/L	96.6	3.1 (0.5)	100	77.3
44.5 mg/L	93.3	3.4 (0.5)	100	14.0

Table A.8. Embryo survival, total survival, and hatching data for the 32-d chronic bioassays with *Pimephales promelas*.

<sup>a</sup> days after initiation of test

<sup>b</sup> total survival = (% embryo survival before thinning/100)\*% survival on day 32.

Table A.9. Boron measurement data from samples collected on 18 occasions throughout the 42-d chronic bioassays with *Hyalella azteca* 

$\frac{110}{42}$ u om	One orous	Suys with 1.	<i>i futetiu uzie</i>	, u			
Nominal	overall	in water	out water	$5^{\text{th}}$	$95^{th}$	min	max
Conc.	mean <sup>a</sup>	mean	mean	%ile	%ile		
Control <sup>b</sup>	0.07	0.05	0.09	0.0	0.2	< 0.02	0.29
3.13 mg/L	3.2	3.3	3.1	3.0	3.4	2.8	3.4
6.25 mg/L	6.6	6.7	6.3	5.9	7.1	5.8	7.2
12.5 mg/L	13.0	13.1	12.8	12.0	14.0	12	14
25 mg/L	25.9	26.2	25.3	24.9	27.2	24	27
50 mg/L	51.1	51.2	50.6	48.0	54.0	48	54

<sup>a</sup> All means are geometric means.

<sup>b</sup> Data shown for controls are means and percentiles of samples that had measureable boron. Five of 18 control samples had boron less than detection limit of 0.02 mg/L.

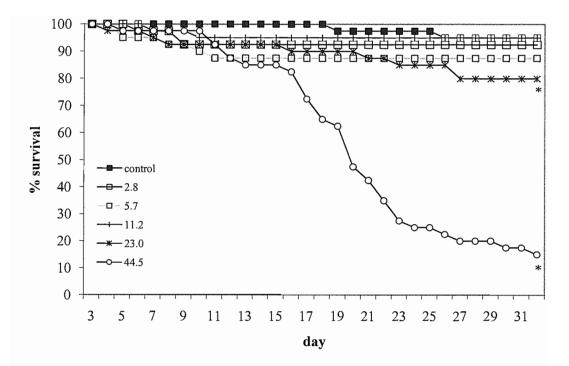


Figure A.1. Mean daily percent survival of fathead minnows (*Pimephales promelas*) in five concentrations of boron plus a control (Hard 100a) in a 32-d chronic, static renewal bioassay. Asterisks indicate mean is significantly different (p < 0.05) from control on day 32.

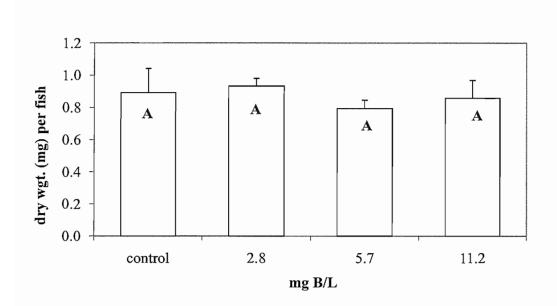


Figure A.2. Mean (error bars = standard deviation) dry weight per 10 fish in three boron concentrations and a control (Hard 100a) at the end of a 32-d chronic, static renewal bioassay with fathead minnows (*Pimephales promelas*). Different capital letters indicate means are significantly different from the control (p < 0.05).

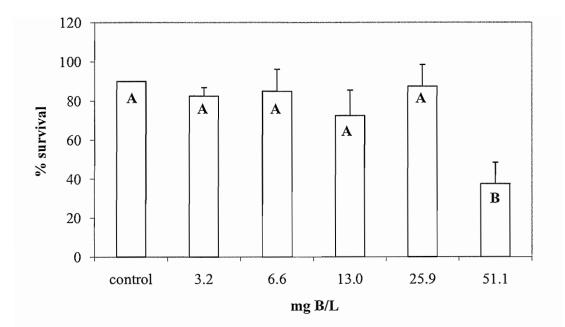


Figure A.3. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control (p < 0.05).

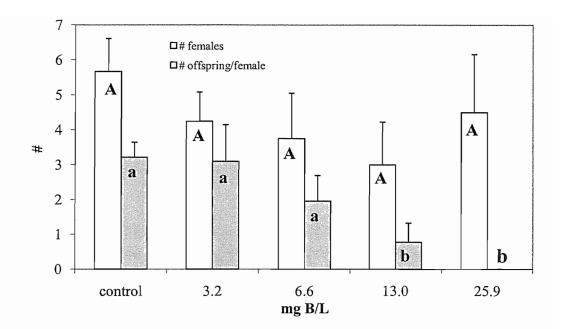


Figure A.4. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

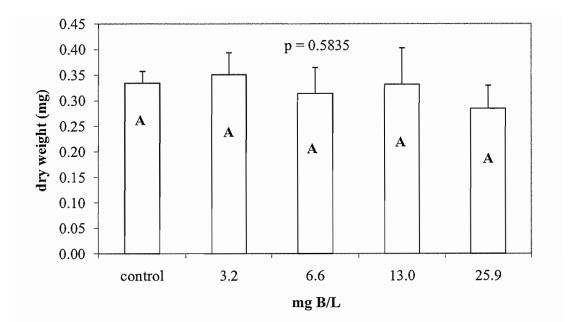


Figure A.5. Mean (error bars = standard deviation) dry weight of individual amphipods in four boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

## **B. FLUORIDE**

#### PURPOSE

The purpose of these experiments was to generate both acute and chronic fluoride toxicity data with *Hyalella azteca* in the same dilution/control water so that an Acute to Chronic Ratio (ACR) can be developed.

## MATERIALS AND METHODS

## Culture of test organisms

The amphipod *Hyalella azteca* was cultured in-house (Soucek laboratory, Illinois Natural History Survey) according to U.S. EPA methods (USEPA 2002) with some modifications. Amphipods were cultured in "Borgmann water" (Borgmann 1996), at 23 °C and a 16:8 (L:D) photoperiod, and were fed ~0.5 mg dry flakes (crushed and sieved to  $<500 \mu$ m) of TetraMin® (TetraWerke, Melle, Germany) daily. Approximately 30 adults were held in a 1-L beaker containing 1 L of Borgmann water. Young were removed at least every week or more frequently when a tighter age range was required.

#### Test chemicals and dilution waters

The fluoride source for both acute and chronic toxicity tests was sodium fluoride (NaF 99+%, CAS # 7681-49-4, Acros Organics, Geel, Belgium). The dilution water for both the acute test and the chronic test was Borgmann water (Table B.1).

#### *Acute test procedures*

Static, non-renewal, acute toxicity tests were conducted according to guidelines detailed in ASTM E729-96 (2002). Treatments were comprised of a 50% dilution series. Five concentrations were tested using Borgmann water (Table B.1) as both the diluent and control with four replicates tested per concentration. Organisms were 7- to 14-d old at the beginning of the test. The test was conducted for 96 h with a 16:8 (L:D) photoperiod at  $23 \pm 1$  °C. Test chambers were 50 ml glass beakers with 40 ml of test solution and a 2by 2-cm piece of nitex mesh was added to each test chamber to provide substrate for these benthic invertebrates. Tests were not fed or aerated. Percent survival in each replicate was recorded every 24 h and at the end of the exposure period. A dissecting microscope was used to assess survival. Acceptable control survival was set at 90%.

Standard water chemistry parameters were measured at both the beginning and the end of the exposure period, including temperature, pH, conductivity, dissolved oxygen, alkalinity and hardness. The pH measurements were made using an Accumet<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet<sup>®</sup> gelfilled combination electrode (accuracy <  $\pm$  0.05 pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments (RDP, Dayton, OH, USA) model 55 meter. Conductivity measurements were made using a Mettler Toledo<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model MC226 conductivity/TDS meter. Alkalinity and

hardness were measured by titration as described in American Public Health Association (APHA) et al. (2005). At both the beginning and end of the acute test, water samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of fluoride concentrations using an automated electrode according to U.S. EPA method 380-75WE. To address the potential need to account for total versus dissolved fluoride, samples from the acute toxicity test were analyzed for both total and dissolved fluoride, samples were filtered using 0.45  $\mu$ m cellulose nitrate filters (Whatman®, Maidstone, England). Total fluoride was determined with unfiltered samples.

## Chronic test procedures

Hyalella azteca -- A 42-d, water only, static-renewal, chronic reproduction bioassay was conducted with H. azteca using recommendations detailed in the U.S. EPA sediment toxicity testing guidelines (USEPA 2000), but with modifications. Treatments included five nominal fluoride concentrations (1.75, 3.5, 7, 14, and 28 mg F/L) and a control with no fluoride added. The control and dilution water was Borgmann water (Table 1). Test chambers were 300-ml, high form beakers and 200 ml of test solution was used per test chamber. Organisms were 7- to 14-d old at the beginning of the test, and we loaded 10 in to each of four replicate chambers per treatment. A 2.5- by 5-cm piece of nitex mesh was added to each test chamber as a substrate, and *Pseudokirchneriella subcapitata* (1 mg dry solid) and 200 µl of a 5 g/L Tetramin<sup>®</sup> suspension (in deionized water) was added each time test solutions were changed. Test solutions were not aerated. Every three to four days, complete water renewals were conducted, with test organisms being transferred to new beakers containing fresh test solutions. After each changeover, "in water" and "out water" samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of fluoride concentrations using an automated electrode according to U.S. EPA method 380-75WE. Survival was evaluated with every changeover. After the first appearance of mating pairs, the number of pairs per test chamber was recorded daily, and discarded tests solutions (after changeovers) were carefully searched for young. Young began to appear on day 28, and the number produced was recorded until the end of the test (day 42). At the end of the test, adult amphipods were sexed and then dried in an oven (60 to 70 °C) for at least 48 h before they were weighed to the nearest 0.001 mg. Endpoints calculated included % survival, mean dry weight (per individual), number of mating pairs, # of young per female.

## Statistical analysis

The LC50 value was calculated using the trimmed Spearman-Karber method (USEPA 2002). For the chronic toxicity test, we followed guidelines detailed in U.S. EPA (2002). Briefly, data for survival, and sub-lethal endpoints (amphipod dry weight, # females, # young per female) were tested for normality using the Shapiro-Wilk's Test, and homogeneity of variance using Bartlett's test. Data that passed both of these tests were analyzed for differences among means using Dunnett's test. Those that did not pass normality or homogeneity of variance tests were analyzed using Steel's Many-One test.

The No Observable Adverse Effects Concentration (NOAEC) was the highest concentration whose mean for a given endpoint was not significantly different from that of the control, and the Least Observable Adverse Effect Concentration (LOAEC) was the lowest concentration whose mean was significantly different from the control. We also calculated Maximum Acceptable Toxicant Concentrations (MATC) as the geometric mean of the LOAEC and the NOAEC, and Acute to Chronic Ratios (ACR) as the LC50 divided by the MATC.

## RESULTS

## Acute toxicity

For the 96-h fluoride toxicity test with *Hyalella azteca*, mean water temperatures remained within 1 °C of the target ( $22.7 \pm 0.1$  SD), the mean pH value was  $8.0 \pm 0.1$ , and mean dissolved oxygen was  $8.0 \pm 0.3$  mg/L. Hardness, measured at the beginning of the test only, decreased with increasing fluoride concentration with the control/dilution water having a hardness of 112 mg/L and the 56 mg F/L nominal treatment having a hardness of 50 mg/L. The geometric mean hardness of all the treatments, excluding the highest fluoride concentration was 104 mg/L as CaCO<sub>3</sub>.

Both total and dissolved fluoride were measured for this test (Table B.2). Ratios of dissolved to total fluoride were higher at the beginning of the test as expected with an overall geometric mean ratio of 1.132 on day zero. The geometric mean of the dissolved to total fluoride ratios at the end of the test was 0.941, with ratios tending to be lower at the higher fluoride concentrations (Table B.2).

In the 96-h fluoride toxicity test with *Hyalella azteca*, control survival was 95% at the end of the test, and the measured 96-h LC50 was 25.8 mg F/L (20.1 - 33.1 95% confidence interval).

## Chronic toxicity

Basic water quality parameters in the 42-d chronic static renewal bioassay with *Hyalella azteca* are provided in table B.3. Temperature variability was within acceptable limits, and dissolved oxygen did not drop below 5.6 mg/L. As was the case with the acute fluoride toxicity test, measured fluoride concentrations were generally similar to nominal concentrations up to nominal concentrations of ~14 mg/L, but in the 28 mg/L nominal treatment, measured fluoride concentrations were consistently lower than nominal, likely due to precipitation (Table B.4). However, variability within treatments was relatively low, particularly in the treatments with fluoride concentrations of 14 mg/L or lower.

At the end of 42 d, % survival of the controls was 90%, and although survival in the four lowest fluoride treatments (measured 1.7, 3.3, 6.7, and 11.7 mg/L) ranged from 70 to 95%, only the highest concentration (16.7 mg/L, 22.5%) had significantly lower survival than the control (Fig. B.1). After excluding the highest treatment (16.7 mg/L) from further analysis because of its lower survival rate, there were no differences among treatments in the number of females present (Fig. B.2). However, there were significant

differences from the control in # offspring produced per female, with both the 11.7 mg/L treatment having a significantly lower mean (Fig. B.2). Analyzing dry weight data for individual amphipods, ANOVA indicated that there was no significant difference among treatment means when the 16.7 mg F/L treatment was excluded because of its significantly lower survival; however, when a post-hoc Dunnett's test was performed comparing the individual treatments to the control, the lowest treatment (1.7 mg/L) was significantly different from the control.

<u>Chronic values</u> –The NOAEC (6.7 mg/L) and LOAEC (11.7 mg/L) values were derived from the number of offspring produced per female. This resulted in an MATC of 8.8 mg/L, and with the 96-h LC50 of 25.8 mg/L, the ACR was 2.9. Because the survival and reproductive data indicated significant differences at much higher fluoride concentrations than did the dry weight data, and because the ANOVA for the weight data was not statistically significant, we suggest, that the significant difference at the lowest fluoride concentration be ignored.

Table B.1. Salt concentrations (mg/L) added to deionized water for generation ofdilution waters used for acute and chronic fluoride toxicity testing with Hyalella azteca.Water nameKClNaHCO3MgSO4(an)CaSO4(an)CaCl2NaBrBorgmann4843001111

Table B.2. Nominal and measured fluoride concentrations (mg F/L) for unfiltered (total F) and filtered<sup>a</sup> (dissolved F) samples from the 96-h acute toxicity test with *Hyalella* azteca.

	1	11 1 1 75	. h	1	1 1 1 1 1		
Nominal	total F	dissolved F	ratio <sup>b</sup>	total F	dissolved F	ratio	
concentration	day 0	day 0	day 0	day 4	day 4	day 4	
Control	< 0.1	0.2	na	< 0.1	< 0.1	na	
3.5	3.5	3.6	1.029	3.7	3.6	0.973	
7.0	7.1	7.1	1.000	6.8	7.1	1.044	
14	11	14	1.273	13	12	0.923	
28	19	23	1.211	17	15	0.882	
56	35	41	1.171	37	33	0.892	
Geometric mean	of day 0 x	values $= 1.132$	and day	4 values =	0.941 Over	all geometric	•

Geometric mean of day 0 values = 1.132, and day 4 values = 0.941. Overall geometric mean = 1.032

<sup>a</sup> samples were filtered with 0.45 µm pore sized cellulose nitrate filters.

<sup>b</sup> ratio = dissolved F divided by total F.

Parameter	mean*	5 <sup>th</sup> %ile	95 <sup>th</sup> %ile	min	max	
Temperature (	°C) 22.7	22.4	22.9	22.0	22.9	
D.O. (mg/L)	7.6	6.6	8.4	5.6	8.8	
pН	7.8	7.4	8.0	7.3	8.2	
Hardness (mg/	/L) 114	100	120	86	124	
Alkalinity (mg	g/L) 55	50	60	50	60	

Table B.3. Water quality data for chronic bioassays with *Hyalella azteca*.

\* Mean of 24 measurements throughout the test.

Table B.4. Fluoride measurement data from samples collected on 22 occasions throughout the 42-d chronic bioassay with *Hyalella azteca* 

unougnout	110 + 2 - 0 01	nome pioas	say with it	uienu uzie	ιu.		
Nominal	overall	in water	out water	$5^{th}$	$95^{th}$	min	max
Conc.	mean <sup>a</sup>	mean	mean	%ile	%ile		
Control <sup>b</sup>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
1.75 mg/L	1.7	1.7	1.7	1.6	1.8	1.6	1.8
3.5 mg/L	3.3	3.3	3.2	3.2	3.5	2.8	3.5
7 mg/L	6.7	6.7	6.6	6.6	6.8	6.5	6.8
14 mg/L	11.7	11.4	12.1	10.0	13.0	10	14
<u>28 mg/L</u>	16.7	15.5	18.5	13.1	21.0	14	24
3							

<sup>a</sup> All means are geometric means.
 <sup>b</sup> Fluoride was never found in detectable concentrations in the control.

Table B.5. Nominal and measured fluoride concentrations (mg F/L) for unfiltered (total
F) and filtered <sup>a</sup> (dissolved F) samples from the 42-d chronic toxicity test with <i>Hyalella</i>
<i>azteca</i> . Both sample 1 and sample 2 were "out" water samples.

				ator sumpre	/0.	
Nominal	total F	dissolved F	ratio <sup>b</sup>	total F	dissolved F	ratio
concentration	sample 1	sample 1	sample 1	sample 2	sample 2	sample 2
Control	< 0.05	< 0.05	na	< 0.05	< 0.05	na
1.75	1.7	1.6	0.941	1.7	1.6	0.941
3.5	3.3	3.2	0.970	3.2	3.2	1.000
7	6.6	6.7	1.015	6.5	6.6	1.015
14	13	13	1.000	12	12	1.000
28	21	20	0.952	20	20	1.000
Geometric mear	ofsample	1  values = 0	075 and a	ample 2 va	$1_{1000} = 0.001$	Overall

Geometric mean of sample 1 values = 0.975, and sample 2 values = 0.991. Overall  $a^{a}$  samples were filtered with 0.45 µm pore sized cellulose nitrate filters. <sup>b</sup> ratio = dissolved F divided by total F. geometric mean = 0.983

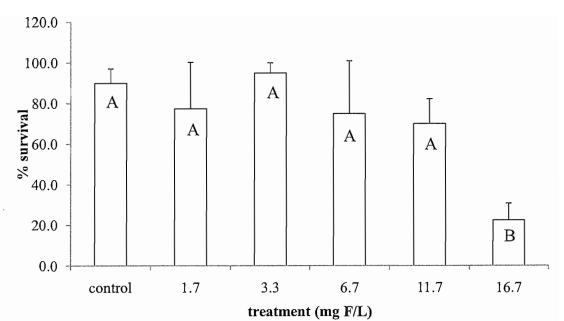


Figure B.1. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five fluoride concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control (p < 0.05).

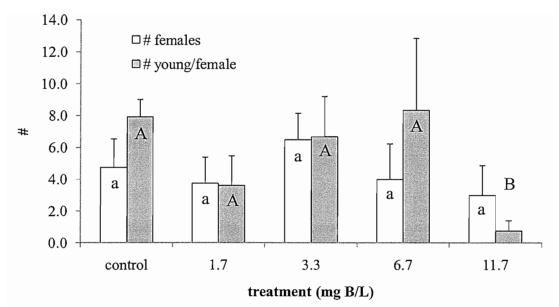


Figure B.2. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four fluoride concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

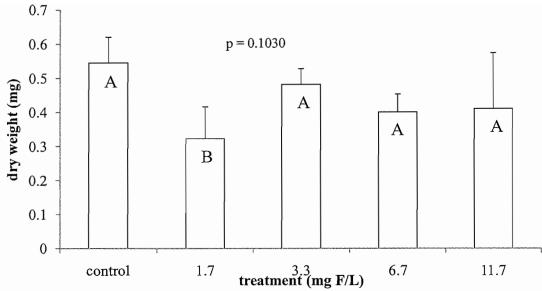


Figure B.3. Mean (error bars = standard deviation) dry weight of individual amphipods in four fluoride concentrations and a control (Borgmann) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

## C. MANGANESE

#### PURPOSE

The purpose of these experiments was to generate both acute and chronic manganese toxicity data with *Hyalella azteca* in the same dilution/control water so that an Acute to Chronic Ratio (ACR) can be developed.

## MATERIALS AND METHODS

#### Culture of test organisms

The amphipod *Hyalella azteca* was cultured in-house (Soucek laboratory, Illinois Natural History Survey) according to U.S. EPA methods (USEPA 2002) with some modifications. Amphipods were cultured in "Borgmann water" (Borgmann 1996), at 23 °C and a 16:8 (L:D) photoperiod, and were fed ~0.5 mg dry flakes (crushed and sieved to <500  $\mu$ m) of TetraMin® (TetraWerke, Melle, Germany) daily. Approximately 30 adults were held in a 1-L beaker containing 1 L of Borgmann water. Young were removed at least every week or more frequently when a tighter age range was required.

#### Test chemicals and dilution waters

The manganese source for both acute and chronic toxicity tests was a combination of manganese sulfate monohydrate ( $MnSO_4 \cdot H_2O$  Certified ACS, CAS # 10034-96-5, Fisher Scientific, Fairlawn, NJ) and manganese chloride tetrahydrate ( $MnCl_2 \cdot 4H_2O$  Certified ACS, CAS # 13446-34-9, Fisher Scientific, Fairlawn, NJ). For both acute and chronic tests, 44% of the Mn was as manganese sulfate, and 56% was as manganese chloride. This combination was used to keep chloride and sulfate concentrations in solution relatively lower than if either salt was used alone. The dilution water for both the acute test and the chronic test was Borgmann water (Table C.1).

## Acute test procedures

Static, non-renewal, acute toxicity tests were conducted according to guidelines detailed in ASTM E729-96 (2002). Treatments were comprised of a 50% dilution series. Five concentrations were tested using Borgmann water (Table C.1) as both the diluent and control with four replicates tested per concentration. Organisms were 7- to 14-d old at the beginning of the test. The test was conducted for 96 h with a 16:8 (L:D) photoperiod at  $23 \pm 1$  °C. Test chambers were 50 ml glass beakers with 40 ml of test solution and a 2by 2-cm piece of nitex mesh was added to each test chamber to provide substrate for these benthic invertebrates. Tests were not fed or aerated. Percent survival in each replicate was recorded every 24 h and at the end of the exposure period. A dissecting microscope was used to assess survival. Acceptable control survival was set at 90%.

Standard water chemistry parameters were measured at both the beginning and the end of the exposure period, including temperature, pH, conductivity, dissolved oxygen, alkalinity and hardness. The pH measurements were made using an Accumet<sup>®</sup> (Fisher

Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet<sup>®</sup> gelfilled combination electrode (accuracy <  $\pm$  0.05 pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments (RDP, Dayton, OH, USA) model 55 meter. Conductivity measurements were made using a Mettler Toledo<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model MC226 conductivity/TDS meter. Alkalinity and hardness were measured by titration as described in American Public Health Association (APHA) et al. (2005). At both the beginning and end of acute tests, water samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of manganese concentrations by inductively couple plasma - atomic emission spectrometry according to U.S. EPA method 200.7 (Martin et al. 1994). To address the potential need to account for total versus dissolved manganese, samples from the acute toxicity test were analyzed for both total and dissolved manganese, samples from the acute toxicity test were analyzed for both total and dissolved manganese, samples from the acute toxicity test were analyzed for both total and dissolved manganese, samples were filtered using 0.45 µm cellulose nitrate filters (Whatman®, Maidstone, England). Total manganese was determined with unfiltered samples.

#### Chronic test procedures

Hyalella azteca -- A 42-d, water only, static-renewal, chronic reproduction bioassay was conducted with H. azteca using recommendations detailed in the U.S. EPA sediment toxicity testing guidelines (USEPA 2000), but with modifications. Treatments included five nominal manganese concentrations (0.38, 0.75, 1.5, 3, and 6 mg Mn/L) and a control with no manganese added. The control and dilution water was Borgmann water (Table 1). Test chambers were 300-ml, high form beakers and 200 ml of test solution was used per test chamber. Organisms were 7- to 14-d old at the beginning of the test, and we loaded 10 in to each of four replicate chambers per treatment. A 2.5- by 5-cm piece of nitex mesh was added to each test chamber as a substrate, and organisms were fed dry flakes (crushed and sieved to <500 µm) of TetraMin® (TetraWerke, Melle, Germany) three times per week. Feeding rates were as follows: week 1 - 1 mg per test chamber, weeks 2 and 3 - 1.25 mg per test chamber, weeks 4, 5, and 6 - 2.5 mg per test chamber. Test solutions were not aerated. Every three to four days, complete water renewals were conducted, with test organisms being transferred to new beakers containing fresh test solutions. After each changeover, "in water" and "out water" samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of manganese concentrations by inductively couple plasma - atomic emission spectrometry according to U.S. EPA method 200.7 (Martin et al. 1994). Survival was evaluated with every changeover. After the first appearance of mating pairs, the number of pairs per test chamber was recorded daily, and discarded tests solutions (after changeovers) were carefully searched for young. Young began to appear on day 28, and the number produced was recorded until the end of the test (day 42). At the end of the test, adult amphipods were sexed and then dried in an oven (60 to 70  $^{\circ}$ C) for at least 48 h before they were weighed to the nearest 0.001 mg. Endpoints calculated included % survival, mean dry weight (per individual), number of mating pairs, # of young per female.

## Statistical analysis

The LC50 value was calculated using the trimmed Spearman-Karber method (USEPA 2002). For the chronic toxicity test, we followed guidelines detailed in U.S. EPA (2002). Briefly, data for survival, and sub-lethal endpoints (amphipod dry weight, # females, # young per female) were tested for normality using the Shapiro-Wilk's Test, and homogeneity of variance using Bartlett's test. Data that passed both of these tests were analyzed for differences among means using Dunnett's test. Those that did not pass normality or homogeneity of variance tests were analyzed using Steel's Many-One test. The No Observable Adverse Effects Concentration (NOAEC) was the highest concentration whose mean for a given endpoint was not significantly different from that of the control, and the Least Observable Adverse Effect Concentration (LOAEC) was the lowest concentration whose mean was significantly different from the control. We also calculated Maximum Acceptable Toxicant Concentrations (MATC) as the geometric mean of the LOAEC and the NOAEC, and Acute to Chronic Ratios (ACR) as the LC50 divided by the MATC.

## RESULTS

## Acute toxicity

For the 96-h acute manganese toxicity test with *Hyalella azteca*, mean water temperatures remained within 1 °C of the target ( $22.7 \pm 0.3$  SD), the mean pH value was  $7.8 \pm 0.1$ , and mean dissolved oxygen was  $8.2 \pm 0.3$  mg/L. Hardness, measured in the controls only because manganese is a divalent cation that interferes with the hardness measurement was 112 mg/L as CaCO<sub>3</sub>.

In the 96-h fluoride toxicity test with *Hyalella azteca*, control survival was 95% at the end of the test, and the measured 96-h LC50 was 11.0 mg Mn/L (8.6 - 14.1 95% confidence interval).

## Chronic toxicity

Basic water quality parameters in the 42-d chronic static renewal bioassay with *Hyalella azteca* are provided in table C.2. Temperature variability was within acceptable limits, and dissolved oxygen did not drop below 6.4 mg/L. Measured manganese concentrations were generally similar to nominal concentrations in all treatments, with relatively little variability (Table C.3). Ratios of dissolved to total manganese concentration were determined on six occasions throughout the 42-d test (Table C.4): three times with "In water" samples or new water to be used for changeovers, and three times with "Out water" samples or water removed from test chambers during a changeover. The geometric mean of ratios (dissolved Mn/total Mn) for "in water" sets was 0.989, and for "out water" sets it was 0.973. The overall geometric mean of ratios throughout the test was 0.981.

At the end of 42 d, % survival of the controls was 92.5%, and survival in the three lowest manganese treatments (measured 0.3, 0.7, and 1.4 mg/L) was relatively high, ranging

from 80 to 94.7%. Both of the highest two concentrations (2.9 and 5.7 mg/L) had significantly lower survival than the control (Fig. C.1). After excluding the highest two treatments from further analysis because of their lower survival rates, there were no differences among treatments in the number of females present, the number of young produced per female (Fig. C.2) or mean dry weight per individual (Fig. C.3).

<u>Chronic values</u> –The NOAEC (1.4 mg/L) and LOAEC (2.9 mg/L) values were derived from the survival data as no significant differences were observed in the sub-lethal endpoints. This resulted in an MATC of 2.0 mg/L, and with the 96-h LC50 of 11.04 mg/L, the ACR was 5.5.

Table C.1. Salt concentrations (mg/L) added to deionized water for generation of dilution waters used for acute and chronic manganese toxicity testing with *Hyalella azteca*.

Water name	KCl	NaHCO <sub>3</sub>	MgSO <sub>4</sub> (an)	CaSO <sub>4</sub> (an)	CaCl <sub>2</sub>	NaBr
Borgmann	4	84	30	0	111	1

Parameter	mean*	5 <sup>th</sup> %ile	95 <sup>th</sup> %ile	min	max	
Temperature (°	C) 22.8	22.1	23.3	22.0	23.6	
D.O. (mg/L)	7.8	7.0	8.4	6.4	8.5	
$_{\rm pH}$	7.8	7.6	8.2	7.5	8.3	
Hardness <sup>a</sup> (mg/	L) 115	112	118	112	125	
Alkalinity (mg/	/L) 52	50	54	50	60	

Table C.2. Water quality data for 42-d chronic Mn bioassay with Hyalella azteca.

\* Mean of 24 measurements throughout the test.

<sup>a</sup> Hardness measured in control only. Mn is a divalent cation and interferes with hardness measurement.

Table C.3. Manganese measurement data from unfiltered (total Mn) samples collected on
24 occasions throughout the 42-d chronic bioassay with Hvalella azteca

24 occasions intolghout the 42-d chrome bloassay with Hydretta azreea							
Nominal	overall	in water	out water	$5^{\text{th}}$	95 <sup>th</sup>	min	max
Conc.	mean <sup>a</sup>	mean	mean	<u>%ile</u>	%ile		
Control <sup>b</sup>	< 0.01	< 0.01	< 0.01	< 0.01	0.011	< 0.01	0.011
0.38 mg/L	0.3	0.4	0.3	0.3	0.4	0.2	0.4
0.75 mg/L	0.7	0.7	0.7	0.6	0.8	0.6	0.8
1.5 mg/L	1.4	1.4	1.4	1.3	1.5	1.3	1.6
3  mg/L	2.9	2.9	2.9	2.8	3.1	2.8	3.1
<u>6 mg/L</u>	5.7	5.7	5.7	5.5	6.1	5.5	6.1

<sup>a</sup> All means are geometric means.

<sup>b</sup> Manganese was detected on one occasion in the control.

<u>Hyalella azteca.</u>	Six differe	ent sets of sar	nples were	e measured	tor total and	dissolved Mn.
Nominal		set 1 (in)			set 2 (out)	
concentration	total	dissolved	ratio <sup>b</sup>	total	dissolved	ratio
Control	< 0.01	< 0.01	na	< 0.01	< 0.01	na
0.38	0.38	0.38	1.000	0.38	0.34	0.895
0.75	0.77	0.76	0.987	0.78	0.71	0.910
1.5	1.6	1.5	0.938	1.5	1.4	0.933
3	3.1	3.0	0.968	3.1	2.8	0.903
6	6.1	6.1	1.000	6.1	5.5	0.902
Nominal		set 3 (in)			set 4 (out)	
concentration	total	dissolved	ratio <sup>b</sup>	total	dissolved	ratio
Control	< 0.01	0.01	na	< 0.01	0.013	na
0.38	0.35	0.35	1.000	0.35	0.35	1.000
0.75	0.70	0.69	0.986	0.68	0.73	1.074
1.5	1.4	1.4	1.000	1.4	1.5	1.071
3	2.8	2.8	1.000	2.9	2.9	1.036
6	5.5	5.5	1.000	5.6	5.9	1.054
Nominal		set 5 (in)			set 6 (out)	
<u>concentration</u>	total	dissolved	ratio <sup>b</sup>	total	dissolved	ratio
Control	< 0.01	0.029	na	< 0.01	0.014	na
0.38	0.36	0.35	0.972	0.23	0.22	0.957
0.75	0.72	0.71	0.986	0.59	0.58	0.983
1.5	1.4	1.4	1.000	1.3	1.3	1.000
3	2.8	2.8	1.000	2.9	2.7	0.931
6	5.7	5.7	1.000	5.7	5.6	0.982
0	. C t C			00 1 C	66 <b>4 4</b>	0.072

Table C.4. Nominal and measured manganese concentrations (mg Mn/L) for unfiltered (total Mn) and filtered<sup>a</sup> (dissolved Mn) samples from the 42-d chronic toxicity test with *Hvalella azteca*. Six different sets of samples were measured for total and dissolved Mn.

Geometric mean of ratios for "in water" sets = 0.989, and for "out water" sets = 0.973. Overall geometric mean of ratios = 0.981

<sup>a</sup> samples were filtered with 0.45  $\mu$ m pore sized cellulose nitrate filters.

<sup>b</sup> ratio = dissolved Mn divided by total Mn.

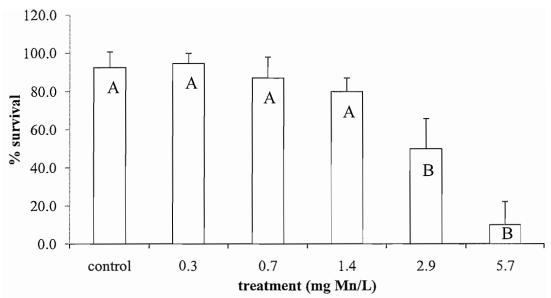


Figure C.1. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five manganese concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control (p < 0.05).

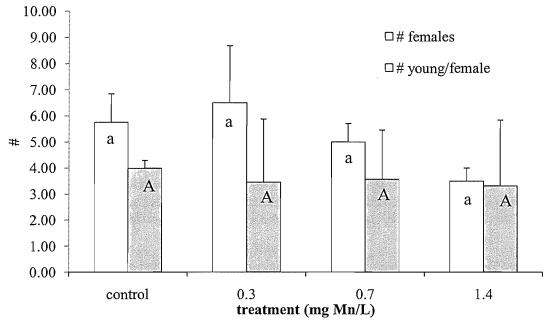


Figure C.2. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four manganese concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

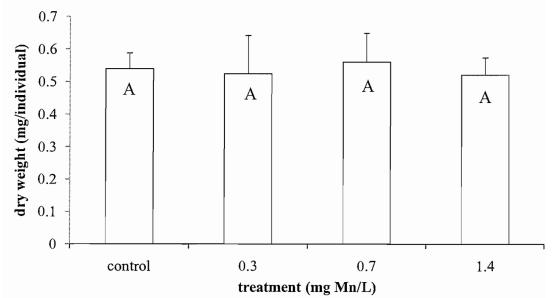


Figure C.3. Mean (error bars = standard deviation) dry weight of individual amphipods in four manganese concentrations and a control (Borgmann) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

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# TOXICITY TEST RESULTS FLUORIDE WATER QUALITY CRITERIA

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3/17/2000

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# 1.0 BACKGROUND

In April 1998, U.S. Steel's National Pollutant Discharge Elimination System (NDPES) permit conditions for Outfall 005 and 010 were modified to include water-quality based effluent limits for fluoride. In July 1998, a Fluoride Work Plan was submitted to the Indiana Department of Environmental Management (IDEM) under Permit Condition Section I.A.3a.AA.1.a for Outfalls 005 and 010. The Work Plan provided details on studies to develop toxicity test data to enable the re-derivation of existing fluoride water quality criteria under Tier I and Tier II guidelines.

#### **1.1 CRITERIA DERIVATION**

At the time the Fluoride Work Plan was submitted, the fluoride water quality criteria used by IDEM were as follows:

- Secondary Acute Value (SAV): 22.29 mg/L
- Acute Aquatic Criterion: (AAC = SMC): 11 mg/L
- Chronic Aquatic Criterion: (CAC = SCC): 1.6 mg/L

These criteria were based on four Genus Mean Acute Value (GMAVs) for five species of aquatic organisms (Appendix 1). Only four taxonomic families were represented in the existing fluoride toxicity database, thus, the Tier II acute value was calculated by dividing the lowest GMAV (156 mg/L) by an assigned Secondary Acute Factor (SAF) of 7.0. The Tier II chronic value was derived using two default Acute-to-Chronic Ratio (ACR) values of 18 and the single measured ACR of 7.91 (Appendix 1).

In June 1999, IDEM re-derived the fluoride water quality criteria after incorporating toxicity data for two genera of juvenile mussels (i.e., *Actinonaias* and *Alasmidonta*). The data were developed by the USEPA Region IV and are not yet published. The fluoride LC50 values generated for the juvenile mussels ranged from 178 mg/L to 347 mg/L. The incorporation of these new data increased the number of families meeting the Minimum Data Requirements (MDRs) to five families and allowed a Secondary Acute Factor (SAF)

of 6.1 to be assigned (327) IAC-1.5-12(g)). The revised fluoride aquatic life criteria (Appendix 2) are as follows:

- Secondary Acute Value (SAV): 25.57 mg/L
- Acute Aquatic Criterion: (AAC = SMC): 13 mg/L
- Chronic Aquatic Criterion: (CAC = SCC): 1.9 mg/L

# 1.2 SCOPE AND OBJECTIVES

The overall objective of the work presented here was to re-derive the Great Lakes fluoride aquatic life criteria by expanding the fluoride toxicity database with aquatic species that meet the required eight family Minimum Data Requirements (MDRs). The anticipated outcome is fluoride acute and chronic criteria that more accurately reflect the toxicity threshold of fluoride on aquatic life. Important tasks in support of the additional fluoride toxicity data include:

- Establish the form of fluoride to be tested; and,
- Identify missing families.

# 1.2.1 Establish the Form of Fluoride to be Tested

The existing fluoride toxicity test database was comprised of tests conducted with sodium fluoride (NaF). It is important that the form of fluoride used in the database tests also adequately represent the fluoride exposure.

Table 1-1 provides the information used to determine the dominant form of fluoride in the treated Coke Plant Effluent (CPE). This information is based on the analytical results from the WWTP pilot-scale study, fluoride solubility data, and the chemistry of solutions in equilibrium. The following steps were used to examine the form of bioavailable fluoride that would be present in the effluent:

 Table 1a lists the sources of the fluoride in coal. Two abundant minerals are listed. Following the coking of the coal and capturing of gases into the flushing liquor, it is assumed that these minerals are broken down to their respectable chemical constituents and occur as the cations and anions in the influent to the Coke Plant WWTP.

- Table 1b lists the major cations and anions present in the treated CPE and the possible forms of fluoride that may be in the effluent. These forms listed are those that would dissociate into ions and hence be bioavailable. Based on Form 2c of the NPDES Permit Modification for Permit No. IN0000281 (Outfall 005), the concentrations of the cations present are as follows: Mg<sup>2+</sup>> Na<sup>+</sup> > CaH > K<sup>+</sup>; and,
- By comparing the relative abundance of the cations, to the their solubility it can be assumed that most probable form of fluoride would be in form of sodium fluoride (NaF).

ADVENT conducted the fluoride aquatic toxicity tests with a pure form of sodium fluoride salt. This form of fluoride represents the permit-related exposure and conforms to the existing toxicity database.

#### 1.2.2 Identifying Missing Families

At the time the Fluoride Workplan was prepared (July 1998), evaluation of the existing fluoride acute toxicity test database indicated that only four of the eight family database requirements were met (see Appendix 1). According to 327 IAC 2.1.6-3 Section 3, the families that were missing from the MDR needed to derive a Tier I acute aquatic life criteria for fluoride or further develop the Tier II acute value were as follows:

- a benthic crustacean (e.g. isopod, amphipod);
- an insect (e.g. mayfly, midge);
- a family in a phylum other than Arthropoda or Chordata (e.g., Annelida or Mollusca); and,
- a family in any order of insect or any phylum not already represented.

Also, evaluation of the fluoride aquatic toxicity test database indicated that only one measured Acute-to-Chronic Ratio (ACR) was available (this value is 7.91 for *Daphnia magna*). The remaining two ACRs utilized consisted of the default ACR value of 18 (see Appendices 1 and 2). The existing Tier II chronic value (i.e., Secondary Chronic Value -

SCV) was derived by dividing the Secondary Acute Value (SAV) by the geometric mean of these three ACRs. It was determined that conducting a chronic toxicity test with fluoride would enable the development of an additional measured ACR.

## 2.0 MATERIALS AND METHODS

#### 2.1 TEST MATERIAL

The test material utilized was 99.9% pure reagent-grade sodium fluoride (NaF) salt. Table 2-1 provides a summary of the physical/chemical properties of the test material. A list of impurities associated with the product is provided in Table 2-2. This list was constructed using information provided by the manufacturer (i.e., Aldrich) and analytical measurements conducted on selected stock solution "lots" of sodium fluoride used during database development. Test material certificates of purity are provided in Appendix 3.

#### 2.2 ACUTE TOXICITY TEST PROCEDURES

Acute toxicity test procedures were established first by what is required by IDEM in applicable sections of Article 2<sup>1</sup>; second by the recommendations of the Final Rule of the Federal GLI<sup>2</sup>; and third by the American Society for Testing and Materials (ASTM) guidelines<sup>3</sup>.

#### 2.2.1 Test Organisms

Species selected for acute toxicity testing were based on the missing family MDRs as well as guidance provided in the ASTM E729, "Standard Guide for Conducting Acute Toxicity Test with Fishes, Macroinvertebrates, and Amphibians" and the Final Rule of the GLI. Table 2-3 presents a summary of the test organisms selected to satisfy the missing MDRs and develop an additional measured ACR. Table 2-3 also indicates the type of test conducted, the scientific name of the test organism utilized, the MDR satisfied, the life stage tested, test duration, temperature and organism source. Appendix 4 provides the

<sup>&</sup>lt;sup>1</sup> Indiana Department of Environmental Management, Article 2, 327 IAC 2-1.5.

<sup>&</sup>lt;sup>2</sup> Federal Great Lake Initiative, 40 CFR 132.

<sup>&</sup>lt;sup>3</sup> American Society for Testing and Materials, 1992. "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians, E729. Vol. II.04. Philadelphia, PA.

taxonomic verifications and organisms history sheets for acute toxicity test conducted with each test species.

Acute toxicity tests with the amphipod *Hyallela azteca* were unsuccessful and are not included in this report. ADVENT was unable to obtain acceptable control survival with this test organism and this organism was eliminated from the testing program.

#### 2.2.2 Acute Toxicity Test Methods

Tables 2-4 through 2-7 provide a summary of the general test design for each organism tested. Important acute toxicity test conditions include the following:

- Static, definitive, 96-hour or 48-hour LC50 value tests;
- LC50 values based on mortality;
- Serial test dilutions of 0.7 (seven exposure concentrations);
- Measured chemical exposure concentrations;
- Daily renewals of test solutions;
- Test with midges will use 2nd or 3rd instar;
- Test with aquatic worms will use organisms similar in size (i.e., 10-30 mm);
- Test with aquatic snails will use organisms similar in size; and,
- Dilution water with less than 5.0 mg/L of Total Organic Carbon (TOC).

#### 2.2.3 Nominal Test Concentrations

Acute toxicity test exposure concentrations were selected by conducting preliminary range finding tests. Test concentrations that facilitate the calculation of a LC50 value included those resulting in partial mortality of the test organism. The concentration range and degree of partial mortality observed for each test species is discussed in

Section 3.0 of this document. Table 2-8 provides a summary of the nominal sodium fluoride concentrations utilized for testing.

#### 2.2.4 Test Dilution Water

Acute toxicity test control and dilution water consisted of reconstituted moderately hard water made up in the ADVENT toxicology laboratory following U.S. EPA specifications (600/4-90/027). Moderately hard water was prepared using four reagent grade salts (NaHCO<sub>3</sub>, MgSO<sub>4</sub>, CaSO<sub>4</sub>, and KCI) and deionized water. De-ionized water was obtained from Nashville city water that was passed through a U.S. Filter, Inc. initial deionization unit equipped with five water purifying cartridges (UF membrane, carbon cartridge, mixed bed ion exchange, mixed bed ion polisher, and organic scavenger).

#### 2.3 CHRONIC TOXICITY TEST PROCEDURES

#### 2.3.1 Test Organism

The fathead minnow fish (*Pimephales promelas*) was the selected test organism for the chronic toxicity test. Test organisms were obtained from ADVENT in-house cultures of this species. The flow-through chronic toxicity test was initiated by placing 25, 1-day old fertilized embryos into each test chamber. The majority of egg hatching (including the controls) occurred toward the end of day 4 of the test (therefore, the control mean day of hatch is day 4). On day 5, fish were removed from each test chamber to reduce the number of fish to fifteen per replicate.

#### 2.3.2 Chronic Toxicity Test Methods

Table 2-9 provides a summary of the general test design to be applied for this test organism. The most important chronic toxicity test attributes observed for testing are summarized below:

Definitive, 28-32 day early life cycle toxicity test for non-salmonid fish;

- Serial test dilutions of 0.5 (five exposure concentrations);
- Flow-through test with concentrations of test material measured at weekly intervals;
- Data analyzed for survival and growth;
- Measured chemical exposure concentrations;
- · Test initiation will begin less than 24 hours after fertilization; and,
- Dilution water with less than 5.0 mg/L of Total Organic Carbon (TOC).

In addition to establishing an early life stage chronic toxicity test with fathead minnows, ADVENT also established a corresponding acute toxicity test with juvenile fathead minnows. Therefore, the acute toxicity test result will be a part of the same study as the chronic toxicity test.

## 2.3.3 Nominal Test Concentrations

Chronic toxicity test exposure concentrations were selected by conducting preliminary range finding tests as well as a 7-day chronic toxicity test (static, daily renewals) with fathead minnows. Based on the results of these preliminary tests, the sodium fluoride concentrations selected for the 28-day flow-through chronic toxicity test were 18.75, 37.5, 75, 150, and, 300 mg/L of sodium fluoride.

#### 2.3.4 Test Dilution Water

Control and dilution water for the flow-through chronic toxicity test consisted of dechlorinated tap water. Dechlorinated tap water was selected for the chronic toxicity test because it was the only source that was available in adequate supply, acceptable to the test organisms and of a somewhat uniform quality. ADVENT has cultured adult and larval fathead minnows in de-chlorinated tap water for over 2 years. The tap water was dechlorinated using a granular activated carbon canister aerated intensively by air stones added to holding containers and prior to preparation of the test stock solution. The success of tap water dechlorination was confirmed by daily analyses of dilution water for total residual chlorine (TRC) (see bench sheet for chronic test).

#### 2.3.5 Test Facilities and Equipment

A Benoit mini-diluter was used to conduct the flow-through chronic toxicity test. The minidiluter system was comprised of the following components: 1) a dilution water holding tank, 2) a dilution water header box, 3) a toxicant mixing chamber, 4) a baffled dilution chamber, 5) flow booster cells and 6) distribution cells. Test dilution water entered the system via the dilution water holding tank. This tank was aerated and equipped with temperature control to maintain adequate dissolved oxygen levels and test temperatures. From the dilution water holding tank, the Benoit mini-diluter utilized an elevated header box so that dilution water was gravity fed into the test chambers. Prior to reaching the testing chambers, dilution water and the toxicant stock solution were combined the toxicant mixing chamber to produce the highest test exposure concentration. The highest test exposure concentration (i.e., 300 mg/L sodium fluoride) was then diluted by the baffled dilution chamber to produce a 0.5 dilution series. The resulting serial dilutions were then delivered via the flow boosters to the corresponding distribution cells that evenly metered the test exposure solutions to each test chamber. The Benoit mini-diluter system was also equipped with a timing device to provide the 16-hour light and 8-hour dark photoperiod.

The flow rate of the toxicant stock solution was set at approximately 6.4 mL per minute to the dilution water flow rate of 64 mL per minute in the toxicant mixing chamber. Since 300 mg/L of sodium fluoride was selected as the highest test exposure concentration, the test stock solution was set at 3,000 mg/L as sodium fluoride. From the toxicant mixing chamber, the metering system was designed to produce a 0.5 serial dilution at a flow rate of 32 mL per minute to each distribution cell. Since each test chamber contained 2.0 L of test solution, the test chamber turnover rate was equivalent to approximately six (6) complete volume exchanges a day. The metering system was calibrated prior to the test initiation and checked a minimum of once a day and adjusted as necessary during the

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2-5

test. The general operation of the entire system was also visually checked daily in the morning and afternoon throughout the test.

# 2.4 ANALYTICAL METHODS

Table 2-10 presents the parameters and selected analytical methods utilized in support of the fluoride toxicity testing. The fluoride analytical tests provided measured fluoride exposure concentrations for each toxicity test. A comparison of measured versus nominal fluoride exposures was conducted to ensure that actual toxicant concentrations did not drop below 70 percent of the target nominal concentrations in the acute toxicity tests or below 80 percent of the target nominal concentrations in the flow-through chronic toxicity test as recommended by IDEM<sup>4</sup>.

During the 96-hour acute toxicity tests, fluoride samples were collected on exposure concentrations at test initiation, at 48-hours, on aged solutions and renewal solutions and at test termination. In the 48-hour acute toxicity tests (conducted with midges only), fluoride samples were collected at test initiation and at test end. Samples of "new" (i.e., initial or renewal) test solutions were collected immediately after test dilutions were established. Samples of "aged" test solutions were collected at the time of test solution renewal and after noting organism survival. Analyses of pH, conductivity, and dissolved oxygen (DO) water quality parameters were performed for each test exposure concentration at 24-hour intervals throughout each test.

In the flow-through chronic toxicity test, fluoride samples were collected for each exposure concentration on days 0, 7, 14, 21, 28, and 32 of the test (approximately weekly). Samples were collected from the center of replicates A and C test vessels. A duplicate fluoride sample was collected from the 150 mg/L exposure concentration only. The toxicant stock solution (concentration of 3,000 mg/L sodium fluoride) was also analyzed each time test exposure samples were collected. Conductivity, pH, and DO were measured on each test exposure concentration at 24-hour intervals throughout the chronic toxicity test. Conductivity was monitored in two replicates (usually only one

Letter from the Indiana Department of Environmental Management dated October 13, 1998.

replicate is required) of each exposure concentration to provide further assurance that the diluter was performing properly.

Other water quality indicator parameters analyzed included: 1) hardness, 2) alkalinity, 3) Total Organic Carbon (TOC), 4) Total Suspended Solids (TSS), and 5) sodium (Table 2-8). In the acute a toxicity tests, samples were collected in the control and highest toxicant exposure concentration (with living organisms) at test initiation and at the 48-hour renewal interval. When enough sample volume was available, some of these analyses were also conducted on "aged" test solutions. In the chronic toxicity test, these water quality indicator parameters were determined for the control and highest exposure concentration (i.e., 300 mg/L) in conjunction with the collection of fluoride samples. These water quality data provided basic test information in support of Quality Assurance and Quality Control (QA/QC) efforts discussed in the Fluoride Work Plan.

#### 2.5 DATA ANALYSIS

Testing and re-derivation calculations were performed in accordance with the Final Rule of the Federal GLI (i.e., ASTM method) as well as the test requirements outlined in IDEM's Article 2 Rule 1.6 "Methodologies for Determination of Tier I and Tier II Aquatic Life Criteria" and Article 2 Rule 1.7 "Methodology for Determination of Human Health and Wildlife Criteria". The acceptability of the acute and chronic toxicity test results was judged in accordance with the U.S. EPA 1985 document entitled "Guidelines for Deriving National Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" as well as the requirement of IDEM Rule 1.6.

Acute toxicity test data were analyzed using the Trimmed Spearman-Karber statistical method to calculate the fluoride concentrations (both nominal and measured) that were lethal to 50 percent of the test organisms (i.e., the LC50 values) and the associated upper and lower 95 percent confidence limits. The Trimmed Spearman-Karber method was applied via the computer program "LC50", obtained from the USEPA in Athens, Georgia<sup>5</sup>.

<sup>&</sup>lt;sup>5</sup> Hamilton, MA., R.C. Russo, and R.V. Thurston, 1977. Trimmed Spearman-Karber Method for Estimating Medium Lethal Concentrations in Toxicity Bioassays. Envir. Sci. Technol. 11(7).

Data from the chronic toxicity test were analyzed using multiple means comparison tests to determine the No Observed Effect Concentration (NOEC) and Lowest Observed Effluent Concentration (LOEC) values for fish survival and growth. These chronic toxicity test endpoints were generated by using the computer program "TOXSTAT"<sup>6</sup> obtained from the University of Wyoming Fish Physiology and Toxicology Lab. The test data (surviving numbers or growth weights) were evaluated using the Shapiro-Wilk's Test for normality and Barlett's Test for homogeneity of variance. Test data that passed the tests for normality and homogeneity used Dunnett's Procedure (for an equal number of replicates) to determine the NOEC and LOEC values (parametric test). If either normality or homogeneity were not met the non-parametric test, Steel's Many One Rank Test (for an equal number of replicates) was used to determine the NOEC and LOEC values. The chronic test IC25 values were calculated using the U.S. EPA program "ICPIN"<sup>7</sup> to estimate the effluent concentration that results in a 25 percent decrease in fish biomass.

<sup>&</sup>lt;sup>6</sup> West, Inc. and David Gulley, University of Wyoming, 1994. TOXSTAT® Version 3.4.

<sup>&</sup>lt;sup>7</sup> Norberg-King, T.J. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. NETAC Technical Report 05-88, U.S. Environmental Protection Agency, Duluth, MN. Document support for Version 2.0.

#### 3.0 RESULTS AND DISCUSSION

Acute and chronic toxicity test results are summarized and discussed in terms of the nominal and average measured fluoride concentrations (i.e., average measured values of freshly prepared and aged test solutions for a given exposure for the entire test period). Appendix 5 shows comparisons of new versus aged test solutions as well as the individual analytical data from which the fluoride analytical summary tables were derived. Select photographs of flow-through test equipment and acute toxicity test set-ups are provided in Appendix 6.

#### 3.1 TOXICITY RESULTS FOR PHYSA SPP. (AQUATIC SNAIL)

Tables 3-1 through 3-4 present the toxicological results and corresponding analytical summaries of the acute toxicity tests conducted with *Physa* spp. The 96-hour nominal and measured LC50 values for both tests with the aquatic snails are summarized below:

TEST RESULTS FOR AQUATIC SNAIL					
Toxicity Endpoint	Test Date	Nominal Fluoride (mg/L)	Measured Fluoride (mg/L)		
96-hr LC50	22Feb99	229.4	231.7		
96-hr LC50	28Apr99	142.3	163.1		

As shown by the table above, nominal and measured fluoride LC50 values corresponded well for each test. The dose response curve for the snail was somewhat irregular for the first test (Table 3-1), but improved considerably for the second test (Table 3-3). Partial mortality (defined here as greater than 10% but less than 100%) occurred in five of the seven test exposure concentrations in the second test. In both tests, complete mortality was observed in the highest exposure concentration (i.e., 1,000 mg/L sodium fluoride), and control survival was 90 percent or greater.

Tables 3-2 and 3-4 show the average measured fluoride and target nominal concentrations at each test exposure. A 30-percent analytical difference between average measured and target nominal fluoride concentrations is allowed by IDEM<sup>4</sup>. The

average measured fluoride values were less than 30 percent different, and generally higher than the target nominal fluoride levels.

# 3.2 TOXICITY RESULTS FOR LUMBRICULUS VARIEGATUS (AQUATIC WORM)

Tables 3-5 through 3-10 present the toxicological results and corresponding analytical summaries of the acute toxicity tests conducted with *Lumbriculus variegatus* (aquatic worm). The 96-hour nominal and measured LC50 values for tests with the aquatic worm are summarized below:

TEST RESULTS FOR AQUATIC WORM						
Toxicity Endpoint	Test Date	Nominal Fluoride (mg/L)	Measured Fluoride (mg/L)			
96-hr LC50	23Feb99	84.9	93.5			
96-hr LC50	31Mar99	91.8	113.1			
96-hr LC50	19Apr99	>135.8	>160.0			

As shown by the table above, nominal and measured fluoride LC50 values corresponded well for each test. As shown by Tables 3-5 and 3-7, the percent mortality observed in each of the test exposure concentrations was very similar. Given this, the dose response curve for these tests were consistent, but also very steep. At the end of the first two tests, almost complete mortality was observed in the 245 mg/L sodium fluoride exposure concentration. In the 172 mg/L exposure concentration, only 20 percent or less mortality was observed. For the first two tests (Tables 3-5 and 3-7), partial mortality occurred in only one of the seven test exposure concentrations.

In an attempt to improve on the dose-response for partial mortality, the range of test exposure concentrations was reduced for the third test (Table 3-9). However, results indicate the aquatic worms used for the third test were less sensitive to sodium fluoride than those used in previous tests. In the 300 mg/L sodium fluoride concentration, only a 40 percent mortality was observed (Table 3-9), compared to 93 percent mortality in

245 mg/L sodium fluoride for Test #2 (Table 3-7), and 95 percent mortality in 245 mg/L sodium fluoride for Test #1 (Table 3-5). Partial mortality did not exceed 50 percent in any test concentration and an actual 96-hour LC50 value could not be calculated.

As shown in Table 3-6, the average measured fluoride concentrations were similar to the target nominal concentrations at each test exposure for the first test. The percent difference between averaged measured and nominal fluoride concentrations were considerably less than the allowed analytical difference of 30 percent or less as specified by IDEM. In fact, the majority of percent differences between target and measured fluoride concentrations were less than 15 percent.

Tables 3-8 and 3-10 indicate that the average measured fluoride concentration differed from the target fluoride concentration at some exposures by more than 30 percent for the second and third toxicity tests conducted with aquatic worms. Based on the range of conductivity values recorded for each test (see Table 3-7 and 3-9), ADVENT believes that several of the measured fluoride values were in error. With the exception of the highest exposure concentration for test #2 (Table 3-8), the majority of the exposures for which the percent difference exceeded 30 percent occurred at exposure concentrations in which the worms were not affected. While the measured fluoride concentrations were consistently higher than the target nominal concentrations, the range of exposure concentrations was not compromised. This suggests that test organisms were at least exposed to the target range of fluoride concentration and did not influence the resulting LC50 values.

#### 3.3 TOXICITY RESULTS FOR CHIRONOMUS TENTANS (AQUATIC INSECT)

Tables 3-11 through 3-14 present the toxicological results and corresponding analytical summaries of the acute toxicity tests conducted with *Chironomus tentans* (aquatic insect). The 48-hour nominal and measured LC50 values for tests with the aquatic insect are summarized below:

3-3

TES	T RESULTS FO	R AQUATIC IN	SECT
Toxicity Endpoint	Test Date	Nominal fluoride (mg/L)	Measured fluoride (mg/L)
48-hr LC50	22May99	96.1	93.1
48-hr LC50	26Aug99	107.1	110.9

As shown by the table above, nominal and measured fluoride LC50 values corresponded well for each test. As shown by Tables 3-11 and 3-13, the percent mortality observed in across the selected test exposure concentrations were similar, therefore, there was good agreement between LC50 values. Partial mortality occurred in three of the seven test exposure concentrations for both tests. At test end, complete mortality was observed in the 500 mg/L sodium fluoride exposure concentration and a good dose response relationship was observed among the other test concentrations (Tables 3-11 and 3-13).

Tables 3-12 and 3-14 show the average measured fluoride concentrations were similar to the target nominal concentrations for each test and at all test exposures. The percent differences between average measured and nominal fluoride concentrations were generally less than ten (10) percent and considerably less than the allowed analytical difference of 30 percent or less as specified by IDEM.

# 3.4 TOXICITY RESULTS FOR PIMEPHALES PROMELAS (FISH)

Both acute and chronic toxicity tests were conducted with fathead minnows. Tables 3-15 through 3-18 present the toxicological results and corresponding analytical summaries of the acute toxicity tests conducted with fathead minnows. The toxicity results are summarized below:

3-4

ACUTE TEST RESULTS FOR FATHEAD MINNOWS					
Toxicity Endpoint	Test Date	Nominal fluoride (mg/L)	Measured fluoride (mg/L)		
96-hr LC50	29June99	127.9	112.2		
96-hr LC50 (*)	10Aug99	209.1	225.1		

Note:

(\*) Used for Acute-to-Chronic Ratio (ACR) value development.

It should be noted that the second fathead minnow acute toxicity test (dated August 10, 1999) corresponded with the 28-day flow-through chronic toxicity test conducted with this species and is used herein for development of an additional Acute-to-Chronic Ratio (ACR).

The results show that for both acute tests, nominal and measured fluoride 96-hr LC50 values were similar. The 96-hour LC50 values for the two tests did not have overlapping confidence intervals (see Tables 3-15 and 3-17). However, the LC50 value of 209 mg/L for the second test shows good agreement with the 180 mg/L LC50 value reported in the current fluoride database (Appendix 2). The reason for the difference may be attributed to the fact that 14-day old fish were used in the second acute test versus more sensitive 8-day old fish for the first acute test.

As shown in the corresponding analytical tables (see Tables 3-16 and 3-18), the average measured fluoride concentrations were similar to the target nominal concentrations for both tests at all test exposures and within the allowed analytical difference of 30 percent or less as specified by IDEM.

Tables 3-19 and 3-20 present the toxicological and analytical summary data for the 28-day flow-through chronic toxicity test conducted with fathead minnows. The chronic toxicity test results are summarized below:

FLOW-THRU TEST RESULTS FOR FATHEAD MINNOWS					
Toxicity Endpoint	Test Date	Nominal fluoride (mg/L)	Measured fluoride (mg/L)		
NOEC Value	22July99	67.9	66.6		
LOEC Value	22July99	135.8	134.3		
Chronic Value (*)	22July99	96.0	94.6		
IC25 Value	22July99	85.4	84.1		

Note:

(\*) Used for Acute-to-Chronic Ratio (ACR) value development.

The results indicate good agreement between the nominal and measured fluoride toxicity test endpoints. As shown in Table 3-19, more than 80 percent mortality was observed in the highest sodium fluoride exposure concentration tested (i.e., 300 mg/L). Essentially no mortality as well as no statistically significant adverse sublethal effects (such as a reduction in average growth weight) were observed for the other test exposure concentrations. Additionally, toxicity test endpoints derived from this 28-day flow-through test with fathead minnows were similar to those determined by a preliminary 7-day chronic toxicity test (daily renewal) also conducted with fathead minnows (see Table 3-21). For example, the fluoride IC25 values for the 28-day flow-through test and the 7-day chronic toxicity test with fathead minnows were 84.1 and 82.8 mg/L, respectively.

It should also be noted that the dilution series for the 28-day flow-through test was established based on the available fluoride acute toxicity test data and the results from the preliminary 7-day chronic toxicity test with fathead minnows. Since the 200 mg/L exposure concentration from the 7-day chronic toxicity test indicated a reduction in fathead minnow growth weights relative to the control, the dilution series for the 28-day flow-through test was selected in anticipation of greater sublethal effects. However, as mentioned above, no such sublethal effects were observed.

Table 3-20 indicates that the average measured fluoride concentrations for the flow through test were similar to the target nominal concentrations. For each test exposure

concentration, the percent differences between average measured and nominal fluoride concentrations were considerably less than the IDEM-allowed analytical difference for chronic tests of 20 percent and were generally less than 10 percent.

# 3.5 WATER QUALITY INDICATORS

Tables 3-22 and 3-23 present a summary of water quality indicator parameters analyzed in support of acute and chronic toxicity testing, respectively. These data verify the quality and consistency of the control and dilution water utilized. Total Organic Carbon (TOC) analyses confirmed that test dilution waters (both moderately hard as well as the dechlorinated tap water) routinely measured less than 5.0 mg/L.

# 3.6 REDERIVATION OF THE FLUORIDE CRITERIA

ADVENT augmented the existing fluoride toxicity database (Appendix 2) with the fluoride acute and chronic toxicity test data for fathead minnow, aquatic snail, aquatic worm, and aquatic insect to re-derive the fluoride aquatic life criteria. ADVENT utilized toxicity test endpoints (i.e., LC50 values, NOEC values) based on the average measured fluoride concentrations only.

Since the acute toxicity tests with amphipods were unsuccessful, there remained only enough data to meet seven of the eight family MDRs. Using the fathead minnow 96-hour LC50 value of 225.1 mg/L fluoride (Table 3-17) divided by the corresponding fathead minnow chronic toxicity test Chronic Value (ChV) of 94.6 mg/L (Table 3-19), a measured Acute-to-Chronic Ratio (ACR) of 2.4 was calculated.

To determine the revised Secondary Chronic Value (SCV), ADVENT utilized the rederived SAV and Secondary Acute Chronic Ratio (SACR). The SACR was calculated by taking the geometric mean of the existing ACR for *Daphnia magna* (7.91), the newly measured ACR for fathead minnows (2.4), and one default ACR of 18. The geometric mean of these three ACRs gives a SACR of 7.0. Table 3-24 presents the revised SAV, SACR, SMC, and SCV values for fluoride and the following re-derived Tier II fluoride aquatic life values:

- Secondary Acute Value (SAV): 23.6 mg/L
- Acute Aquatic Criterion: (AAC = SMC): 11.8 mg/L
- Chronic Aquatic Criterion: (CAC = SCC): 3.38 mg/L

# 4.0 CONCLUSION

The incorporation of the recently developed data and re-derivation of the fluoride water quality criteria results in the following new Tier II criteria:

- Secondary Acute Value (SAV): 23.6 mg/L
- Acute Aquatic Criterion: (AAC = SMC): 11.8 mg/L
- Chronic Aquatic Criterion: (CAC = SCC): 3.38 mg/L

The use of these new fluoride Tier II water quality criteria would be protective of aquatic life. The data developed as well as the process utilized to re-derive the criteria should be acceptable to IDEM.

#### TABLE 1-1. DETERMINATION OF DOMINANT FLUORIDE FORM IN COKE PLANT EFFLUENT

#### A. SOURCES OF FLUORIDE IN COAL

MINERAL	FORMULA
Fluorspar	CaF₂
Cryolite	Na₃AIF₅

#### B. MAJOR CATIONS AND ANIONS PRESENT IN TREATED COKE PLANT EFFLUENT AND POSSIBLE FORMS OF FLUORIDE

CATIONS	ANIONS	ASSOCIATED CATIONS
Са	CI	Na, K, Mg, Ca
ĸ	F	Na, K, Ca
Na	NO <sub>3</sub>	Na, K, Mg, Ca
Mg	SO4	K, Mg, Ca
Fe	CO3	Na, K, Ca

#### C. SOLUBILITIES OF POSSIBLE FORMS OF FLUORIDE

COMPOUND	SOLUBILITY (g/ 100 mL)
CaF₂	0.0017
CaF <sub>2</sub> Na <sub>3</sub> AlF <sub>6</sub>	0.4
KF	92
NaF*	4.22
MgF <sub>2</sub>	0.0076
MgF <sub>2</sub> FeF <sub>2</sub> <sup>b</sup>	slightly

#### Notes:

a) Na\* concentration higher than K\* in effluent, dominant form in effluent most likely is NaF.

(As per permit modification application: Daily Maximum for F 19 mg/L; and Long Term Average for F 17 mg/L.).b) As per ADVENT report "Evalution of Iron in the USS CPE", Iron primarily exists as cyanide complexes.

TABLE 2-1. LIST OF CHEMICAL AND PHYSICAL PROPERTIES FOR SODIUM FLUORIDE (99.9% PURE)

SODIUM	CHEMICAL/ PHYSICAL PROPERTIES					
FLUORIDE	FORMULA WEIGHT	SPECIFIC GRAVITY	MELTING POINT	BOILING POINT	WATER SOLUBILITY	COMMENTS
NaF (A.C.S. Reagent)	41.99	2.78	993°C	1704°C	4.3 g/100mL	White Powder

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TABLE 2-2.	LIST OF IMPURITIES FOR	SODIUM FLUORIDE (99.9% PURE)
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	UNITS	STOCK SOLUTION CONCENTRATIONS (mg/L) (a)		MANUFACTURE SPECIFICATIONS (mg/L)	
PARAMETER		500 24-May-99	3000 29-Jun-99	LOT NO. 15402BN	
<u>Metals, Total</u>					
Aluminum	mg/L	ND	ND	<1.0	
Antimony	mg/L	0.0043	<0.0020	4.0	
Arsenic	mg/L	<0.005	<0.0050	N	
Barium	mg/L	ND	ND	1.0	
Beryllium	mg/L	<0.002	<0.0020	NE	
Boron	mg/L	ND	ND	20.0	
Cadmium	mg/L	<0.002	<0.0020	N	
Chromium	mg/L	0.0033	0.0058	N	
Copper	mg/L	0.0130	<0.010	<1.0	
Iron	mg/L	ND	ND	<1.0	
Lead	mg/L	<0.005	<0.0050	10.0	
Magnesium	mg/L	ND	ND	1.0	
Manganese	mg/L	ND	ND	NC	
Mercury	mg/L	<0.0002	ND	NC	
Nickel	mg/L	<0.01	<0.010	NE	
Selenium	mg/L	<0.005	<0.0050	NC	
Silver	mg/L	<0.002	<0.0020	NE	
Sodium	mg/L	270.0	ND	NE	
Thallium	mg/L	0.0110	0.0017	NE	
Zinc	mg/L	0.0530	0.0180	NE	

ND = No Data · Not Analysed (a) · Stock solutions from actual tests with sodium fluoride used.

# TABLE 2-3. LIST OF TEST ORGANISMS SELECTED FOR FLUORIDE DATABASE DEVELOPMENT

TEST ORGANISMS	MINIMUM DATABASE REQUIREMENT (MDR) SATISFIED	LIFE STAGE	TEST TEMPERATUR	ORGANISM SOURCE (a)
Acute Toxicity Tests				
Hyallela azteca (amphipod); or, Gammarus pseudolimneus (amphipod -second choice)	a benthic crustacean	early instar (7-10 days old)	25°C	ECT
Chironomus tentans (midge)	an insect	3rd instar	25°C	ECT
Lumbriculus variegatus (worm)	a family in a phylum other than Arthropoda or Chordata	10 to 30 mm in length	20°C	ARO
Physa spp. (aquatic snail)	a family in any order of insect or phylum not already represented	early life stage all similar size	25°C	ARO
Pimephales promelas (fathead minnows)	used to determine an additional acute to chronic ratio	1 to 14 days in age	25°C	ADVENT
Chronic Toxicity Test			1	1.00
Pimephales promelas (fathead minnows)	for an additional acute to chronic ratio removal	embryonic stage	25°C	ADVENT

Notes:

(a) Organisms obtained from Aquatic Research Organisms (ARO) of New Hampshire, Environmental Consulting and Testing (ECT) of Wisconsin, or ADVENT in-house cultures.

#### TABLE 2-4. SUMMARY OF ACUTE TOXICITY TEST CONDITIONS FOR PHYSA SPP.

TEST CONDITION	SPECIFICATION
1. Temperature (°C):	25 degrees C
2. Light quality	Ambient laboratory illumination
3. Light intensity:	10-20 uE/m2/s (50-100 ft-c) (ambient laboratory levels)
4. Photoperiod:	16 hrs light, 8 hrs darkness
5. Size of test vessels:	250 mL
6. Volume of test solution:	200 mL
7. Age of organisms:	Early life stage - similar size
<ol> <li>Number of organisms per test chamber:</li> </ol>	Ten (10)
<ol> <li>Number of replicate test chambers per concentration:</li> </ol>	Two (2)
<ol> <li>Number of organisms per concentration:</li> </ol>	Twenty (20)
11. Feeding regime:	Food was available a minimum of 2 hours prior to testing; organisms were not fed after test initiation.
12. Aeration:	Aeration is not required unless DO <60%
13. Dilution water:	USEPA Moderately hard sythetic water
14. Test Type:	Static, daily renewal tests
15. Test duration:	96 hours
16. Effect measured:	Mortality
17. Endpoint:	LC50 value
18. Test acceptability criterion:	90% or greater survival in controls
19. Special conditions:	None

## TABLE 2-5. SUMMARY OF ACUTE TOXICITY TEST CONDITIONS FOR LUMBRICULUS SPP.

TEST CONDITION	SPECIFICATION
1. Temperature (°C):	20 degrees C
2. Light quality	Ambient laboratory illumination
3. Light intensity:	10-20 uE/m2/s (50-100 ft-c) (ambient laboratory levels)
4. Photoperiod:	16 hrs light, 8 hrs darkness
5. Size of test vessels:	500 mL
6. Volume of test solution:	450 mL
7. Age of organisms:	Early life stage - 30 mm in length
8. Number of organisms per test chamber:	Ten (10)
<ol> <li>Number of replicate test chambers per concentration:</li> </ol>	Two (2) to Three (3)
<ol> <li>Number of organisms per concentration:</li> </ol>	Twenty (20) to Thirty (30)
11. Feeding regime:	Food was available a minimum of 2 hours prior to testing; organisms were not fed after test initiation.
12. Aeration:	Aeration was not required
13. Dilution water:	USEPA Moderately hard sythetic water
14. Test Type:	Static, daily renewal tests
15. Test duration:	96 hours
16. Effect measured:	Mortality
17. Endpoint:	LC50 value
<ol><li>Test acceptability criterion:</li></ol>	90% or greater survival in controls
19. Special conditions:	None

#### TABLE 2-6. SUMMARY OF ACUTE TOXICITY TEST CONDITIONS FOR CHIRONOMUS TENTANS

TEST CONDITION	SPECIFICATION
1. Temperature (*C);	25 degrees C
2. Light quality	Ambient laboratory illumination
3. Light intensity:	10-20 uE/m2/s (50-100 ft-c) (ambient laboratory levels)
4. Photoperiod:	16 hrs light, 8 hrs darkness
5. Size of test vessels:	250 mL
6. Volume of test solution:	200 mL
7. Age of organisms:	3rd instar
<ol> <li>Number of organisms per test chamber;</li> </ol>	Five (5)
<ol> <li>Number of replicate test chambers per concentration:</li> </ol>	Four (4)
<ol> <li>Number of organisms per concentration:</li> </ol>	Twenty (20)
1. Feeding regime:	Food was available a minimum of 2 hours prior to testing; organisms were not fed after test initiation.
2. Aeration:	Aeration was not required
.3. Dilution water:	USEPA Moderately hard sythetic water
4. Test Type:	Static, daily renewal tests
.5. Test duration:	48 hours
.6. Effect measured:	Mortality
7. Endpoint:	LC50 value
8. Test acceptability criterion:	90% or greater survival in controls
9. Special conditions:	Used sterile sand in bottom of test chambers

TEST CONDITION	SPECIFICATION
1. Temperature (°C):	25 degrees C
2. Light quality	Ambient laboratory illumination
3. Light intensity:	10-20 uE/m2/s (50-100 ft-c) (ambient laboratory levels)
4. Photoperiod:	16 hrs light, 8 hrs darkness
5. Size of test vessels:	250 mL
6. Volume of test solution:	200 mL
7. Age of organisms:	1 to 14 days in age
<ol> <li>Number of organisms per test chamber:</li> </ol>	Ten (10)
<ol> <li>Number of replicate test chambers per concentration:</li> </ol>	Two (2)
0. Number of organisms per concentration:	Twenty (20)
1. Feeding regime:	Food was available a minimum of 2 hours prior to testing; organisms were not fed after test initiation.
2. Aeration:	Aeration was not required
3. Dilution water:	Dechlorinated Tap Water (same as for chronic test)
4. Test Type:	Static, daily renewal tests
5. Test duration:	96 hours
6. Effect measured:	Mortality
7. Endpoint:	LC50 value
8. Test acceptability criterion:	90% or greater survival in controls
9. Special conditions:	None

# TABLE 2-7. SUMMARY OF ACUTE TOXICITY TEST CONDITIONS FOR FATHEAD MINNOWS

TABLE 2-8. TEST EXPOSURE CONCENTRATIONS FOR ACUTE TOXICITY TESTS WITH SODIUM FLUORIDE

TEST SPECIES	TEST DURATION	EXPOSURE CONCENTRATIONS (mg/L NaF)	TEST RENEWAL	TEST REPS	ANALYTICAL
Physa physa (Aquatic snail)	96-hr	1000, 700, 490, 343, 240, 168, 118, 0	Daily (a)	2 reps/10	(b)
Lumbriculus varigateus (Aquatic worm)	96-hr	500, 350, 245, 172, 120, 84, 59, 0 300, 260, 220, 180, 140, 100, 60, 0	Daily (a)	3 reps/10	(b)
Chironomus tentans (Midge - Aquatic insect)	48-hr	500, 350, 245, 172, 120, 84, 59, 0	Daily (a)	4 reps/5	(b)
Pimephales promelas (Fathead minnows)	96-hr	500, 400, 300, 200, 100, 50, 0 615, 430, 301, 211, 148, 103, 73, 0	Daily (a)	2 reps/10	(b)

Notes:

(a) Daily renewals at 24 hour intervals made from stock solutions; New stock solutions at T=0 hrs and T=48 hrs.

(b) Collected fluoride samples at all exposure concentrations; collected all others on control and highest exposure.

For fluoride = Collected new at T=0 hrs, collected old and new at T=48 hrs and collected old at T=96 hrs.

For other water quality indicators = Collected new at T=0 hrs and collected new at T=48 hrs.

Water quality indicators = sodium, TSS, TOC, hardness and alkalinity.

TEST CONDITION	SPECIFICATION
1. Temperature (°C):	25°C ± 1°C
2. Light quality	Ambient laboratory illumination
3. Light intensity:	10-20 uE/m2/s (50-100 ft-c) (ambient laboratory levels)
4. Photoperiod:	16 hrs light, 8 hrs darkness
5. Size of test vessels:	3.0 L
6. Volume of test solution:	2.0 L
7. Age of organisms:	Less than 24 hours after fertilization; embryos
<ol> <li>Number of organisms per test chamber:</li> </ol>	Fifteen (15)
<ol> <li>Number of replicate test chambers per concentration:</li> </ol>	Four (4)
<ol> <li>Number of embryos per concentration:</li> </ol>	Sixty (60)
<ol> <li>Feeding regime:</li> </ol>	0.15 mL twice daily, 6 hours between feedings (at the beginning of the work day prior to renewal, and at the end of the work day following renewal). Sufficient larvae are added to provide an excess. Larvae are not fed during the final 12 hours of the test
12. Aeration:	Dilution water holding tank was aerated Dissolved oxygen levels were checked daily
13. Dilution water:	USEPA Moderately hard synthetic water ? DE-CELLE TUP HID.
14. Test Type:	USEPA Moderately hard synthetic water DE - CELLER TEPHNO. Flow-through tests 32 days (Mean date of hatch 4.5 days)
15. Test duration:	32 days (Mean date of hatch 4.5 days)
l6. Endpoint:	Survival and growth (weight)
7. Test acceptability:	80 percent control survival
8. Special conditions:	None

# TABLE 2-9. SUMMARY OF CHRONIC TOXICITY TEST CONDITIONS FOR FATHEAD MINNOWS

# TABLE 2-10 . PARAMETERS AND ANALYTICAL METHODS UTILIZED FOR TESTING

PARAMETERS	USEPA METHOD <sup>(1)</sup>	MDL (mg/L)	RECOMMENDED SAMPLE VOLUME	SAMPLE CONTAINER	PRESERVATIVE	HOLD TIME
Each Test Exposure Concentration						
Fluoride	340.2	0.01	500 mL	Р	4°C	28 Days
			1.1.1		4°C, H <sub>2</sub> SO <sub>4</sub> or HNO <sub>3</sub> to	
Dissolved Oxygen	360.1/2		500 mL	G - Bottle and Top	pH<2	Immediate
Specific Conductance	120.1		100 mL	P	4°C	28 Days
pH	150.1	-	100 mL	P, G	1.1.1	Immediate
Control & Highest Test Exposure		_				
Sodium	273.1	- 2-	100 mL	P, G	4°C, HNO3 to pH<2	6 Months
	200.7	0.72	100 mL	P, G	4°C, HNO <sub>3</sub> to pH<2	6 Months
тос	415.1	0.22				
TSS	160.2	0.87	100 mL	P, G		7 Days
			100.00		4°C, H <sub>2</sub> SO <sub>4</sub> or HNO <sub>3</sub> to	1.000
Hardness	130.2		250 mL	P, G	pH<2	6 Months
Alkalinity	310.1/2		100 mL	P, G	4°C	14 Days

Note:

(1) USEPA "Methods for Chemical Analyses of Water and Wastes"

# TABLE 3-1. ACUTE TOXICITY RESULTS FOR PHYSA PHYSA (AQUATIC SNAIL) - Test #1

TEST DATE:	02/22/99
TEST LOG NUMBER:	4360
SAMPLE IDENTIFICATION:	Sodium Fluoride (NaF) Pure Chemical Test
PROJECT NAME:	USS - Tier II Development

PARAMETER	EPA		NOMI	AL SODIUM	FLUORIDE CO	NCENTRATIO	NOMINAL SODIUM FLUORIDE CONCENTRATIONS									
	MOD HARD CONTROL	118 (mg/L)	168 (mg/L)	240 (mg/L)	343 (mg/L)	490 (mg/L)	700 (mg/L)	1000 (mg/L)								
Number of Individuals		-						1								
(Test Chamber ID)								-								
Α	10	10	10	10	10	10	10	10								
В	10	10	10	10	10	10	10	10								
Individuals Surviving at 96 Hours							_									
А	8	6	8	6	6	7	1	0								
B	10	6	7	6	8	6	1	0								
Percent Survival	90	60	75	60	70	65	10	0								
Chemical/Physical Parameters																
Temperature (*C)	24.0 - 25.5	24.0 - 25.5	24.0 - 25.5	24.0 - 25.5	24.0 - 25.5	24.0 - 25.5	24.0 - 25.5	24.0 - 25.								
Dissolved Oxygen (mg/L)	7.8 - 8.6	7.0 - 8.8	7.6 - 8.7	8.0 - 8.7	7.8 - 8.8	7.6 - 8.8	7.8 - 8.8	8.4 - 8.4								
pH (s.u.)	7.23 - 7.71	7.28 - 7.69	7.35 - 7.69	7.31 - 7.72	7.38 - 7.75	7.39 - 7.78	7.45 - 7.81	7.57 - 7.7								
Conductivity (µmhos/cm)	319 - 348	573 - 638	689 - 717	820 - 851	1010 - 1065	1317 - 1373	1740 - 1820	2335 - 2460								
NOMINAL FLUORIDE (mg/L)	0.0	53.4	76.0	108.6	155.2	221,7	316.8	452.5								
Measured Sodium Fluoride (mg/L)																
Initial Fluoride Concentration	<0.10	58	80	120	170	230	330	430								
48-hr Aged Fluoride Concentration	<0.10	51	83	110	150	240	300									
48-hr New Fluoride Concentration	<0.10	52	83	110	150	210	310									
96-hr Aged Fluoride Concentration	<0.10	62	83	120	170	210	340									
Statistical Analysis:	NOMINAL				MEASURED											
Trimmed Spearman-Karber	FLUORIDE	Lower	Upper		FLUORIDE	Lower	Upper									
	LC50 (mg/L)	Limit	Limit		LC50 (mg/L)	Limit	Limit									
24 Hour	and the second	293.3	354.5	24 Hour	NA I	NA	NA									
48 Hour	279.6	249.3	313.6	48 Hour	NA	NA	NA									
72 Hour	217.4	176.3	267.9	72 Hour	NA	NA	NA									
96 Hour	229.4	156.5	336.3	96 Hour	231.7	161.8	331.9									

NR - Not Recorded

NA - Not Applicable

NC - Not Calculable

TABLE 3-2. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR PHYSA PHYSA - TEST #1

TARGET NOMINAL NaF Conc. F Conc. (mg/L) <sup>(a)</sup> (mg/L)	TARGET	MEASU	SAMPLING RED FLUORIDE		AVERAGE MEASURED FLUORIDE CONC. <sup>(c)</sup> (mg/L)	AVERAGE	STATUS OF FLUORIDE EXPOSURE <sup>(d)</sup>	
	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	New (T=48) F Conc. (mg/L)	Aged (T=96) F Conc. (mg/L)		PERCENT OFF NOMINAL (%) <sup>(e)</sup>		
118	53.4	58.0	51.0	52.0	62.0	55.8	4.4%	more
168	76.0	80.0	83.0	83.0	83.0	82.3	8.2%	more
240	108.6	120.0	110.0	110.0	120.0	115.0	5.9%	more
343	155.2	170.0	150.0	150.0	170.0	160.0	3.1%	more
490	221.7	230.0	240.0	210.0	210,0	222.5	0.3%	more
700	316.8	330.0	300.0	310.0	340.0	320.0	1.0%	more
1000	452.5	430.0			-	430.0	-5.0%	less

#### Notes:

(a) Test initiation date was February 22, 1999.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(e) Thirty percent difference allowed by IDEM.

# TABLE 3-3. ACUTE TOXICITY RESULTS FOR PHYSA PHYSA (AQUATIC SNAIL) - TEST #2

TEST DATE:	04/28/99
TEST LOG NUMBER:	4997
SAMPLE IDENTIFICATION:	Sodium Fluoride (NaF) Pure Chemical Test
PROJECT NAME:	USS - Tier II Development

PARAMETER		NOMINAL SODIUM FLUORIDE CONCENTRATIONS							
	EPA MOD HARD CONTROL	118 (mg/L)	168 (mg/L)	240 (mg/L)	343 (mg/L)	490 (mg/L)	700 (mg/L)	1000 (mg/L)	
Number of Individuals									
(Test Chamber ID)									
A	10	10	10	10	10	10	10	10	
В	10	10	10	10	10	10	10	10	
Individuals Surviving at 96 Hours									
А	10	10	9	5	6	3	2	0	
B	10	9	7	3	5	3	3	0	
Percent Survival	100	95	80	40	55	30	25	0	
Chemical/Physical Parameters									
Temperature (*C)	24.6 -25.0	24.6 -25.0	24.6 -25.0	24.6 -25.0	24.6 -25.0	24.6 -25.0	24.6 -25.0	24.6 -25.	
Dissolved Oxygen (mg/L)	6.6 - 8.3	7.0 - 8.3	7.1 - 8.3	6.9 - 8.3	6,9 - 8,3	6.9 - 8.3	6.9 - 8.3	7.0 - 8.	
pH (s.u.)	7.42 - 7.77	7.45 - 7.80	7.48 - 7.74	7.36 - 7.79	7.46 - 7.81	7.58 - 7.92	7:59 - 8.04	7.51 - 8.1	
Conductivity (µmhos/cm)	330 - 347	582 - 604	690 - 716	830 - 875	1036 - 1066	1332 - 1377	1796 - 1849	2360 - 2420	
NOMINAL FLUORIDE (mg/L)	0.0	53.4	76.0	108.6	155.2	221.7	316.8	452.5	
Measured Fluoride Concentration (r	ng/L)								
Measured Fluoride Concentration (r	ng/L) <0.10	57	80	110	190	280	370	510	
		57	80 80	110	190 170	280 250	370 370	510	
Initial Fluoride Concentration	<0.10					77.9.3		1.70,70	
Initial Fluoride Concentration 48-hr Aged Fluoride Concentration	<0.10 <0.10	57	80	120	170	250	370	530	
Initial Fluoride Concentration 48-hr Aged Fluoride Concentration 48-hr New Fluoride Concentration	<0.10 <0.10 <0.10	57 57	80 82	120 110	170 190	250 260	370 360	530 500	
Initial Fluoride Concentration 48-hr Aged Fluoride Concentration 48-hr New Fluoride Concentration	<0.10 <0.10 <0.10	57 57	80 82	120 110	170 190	250 260	370 360	530 500	
Initial Fluoride Concentration 48-hr Aged Fluoride Concentration 48-hr New Fluoride Concentration 96-hr Aged Fluoride Concentration	<0.10 <0.10 <0.10 0.10	57 57	80 82	120 110	170 190 190	250 260	370 360	530 500	
Initial Fluoride Concentration 48-hr Aged Fluoride Concentration 48-hr New Fluoride Concentration 96-hr Aged Fluoride Concentration Statistical Analysis:	<0.10 <0.10 <0.10 0.10 NOMINAL	57 57 63	80 82 120	120 110	170 190 190 MEASURED	250 260 250	370 360 350	530 500	
Initial Fluoride Concentration 48-hr Aged Fluoride Concentration 48-hr New Fluoride Concentration 96-hr Aged Fluoride Concentration Statistical Analysis:	<0.10 <0.10 <0.10 0.10 NOMINAL FLUORIDE	57 57 63 Lower	80 82 120 Upper	120 110	170 190 190 MEASURED FLUORIDE	250 260 250 Lower	370 360 350 Upper	530 500	
Initial Fluoride Concentration 48-hr Aged Fluoride Concentration 48-hr New Fluoride Concentration 96-hr Aged Fluoride Concentration Statistical Analysis: Trimmed Spearman-Karber	<0.10 <0.10 <0.10 0.10 NOMINAL FLUORIDE LC50 (mg/L)	57 57 63 Lower Limit	80 82 120 Upper Limit	120 110 130	170 190 190 MEASURED FLUORIDE LC50 (mg/L)	250 260 250 Lower Limit	370 360 350 Upper Limit	530 500	
Initial Fluoride Concentration 48-hr Aged Fluoride Concentration 96-hr Aged Fluoride Concentration 96-hr Aged Fluoride Concentration Statistical Analysis: Trimmed Spearman-Karber 24 Hour	<0.10 <0.10 <0.10 0.10 NOMINAL FLUORIDE LC50 (mg/L) 359.4	57 57 63 Lower Limit 305.5	80 82 120 Upper Limit 422.8	120 110 130 24 Hour	170 190 190 MEASURED FLUORIDE LC50 (mg/L) NA	250 260 250 Lower Limit NA	370 360 350 Upper Limit NA	530 500	

NR - Not Recorded

NA - Not Applicable

NC - Not Calculable

TARGET NOMINAL NaF Conc. (mg/L) <sup>(a)</sup> F Conc. (mg/L)	MEASUR	SAMPLING RED FLUORIDE		AVERAGE MEASURED	AVERAGE			
	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	New (T=48) F Conc. (mg/L)	Aged (T=96) F Conc. (mg/L)	FLUORIDE CONC. <sup>(c)</sup> (mg/L)	PERCENT OFF NOMINAL (%) <sup>(e)</sup>	STATUS OF FLUORIDE EXPOSURE <sup>(d)</sup>	
Control	0.1	0.1	0.1	0,1	0.1	0.1	0.0%	equal
118	53.4	57.0	57.0	57.0	63.0	58.5	9.6%	more
168	76.0	80.0	80.0	82.0	120.0	90.5	19.0%	more
240	108.6	110.0	120.0	110.0	130.0	117.5	8.2%	more
343	155.2	190.0	170.0	190.0	190.0	185.0	19.2%	more
490	221.7	280.0	250.0	260.0	250.0	260.0	17.3%	more
700	316.8	370.0	370.0	360.0	350.0	362.5	14.4%	more
1000	452.5	510.0	530.0	500.0	490.0	507.5	12.2%	more

TABLE 3-4. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR PHYSA PHYSA - TEST #2

#### Notes:

(a) Test initiation date was April 28, 1999; Test log number 4997.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(e) Thirty percent difference allowed by IDEM.

#### TABLE 3-5. ACUTE TOXICITY RESULTS FIR LUMBRICULUS VARIEGATUS (AQUATIC WORM) - TEST #1

TEST DATE:	02/23/99
TEST LOG NUMBER:	4363
SAMPLE IDENTIFICATION:	Sodium Fluoride Pure Chemical Test
PROJECT NAME:	USS - Tier II development

PARAMETER	EPA		NOM	INAL SODIUN	I FLUORIDE COI	NCENTRATIO	NS	
	MOD HARD CONTROL	59 (mg/L)	84 (mg/L)	120 (mg/L)	172 (mg/L)	245 (mg/L)	350 (mg/L)	500 (mg/L)
Number of Individuals								
(Test Chamber ID)								
A	10	10	10	10	10	10	10	10
В	10	10	10	10	10	10	10	10
Individuals Surviving at 96 Hours								
A	10	10	10	10	8	1	0	(
В	10	10	10	8	8	0	0	(
Percent Survival	100	100	100	90	80	5	0	C
Chemical/Physical Parameters Temperature (*C)	19.2 - 21.7	19.2 - 21.7	19.2 - 21.7	19.2 - 21.7	19.2 - 21.7	19.2 - 21.7	19.2 - 21.7	19.2 - 21.
Dissolved Oxygen (mg/L)	8.0 - 9.0	8.0 - 9.0	7.9 - 9.0	8.0 - 9.0	7.9 - 9.0	7.6 - 9.0	7.6 - 9.0	8.0 - 9
pH (s.u.)	7.26 - 7.86	7.05 - 7.43	7.05 - 7.48	7.06 - 7.45	7.09 - 7.47	7.05 - 7.51	7.16 - 7.56	7.38 - 7.7
Conductivity (umhos/cm)	321 - 340	439 - 461	495 - 523	577 - 603	669 - 708	800 - 836	1015 - 1052	1289 - 134
NOMINAL FLUORIDE (mg/L)	0.0	26.7	38.0	54.3	77.8	110.9	158.4	226.
Measured Fluoride Concentration (n	100							
Initial Fluoride Concentration	<0.10	27	42	58	79	110	180	25
48-hr Aged Fluoride Concentration	<0.10	28	40	61	87	150	170	
48-hr New Fluoride Concentration	<0.10	32	43	52	78	140	340	
96-hr Aged Fluoride Concentration	<0.10	29	43	69	79	120	160	-
Statistical Analysis: Trimmed Spearman-Karber	NOMINAL FLUORIDE	Lower	Иррег		MEASURED	Lower	Upper	
	LC50 (mg/L)	Limit	Limit	1000	LC50 (mg/L)	Limit	Limit	
24 Hour	>226	NC	NC	24 Hour	NA	NA	NA	
48 Hour	178.4	165.3	192.5	48 Hour	NA	NA	NA	
72 Hour	108.9	99.3	119.6	72 Hour	NA	NA	NA	
96 Hour	84.9	77.9	92.7	96 Hour	93.5	85.5	102.3	

NR - Not Recorded NA - Not Applicable

NC - Not Calculable

TABLE 3-6. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR LUMBRICULUS VARIEGATUS (AQUATIC WORM) - TEST #1

TARGET	TARGET	MEAS	SAMPLING URED FLUORIDE		AVERAGE	AVERAGE		
NOMINAL NaF Conc. (mg/L) <sup>(a)</sup>	NOMINAL F Conc. (mg/L)	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	New (T=48) F Conc. (mg/L)	Aged (T=96) F Conc. (mg/L)	FLUORIDE CONC. <sup>(c)</sup> (mg/L)	PERCENT OFF NOMINAL (%) <sup>(e)</sup>	STATUS OF FLUORIDE EXPOSURE <sup>(d</sup>
Control	0.1	0.1	0.1	0.1	0.1	0.1	0.0%	equal
59	26.7	27.0	28.0	32.0	29.0	29.0	8.6%	more
84	38.0	42.0	40.0	43.0	43.0	42.0	10.5%	more
120	54.3	58.0	61.0	52.0	69.0	60.0	10.5%	more
172	77.8	79.0	87.0	78.0	79.0	80.8	3.8%	more
245	110.9	110.0	150.0	140.0	120.0	130.0	17.3%	more
350	158.4	180.0	170.0	340.0	160.0	170.0	7.3%	more
500	226.3	250.0	270.0		-	260.0	14.9%	more

#### Notes:

(a) Test initiation date was February 23, 1999; Test log number 4363.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval; Averages used to calculate the LC50 value.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(e) Thirty percent difference allowed by IDEM.

TEST LOG NUMBER: SAMPLE IDENTIFICATION: PROJECT NAME:	4962 Sodium Fluride F USS - Tier II dev		Test		TEST DATE:	31-Mar-99		
PARAMETER	EPA		NOM	INAL SODIUN	M FLUORIDE CO	NCENTRATIO	NS	
	MOD HARD CONTROL	59 (mg/L)	84 (mg/L)	120 (mg/L)	172 (mg/L)	245 (mg/L)	350 (mg/L)	500 (mg/L)
Number of Individuals				_			_	
(Test Chamber ID)								
A	10	10	10	10	10	10	- 10	10
В	10	10	10	10	10	10	10	10
C	10	10	10	10	10	10	10	10
Individuals Surviving at 96 Hours								
A	10	10 [	10	9	10	1]	0	0
B	10	10	10	10	10	0	0	0
c	10	10	10	10	8	1	0	0
Percent Survival	100	100	100	97	93	7	0	0
5.51.7 (0.1 × 1.1	1		153.1				5.1	
Chemical/Physical Parameters								
Temperature (°C)	19.0 - 20.8	19.0 - 20.8	19.0 - 20.8	19.0 - 20.8	19.0 - 20.8	19.0 - 20.8	19.0 - 20.8	19.0 - 20.8
Dissolved Oxygen (mg/L)	8.8 - 9.4	8.8 - 9.4	8.8 - 9.4	8.8 - 9.4	8.8 - 9.3	8.1 - 9.3	7.4 - 9.2	8.0 - 9.1
pH (s.u.)	7.11 - 7.75	6.87 - 7.71	6.97 - 7.72	6.98 - 7.71	7.09 - 7.74	7.10 - 7.72	7.17 - 7.76	7.32 - 7.89
Conductivity (µmhos/cm)	317 - 326	447 - 455	504 - 510	580 - 591	674 - 693	789 - 815	1023 - 1053	1331 - 1358
NOMINAL FLUORIDE (mg/L)	0.0	26.7	38.0	54.3	77.8	110.9	158.4	226.3
Measured Fluoride Concentration	Contraction of the second s							
Initial Fluoride Concentration	<0.10	31	43	62	77	115	170	250
48-hr Aged Fluoride Concentration	1.10	38	58	80	100	160	200	350
48-hr New Fluoride Concentration	<0.10	43	57	76	100	140	200	300
96-hr Aged Fluoride Concentration	<0.10	42	60	80	110	120	200	350
Statistical Analysis:	NOMINAL				MEASURED			
Trimmed Spearman-Karber	FLUORIDE	Lower	Upper		FLUORIDE	Lower	Upper	
- search - by the branching of states and	LC50 (mg/L)	Limit	Limit		LC50 (mg/L)	Limit	Limit	
24 Hour		NC	NC	24 Hour	NA	NA	NA	
48 Hour	>226.3	NC	NC	48 Hour	NA	NA	NA	
72 Hou	126.4	117.6	135.9	72 Hour	NA	NA	NA	

NR - Not Recorded

NA - Not Applicable

NC - Not Calculable

TABLE 3-8. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR LUMBRICULUS VARIEGATUS (AQUATIC WORM) - TEST #2

TARGET	TARGET	MEASU	SAMPLING RED FLUORIDE		AVERAGE MEASURED	AVERAGE		
NOMINAL NaF Conc. (mg/L) <sup>(a)</sup>	NOMINAL F Conc. (mg/L)	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	New (T=48) F Conc. (mg/L)	Aged (T=96) F Conc. (mg/L)	FLUORIDE CONC. <sup>(c)</sup> (mg/L)	PERCENT OFF NOMINAL (%) <sup>(e)</sup>	STATUS OF FLUORIDE EXPOSURE <sup>(d</sup>
Control	0.1	0.1	1.1	0.1	0.1	0.1	0.0%	more
59	26.7	31.0	38.0	43.0	42.0	38.5	44.2%	more
84	38.0	43.0	58.0	57.0	60.0	54.5	43.4%	more
120	54.3	62.0	80.0	76.0	80.0	74.5	37.2%	more
172	77.8	77.0	100.0	100.0	110.0	96.8	24.3%	more
245	110.9	115.0	160.0	140.0	120.0	133.8	20.6%	more
350	158.4	170.0	200.0	200.0	200.0	190.0	20.0%	more
500	226.3	250.0	350.0	300.0	350.0	300.0	32.6%	more

Notes:

(a) Test initiation date was March 31, 1999; Test log number 4962.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval; Averages used to calculate the LC50 value.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(e) Thirty percent difference allowed by IDEM.

#### TABLE 3-9, ACUTE TOXICITY RESULTS FOR LUMBRICULUS VARIEGATUS (AQUATIC WORM) - TEST #3

TEST LOG NUMBER: SAMPLE IDENTIFICATION: PROJECT NAME: 4989 Sodium Fluoride Pure Chemical Test USS - Tier II development TEST DATE: 19-Apr-99

PARAMETER	1.5.5		NOM	NAL SODIUN	FLUORIDE CO	NCENTRATIO	NS	
	EPA MOD HARD CONTROL	60 (mg/L)	100 (mg/L)	140 (mg/L)	180 (mg/L)	220 (mg/L)	260 (mg/L)	300 (mg/L)
Number of Individuals			199					
(Test Chamber ID)								
A	10	10	10	10	10	10	10	10
В	10	10	10	10	10	10	10	10
C	10	10	10	10	10	10	10	10
Individuals Surviving at 96 Hours								
A	10	10	10	10	10	9	5	4
В	10	10	10	10	10	8	9	5
C	10	10	10	10	10	8	7	9
Percent Survival	100	100	100	100	100	83	70	60
Chemical/Physical Parameters Temperature (*C)	20.2 - 21.0	20.2 - 21.0	20.2 - 21.0	20.2 - 21.0	20.2 - 21.0	20.2 - 21.0	20.2 - 21.0	20.2 - 21.0
Dissolved Oxygen (mg/L)	8.0 - 9.0	8.0 - 9.0	8.0 - 9.0	8.0 - 9.0	8.1 - 9.0	8.0 - 9.0	7.4 - 9.0	7.0 - 9.0
pH (s.u.)	7.55-7.70	7.28-7.73	7,35-7.73	7.22-7.74	7.28-7.61	7.34-7.60	7.37-7.60	7.41-7.75
Conductivity (µmhos/cm)	308-323	437-458	517-541	573-624	665-711	745-797	831-868	919-955
NOMINAL FLUORIDE (mg/L)	0.0	27.2	45.3	63.4	81.5	99.6	117.7	135.8
Measured Sodium Fluoride (mg/L)								
Initial Fluoride Concentration	0.12	36	52	76	95	130	140	150
48-hr Aged Fluoride Concentration	<0.10	35	62	77	99	130	150	170
48-hr New Fluoride Concentration	<0.10	39	59	77	96	130	190	190
96-hr Aged Fluoride Concentration	<0.10	44	53	82	93	130	150	170
Statistical Analysis: Trimmed Spearman-Karber	NOMINAL FLUORIDE LC50 (mg/L)	Lower Limit	Upper Limit		MEASURED FLUORIDE LC50 (mg/L)	Lower Limit	Upper Limit	
24 Hour	>135.8	NC	NC	24 Hour	NA	NA	NA	
48 Hour	>135.8	NC	NC	48 Hour	NA	NA	NA	

NR - Not Recorded

72 Hour

96 Hour

>135.8

>135.8

NC

NC

NC

NC

72 Hour

96 Hour

NA

>160.0

NA

NC

NA

NC

NA - Not Applicable

NC - Not Calculable

TABLE 3-10. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR LUMBRICULUS VARIEGATUS (AQUATIC WORM) - TEST #3

	TARGET	MEASU	SAMPLING RED FLUORIDE		ONS (b)	AVERAGE MEASURED	AVERAGE	
NOMINAL NaF Conc. (mg/L) <sup>(a)</sup>	NOMINAL F Conc. (mg/L)	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	New (T=48) F Conc. (mg/L)	Aged (T=96) F Conc. (mg/L)	FLUORIDE CONC. <sup>(c)</sup> (mg/L)	PERCENT OFF NOMINAL (%) <sup>(%)</sup>	STATUS OF FLUORIDE EXPOSURE <sup>(d</sup>
Control	0.1	0.12	0.1	0.1	0.1	0.1	6.7%	more
60	27.2	36.0	35.0	39.0	44.0	38.5	41.8%	more
100	45.3	52.0	62.0	59.0	53.0	56.5	24.9%	more
140	63.4	76.0	77.0	77.0	82.0	78.0	23.1%	more
180	81.5	95.0	99.0	96.0	93.0	95.8	17.6%	more
220	99.6	130.0	130.0	130.0	130.0	130.0	30.6%	more
260	117.7	140.0	150.0	190.0	150.0	146.7	24.7%	more
300	135.8	150.0	170.0	190.0	170.0	160.0	17.9%	more

#### Notes:

(a) Test initiation date was April 19, 1999; Test log number 4989.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval; Averages used to calculate the LC50 value.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(e) Thirty percent difference allowed by IDEM.

#### TABLE 3-11. ACUTE TOXICITY RESULTS FOR CHIRONOMUS TENTANS - TEST #1

TEST INITIATION DATE: TEST LOG NUMBER: SAMPLE IDENTIFICATION: PROJECT NAME: 22-May-99 5028 Sodium Fluoride (NaF) Pure Chemical Test USS - Tier II Development

PARAMETER	14 . Ca 11		NOMIN	AL SODIUN	FLUORIDE CO	NCENTRAT	IONS	
	EPA MOD HARD CONTROL	59 (mg/L)	84 (mg/L)	120 (mg/L)	172 (mg/L)	245 (mg/L)	360 (mg/L)	600 (mg/L)
Number of Individuals		-						
(Test Chamber ID)								
A	5	5	5	5	5	5	5	1
В	5	5	5	5	5	5	5	1
C	5	5	5	5	5	5	5	1
D	5	5	5	5	5	5	5	
Individuals Surviving at 48 Hours								
A	5	5	5	3	4	3	0	1
В	5	5	5	5	4	2	0	1
C	5	4	5	5	2	2	0	1
D	5	5	5	5	2	4	1	1
Percent Survival	100	95	100	90	60	55	5	- 4
Chemical/Physical Parameters								
Temperature (°C)	24.4-25.8	24.4-25.8	24.4-25.8	24.4-25.8	24.4-25.8	24.4-25.8	24.4-25.8	24.4-25
Dissolved Oxygen (mg/L)	7.2-8.3	7.2-8.3	7.2-8.3	7.2-8.3	7.2-8.3	7.2-8.3	7.2-8.3	7.2-8
pH (s.u.)	8.01-8.20	8.04-8.22	8.03-8.20	8.10-8.23	8.02-8.30	8.04-8.23	8.02-8.23	8.07-8.3
Conductivity (umhos/cm)	328-351	459-477	511-529	588-610	692-712	818-854	1034-1072	1313-136
NOMINAL FLUORIDE (mg/L)	0.0	26.7	38.0	54.3	77.8	110.9	158.4	226
Measured Sodium Fluoride (mg/L)								
Initial Fluoride Concentration	0.10	20	35	56	74	120	170	22
48-hr Aged Fluoride Concentration	0.19	30	34	49	72	93	149	22
48-hr New Fluoride Concentration	NA	NA	NA	NA	NA	NA	NA	N
96-hr Aged Fluoride Concentration	NA	NA	NA	NA	NA	NA	NA	N
Statistical Analysis: Trimmed Spearman-Karber	NOMINAL FLUORIDE LC50 (mg/L)	Lower Limit	Upper Limit		MEASURED FLUORIDE LC50 (mg/L)	Lower Limit	Upper Limit	
24 Hour	>226.3	NC	NC	24 Hour	NA	NA	NA	
48 Hour	96.1	84.2	109.7	48 Hour	93.1	81.1	106.9	
72 Hour	NA	NA	NA	72 Hour	NA	NA	NA	
96 Hour	NA	NA	NA	96 Hour	NA	NA	NA	
NR - Not Recorded								
NA - Not Applicable								
NC - Not Calculable								

Notes:

(a) Based on averaged measured values.

TABLE 3-12. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR CHIRONOMID - TEST #1

	TARGET	SAMPLING INT MEASURED FLUORIDE CC	and the second	AVERAGE	AVERAGE	
NOMINAL NaF Conc. (mg/L) <sup>(a)</sup>	NOMINAL F Conc. (mg/L)	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	FLUORIDE CONC. <sup>(c)</sup> (mg/L)	PERCENT OFF NOMINAL (%) <sup>(e)</sup>	STATUS OF FLUORIDE EXPOSURE <sup>(d</sup>
Control	0.1	0.1	0.19	0.1	0.0%	equal
59	26.7	20.0	30.0	25.0	-6.4%	less
84	38.0	35.0	34.0	34.5	-9.2%	less
120	54.3	56.0	49.0	52.5	-3.3%	less
172	77.8	74.0	72.0	73.0	-6.2%	less
245	110.9	120.0	93.0	106.5	-3.9%	less
350	158.4	170.0	149.0	159.5	0.7%	more
500	226.3	220.0	220.0	220.0	-2.8%	less

Notes:

(a) Test initiation date was May 22, 1999; Test log number 5028.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval; Averages used to calculate the LC50 value.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(e) Thirty percent difference allowed by IDEM.

#### TABLE 3-13. ACUTE TOXICITY RESULTS FOR CHIRONOMUS TENTANS - TEST #2

TEST INITIATION DATE:	26-Aug-99
TEST LOG NUMBER:	5130
SAMPLE IDENTIFICATION:	Sodium Fl
PROJECT NAME:	USS - Tier

25-Aug-99 5130 Sodium Fluoride (NaF) Pure Chemical Test USS - Tier II Development

MOD HARD CONTROL         59         84         120         172         245         360         60           Number of Individuals (Test Chamber ID)	PARAMETER	1.1		NOM	INAL SODIU	M FLUORIDE CO	NCENTRATI	ONS	
Itest Chamber ID)         A         5         4         2         1         1         0		Contraction of the contraction of the	100	- 1 - C		16.8.100	17 C	1.4.4	500 (mg/L)
A         5         1         1         0         0         5         5         5         5         4         2         1         1         0         0         5         5         5         4         2         1	Number of Individuals				-	_			
B         S	(Test Chamber ID)	· · · · · · · · · · · · · · · · · · ·							
C         5         4         2         1         1         0         5         5         5         5         5         4         2         1         1         0         5         5         5         4         2         1         1         0         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1	A	5	5	5	5	5	5	5	÷
D         5         6         25.2-25.6 <td>В</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td>	В	5	5	5	5	5	5	5	5
Individuals Surviving at 48 Hours           A         4         5         5         4         4         0           B         4         5         3         5         4         2         0           C         5         5         5         4         2         0           C         5         5         5         4         2         1           D         5         5         5         4         2         3           Percent Survival         90         100         90         95         85         50         20           Chemical/Physical Parameters         2         25.2-25.6 <td>C</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td>	C	5	5	5	5	5	5	5	5
A         4         5         5         4         4         0           B         4         5         3         5         4         2         0           C         5         5         5         5         4         2         1           D         5         5         5         4         5         2         3           Percent Survival         90         100         90         95         85         50         20           Chemical/Physical Parameters         Temperature (*C)         252-25.6         25.2-	D	5	5	5	5	5	5	5	5
B         4         5         3         5         4         2         0           C         5         5         5         5         4         2         1           D         5         5         5         5         4         2         1           D         5         5         5         4         5         2         3           Percent Survival         90         100         90         95         85         50         20           Chemical/Physical Parameters         Temperature (*C)         25.2-25.6 <td>Individuals Surviving at 48 Hours</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	Individuals Surviving at 48 Hours								
C         5         5         5         4         2         1           D         5         5         5         5         4         5         2         3           Percent Survival         90         100         90         95         85         50         20           Chemical/Physical Parameters         2         2         3         2         3         2         3           Chemical/Physical Parameters         2         2         2         2         2         3         3           Chemical/Physical Parameters         2         3	A	4	5	5	5	4	4	0	0
D         5         5         4         5         2         3           Percent Survival         90         100         90         95         85         50         20           Chemical/Physical Parameters         2         2         3         20         20         20           Chemical/Physical Parameters         2         25.2-25.6 <th< td=""><td>В</td><td>4</td><td>5</td><td>3</td><td>5</td><td>4</td><td>2</td><td>0</td><td>0</td></th<>	В	4	5	3	5	4	2	0	0
Percent Survival         90         100         90         95         85         50         20           Chemical/Physical Parameters           Temperature (*C)         25.2-25.6         25	C	5	5	5	5	4	2	1	0
Chemical/Physical Parameters           Temperature (*C)         25.2-25.6	D	5	5	5	4	5	2	З	0
Temperature (*C)         25.2-25.6         26.2-25.6         26.2-25.6	Percent Survival	90	100	90	95	85	50	20	0
Dissolved Oxygen (mg/L)         8.2 - 8.4         7.6 - 8.4         7.6 - 8.4         7.6 - 8.4         7.5 - 8.4         7.5 - 8.4         7.5 - 8.4         7.6 - 8.4         7.7 - 8.4         7.5 - 8.4         7.6 - 8.4         7.7 - 8.4         7.5 - 8.4         7.6 - 8.4         7.7 - 8.4         7.5 - 8.4         7.6 - 8.4         7.5 - 8.4         7.5 - 8.4         7.6 - 8.4         7.5 - 8.4         7.5 - 8.4         7.6 - 8.4         7.7 - 7.78         7.3 - 7.77	Chemical/Physical Parameters								
pH (s.u.)         7.57 - 7.76         7.28 - 7.81         7.40 - 7.79         7.28 - 7.22         7.40 - 7.78         7.37 - 7.77         7.34 - 7.77         7.44 - 7.77         7.44           Conductivity (µmhos/cm)         321 - 331         441 - 454         495 - 514         581 - 615         685 - 705         822 - 866         1045 - 1118         1352           NOMINAL FLUORIDE (mg/L)         0.0         26.7         38.0         54.3         77.8         110.9         158.4           Measured Sodium Fluoride (mg/L)         Initial Fluoride Concentration         0.10         27         38         55         77         110         150           48-hr Aged Fluoride Concentration         0.10         27         38         55         77         110         150           48-hr New Fluoride Concentration         0.10         31         49         61         83         130         170           48-hr New Fluoride Concentration         NA         NA         NA         NA         NA         NA           96-hr Aged Fluoride Concentration         NA         NA         NA         NA         NA         NA           Statistical Analysis:         FLUORIDE         Lower         Upper         LC50 (mg/L)         Limit         Limit<	Temperature (*C)	25.2-25.6	25.2-25.6	25.2-25.6	25.2-25.6	25.2-25.6	25.2-25.6	25.2-25.6	25.2-25.6
Conductivity (µmhos/cm)         321 - 331         441 - 454         495 - 514         581 - 615         685 - 705         822 - 866         1045 - 1118         1352           NOMINAL FLUORIDE (mg/L)         0.0         26.7         38.0         54.3         77.8         110.9         158.4           Measured Sodium Fluoride (mg/L)	Dissolved Oxygen (mg/L)	8.2 - 8.4	7,6 - 8.4	7.6 - 8.4	7.6 - 8.4	7.5 - 8.4	7.5 - 8.4	7.6 - 8.4	7.6 - 8.4
NOMINAL FLUORIDE (mg/L)         0.0         26.7         38.0         54.3         77.8         110.9         158.4           Measured Sodium Fluoride (mg/L)         Initial Fluoride Concentration         0.10         27         38         55         77         110         150           Measured Sodium Fluoride Concentration         0.10         27         38         55         77         110         150           Measured Sodium Fluoride Concentration         0.10         31         49         61         83         130         170           Measured Sodium Fluoride Concentration         NA	pH (s.u.)	7.57 - 7.76	7.28 - 7.81	7.40 - 7.79	7.28 - 7.72	7.40 - 7.78	7.37 - 7.77	7.34 - 7.77	7.45 - 7.8
Measured Sodium Fluoride (mg/L)           Initial Fluoride Concentration         0.10         27         38         55         77         110         150           48-hr Aged Fluoride Concentration         0.10         31         49         61         83         130         170           48-hr New Fluoride Concentration         NA         NA         NA         NA         NA         NA         NA           96-hr Aged Fluoride Concentration         NA         NA         NA         NA         NA         NA         NA           96-hr Aged Fluoride Concentration         NA         NA         NA         NA         NA         NA           Statistical Analysis:         NOMINAL         MEASURED         FLUORIDE         Lower         Upper         FLUORIDE         Lower         Upper           LC50 (mg/L)         Limit         Limit         Limit         Limit         Limit         Limit         110.9         98.2         125.1           72 Hour         NA         NA         NA         NA         NA         NA         NA	Conductivity (µmhos/cm)	321 - 331	441 - 454	495 - 514	581 - 615	685 - 705	822 - 866	1045 - 1118	1352 - 1398
Initial Fluoride Concentration         0.10         27         38         55         77         110         150           48-hr Aged Fluoride Concentration         0.10         31         49         61         83         130         170           48-hr New Fluoride Concentration         NA         NA         NA         NA         NA         NA         NA           96-hr Aged Fluoride Concentration         NA         NA         NA         NA         NA         NA         NA           96-hr Aged Fluoride Concentration         NA         NA         NA         NA         NA         NA         NA           Statistical Analysis:         NOMINAL         FLUORIDE         Lower         Upper         FLUORIDE         Lower         Upper           LC50 (mg/L)         Limit         Limit         Limit         Limit         Limit         Limit         Limit         Limit         110.9         98.2         125.1           72 Hour         NA         NA         NA         NA         NA         NA         NA	NOMINAL FLUORIDE (mg/L)	0.0	26.7	38.0	54,3	77.8	110.9	158.4	226.3
48-hr Aged Fluoride Concentration         0.10         31         49         61         83         130         170           48-hr New Fluoride Concentration         NA         Init         LC50 (mg/L)         Limit         Limit         Limit         Limit         110.9         98.2         125.1         110.9         98.2         125.1         NA         NA         NA         NA         NA         NA         NA         NA         NA	Measured Sodium Fluoride (mg/L)								
48-hr New Fluoride Concentration     NA     NA     NA     NA     NA       96-hr Aged Fluoride Concentration     NA     NA     NA     NA     NA     NA       96-hr Aged Fluoride Concentration     NA     NA     NA     NA     NA     NA     NA       Statistical Analysis:     NOMINAL     MEASURED       Trimmed Spearman-Karber     FLUORIDE     Lower     Upper     FLUORIDE     Lower     Upper       LC50 (mg/L)     Llmit     Llmit     LC50 (mg/L)     Llmit     Limit       24 Hour     >226.3     NC     NC     NA     NA       48 Hour     107.1     93.9     122.1     110.9     98.2     125.1       72 Hour     NA     NA     NA     NA     NA     NA	Initial Fluoride Concentration	0.10	27	38	55	77	110	150	200
48-hr New Fluoride Concentration     NA     NA     NA     NA     NA       96-hr Aged Fluoride Concentration     NA     NA     NA     NA     NA     NA       96-hr Aged Fluoride Concentration     NA     NA     NA     NA     NA     NA     NA       Statistical Analysis:     NOMINAL     MEASURED       Trimmed Spearman-Karber     FLUORIDE     Lower     Upper     FLUORIDE     Lower     Upper       LC50 (mg/L)     Llmit     Llmit     LC50 (mg/L)     Llmit     Limit       24 Hour     >226.3     NC     NC     NA     NA       48 Hour     107.1     93.9     122.1     110.9     98.2     125.1       72 Hour     NA     NA     NA     NA     NA     NA	48-hr Aged Fluoride Concentration	0.10	31	49	61	83	130	170	210
Statistical Analysis:     NOMINAL     MEASURED       Trimmed Spearman-Karber     FLUORIDE     Lower     Upper       LC50 (mg/L)     Limit     Limit     LC50 (mg/L)       24 Hour     >226.3     NC     NC       48 Hour     107.1     93.9     122.1       72 Hour     NA     NA     NA	48-hr New Fluoride Concentration	NA	NA	NA	NA	NA	NA	NA	NA
Trimmed Spearman-KarberFLUORIDE <bh>Lower LC50 (mg/L)Upper LImitFLUORIDE<bh>Lower LC50 (mg/L)Upper LImit24 Hour&gt;226.3NCNCNANANA24 Hour&gt;226.3NCNCNANANA48 Hour107.193.9122.1110.998.2125.172 HourNANANANANANA</bh></bh>	96-hr Aged Fluoride Concentration	NA	NA	NA	NA	NA	NA	NA	NA
Trimmed Spearman-KarberFLUORIDE <bh>Lower LC50 (mg/L)Upper LImitFLUORIDE<bh>Lower LC50 (mg/L)Upper LImit24 Hour&gt;226.3NCNCNANANA24 Hour&gt;226.3NCNCNANANA48 Hour107.193.9122.1110.998.2125.172 HourNANANANANANA</bh></bh>			-						
LC50 (mg/L)         Llmit         Limit         LC50 (mg/L)         Llmit         LImit           24 Hour         >226.3         NC         NC         NA         NA         NA           48 Hour         107.1         93.9         122.1         110.9         98.2         125.1           72 Hour         NA         NA         NA         NA         NA			a	10.00			12355.4	1000	
24 Hour         >226.3         NC         NC           48 Hour         107.1         93.9         122.1         110.9         98.2         125.1           72 Hour         NA         NA         NA         NA         NA	Trimmed Spearman-Karber		(B41)44					and the second second	
48 Hour         107.1         93.9         122.1         110.9         98.2         125.1           72 Hour         NA         NA         NA         NA         NA									
72 Hour NA NA NA NA NA		1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1							
	and the second se	1-131	****	100 M 100 M			10.001		
96 Hour NA NA NA NA NA NA									
	96 Hour	NA	NA	NA	1	NA	NA	NA	

NR - Not Recorded

NA - Not Applicable

NC - Not Calculable

TABLE 3-14. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR CHIRONOMID - TEST #2

	TARGET	SAMPLING INT		AVERAGE	AVERAGE	
NOMINAL NaF Conc. (mg/L) (a)	NOMINAL F Conc. (mg/L)	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	FLUORIDE CONC. (c) (mg/L)	PERCENT OFF NOMINAL (%)	STATUS OF FLUORIDE EXPOSURE (d
Control	0.1	0.1	0.1	0.1	0.0%	equal
59	26.7	27.0	31.0	29.0	8.6%	more
84	38.0	38.0	49.0	43.5	14.4%	more
120	54.3	55.0	61.0	58.0	6.8%	more
172	77.8	77.0	83.0	80.0	2.8%	more
245	110.9	110.0	130.0	120.0	8.2%	more
350	158.4	150.0	170.0	160.0	1.0%	more
500	226.3	200.0	210.0	205.0	-9.4%	less

Notes:

(a) Test initiation date was August 26, 1999; Test log number 5130.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval; Averages used to calculate the LC50 value.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

## TABLE 3-15. ACUTE TOXICITY RESULTS FOR PIMEPHALES PROMELAS (FATHEAD MINNOWS) - TEST #1

TEST DATE:	June 29, 1999
TEST LOG NUMBER:	5073
SAMPLE IDENTIFICATION:	Sodium Fluoride (NaF) Pure Chemical Test
PROJECT NAME:	USS - Tier II Development

PARAMETER	EPA	1.17	NOMI	NAL SODIUM	I FLUORIDE CO	ONCENTRA	TIONS
	MOD HARD CONTROL	50 (mg/L)	100 (mg/L)	200 (mg/L)	300 (mg/L)	400 (mg/L)	500 (mg/L)
Number of Individuals							
(Test Chamber ID)		_				_	
A	10	10	10	10	10	10	10
B	10	10	10	10	10	10	10
Individuals Surviving at 96 Hours							
A	9	10	10	8	8	0	0
В	10	10	10	9	5	0	0
Percent Survival	95	100	100	85	65	0	0
Chemical/Physical Parameters							
Temperature (°C)	24.2 - 25.2	24.2 -25.2	24.2 -25.2	24.2 -25.2	24.2 -25.2	24.2 -25.2	24.1 -25.2
Dissolved Oxygen (mg/L)	7.6 - 8.2	7.4 - 8.2	7.4 - 8.2	7.2 - 8.2	7.4 - 8.2	7.5 - 8.0	200 - 20 - 20 - 20 - 20 - 20 - 20 - 20
pH (s.u.)	7.44 - 8.08	7.55 - 8.15	7.61 - 8.18	7.62 - 8.20	7.72 - 8.20	7.78 - 7.92	A NOR CONTRACTOR
Conductivity (µmhos/cm)	255 - 292	365 - 397	474 - 521	631 - 699		1013 - 1018	
NOMINAL FLUORIDE (mg/L)	0.0	22.6	45.3	90.5	135.8	181.0	226.3
Measured Sodium Fluoride (mg/L)							
Initial Fluoride Concentration	1.10	23	42	88	98	150	200
48-hr Aged Fluoride Concentration	1,10	23	45	80	110	100	-
48-hr New Fluoride Concentration	1,10	26	47	64	120	-	
96-hr Aged Fluoride Concentration	1.30	28	51	88	140		
Statistical Analysis:	NOMINAL				MEASURED		
Trimmed Spearman-Karber	FLUORIDE	Lower	Upper		FLUORIDE	Lower	Upper
	LC50 (mg/L)	Limit	Limit		LC50 (mg/L)	Limit	Limit
24 Hour	146.3	137.5	155.6	24 Hour	NA	NA	NA
48 Hour	143.7	134.4	153.7	48 Hour	NA	NA	NA
72 Hour	139.9	128.3	152.4	72 Hour	NA	NA	NA
72 HOUL							

NR - Not Recorded

NA - Not Applicable

NC - Not Calculable

#### Notes:

Used dechlorinated tap water as control and dilution water.

#### TABLE 3-16. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR PIMEPHALES PROMOLAS (FATHEAD MINNOW) ACUTE TEST #1

	TARGET	MEAS	SAMPLING I JRED FLUORIDE (		S (b)	AVERAGE	AVERAGE	
NOMINAL NaF Conc. (mg/L) (a)	NOMINAL F Conc. (mg/L)	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	New (T=48) F Conc. (mg/L)	Aged (T=96) F Conc. (mg/L)	FLUORIDE CONC. (c) (mg/L)	PERCENT OFF NOMINAL (%)	STATUS OF FLUORIDE EXPOSURE (d
Control	1.0	1,1	1.1	1.1	1.3	1.2	15.0%	more
50	22.6	23.0	23.0	26.0	28.0	25.0	10.5%	more
100	45.3	42.0	45.0	47.0	51.0	46.3	2.2%	more
200	90.5	88.0	80.0	64.0	88.0	80.0	-11.6%	less
300	135.8	113.0	110.1	120.0	140.0	120.8	-11.0%	less
400	181.0	150.0	-	-	-	150.0	-17.1%	less
500	226.3	200.0	-	-	- 1	200.0	-11.6%	less
3000	1357.5	1500.0	-	1500.0	-	1500.0	10.5%	more

#### Notes:

(a) Test initiation date was June 29, 1999; Test log number 5073.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(-) Not analyzed.

Dechlorinated tap water used as control and dilution water.

#### TABLE 3-17. ACUTE TOXICITY RESULTS FOR PIMEPHALES PROMELAS (FATHEAD MINNOWS) (ACR)

TEST DATE: TEST LOG NUMBER: SAMPLE IDENTIFICATION: PROJECT NAME: August 10, 1999 5117 - ACR test Sodium Fluoride (NaF) Pure Chemical Test USS - Tier II Development

PARAMETER			NOMI	NAL SODIUM	FLUORIDE CO	NCENTRA	TIONS	
	EPA MOD HARD CONTROL	73 (mg/L)	103 (mg/L)	148 (mg/L)	211 (mg/L)	301 (mg/L)	430 (mg/L)	615 (mg/L)
Number of Individuals								-
(Test Chamber ID)			_	_				
A	10	10	10	10	10	10	10	10
В	10	10	10	10	10	10	10	10
Individuals Surviving at 96 Hours								
A	10	10	10	10	9	9	8	0
В	10	10	10	10	10	10	8	0
Percent Survival	100	100	100	100	95	95	80	0
Chemical/Physical Parameters					1			
Temperature (°C)	24.0 - 24.9	24.0 - 24.9	24.0 - 24.9	24.0 - 24.9	24.0 - 24.9	24.0 - 24.9	24.0 - 24.9	24.0 - 24.9
Dissolved Oxygen (mg/L)	7.4 - 8.6	7.5 - 8.6	7.3 - 8.6	7.0 - 8.6	7.1 - 8.6	7.0 - 8.6	7.0 - 8.6	7.4 - 8.1
pH (s.u.)	7.43 - 7.86	7.49 - 7.91	7.26 - 7.94	7.38 - 8.01	7.44 - 8.02	7.49 - 8.30	7.54 - 8.13	7.71 - 8.21
Conductivity (µmhos/cm)	228 - 258	383 - 415	449 - 481	541 - 580	675 - 713	865 - 920	1074 - 1174	1380 - 1443
NOMINAL FLUORIDE (mg/L)	0.0	33.0	46.6	67.0	95.5	136.2	194.6	278.3
Measured Sodium Fluoride (mg/L)								
Initial Fluoride Concentration	1.20	44	50	66	93	140	230	300
48-hr Aged Fluoride Concentration	1.30	39	56	69	110	140	200	300
48-hr New Fluoride Concentration	1,30	38	53	67	110	140	230	500
96-hr Aged Fluoride Concentration	1.10	38	29	70	110	150	180	
	100	-						
Statistical Analysis:	NOMINAL				MEASURED			
Trimmed Spearman-Karber	FLUORIDE	Lower	Upper		FLUORIDE	Lower	Upper	
	LC50 (mg/L)	Limit	Limit		LC50 (mg/L)	Limit	Limit	
24 Hour	232.7	226.4	239.2	24 Hour	NA	NA	NA	
48 Hour	224.6	213,8	235.9	48 Hour	NA	NA	NA	
	and a first sector			con vie al al	the second second second	1.7.4		

235.9

226.6

72 Hour

96 Hour

NA

225,1

NA

207.4

NA

244.3

NR - Not Recorded

NA - Not Applicable

NC - Not Calculable

#### Notes:

Used dechlorinated tap water as control and dilution water. Fathead minnows were 14 days old at test initiation.

72 Hour

96 Hour

224.6

209.1

213.8

192.9

#### TABLE 3-18. TARGET VS. AVERAGE MEASURED FLUORIDE VALUES FOR PIMEPHALES PROMELAS (FATHEAD MINNOW) ACUTE TEST ACR

	TARGET	MEASU	SAMPLING RED FLUORIDE		ONS (b)	AVERAGE MEASURED	AVERAGE	
NOMINAL NaF Conc. (mg/L) <sup>(a)</sup>	NOMINAL F Conc. (mg/L)	Initial (T=0) F Conc. (mg/L)	Aged (T=48) F Conc. (mg/L)	New (T=48) F Conc. (mg/L)	Aged (T=96) F Conc. (mg/L)	FLUORIDE CONC. <sup>(c)</sup> (mg/L)	PERCENT OFF NOMINAL (%) <sup>(e)</sup>	STATUS OF FLUORIDE EXPOSURE <sup>(d</sup>
Control	1.0	1.2	1.3	1.2	1.1	1.2	20.0%	more
73	33.0	44.0	39.0	38.0	38.0	39.8	20.3%	more
103	46.6	50.0	56.0	53.0	29.0	47.0	0.8%	more
148	67.0	66.0	69.0	67.0	70.0	68.0	1.5%	more
211	95.5	93.0	110.0	110.0	110.0	105.8	10.8%	more
301	136.2	140.0	150.0	140.0	150.0	145.0	6.5%	more
430	194.6	230.0	200.0	230.0	180.0	210.0	7.9%	more
615	278.3	-	300.0		-	300.0	7.8%	more

#### Notes:

(a) Test initiation date was August 10, 1999; Test log number 5117; Used for ACR development.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(e) Thirty percent difference allowed by IDEM.

(-) Not analyzed.

Dechlorinated tap water used as control and dilution water.

#### TABLE 3-19. 28-DAY FLOW-THROUGH CHRONIC TOXICITY RESULTS - PIMEPHALES PROMELAS

TEST DATE: TEST LOG NUMBER: SAMPLE IDENTIFICATION: PROJECT NAME: July 22, 1999 5101 SODIUM FLUORIDE USS - TIER II DEVELOPMENT

	Same I		NOMINAL SODIU	M FLUORIDE CONC	ENTRATIONS		
PARAMETER	CONTROL WATER	18.75 (mg/L)	37.5 (mg/L)	75 (mg/L)	160 (mg/L)	300 (mg/L)	STOCK 3000 (mg/L)
Number of Individuals							
(Test Chamber ID)							
A	15	15	15	15	15	15	
8	15	15	15	15	15	15	_
C	15	15	15	15	15	15	
D	15	15	15	15	15	15	
Survival on Day 32, %							
A	80	100	100	100	100	13	
B	100	100	100	100	100	13	
0		100	100	100	100	20	
C	100	100	100				
	100	100	100	100	100		
C D Average Average Dry Weight	and the second data was a second data w	the second se				27 18	
C D Average Average Dry Weight per Individual, mg	93	100	100	100	100	27	
C O Average Average Dry Weight	93	100	100	100 100	100 100	27	
C D Average Average Dry Weight per Individual, mg A	93 93 4.503	100 100 3.940	100 100 3.148	100 100 3.660	100 100 3.123	27 18 NA	
C D Average Average Average Individual, mg A B	93 93 4,503 2,971	100 100 3.940 3.115	100 100 3.148 2.860	100 100 3.660 3.889	100 100 3.123 3.351	27 18 NA NA	
C D Average Average Average Average A A B B C D	4.503 2.971 3.185 4.447	100 100 3.940 3.115 4.166	100 100 3.148 2.860 4.149	100 100 3.860 3.889 3.425	100 100 3.123 3.351 3.778	27 18 NA NA	
C D Average Average Average Dry Weight A B C C D Average Dry Weight	4.503 2.971 3.185	100 100 3,940 3,115 4,166 3,861	100 100 3.148 2.880 4.149 3.991	100 100 3.660 3.889 3.425 3.031	100 100 3.123 3.351 3.778 3.673	27 18 NA NA NA	
C D Average Average A A A B C C D Average Dry Weight C Average Dry Weight	93 93 2.971 3.185 4.447 3.777	100 100 3.940 3.115 4.166 3.861 3.771	100 100 3.148 2.860 4.149 3.991 3.537	100 100 3.660 3.889 3.425 3.031 3.501	100 100 3.123 3.351 3.778 3.673 3.481	27 18 NA NA NA NA	
C D Average Average Average Average Average Average C D Average Dry Weight C C D Average Dry Weight C C D D C D D D D D D D D D D D D D	83 93 93 4.503 2.971 3.185 4.447 3.777 24.0 - 26.0	100 100 3,940 3,115 4,166 3,861	100 100 3.148 2.880 4.149 3.991	100 100 3.660 3.889 3.425 3.031	100 100 3.123 3.351 3.778 3.673	27 18 NA NA NA	
C D Average Average Average Average Dry Weight A B C D Average Dry Weight C D Average Dry Weight C D D Average Dry Weight	83 93 93 4.503 2.971 3.185 4.447 3.777 24.0 - 26.0 YES	100 100 3.940 3.115 4.156 3.861 3.771 24.0 - 28.0	100 100 3.148 2.880 4.149 3.991 3.537 24.0 - 28.0	100 100 3.860 3.889 3.425 3.031 3.501 24.0 - 28.0	100 100 3.123 3.351 3.778 3.673 3.481 24.0 - 28.0	27 18 NA NA NA NA NA 24.0 - 26.0 YES	
C D Average Average Average Dry Weight per Individual, mg A B C D Average Dry Weight C bemical/Physical Parameters "emperature (*C) "issolved Oxygen (mg/L) in an Acceptable Range? H (s.u.) in an Acceptable Range?	83 93 93 4.503 2.971 3.185 4.447 3.777 24.0 - 26.0	100 100 3.940 3.115 4.166 3.861 3.771 24.0 - 26.0 YES	100 100 3.148 2.860 4.149 3.991 3.537 24.0 - 26.0 YES	100 100 3.860 3.889 3.425 3.031 3.501 24.0 - 26.0 YES	100 100 3.123 3.351 3.778 3.673 3.481 24.0 - 28.0 YES	27 18 NA NA NA NA 24.0 - 26.0	
C D Average Average Average Dry Weight per Individual, mg A B C D Average Dry Weight C bemical/Physical Parameters issolved Oxygen (mg/L) in an Acceptable Range? H (s.u.) in an Acceptable Range? Conductivity (umhos/cm) - Available from Raw Datasheets.	83 93 93 4.503 2.971 3.185 4.447 3.777 24.0 - 26.0 YES	100 100 3.940 3.115 4.166 3.861 3.771 24.0 - 26.0 YES	100 100 3.148 2.860 4.149 3.991 3.537 24.0 - 26.0 YES	100 100 3.860 3.889 3.425 3.031 3.501 24.0 - 26.0 YES	100 100 3.123 3.351 3.778 3.673 3.481 24.0 - 28.0 YES	27 18 NA NA NA NA NA 24.0 - 26.0 YES	
C D Average Average Average Dry Weight per Individual, mg A B C D Average Dry Weight Chemical/Physical Parameters remperature (*C) Vissolved Oxygen (mg/L) in an Acceptable Range? H (s.u.) in an Acceptable Range? Conductivity (unhos/cm) - Available from Raw Datasheets. Total Hardness (mg/L as CaCO3)	83 93 93 4.503 2.971 3.185 4.447 3.777 24.0 - 26.0 YES YES	100 100 3.940 3.115 4.166 3.861 3.771 24.0 - 26.0 YES	100 100 3.148 2.860 4.149 3.991 3.537 24.0 - 26.0 YES	100 100 3.860 3.889 3.425 3.031 3.501 24.0 - 26.0 YES	100 100 3.123 3.351 3.778 3.673 3.481 24.0 - 28.0 YES	27 18 NA NA NA NA NA NA 24.0 - 26.0 YES YES	
C D Average Average Average Average A Per Individual, mg A B C C D	83 93 93 4,503 2,971 3,185 4,447 3,777 24.0 - 26.0 YES YES 89 - 96	100 100 3.940 3.115 4.166 3.861 3.771 24.0 - 26.0 YES	100 100 3.148 2.860 4.149 3.991 3.537 24.0 - 26.0 YES	100 100 3.860 3.889 3.425 3.031 3.501 24.0 - 26.0 YES	100 100 3.123 3.351 3.778 3.673 3.481 24.0 - 28.0 YES	27 18 NA NA NA NA NA NA NA NA NA NA	

	CONCENT	RATIONS	CONCEN	RATIONS
	Survival (mg/L)	Growth (mg/L)	Survival (mg/L)	Growth (mg/L)
Shapiro-Wilk's (Normality)	FAIL	PASS	FAIL	PASS
Bartlett's (Homogeneity of Variance)	FAIL	PASS	FAIL	PASS
Test Used	STEEL'S	DUNNETT'S	STEEL'S	DUNNETTS
NOEC Value	67.9	67.9	66.6	66.6
LOEC Value	135.8	135.8	134.3	134.3
Mean (geometric)-Chronic Value	96.0	96.0	94.6	94.6
Chronic Toxicity Units (TUc)	NA	NA	NA	NA
IC25 Point Estimation (Biomass)		85.4		84.1

NC - Not Applicable

NC - Not Calculable

	TARGET	м		SAMPLING FLUORIDE			ь)	AVERAGE MEASURED	AVERAGE	
NOMINAL NaF Conc. (mg/L) (a)	NOMINAL F Conc. (mg/L)	Initial F Conc. (mg/L)	Week 1 F Conc. (mg/L)	Week 2 F Conc. (mg/L)	Week 3 F Conc. (mg/L)	Week 4 F Conc. (mg/L)	Test End F Conc. (mg/L)	FLUORIDE CONC. (c) (mg/L)	AVERAGE PERCENT OFF NOMINAL (%)	STATUS OF FLUORIDE EXPOSURE (d
Control	1.0	1.2	1.1	1.1	1.2	1.2	1.1	1.2	15.0%	more
18.75	8.5	7.3	9.2	8.2	9.0	7.9	8.5	8.4	-1.6%	less
37.5	17.0	15.0	18.8	15.0	16.0	16.0	14.0	15.8	-6.9%	less
75	33.9	34.0	43.8	31.0	38.0	39.0	37.0	37.1	9.4%	more
150	67.9	58.0	67.4	69.1	64.0	65.0	63.0	64.4	-5.1%	less
150	67.9	57.0	69.2	72.5	68.0	70.0	63.0	66.6	-1.9%	less
300	135.8	150.0	137.0	134.0	130.0	130.0	125.0	134.3	-1.0%	less
3000	1357.5	1500.0	-	1650.0	1700.0	1350.0		1550.0	14.2%	more
ARGET ANALYTIC	AL VALUE:								<20.0%	

TABLE 3-20. TARGET VS. AVERAGED MEASURED FLUORIDE VALUES FOR FATHEAD MINNOW FLOW-THROUGH TEST

Notes:

(a) Test initiation date was July 22, 1999; Test log number 5101; Used for ACR development.

(b) Bolded values are not included in the test average.

(c) Average of measured fluoride concentrations from each sampling interval.

(d) Indicates the percentage higher (more) or lower (less) than the target fluoride concentration.

(-) Not analyzed.

Dechlorinated tap water served as control and test dilution water.

#### TABLE 3-21. SUMMARY OF 7-DAY CHRONIC TOXICITY RESULTS - PIMEPHALES PROMELAS

TEST DATE:	17-May-99
TEST LOG NUMBER:	5023
SAMPLE IDENTIFICATION:	SODIUM FLUORIDE
PROJECT NAME:	US STEEL - 7 DAY CHRONIC SCREEN TEST

	MOD HARD CONTROL	HARD	1.5	NOMINAL SODI	UM FLUORIDE CO	NCENTRATIONS	100	ANALYTICAL
PARAMETER	(Primary)	(Secondary)	12.5 (mg/L)	25 (mg/L)	50 (mg/L)	100 (mg/L)	200 (mg/L)	100%
Number of Individuals								
(Test Chamber ID)								
A	10		10	10	10	10	9	
8	10		10	10	10	10	10	
C	10		9	10	10	10	9	
Survival, %			_			10 A		
A	100		100	100	100	100	90	
8	100		100	100	100	100	100	
C	100		90	100	100	100	90	
Average	100	and the	97	100	100	100	93	
Average Dry Weight per Individual, mg								
A	0.481		0.445	0.437	0.472	0.465	0.371	C
В	0.439		0.523	0.540	0.450	0.516	0.382	
C	0.451		0.592	0.509	0.569	0.572	0.352	
Average	0.457		0.520	0.495	0.497	0.518	0.368	
Chemical/Physical Parameters								
Temperature (*C)	24.2 - 25.5		24.2 - 25.5	24.2 - 25.5	24.2 - 25.5	24.2 - 25.5	24.2 - 25.5	
Dissolved Oxygen (mg/L) in an Acceptab	le Range?		YES	YES	YES	YES	YES	
oH (s.u.) in an Acceptable Range?	YES		YES	YES	YES	YES	YES	
Conductivity (umhos/cm) Available from F	Raw Datasheets.							
Total Hardness (mg/L as CaCO3)	80 - 100							
Total Alkalinity (mg/L as CaCO3)	45 - 75		1		2			-
Total Residual Chionine (mg/L as Cl2)	NA						1	
NOMINAL FLUORIDE CONC.	0		5.7	11.3	22.6	45.3	90.5	
Statistical Analysis: Shapiro-Wilk's (Normality) Bartiett's (Homogeneity of Variance)	NOMINAL FLUOR Survival (mg/L) FAIL FAIL	IDE CONC. Growth (mg/L) PASS PASS				Endpointa: LC50 VALUES (F) Trimmed Spearman 24-hr LC50	⊢Karber (mg/L) >90.5 >90.5	
Test Used	WILCOXON	DUNNETTS			5	72-hr LC50	>90.5	
NOEC	90.5	45.3			1	96-hr LC50	>90.5	
LOEC	>90.5	>90.5				120-hr LC50	>90.5	
Mean (geometric)-Chronic Value	>90.5	64.0				144-hr LC50	>90.5	
Chronic Toxicity Units (TUc)	NA	NA				168-hr LC50	>90.5	
IC25 Point Estimation (Biomass)		82.8	1					
		_		C - Not Applicat	ole .	NC - Not Calculabl	0	
REFERENCE TOXICANT TEST DATA 1	998-1999				TEST A	CCEPTANCE CRIT	TERIA	
		VaCI			TEST A Control survival o		ERIA	_
Reference Toxicant:		VaCI			Control survival o	f 80% or more		
Reference Toxicant: Number of Tests Completed:		15			Control survival o			
Reference Toxicant: Number of Tests Completed: Central Tendency (g/L):		15 1.4			Control survival o	f 80% or more		
REFERENCE TOXICANT TEST DATA 1 Reference Toxicant: Number of Tests Completed: Central Tendency (g/L): Range of Acceptability (g/L): Date of Last Test:		15 1.4 0.75 to 3.0			Control survival o	f 80% or more		
Reference Toxicant: Number of Tests Completed: Central Tendency (g/L):		15 1.4			Control survival o	f 80% or more		

#### TABLE 3-22. SUMMARY OF MEASURED WATER QUALITY INDICATORS FOR ACUTE TOXICITY TESTS

	SAMPLE	SAMPLE COLLECTION	SOD	UM	ALKALI	NITY	HARD	NESS	TOTA SUSPENDE	Sector Sector	TOT.	Wie and and
TEST SPECIES	DATE	TIME	Control (mg/L)	Highest (mg/L)	Control (mg/L)	Highest (mg/L)	Control (mg/L)	Highest (mg/L)	Control (mg/L)	Highest (mg/L)	Control (mg/L)	Highest (mg/L)
<u>Physa spp.</u>	2/22/99	New=0 hrs	27	520	67	260	90	34		55		3.0
the second second	2/24/99	New=48 hrs	25	340	67	220	92	61	11.7.	34		
	4/28/99	New=0 hrs	28	560	61	300	94	45		54		
	4/30/99	New=48 hrs	76	510	55	300	95	29	2.0	93	and the second se	3.0
	5/2/99	Aged=96 hrs	28	530	56	300	93	38	< 1.0	59	< 2.0	< 2.0
Average			37	492	61	276	93	41	1.2	59	2,0	2.4
Standard Deviation			21.9	87.0	5.8	35.8	1.9	12.4	0.4	60	0.0	0.5
Lumbriculus spp.	2/23/99	New=0 hrs		200	63	190	92	49		37		
	2/25/99	New=48 hrs	27	290 220	66	190	92	48 51		31	a second s	
	3/31/99	New=0 hrs	20	220	65	210	the second se	69		31	< 2.0	
	4/2/99	New=48 hrs	27	300	71	170	96	57		30		2.0
States and the states of the	4/19/99	New=0 hrs	28	170	64	130		66			< 2.0	
	4/21/99	New=48 hrs	28	180	65	120	99	72	100 CT	28		
	4/25/99	Aged=96 hrs	28	180	52	140	96	63	and the second s		< 2.0	5.4
Average			28	233	64	159	96	61	1.0	23	2.0	2.5
Standard Deviation	1		0.7	58.8	5.8	32.9	3.1	9.1	0.0	13.9		1.3

1

TABLE 3-23. SUMMARY OF MEASURED WATER QUALITY INDICATORS FOR THE FLOW-THROUGH TOXICITY TEST

	SAMPLE SAMPLE COLLECTION	SODIUM		ALKALINITY		HARDNESS		TOTAL SUSPENDED SOLIDS		TOTAL ORGANIC CARBON		
TEST SPECIES	DATE	TIME	Control (mg/L)	Highest (mg/L)	Control (mg/L)	Highest (mg/L)	Control (mg/L)	Highest (mg/L)	Control (mg/L)	Highest (mg/L)	Control (mg/L)	Highes (mg/L)
Fathead minnows	7/22/99	Test Start	13	160	88	190	96	67	1.0	2.0	2.4	2.
	7/29/99	Week 1	8.3	150	76	140	94	61	9.0	2.0	< 2.0	< 2.
	8/4/99	Week 2	6.2	170	100	150	89	71	1.0	3.0	< 2.0	< 2.
	8/12/99	Week 3	13	140	75	180	96	70	< 1.0	4.0	< 2.0	< 2.
2010 ISBN 1000	8/19/99	Week 4	12	160	74	140	89	64	< 1.0	3.0	< 2.0	< 2
	8/23/99	Test End		-	-		-		-	-	-	
Average			10.5	156	83	160	93	67	2.6	2.8	2.1	2.
standard Deviation			3.1	11.4	11.3	23.5	3.6	4.2	3.6	0.8	0.2	0

Notes:

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# TABLE 3-24. ADVENT-DERIVED GLI AQUATIC LIFE VALUES FOR FLUORIDE - TIER II VALUE CALCULATIONS

GENUS SPECIES	COMMON NAME	LC50 VALUE (µg/L)	SMAV (µg/L)	EXISTING GMAV (a) (µg/L)	REVISED GMAV (b) (µg/L)
Gasterosteus	Stickleback	340,000	340,000	340,000	340,000
Alasmidonta raveneliana	Mussel, juvenile	303,000	303,000	303,000	303,000
Actinonaias pectorosa	Mussel, juvenile	347,000	347,000	265,000	265,000
Actinonaias pectorosa	Mussel, juvenile	300,000	300,000		
Actinonaias pectorosa	Mussel, juvenile	178,000	178,000		
Daphnia magna	Cladoceran	250,000	250,000	250,000	250,000
Pimephales promelas	Fathead minnow	180,000	180,000	180,000	165,658
Pimephales promelas	Fathead minnow	112,200	112,200		
Pimephales promelas	Fathead minnow	225,100	225,100		
Salmo trutta	Brown trout	164,500	164,500	156,000	156,000
Salmo gairdneri	Rainbow trout	147,000	147,000		
Physa spp.	Aquatic snail	231,700	194,397		194,397
Physa spp.	Aquatic snail	163,100			
Lumbriculus variegatus	Aquatic worm	93,500	119,160		119,160
Lumbriculus variegatus	Aquatic worm	113,100			
Lumbriculus variegatus	Aquatic worm	160,000			
Chironomus tentans	Midge (Aquatic insect)	93,100	101,611		101,611
Chironomus tentans	Midge (Aquatic insect)	110,900			

SECONDARY CONTINUOUS CONCENTRATION	(SCC) =		3,380
SECONDARY MAXIMUM CONCENTRATION (SI	WC) =		11,815
SECONDARY CHRONIC VALUE (SCV) =		SAV/SACR =	3,380
ACUTE-TO-CHRONIC RATIO (ACR) =	7.0		
SECONDARY ACUTE VALUE (SAV) = LOWEST	GMAV/ SAF =		23,630
SECONDARY ACUTE FACTOR = 4.3 (p		Table A-1)	(µg/L)

#### Notes:

(a) IDEM's most recent Tier II aquatic life values for fluoride.(b) Includes the fluoride toxicity test data developed during this study.

# APPENDIX1

# INITIAL FLUORIDE CRITERIA



## TIER II ACUTE AND CHRONIC AQUATIC LIFE VALUES FOR FLUORIDE

#### Standard:

The procedures described in the Tier II methodology indicate that, except possibly where a locally important species is very sensitive, aquatic organisms should not be affected unacceptably if the four (4) day average concentration of fluoride does not exceed 1.6 mg/L more than once every three (3) years on the average and if the one (1) hour average concentration does not exceed 11 mg/L more than once every three (3) years on the average.

#### Calculations:

Acute Aquatic Life:

SAV = lowest GMAV/SAF Lowest GMAV = 156 mg/L SAF = 7.0 SAV = 156/7.0 = 22.29 mg/L SMC = SAV/2 = 22.29/2 = 11 mg/L

Chronic Aquatic Life:

SCV = SAV/SACR SACR = 13.7 (Geometric mean of 18, 18, 7.91) SCV = 22.29/13.7 = 1.6 mg/L

Calculation of ACR's

Daphnia magna

NOEC = 25 mg/L LOEC = 40 mg/L

CV = Geometric Mean of 25 and 40 = 31.62 ACR = 250/31.62 = 7.91

# Notes:

The datum from Dave 1984 (9.8 mg/L) for <u>Daphnia magna</u> was not used since this varied by more than an order of magnitude from the other two data points for this genus, and because the reported EC<sub>50</sub> was well below a reported NOEC of 25 mg/L (21-day chronic study).

Genus Mean	Table 1. GMAVs an	d SMAVs for Fluor Species Mear			
Acute Value (mg/L)	Species	Acute Value (mg/L)		Reference Number	
156	Rainbow Trout 147 Salmo gairdneri	a second contract of the second se		1,4	
	Brown Trout Salmo trutta	164.5		t	
180	Fathead Minnow Pimephales promelas	180		4	
250	Cladoceran Daphnia magna	250	7.91	2,3	
340	Stickleback Gasterosteus	340		4	

# DRAFT

## References:

- Camargo, J.A. and J.V. Tarazona 1991. Short-term toxicity of fluoride ion (F-) in soft water to rainbow trout (<u>Salmo gairdneri</u>) and brown trout (<u>Salmo trutta fario</u>). Fluoride 24(2): 76-83.
- Fieser, A.H., J.L. Sykora, M.S. Kostalos 1986. Effect of fluorides on survival and reproduction of <u>Daphnia magna</u>. J. Water Pollut. Contr. Fed. 58(1): 82-86.
- LeBlanc, G.A. 1980. Acute toxicity of priority pollutants to water flea (<u>Daphnia magna</u>). Bull. Environ. Contam. Toxicol. 24(5): 684-691.
- Smith, L.R., T.M. Holsen, N.C. Ibay 1985. Studies on the acute toxicity of fluoride ion to stickleback, fathead minnow and rainbow trout. Chemosphere 14: 1383-1389.

#### Not used:

Dave, G. 1984. Effects of fluoride on growth, reproduction and survival in <u>Daphnia magna</u>. Comp. Biochem. Physiol. 78(12): 425-431.

Last modified: January 22, 1997

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# APPENDIX 2 CURRENT FLUORIDE CRITERIA AG

#### TIER II ACUTE AND CHRONIC AQUATIC LIFE VALUES FOR FLUORIDE

#### Standard:

The procedures described in the Tier II methodology indicate that, except possibly where a locally important species is very sensitive, aquatic organisms should not be affected unacceptably if the four (4) day average concentration of fluoride does not exceed 1,900 ig/L more than once every three (3) years on the average and if the one (1) hour average concentration does not exceed 13,000 ig/L more than once every three (3) years on the average.

#### Calculations:

Acute Aquatic Life:

SAV = lowest GMAV/SAF

Lowest GMAV = 156,000 µg/L

SAF = 6.1

SAV = 156,000/6.1 = 25,574 µg/L

SMC = SAV/2 = 25,574/2 = 13,000 µg/L

Chronic Aquatic Life:

SCV = SAV/SACR

SACR = 13.7 (Geometric mean of 18, 18, 7.91)

SCV = 25,574/13.7 = 1,900 µg/L

Calculation of ACR's

Daphnia magna

NOEC = 25 mg/L LOEC = 40 mg/L

CV = Geometric Mean of 25 and 40 = 31.62 ACR = 250/31.62 = 7.91

#### Notes:

The datum from Dave 1984 (9.8 mg/L) for <u>Daphnia magna</u> was not used since this varied by more than an order of magnitude from the other two data points for this genus, and because the reported EC<sub>50</sub> was well below a reported NOEC of 25 mg/L (21-day chronic study).

#### Table 1. GMAVs and SMAVs for Fluoride

Genus Mean Acute Value (µg/L) 156,000	Species Rainbow Trout	Species Mean Acute Value (μg/L) 147,000	Acute- Chronic Ratio	Reference <u>Number</u> 1,4	
100,000	<u>Salmo gairdneri</u>				
	Brown Trout Salmo trutta	164,500		1	
180,000	Fathead Minnow Pimephales promelas	180,000		4	
250,000	Cladoceran <u>Daphnia magna</u>	250,000	7.91	2,3	
340,000	Stickleback Gasterosteus	340,000		4	
265,000	Mussel, juvenile <u>Actinonaias pectorosa</u>	347,000		5	
	Mussel, juvenile <u>Actinonaias pectorosa</u>	178,000		5	
	Mussel, juvenile <u>Actinonaias pectorosa</u>	300,000		5	
303,000	Mussel, juvenile <u>Alasmidonta raveneliana</u>	303,000		5	

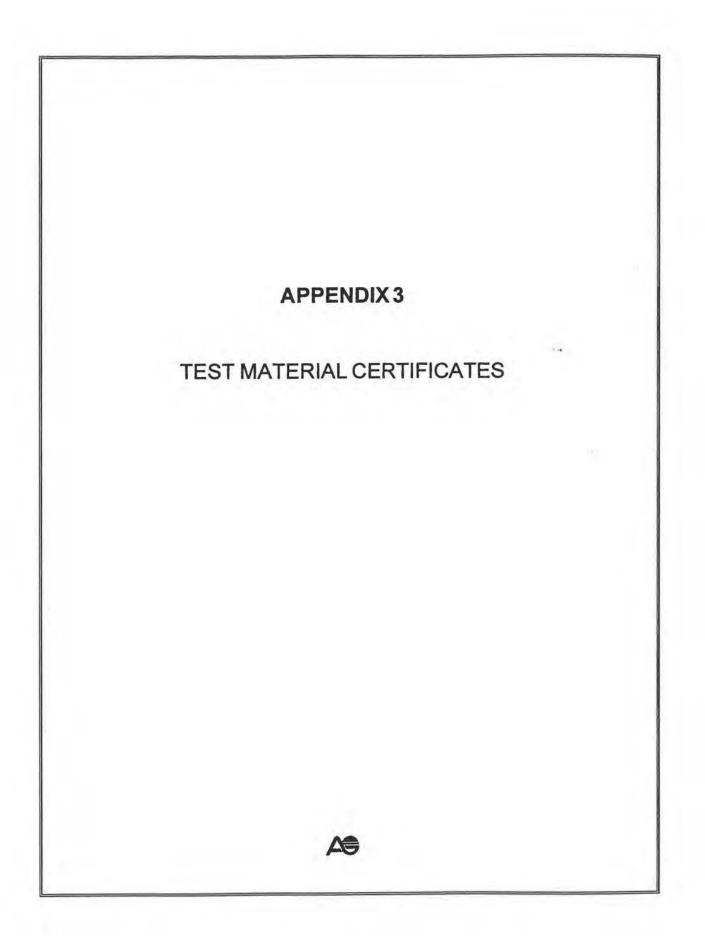
#### References:

- Camargo, J.A. and J.V. Tarazona 1991. Short-term toxicity of fluoride ion (F-) in soft water to rainbow trout (<u>Salmo gairdneri</u>) and brown trout (<u>Salmo trutta fario</u>). Fluoride 24(2): 76-83.
- Fieser, A.H., J.L. Sykora, M.S. Kostalos 1986. Effect of fluorides on survival and reproduction of <u>Daphnia magna</u>. J. Water Pollut. Contr. Fed. 58(1): 82-86.
- LeBlanc, G.A. 1980. Acute toxicity of priority pollutants to water flea (<u>Daphnia magna</u>). Bull. Environ. Contam. Toxicol. 24(5): 684-691.
- Smith, L.R., T.M. Holsen, N.C. Ibay 1985. Studies on the acute toxicity of fluoride ion to stickleback, fathead minnow and rainbow trout. Chemosphere 14: 1383-1389.
- 5. Keller, A. 1999. USEPA Region IV. Unpublished data.

# References not used:

Dave, G. 1984. Effects of fluoride on growth, reproduction and survival in <u>Daphnia magna</u>. Comp. Biochem. Physiol. 78(12): 425-431.

Last modified: June 17, 1999



# CERTIFICATE OF ANALYSIS

2/9/98 0PNiD 198 12/9/98 12/29/98 EMS Rin7

PRODUCT NUMBER: 21530-9

LOT NUMBER: 15402BN

PRODUCT NAME: SODIUM FLUORIDE, 99.99%

FORMULA: NAF

FORMULA WEIGHT: 41.99

APPEARANCE

WHITE POWDER

V

45.0% F (WITH LEAD ACETATE)

FE	10	PPM
BA	1	PPM
MG	1	PPM
SR	0.6	PPM

ICP ASSAY

CONFIRMS NA COMPONENT

SUITABLE ALTERNATIVE TO XRAY POWDER PATTERN FOR IDENTIFICATION SPEC.

ATOMIC ABSORPTION

GRAVIMETRIC ASSAY

TRACE ANALYSIS. ICP

K 3 PPM CS 2 PPM RB 1 PPM

> ALDRICH CHEMICAL COMPANY DAVID SWESSEL JUNE 14, 1995

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P.O. Box 355, Milwaukee, Wisconsin 53201 USA • (414) 273-3850 • FAX (414) 273-4979

# CERTIFICATE OF ANALYSIS

PRODUCT NUMBER: 21530-9	LOT NUMBER: 15402BN
PRODUCT NAME: SODIUM FLUORIDE,	99.99%
FORMULA: NAF	FORMULA WEIGHT: 41.99
APPEARANCE	WHITE POWDER
GRAVIMETRIC ASSAY	45.0% F (WITH LEAD ACETATE)
TRACE ANALYSIS, ICP	FE 10 PPM BA 1 PPM MG 1 PPM SR 0.6 PPM
ICP ASSAY	CONFIRMS NA COMPONENT
	SUITABLE ALTERNATIVE TO XRAY POWDER PATTERN FOR IDENTIFICATION SPEC.
ATOMIC ABSORPTION	К 3 РРМ CS 2 РРМ RB 1 РРМ
Both Both No. 3	5 NO.4
	ALDRICH CHEMICAL COMPANY David Swessel June 14, 1995
aldrich chemists in resear	in this and other Aldrich publications. Purchaser must determine the

P.O. Box 355, Milwaukaa, Wisconsin 53201 USA + (414) 273-3850 + FAX (414) 273-4979

505

# CERTIFICATE OF ANALYSIS

PRODUCT NUMBER: 21530-9	LOT NUMBER: 15402BN
PRODUCT NAME: SODIUM FLUORIDE.	99.99%
FORMULA: NAF	FORMULA WEIGHT: 41.99
APPEARANCE	WHITE POWDER
GRAVIMETRIC ASSAY	45.0% F (WITH LEAD ACETATE)
TRACE ANALYSIS, ICP	FE 10 PPM BA 1 PPM MG 1 PPM SR 0.6 PPM
ICP ASSAY	CONFIRMS NA COMPONENT SUITABLE ALTERNATIVE TO XRAY POWDER PATTERN
	FOR IDENTIFICATION SPEC.
ATOMIC ABSORPTION	К 3 РРМ CS 2 РРМ RB 1 РРМ

ALDRICH CHEMICAL COMPANY DAVID SWESSEL JUNE 14, 1995



ALDRICH warrants that its products conform to the information contained in this and other Aldrich publications. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing sign for additional terms and conditions of sale.

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# APPENDIX4 TAXONOMIC VERIFICATION AND ORGANISM HISTORIES A

II. Taxonomic Verification

Taxonomic identification was made on July 16, 1986, by Dr. Robert O. Brinkhurst, Ocean Ecology Department Chief, Institute of Ocean Sciences, Sidney, British Columbia, Canada. The taxonomy of this organism was determined to be as follows:

> Phylum - Annelida Class - Oligochaeta Order - Lumbriculida Family - Lumbriculidae Genus - <u>Lumbriculus</u> Species - <u>variegatus</u> (Müller)

#### III. Biology

Aquatic oligochaetes have the same fundamental structure as the common terrestrial earthworm, i.e., body construction is on a tube-within-tube plan. The body wall is soft, muscular, and covered with a very thin cuticle. The specialized digestive tract has a terminal mouth and anus and is supported in the coelom by thin transverse septa that mark the internal segmental divisions. Segmentation is homologous. The circulatory systems are closed. Reproduction is syngamic, and individuals are hermaphroditic. Cross-fertilization is the rule.

Usually locomotion is a crawling movement on or in the superficial layers of the substrate. It is similar to that of the earthworm and involves contractions of the muscular body wall and obtaining a purchase with the setae.

The great majority of aquatic oligochaetes obtain nutrients by ingesting quantities of the substrate after the manner of the earthworms, the organic component being digested as it passes through the alimentary canal. Because the body wall is thin and often well supplied with capillaries, most of the carbon dioxide and oxygen exchange occurs through the general body surface.

Aquatic oligochaetes occupy a niche equivalent to that occupied by terrestrial species of earthworms, and they feed on bottom mud and mix it much as earthworms effectively mix the surface layers of garden and meadow soils. The Lumbriculidae are restricted to temperate and cold-temperate zones.

-2-



## **Environmental Consulting & Testing**

1423 N. 8th St. Suite 118 . Superior, WI 54880

Nov. 10, 1999

Rick Lockwood Advent Group 201 Summit View Dr. Brentwood, TN

Dear Sir,

Environmental Consulting and Testing has reared and cultured the Diptera, <u>Chironomus tentans</u> since 1991. Original culture was obtained from the USEPA Laboratory, Duluth, MN. Organisms were verified as <u>Chironomus tentans</u> using one or more of the following taxonomic references: Johannsen, O.A. and L.C. Thomsen. 1937 "Aquatic Diptera". Pennak, R.W. 1953. "Freshwater Invertebrates of the United States ". Ward and Whipple. "Freshwater Biology". Wiederholm, T. 1983. "Chironomidae of Holartic Region".

Respectfully,

Steven N. Gries

Steven H. Poirier

		DATA SHEET
	Organism	History
	Species:	Physa SP-
	Source:	Lab reared Hatchery reared Field collected
		Hatch date Mixed Aley Receipt date
		Lot number 052399PS Strain
		Brood Origination FL
п.	Water Qu	ality
		Temperature 23 °C Salinity 22 ppt DO SET
		pH_7.5 Hardness 80 220 ppm
ш.	Culture C	onditions
		System: FW HEATED FLOW
		Diet: Flake Food // Phytoplankton Trout Chow //
		Brine Shrimp Rotifers Other
		Prophylactic Treatments:
		Prophylactic Treatments:
		Prophylactic Treatments:
	OLISSI	Comments:
IV.	Shipping	Comments:
IV.	Shipping	Comments: Information Client:_ <u>ADVENT_GRUP</u> # of Organisms:_ <u>180</u>
IV.	Shipping	Comments:

1 - 800 - 927 - 1650

a shi	Aquatic Research Organisms
EV.	DATA SHEET m History peulot 22°
Organis	m History New 270
Species:	
Source:	Lab reared Hatchery reared Field collected
	Hatch date MIXEO Receipt date
	Lot number 042799 PS Strain BRO
	Brood Origination
I. Water (	Quality
	Temperature 23 °C Salinity ppt DO SH
	pH_ $75$ Hardness $180^{+}$ ppm
II. Culture	pH_75 Hardness=180 <sup>+</sup> ppm
II. Culture	pH_75 Hardness=180 <sup>+</sup> ppm
II. Culture	pH_75 Hardness 180 <sup>+</sup> ppm Conditions
I. Culture	pH_75 Hardness-180 <sup>+</sup> ppm Conditions System: FW SPATIC REWEAGE
II. Culture	pH_75 Hardness_180 <sup>+</sup> ppm Conditions System: <u>FW SPATIC REWEAR</u> Diet: Flake Food <u>V</u> Phytoplankton <u>V</u> Trout Chow <u>V</u>
II. Culture	pH_75 Hardness 180 <sup>+</sup> ppm Conditions System: <u>FW SPATIC REWEAR</u> Diet: Flake Food <u>V</u> Phytoplankton <u>V</u> Trout Chow <u>V</u> Brine Shrimp Rotifers Other
	pH_75 Hardness 180 <sup>+</sup> ppm Conditions System: <u>FW SPANC REWEAR</u> Diet: Flake Food <u>V</u> Phytoplankton <u>V</u> Trout Chow <u>V</u> Brine Shrimp Rotifers Other Prophylactic Treatments: Comments:
	pH_75_ Hardness_180 <sup>±</sup> ppm Conditions System: <u>FW SPAR Reway</u> Diet: Flake Food <u>V</u> Phytoplankton <u>V</u> Trout Chow <u>V</u> Brine Shrimp Rotifers Other <u>Prophylactic Treatments</u> Comments: <u>Comments</u>
	pH_75 Hardness 180 <sup>±</sup> ppm Conditions System: <u>FW SPATIC REMEAR</u> Diet: Flake Food <u>V</u> Phytoplankton <u>V</u> Trout Chow <u>V</u> Brine Shrimp Rotifers Other <u>Prophylactic Treatments</u> Comments: <u>Comments</u> <u>Guident</u> <u>Howkov</u> # of Organisms: <u>180<sup>±</sup></u>
II. Culture	pH_75_ Hardness_180 <sup>±</sup> ppm Conditions System: <u>FW SPAR Reway</u> Diet: Flake Food <u>V</u> Phytoplankton <u>V</u> Trout Chow <u>V</u> Brine Shrimp Rotifers Other <u>Prophylactic Treatments</u> Comments: <u>Comments</u>

1 - 800 - 927 - 1650

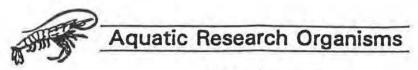
E.F.	Aquatic	Research	Organisms
SI.	Aquatic	Research	Organish

DATA SHEET

I.	Organism	History
	Species:	Physa SP.
	Source:	Lab reared Hatchery reared Field collected
		Hatch date N.A. Receipt date
		Lot number 020099PS Strain Allo
		Brood OriginationFL
п.	Water Qu	ality Temperature <u>24/</u> °C Salinity ppt DO <u>2.6</u> pH <u>2.9</u> Hardness 180 ppm Fonditions
		Temperature 24/°C Salinity ppt DO 2.6
		pH. 7.9 Hardness 180 ppm
III.	Culture C	conditions #797
		System: FU RECIRC
		Diet: Flake Food Phytoplankton Trout Chow
		Brine Shrimp Rotifers Other
		Prophylactic Treatments:
		Comments:
		comments
IV	Shinning	Information
11.	Subbug	Client: ACULT # of Organisms: 160+
		Carrier: Pro EX Date Shipped: 2-17-19
		1- 1-1

1 - 800 - 927 - 1650

ORGANISM RECORD						
DATE	PORCIES	DATON	COLIDCE	ACE	77.00	TOTIOCA
DATE 1/6/99	SPECIES	The second se		the second s		TEST LOG #'s
5/5-195		2001	EUT	600	102 AF	Defter - Edin
45/94	FHM	240+	ECT ECT	el	17.8	lifter
214 199	Lymbricalus	29	ARO	Fre	NOTUSIT	
2/10/99	Lumbriculus	29	ARO	10-	18.5	3-10 mm
2/16/55	FAMIL	100	ECT	624	14.2	SPA DULL
2/16/95	FHILL	170	SUT	Cole	11	N'E EXIN
2/18	Physic	110	420	Alit		
	limber i		ARO	1. 47		220
2/18	FALL	in	Alad	Fizde		AIRL
415	Selenestrum	49601	FUT	11400	de la	nue -
alis	E.Assbia Magnia		ECT	MX		
	C. dubia	Inc	-	Tid F		
2/15		200	ECT	7de		USS
2/25	FHIN	440	SCT	2.24	17.4	67002
2/25	Haylera	200+	eat	7le	1.1.1	USS
3/1	11	100	21	2.8		Carton terks
2/1	n	200	u	11		USS
3 15-	FHUL	240	Al. A	624	Anifi	Reft :
3/9	FAIN	120	Alut	9d	Ting	mil
3/01	FHR	480	Adit	SUV		64 001 Fill Bu
3/11	FHUN	240	Eet	224		AL UV
3/16	FMIN	120	Adul	224		Ramack 20
3/16	Fim	850	SCT .	Kay		Frans
3/17	Finn	120	SUT	Sde.	132	NC/specienzel
\$1.8	Hille	340	SCT	RE	16.50	Sizif
3/23	FHM	450	Adant	<24	24.5	benja PACIJEC
121.4	Form	240		6de	RO	Sec
3125	FM	240		Tdays		GA Pratic
3/30	LV	29	ARO	mixed	170	US Steel Nat
3/36	Selenastran	1000	ECT		· NA	Feeding
3/36	YCT	250	ECT	3/5		Freeding
3/31	FHL	120	Adul		25.0	Refer Vin
4/1	FHM	120	Adud		25:0	nelty Adut
41.	Ethn	240	Adut	224		Refer and
4/4/99	FIM	4904	ECT		21.4	54002
4/7/99	Musids	200+	ARO			Burshive
4/7/99	Speephead.	200+				Bergher
4/8/99		2001	ECT	1	Aliot	Feeding
VIL/50		320	Aluet	1746	ALNT	Metri / COF
4/2:0/99		120	Advent	4-23	26.0	
4/26/99	FHUL	80	Adult	×244		LOF .
41, 5/97	2145	1XUT	ARC		2220	isster l
4/20199	Physic	100+	ELT		19.2%	(MARIE IN
5/4/09	Magna Pinysa	land	GRO	minud	2200	: USStel NAF
5/4/99	Fitter Ftter Selenastrum	120	Alut	Col	4	fulling



#### DATA SHEET

I. Organism History

÷

Spec	ies: LUMBRICULUS VARIA gATUS
Sour	ce: Lab reared <u> Hatchery reared</u> Field collected
	Hatch date <u>mixes</u> Receipt date
	Lot number_03009911 Strain
	Brood Origination ROGHESTER NY
I. Wat	er Quality
	Temperature 23 °C Salinity ppt DO 76
	pH $\frac{7.5}{17.0}$ Hardness $\frac{-100}{17.0}$ ppm $17.0$
III. Cult	pH <u>75</u> Hardness <u>-180</u> ppm 17.0 ure Conditions
	System: Flat CLOW THIS OUGH
	Diet: Flake Food Phytoplankton Trout Chow
	Brine Shrimp Rotifers Other
	Prophylactic Treatments:
	Comments:
IV. Ship	pping Information
	Client: ACULT # of Organisms: 26
	Carrier: FO ZX Date Shipped: 3/29/99
	H DED
Biologist	- Aur Subski

1 - 800 - 927 - 1650

Aquatic Research Organisms

#### DATA SHEET

I. Organism History

	Species:	LUMBRICULUS VARIEGATUS
	Source:	Lab reared Hatchery reared Field collected
		Hatch date Receipt date
		Lot number 110098LV Strain
		Brood Origination Rochester NY
II.	Water Qu	ality
		Temperature 25 °C Salinity
		pH_7.5 Hardnessppm 11
ш.	Culture C	conditions A7
		System: Flo Theu
		Diet: Flake Food Phytoplankton Trout Chow
		Brine Shrimp Rotifers Other Rabbit Pellet
		Prophylactic Treatments:
		Comments:
		Comments
11/	Chinaina	
1.	Snipping	Information
		Client: <u>AAVENT Graup</u> # of Organisms: <u>2+72</u>
		Carrier: EAX Date Shipped: 2-17-99
<b>D</b> :	lacian	Mark Toungert

1 - 800 - 927 - 1650

Log No.	Date	Species	Batch	Source	Age	Temp	Used for:
she	sheles	C. tenfort	ZUDE	Ect	HDShak	25-8	USS Plaoricle
899	2/2/79	FM	aut	Friveret	1 1 1 1	924.8	EPA DMR Chrom
100	8-27-99	FM		Advent	8-18-99	24.6	58 A OMR-ACUTE
101	9-2-29	EM.		Advent	\$9-299	24.6	Naci Reptor
702	9/7/85	Fthen	170	Al the	16/45	14.5-	NIASCO
903	\$1249	FHILL	360	Adut	40850 55	54.5-	2015 Refter Minster
404	9/14	FHM	240+140	Heref	224	24.5	ET MW
905	9/22	FHM	120	Adut	He Dock	2.4-5-	ACS oil away
206	9/21	Film	280	Adul	224	240	Annes
907	6/28	FHUL	240	Ect	<24	23.9	USS COS-
908	9/28	FHIN	40	Acht	224	24.5-	DUNION
909	10/5	FHA	1100+	ECT	10-4-99	16.3	Febrain Pacific, Dyr
310	10/5	FHM.530	150	Advent	10-5-99	24.5	MW Street
11	10/17	FHM	120	Ada &	9 de	24 5-	Nipsco
2/2	10/55	phin	120	Art	HO w/ 4 des	24.5	HI COA:
113	10/10	FHIM	240	Alt	8dg	24,3	Reft ,
914	10/12	FHM	720	SUT	124	195-	Nipsen / Robins / Reft
9.5	10/19	FHM	850+	ECT	L24	18.7	677min
716	10/21	FHIM	240	Adut.	HO W/12	24.0	Dincers Acities
317	16/22	FHin	480	Adat	224	24.1-	ALY OUL
18	10/24	FAM	240	Adult	<24	24.5	Nipsces
119	10/26	FAIN	480	ELT	514	160	Metro
920	10/26	Etter	12	Elt	Aduts	-	Boodste
721	10/27	FHM	480	Ett	57	168	- Musteel
922	11/2	FHM	700	Adut	<24	24.5	MW steel
923	(1/3	FAN	300	EUT	524	14.5	500 anustel
924	11/2,3	Ffin	100	Arlet	HUDIGHY	24.5	SET/CEL
		1=Hun	240	Achit	HUICAE		Dynerrip
926	11/5-	FALL	480	ELT	LAY		K4002
27	11/16/99	FHM	280	ECT	424 hrs	14.5	MW Steel
28	11/18/99	FHM	240+	Advento	424hrs		TOCCOL, GA,
129	11/8/99	FAM	240	Adut	Kd Yhy	24.0	Astro Dry Gh
330	11/23	FAM	240	Acht	~27	24.5-	Nepty -
931	11/30/99	Selenistrum		ECT	11-24-99		
932	11/30/99	FRIM	482+	ECT	11-29	18.02	
933	12/2 FASC	FHIN	50	Alut	RD X/26	24.5-	AURI
734	12/7	FHL	200	Adres	<jy< td=""><td>24.5-</td><td></td></jy<>	24.5-	



## **Environmental Consulting & Testing**

1423 N. 8th St. Suite 118 . Superior, WI 54880

SHIPPING DATE: 05-27-99 FROM: ECT SUPERIOR, WI. TO: ADVENT SPECIES: MIDGE (<u>C. tentans</u>) HATCH DATE: 05-19-99 NUMBER: 200 + TEMP. AT SHIPPING: 23.2 C P.O.#: RICK SHIPPED BY: FEDERAL EX.

WATER CHEMISTRY RECORDS

TEMPERATURE	22 to	24	AS	Co
CONDUCTIVITY	100 to	200	AS	UMHOS/CM
T.HARDNESS	60 to	80	AS	CACO3
T.ALKALINITY	40 tc	60	AS	CAC03
рН	7.3 to	7.8		

Originally obtained from culture at EPA Duluth Lab, MN. Fed Blend of Tetra flake food Substrate of solvent rinsed paper towels REF. TOX NACL N=14 MEAN = 7.26 G/L S.D = 0.88 G/L



## **Environmental Consulting & Testing**

1423 N. 8th St. Suite 118 • Superior, WI 54880

SHIPPING DATE: 05-20-99 FROM: ECT SUPERIOR, WI. TO: ADVENT SPECIES: MIDGE (<u>C. tentans</u>) HATCH DATE: 05-09-99 J2 days on NUMBER: 200 + dl TEMP. AT SHIPPING: 22.8 C P.O.#: RICK SHIPPED BY: FEDERAL EX. WATER CHEMISTRY RECORDS TEMPERATURE <u>22 to 24</u> AS CO CONDUCTIVITY 100 to 200 AS UMHOS/CM T.HARDNESS <u>60 to 80</u> AS CACO3

T.ALKALINITY 40 to 60 AS CACO3

7.3 to 7.8

pH

origi	na	11	y c	bt	ain	eđ	from	cul	ture	at	EPA	Duluth	Lab.	MN.
Fed H													and the second	
Subst	ra	ate	of	Es	olv	ent	rin	sed	pape	r to	owels	5		
REF.	TC	xc	NAC	CL										
N=14														
MEAN	=	7.	26	G/	'L									
S.D	=	0.	88	G/	L									

## APPENDIX 5

## FLUORIDE TABLES

## (Individual Analytical Comparisons)



Sampling Intervals	Nominal NaF Conc. (mg/L)	Measured New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	118	58.0	51.0	-12.8%	less
	168	80.0	83.0	3.7%	more
	240	120.0	110.0	-8.7%	less
	343	170.0	150.0	-12.5%	less
	490	230.0	240.0	4.3%	more
	700	330.0	300.0	-9.5%	less
	1000	430.0	-		
48/96 hours	118	52.0	62.0	17.5%	more
	168	83.0	83.0	0.0%	equal
	240	110.0	120.0	8.7%	more
	343	150.0	170.0	12.5%	more
	490	210.0	210.0	0.0%	equal
	700	310.0	340.0	9.2%	more

#### TABLE A5-1. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH PHYSA SPP. (#1)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Nominal F Conc, (mg/L)	Measured F Conc. (mg/L)	Percent Off Nominal (%)	Status of Total Fluoride
0 hours	118	53.4	58.0	8.6%	more
	168	76.0	80.0	5.2%	more
	240	108.6	120.0	10.5%	more
	343	155.2	170.0	9.5%	more
	490	221.7	230.0	3.7%	more
	700	316.8	330.0	4.2%	more
	1000	452.5	430.0	-5.0%	less
48 hours (old)	118	53.4	51.0	-4.5%	less
a server a second provide the second second	168	76.0	83.0	9.2%	more
	240	108.6	110.0	1.3%	more
	343	155.2	150.0	-3.4%	less
	490	221.7	240.0	8.2%	more
	700	316.8	300.0	-5.3%	less
	1000	452.5 -			
48 hours (new)	118	53.4	52.0	-2.6%	less
	168	76.0	83.0	9.2%	more
	240	108.6	110.0	1.3%	more
	343	155.2	150.0	-3.4%	less
	490	221.7	210.0	-5.3%	less
	700	316.8	310.0	-2.1%	less
	1000	452.5			
96 hours (aged)	118	53.4	62.0	16.1%	more
	168	76.0	83.0	9.2%	more
	240	108.6	120.0	10.5%	more
	343	155.2	170.0	9.5%	more
	490	221.7	210.0	-5.3%	less
	700	316.8	340.0	7.3%	more
	1000	452.5	0.0.0		

TABLE A5-1. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH PHYSA SPP. (#1)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Measured New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	118	57.0	57.0	0.0%	equal
	168	80.0	80.0	0.0%	equal
	240	110.0	120.0	8.7%	more
	343	190.0	170.0	-11.1%	less
	490	280.0	250.0	-11.3%	less
14.	700	370.0	370.0	0.0%	equal
	1000	510.0	530.0	3.8%	more
48/96 hours	118	57.0	63.0	10.0%	more
	168	82.0	120.0	37.6%	more
	240	110.0	130.0	16.7%	more
	343	190.0	190.0	0.0%	equal
	490	260.0	250.0	-3.9%	less
	700	360.0	350.0	-2.8%	less
	1000	500.0	490.0	-2.0%	less

#### TABLE A5-2. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH PHYSA SPP. (#2)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervais	Nominal NaF Conc. (mg/L)	Nominal F Conc. (mg/L)	Measured F Conc. (mg/L)	Percent Off Nominal (%)	Status of Total Fluoride
0 hours	118	53.4	57.0	6.8%	more
2012-01-01	168	76.0	80.0	5.2%	more
	240	108.6	110.0	1.3%	more
	343	155.2	190.0	22.4%	more
	490	221.7	280.0	26.3%	more
	700	316.8	370.0	16.8%	more
	1000	452.5	510.0	12.7%	more
48 hours (old)	118	53.4	57.0	6.8%	more
	168	76.0	80.0	5.2%	more
	240	108.6	120.0	10.5%	more
	343	155.2	170.0	9.5%	more
	490	221.7	250.0	12.8%	more
	700	316.8	370.0	16.8%	more
	1000	452.5	530.0	17.1%	more
48 hours (new)	118	53.4	57.0	6.8%	more
io nouro (non)	168	76.0	82.0	7.9%	more
	240	108.6	110.0	1.3%	more
	343	155.2	190.0	22.4%	more
	490	221.7	260.0	17.3%	more
	700	316.8	360.0	13.7%	more
	1000	452.5		-	
96 hours (aged)	118	53.4	63.0	18.0%	more
to hours (adoa)	168	76.0	120.0	57.9%	more
	240	108.6	130.0	19.7%	more
	343	155.2	190.0	22.4%	more
	490	221.7	250.0	12.8%	more
	700	316.8	350.0	10.5%	more
	1000	452.5			

#### TABLE A5-2. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH PHYSA SPP. (#2)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Measured New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	59	27.0	28.0	3.6%	more
	84	42.0	40.0	-4.9%	less
	120	58.0	61.0	5.0%	more
	172	79.0	87.0	9.6%	more
	245	110.0	150.0	30.8%	more
	350	180.0	170.0	-5.7%	less
	500	250.0	270.0	7.7%	more
48/96 hours	59	32.0	29.0	-9.8%	less
	84	43.0	43.0	0.0%	equal
	120	52.0	69.0	28.1%	more
	172	78.0	79.0	1.3%	more
	245	140.0	120.0	-15.4%	less
	350	340.0	160.0	-72.0%	less
	500	-	-	-	-

#### TABLE A5-3. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH LUMBRICULUS (#1)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

	Nominal	Nominal	Measured	Percent	
Sampling Intervals	NaF Conc. (mg/L)	F Conc. (mg/L)	F Conc. (mg/L)	Off Nominal (%)	Status of Total Fluoride
0 hours	59	26.7	27.0	1.1%	more
	84	38.0	42.0	10.5%	more
	120	54.3	58.0	6.8%	more
	172	77.8	79.0	1.5%	more
	245	110.9	110.0	-0.8%	less
	350	158.4	180.0	13.7%	more
	500	226.3	250.0	10.5%	more
48 hours (old)	59	26.7	28.0	4.9%	more
12 manufarmer	84	38.0	40.0	5.2%	more
	120	54.3	61.0	12.3%	more
	172	77.8	87.0	11.8%	more
	245	110.9	150.0	35.3%	more
	350	158.4	170.0	7.3%	more
	500	226.3	270.0	19.3%	more
48 hours (new)	59	26.7	32.0	19.9%	more
in the second design of the second se	84	38.0	43.0	13.1%	more
	120	54.3	52.0	-4.2%	less
	172	77.8	78.0	0.2%	more
	245	110.9	140.0	26.3%	more
	350	158.4	340.0	114.7%	more
	500	226.3		-	
96 hours (aged)	59	26.7	29.0	8.6%	more
er under a fallant	84	38.0	43.0	13.1%	more
	120	54.3	69.0	27.1%	more
	172	77.8	79.0	1.5%	more
	245	110.9	120.0	8.2%	more
	350	158.4	160.0	1.0%	more
	500	226.3	100.0		

#### TABLE A5-3. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH LUMBRICULUS (#1)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Measured New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	59	31.0	38.0	20.3%	more
	84	43.0	58.0	29.7%	more
	120	62.0	80.0	25.4%	more
	172	77.0	100.0	26.0%	more
	245	115.0	160.0	32.7%	more
	350	170.0	200.0	16.2%	more
	500	250.0	350.0	33.3%	more
48/96 hours	59	43.0	42.0	-2.4%	less
	84	57.0	60.0	5.1%	more
	120	76.0	80.0	5.1%	more
	172	100.0	110.0	9.5%	more
	245	140.0	120.0	-15.4%	less
	350	200.0	200.0	0.0%	equal
	500	300.0	350.0	15.4%	more

#### TABLE A5-4. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH LUMBRICULUS (#2)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Nominal F Conc. (mg/L)	Measured F Conc. (mg/L)	Percent Off Nominal (%)	Status of Total Fluoride
0 hours	59	26.7	31.0	16.1%	more
	84	38.0	43.0	13.1%	more
	120	54.3	62.0	14.2%	more
	172	77.8	77.0	-1.1%	less
	245	110.9	115.0	3.7%	more
	350	158.4	170.0	7.3%	more
	500	- 226.3	250.0	10.5%	more
48 hours (old)	59	26.7	38.0	42.3%	more
	84	38.0	58.0	52.6%	more
	120	54.3	80.0	47.3%	more
	172	77.8	100.0	28.5%	more
	245	110.9	160.0	44.3%	more
	350	158.4	200.0	26.3%	more
	500	226.3	350.0	54.7%	more
48 hours (new)	59	26.7	43.0	61.1%	more
the opening friends	84	38.0	57.0	50.0%	more
	120	54.3	76.0	40.0%	more
	172	77.8	100.0	28.5%	more
	245	110.9	140.0	26.3%	more
	350	158.4	200.0	26.3%	more
	500	226.3			
96 hours (aged)	59	26.7	42.0	57.3%	more
	84	38.0	60.0	57.9%	more
	120	54.3	80.0	47.3%	more
	172	77.8	110.0	41.3%	more
	245	110.9	120.0	8.2%	more
	350	158.4	200.0	26.3%	more
	500	226.3	200.0	20.076	inor o

TABLE A5-4. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH LUMBRICULUS (#2)

At 95% Confidence, USEPA Relative Percent Difference = 14%; Stendard Matheda BBD = 0.6%

... Standard Methods RPD = 9.6%.

...

Sampling Intervals	Nominal NaF Conc. (mg/L)	Measured New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	60	36.0	35.0	-2.8%	less
	100	52.0	62.0	17.5%	more
	140	76.0	77.0	1.3%	more
	180	95.0	99.0	4.1%	more
	220	130.0	130.0	0.0%	equal
	260	140.0	150.0	6.9%	more
	300	150.0	170.0	12.5%	more
48/96 hours	60	39.0	44.0	12.0%	more
	100	59.0	53.0	-10.7%	less
	140	77.0	82.0	6.3%	more
	180	96.0	93.0	-3.2%	less
	220	130.0	130.0	0.0%	equal
	260	190.0	150.0	-23.5%	less
	300	190.0	170.0	-11.1%	less

#### TABLE A5-5. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH LUMBRICULUS (#3)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

	Nominal	Nominal	Measured	Percent	
Sampling Intervals	NaF Conc. (mg/L)	F Conc. (mg/L)	F Conc. (mg/L)	Off Nominal (%)	Status of Total Fluoride
0 hours	60	27.2	36.0	32.6%	more
	100	45.3	52.0	14.9%	more
	140	63.4	76.0	20.0%	more
	180	81.5	95.0	16.6%	more
	220	99.6	130.0	30.6%	more
	260	117.7	140.0	19.0%	more
	300	135.8	150.0	10.5%	more
48 hours (old)	60	27.2	35.0	28.9%	more
	100	45.3	62.0	37.0%	more
	140	63.4	77.0	21.5%	more
	180	81.5	99.0	21.5%	more
	220	99.6	130.0	30.6%	more
	260	117.7	150.0	27.5%	more
	300	135.8	170.0	25.2%	more
48 hours (new)	60	27.2	39.0	43.6%	more
io nouro (non)	100	45.3	59.0	30.4%	more
	140	63.4	77.0	21.5%	more
	180	81.5	96.0	17.9%	more
	220	99.6	130.0	30.6%	more
	260	117.7	190.0	61.5%	more
	300	135.8		-	
96 hours (aged)	60	27.2	44.0	62.1%	more
oo nours (agos)	100	45.3	53.0	17.1%	more
	140	63.4	82.0	29.4%	more
	180	81.5	93.0	14.2%	more
	220	99.6	130.0	30.6%	more
	260	117.7	150.0	27.5%	more
	300	135.8		120/23/55	

#### TABLE A5-5. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH LUMBRICULUS (#3)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

... Standard Methods RPD = 9.6%.

.....

Sampling Intervals	Nominal NaF Conc. (mg/L)	Measured New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	59	20.0	30.0	40.0%	more
	84	35.0	34.0	-2.9%	less
	120	56.0	49.0	-13.3%	less
	172	74.0	72.0	-2.7%	less
	245	120.0	93.0	-25.4%	less
	350	170.0	149.0	-13.2%	less
	500	220.0	220.0	0.0%	
48/96 hours	59			-	
	84			-	
	120		-	-	
	172		-	-	
	245	-	-	-	
	350 500	-	-	+	

#### TABLE A5-6. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH CHIRONOMIDS (#1)

At 95% Confidence, USEPA Relative Percent Difference = 14%; ....Standard Methods RPD = 9.6%.

Sampling Intervals	Nominal NaF Conc. (mg/L)	Nominal F Conc. (mg/L)	Measured F Conc. (mg/L)	Percent Off Nominal (%)	Status of Total Fluoride
0 hours	59	26.7	20.0	-25.1%	less
	84	38.0	35.0	-7.9%	less
	120	54.3	56.0	3.1%	more
	172	77.8	74.0	-4.9%	less
	245	110.9	120.0	8.2%	more
	350	158.4	170.0	7.3%	more
	500	226.3	220.0	-2.8%	less
48 hours (old)	59	26.7	30.0	12.4%	more
	84	38.0	34.0	-10.5%	less
	120	54.3	49.0	-9.8%	less
	172	77.8	72.0	-7.5%	less
	245	110.9	93.0	-16.1%	less
	350	158.4	149.0	-5.9%	less
	500	226.3	220.0	-	

#### TABLE A5-6. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH CHIRONOMIDS (#1)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Measured New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	59	26.0	26.0	0.0%	equal
	84	34.0	35.0	2.9%	more
	120	52.0	55.0	5.6%	more
	172	71.0	72.0	1.4%	more
	245	95.0	94.0	-1.1%	less
,	350	170.0	150.0	-12.5%	less
	500	220.0	230.0	4,4%	more
48/96 hours	59		-		
	84	-	-	-	
	120		-	-	
	172	-	-	-	
	245	-	-	-	
	350	-	-	-	
	500		-	-	

#### TABLE A5-7. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH CHIRONOMIDS (#2)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Nominal F Conc. (mg/L)	Measured F Conc. (mg/L)	Percent Off Nominal (%)	Status of Total Fluoride
0 hours	59	26.7	26.0	-2.6%	less
	84	38.0	34.0	-10.5%	less
	120	54.3	52.0	-4.2%	less
	172	77.8	71.0	-8.8%	less
	245	110.9	95.0	-14.3%	less
	350	158.4	170.0	7.3%	more
	500	226.3	220.0	-2.8%	less
48 hours (old)	59	26.7	26.0	-2.6%	less
	84	38.0	35.0	-7.9%	less
	120	54.3	55.0	1.3%	more
	172	77.8	72.0	-7.5%	less
	245	110.9	94.0	-15.2%	less
	350	158.4	150.0	-5.3%	less
	500	226.3	230.0	1.7%	more

#### TABLE A5-7. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH CHIRONOMIDS (#2)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Measured New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	50 100 200 300 400 500	23.0 42.0 88.0 113.0 150.0 200.0	23.0 45.0 80.0 110.1 -	0.0% 6.9% -9.5% -2.6% -	equal more less less
48/96 hours	50 100 200 300 400 500	26.0 47.0 64.0 120.0	28.0 51.0 88.0 140.0 -	7.4% 8.2% 31.6% 15.4% -	more more more more

#### TABLE A5-8. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH FISH ACUTE TEST (#1)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	Nominal F Conc. (mg/L)	Measured F Conc. (mg/L)	Percent Off Nominal (%)	Status of Total Fluoride
0 hours	50	22.6	23.0	1.7%	more
	100	45.3	42.0	-7.2%	less
	200	90.5	88.0	-2.8%	less
	300	135.8	113.0	-16.8%	less
	400	181.0	150.0	-17.1%	less
	500	226.3	200.0	-11.6%	less
48 hours (old)	50	22.6	23.0	1.7%	more
	100	45.3	45.0	-0.6%	less
	200	90.5	80.0	-11.6%	less
	300	135.8	110.1	-18.9%	less
	400	181.0	-		
	500	226.3	-	-	
48 hours (new)	50	22.6	26.0	14.9%	more
to nours (new)	100	45.3	47.0	3.9%	more
	200	90.5	64.0	-29.3%	less
	300	135.8	120.0	-11.6%	less
	400	181.0			
	500	226.3		-	
96 hours (aged)	50	22.6	28.0	23.8%	more
ee noure (aged)	100	45.3	51.0	12.7%	more
	200	90.5	88.0	-2.8%	less
	300	135.8	140.0	3.1%	more
	400	181.0		-	
	500	226.3		-	

TABLE A5-8. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH FISH ACUTE TEST (#1)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	Nominal NaF Conc. (mg/L)	New F (mg/L)	Measured Aged F (mg/L)	Aged vs. New Relative Difference (%)	Status of Total Fluoride
0/48 hours	73	44.0	39.0	-12.0%	less
	103	50.0	56.0	11.3%	more
	148	66.0	69.0	4.4%	more
	211	93.0	110.0	16.7%	more
	301	140.0	150.0	6.9%	more
	430	230.0	200.0	-14.0%	less
	615	-	300.0		
48/96 hours	73	38.0	38.0	0.0%	equal
	103	53.0	29.0	-58.5%	less
	148	67.0	70.0	4.4%	more
	211	110.0	110.0	0.0%	equal
	301	140.0	150.0	6.9%	more
	430	230.0	180.0	-24.4%	less
	615	-	-	-	

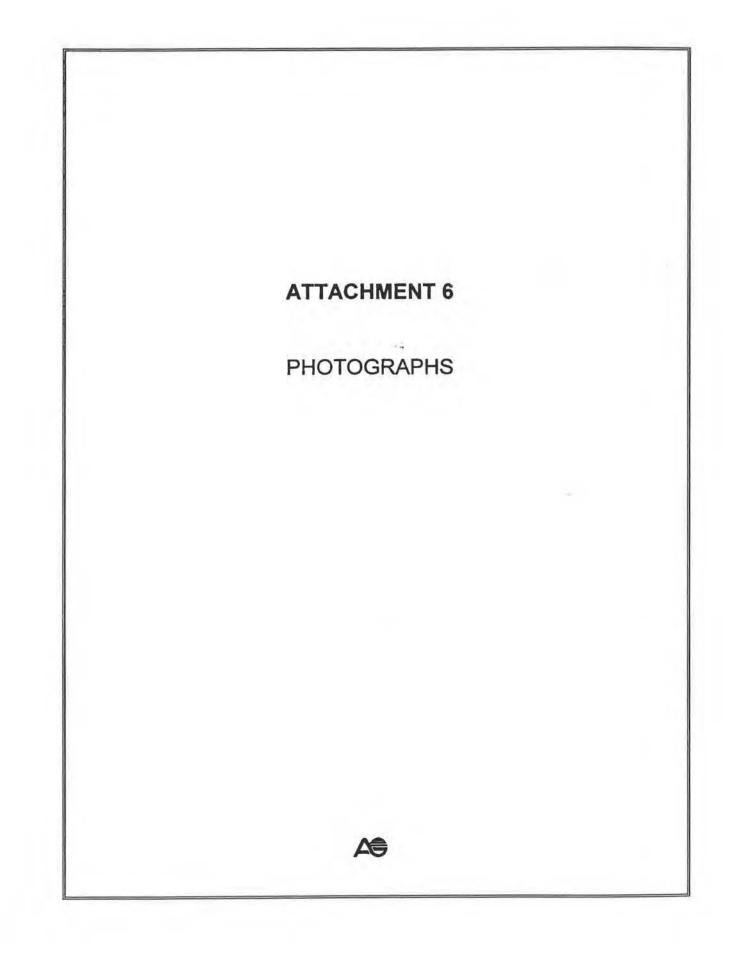
#### TABLE A5-9. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH FISH ACUTE TEST (ACR)

At 95% Confidence, USEPA Relative Percent Difference = 14%;

Sampling Intervals	NaF Conc. F C	Nominal F Conc.	Measured F Conc. (mg/L)	Percent Off Nominal (%)	Status of Total Fluoride
		(mg/L)			
0 hours	73	33.0	44.0	33.2%	more
17.2.3	103	46.6	50.0	7.3%	more
	148	67.0	66.0	-1.4%	less
	211	95.5	93.0	-2.6%	less
	301	136.2	140.0	2.8%	more
	430	194.6	230.0	18.2%	more
	615	278.3			
48 hours (old)	73	33.0	39.0	18.1%	more
	103	46.6	56.0	20.2%	more
	148	67.0	69.0	3.0%	more
	211	95.5	110.0	15.2%	more
	301	136.2	150.0	10.1%	more
	430	194.6	200.0	2.8%	more
	615	278.3	300.0	7.8%	more
48 hours (new)	73	33.0	38.0	15.0%	more
	103	46.6	53.0	13.7%	more
	148	67.0	67.0	0.0%	more
	211	95.5	110.0	15.2%	more
	301	136.2	140.0	2.8%	more
	430	194.6	230.0	18.2%	more
	615	278.3			
96 hours (aged)	73	33.0	38.0	15.0%	more
	103	46.6	29.0	-37.8%	less
	148	67.0	70.0	4.5%	more
	211	95.5	110.0	15.2%	more
	301	136.2	150.0	10.1%	more
	430	194.6	180.0	-7.5%	less
	615	278.3			

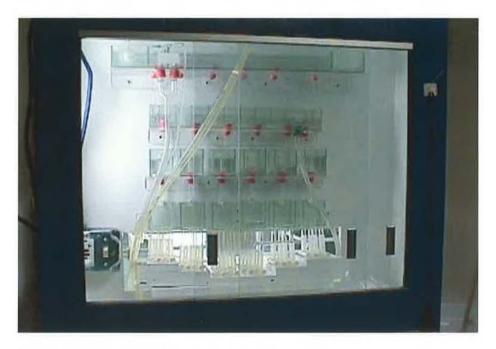
TABLE A5-9. ANALYTICAL SUMMARIES FOR SODIUM FLUORIDE TEST WITH FISH ACUTE TEST (ACR)

At 95% Confidence, USEPA Relative Percent Difference = 14%;





A6-1. Side view of Benoit Mini-Diluter.



A6-2. Dilution cell for flow-through test.



A6-3. Test chambers for flow-through test.



A6-4. Chironomid acute toxicity test setup. Temperature and light controlled room.



A6-5. Preparing to monitor standard water quality parameters.



A6-6. Close-up of chironomid test chambers with sterile sand on cup bottoms.

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# FACTORS INFLUENCING THE BIOAVAILABILITY OF FLUORIDE FROM CALCIUM-RICH, HEALTH-FOOD PRODUCTS AND CaF<sub>2</sub> IN MAN

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Summary—After single, oral doses, 8 h profiles of fluoride (F) concentrations in plasma were determined in healthy human volunteers. Bioavailability of F from bone-meal, calcium and  $CaF_2$  tablets was evaluated in relation to that of NaF. Tablets were administered either whole or as a finely-ground powder, either to fasting subjects or with breakfast. Availability was lowest from whole tablets taken by fasting subjects, and highest from powdered substances given with breakfast. Mean F availabilities ranged between 7.2 and 39 per cent with bone-meal tablets, between 20 and 59 per cent with Ca tablets, and between 0 and 47 per cent with  $CaF_2$  tablets.

#### INTRODUCTION

Several health-food products, because of their high Ca content are especially recommended for infants and children. No declaration of their F content is given, but some of these products have F contents between 100 and 850 mg/kg (Siebert and Trautner, 1985). The recommended daily dose gives an F-intake between 0.9 and 2.9 mg/day; together with F from dietary and other sources (F tablets, dentifrices etc.), this would give a total F-intake much higher than recommended for dental-caries prevention (Bergmann et al., 1983; Driscoll and Horowitz, 1978).

We showed the high bioavailability of F from some of these products when taken by human subjects (Trautner and Einwag, 1986a). These results contrast with findings that concomitant ingestion of F and Ca markedly reduces bioavailability of F in the rat as well as in man (Patz and Fickenscher, 1977; Jowsey and Riggs, 1978). Furthermore, after intake of CaF, tablets by human volunteers almost no increase of plasma F concentration was observed (Afseth, Ekstrand and Hagelid, 1985). This discrepancy might be due, at least in part, to differences in modes of application: the low bioavailability was found when F- and Ca-containing substances were given to fasting subjects, whereas in our experiments (Trautner and Einwag, 1986a) the subjects took them under normal living conditions, i.e. with meals. Findings by Machle and Largent (1943) and McClure et al. (1945) seem to support this idea; they fed CaF, or bone meal to human subjects as part of their diet and reported F availabilities of between 37 and 62 per cent.

In order to determine which factors influence F bioavailability from Ca-rich substances and which might be responsible for the differences outlined above, we have performed an intra-individual crossover study on human volunteers. They were given Fand Ca-rich substances either on a fasting stomach or with a standard breakfast, and either as whole tablets or in a powdered form. The F bioavailability was determined by measuring the area under the plasma F-concentration-versus-time curve and, for NaF tablets only, by measuring urinary F excretion. The term bioavailability is defined by Ritschel (1984) as the rate and extent to which F is absorbed from a product and reaches the systemic circulation.

#### MATERIALS AND METHODS

#### Test substances

NaF tablets (Zymafluor-Tabletten, Zyma GmbH, München, F.R.G.; 1 mg F/tablet) were used as a reference. Test substances were bone-meal tablets (Bone Meal Plus, Healthcraft, Associated Healthfood, Byfleet, England; 0.27 mg F/tablet), Ca tablets (Sanddorn-Kalk-Tabletten. Natura-Werk, Hannover, F.R.G.; 0.19 mg F/tablet), and CaF<sub>2</sub> tablets (gift of Fresenius A.G., Oberursel/Ts., F.R.G.; 2 mg F/tablet). The quantities given were two, six, six or one tablets, respectively, at each experiment, containing 2, 1.62, 1.14 or 2 mg F, and 0, 690, 1030 and 2.1 mg Ca, respectively. The Ca contents were calculated from the data on the labels. Either whole tablets were used or they were ground in a ball mill to a fine powder. Powdering was preferred to chewing by the subjects as it gave uniform conditions.

#### Subjects

Six healthy subjects, three males and three females, aged between 16 and 18 years, participated as volunteers. The purpose of the experiments was described to the subjects and their parents; written consent was obtained from all parents.

#### Experimental procedure

The subjects fasted for 10 h (overnight) before the day of the experiment. All experiments began at 0800 h; there were four different groups of experiments with respect to intake of food and consistency of the test substance. In groups 1 and 2, the substances were taken on a fasting stomach either as whole tablets (1) or as a fine powder (2). They were swallowed with 100 ml of tap water (F concentration  $<0.2 \text{ parts}/10^6$ ). No food or drink was allowed until 1100 h, when a standard breakfast was taken. In groups 3 and 4, the standard breakfast was taken at the beginning of the experiment and the substances were ingested at the end of the meal, either as whole tablets (3) or as powder (4). No food or drink was allowed for 3 h. The breakfast consisted of rolls, butter, bacon, jam and coffee without milk. Its Fand Ca-content as calculated from food-composition tables (Souci, Fachmann and Kraut, 1981) were between 90 and  $120 \mu g$  F and 60 and 80 mg Ca, respectively. During the days of experimentation, the same diet was given to all subjects. For determination of F availability by measuring net urinary F output, the subjects took the NaF tablets either with breakfast or on a fasting stomach and they were not allowed to eat or drink for the next 3 h. Some of these experiments were done unsupervised at home, because with the greater variation in this method, each experiment had to be performed several times. For technical reasons, these experiments had to be limited to NaF tablets.

#### Collection of blood and urine

For each experiment, a total of 18 blood samples was taken from the cubital vein. Sampling was done before the start of the experiment (control), then at 15-min intervals for 1 h, at 30-min intervals up to 6 h and at 7 h and 8 h after administration of the substance. The samples were centrifuged and plasma was stored at 4-6°C in polyethylene tubes for analysis within 72 h. Urine was collected over 24 h and samples were stored at -18°C in polyethylene tubes until analysed. Samples from the second day after an experiment until the day of the next served as controls for determination of the mean daily F output (background). The number of control samples was not less than 18 for each subject.

#### Analytical procedure

Plasma and urinary F levels were measured using an F-ion-sensitive electrode (model 96-09; Orion Research Corp., Cambridge, Massachusetts, U.S.A.), according to the method of Cowell (1975). In addition, part of the serum samples was analysed by gas chromatography according to the method of Fresen, Cox and Witter (1968). For gas chromatography, the powdered tablets were extracted with 1 M HCl; this method allows determination of total F. Details of the analytical procedure are described by Siebert and Trautner (1985).

#### Calculations

Net F concentrations were calculated by subtraction of the background F levels (control) from the F data obtained after intake of the test substances. The area under the plasma concentration-versus-time curve (AUC) was calculated by the trapezoidal rule; it also included the area after the 8-h sampling point.

The bioavailability (B) of F from different substances (sub) was calculated from the plasma data as

$$B\% = \frac{AUC_{sub} \times D_{NaF} \times 100}{AUC_{NaF} \times D_{sub}}$$

where D is the quantity of F in the substance given or in the NaF sample (reference), respectively.

The calculation of bioavailabilities from values of urinary F was:

$$B\% = \frac{U_{sub} \times D_{NaF} \times 100}{U_{NaF} \times D_{sub}}$$

where U is the net amount of F excreted in the urine during 24 h.

All data were expressed as  $\bar{x} \pm SD$ . Significance of differences were assessed by Student's *t*-test. All data in the text, figures and tables are given as net F-concentrations, i.e. with background subtracted. Complete bioavailability of F administered as NaF tablet was assumed (Ekstrand, Ehrnebo and Boreus, 1978).

#### RESULTS

The intake of 2 mg F as NaF tablets either by fasting subjects or together with food gave similar plasma F-concentration curves (Fig. 1). When taken with food, however, the peak level was delayed when compared to the fasting subjects. The AUC data showed remarkable between-individual variation (Table 1). On an intra-individual basis, however, the maximum difference between the two experiments was 6.3 per cent. Furthermore, the way of taking the NaF tablets did not alter the net urinary F output of the individuals (Table 2). The intra-individual variation was below  $\pm 5$  per cent of the given dose under both experimental conditions.

Figures 2 and 3 show the plasma F-concentration curves after intake of bone-meal and Ca tablets, respectively, by subject 4; the curves for the other subjects were similar. In contrast to NaF, the ingestion of these resulted in more flattened curves with maximum levels between 2 and 4 h. When given to fasting subjects maximum levels were reached in most cases earlier than when given with food. The AUC



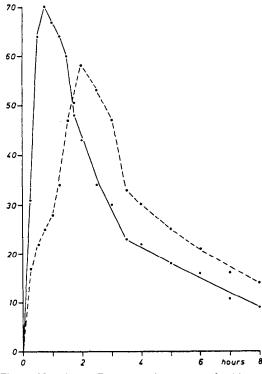


Fig. 1. Net plasma F-concentration curves of subject 4 following ingestion of NaF tablets (2 mg F) on a fasting stomach ( $\bigcirc$ ——) or together with breakfast (\*----\*).

Table 1. Net area under the plasma F-concentration curve following intake of different substances

	Substances								
Subject: Age (years):	1(f) 18	2(f) 16	3(f) 17	4(m) 18	5(m) 16	6(m) 17			
AUC									
NaF tablets (2 mg F)									
Fasting, whole Breakfast, whole	291 303	253 237	292 274	219 208	276 284	224 226			
Bone-meal tablets (1.62	(mg F)								
Fasting, whole	17.0	11.7	10.9	13.4	20.9	15.3			
Fasting, powdered	29.9	15.1	32.6	23.1	55.2	38.1			
Breakfast, whole	54.0	33.7	50.2	44.5	65.0	34.9			
Breakfast, powdered	92.3	77.3	87.0	77.0	109	52.2			
Ca tablets (1.14 mg F)									
Fasting, whole	29.3	32.8	22.9	28.3	30.6	23.7			
Fasting, powdered	—	33.1	22.0	27.0	34.8	23.5			
Breakfast, whole	49.8	54.5	31.4	45.4	47.3	30.1			
Breakfast, powdered	—	50.1	39.7	50.1	45.1	31.5			
CaF <sub>2</sub> tablets (2 mg F)									
Fasting. whole	<10	<10	<10	<10	<10	<10			
Breakfast, powdered	137		76.0	87.3	83.5	57.2			

f = female subjects; m = male subjects. — = Experiment not done. AUC = area under the plasma F-concentration-versus-time curve ( $h \times ng \times ml^{-1}$ ); fasting = substance given to fasting subjects; breakfast = substance given together with breakfast; whole = whole tablets; powdered = powdered tablets.

Table 2. Net urinary F output after administration of 2 mg F as NaF (percentage of administered dose)

Subject	1	2	3	4	5	6
Fasting stomach <sup>*</sup>	43.9 + 5.8	23.4 + 4.8	21.3 + 2.7	42.0 + 4.6	37.6 + 1.6	35.1 + 3.1
With breakfast <sup>†</sup>	39.2 + 2.7	21.0 + 1.5	21.8 + 3.6	46.1 + 4.7	33.1 + 2.8	39.6 + 3.9

Data given as  $\bar{x} \pm SD$ . \*Mean of five experiments. †Mean of three experiments.

data again showed remarkable inter-individual differences. The plasma F profile after intake of  $CaF_2$  tablets (profile not shown) corresponded to that observed after intake of Ca tablets. Ingestion of  $CaF_2$  tablets by fasting subjects caused only a negligible increase in plasma F concentrations. The resulting AUC data were less than 5 per cent of those obtained after ingestion of the same quantity of F as NaF.

The mean F availability from bone-meal tablets given to fasting subjects was only 7.2 per cent; this rose to 15.7 per cent when it was given as a fine powder. When given with food the figures were 22.6 and 39.6 per cent, respectively. All differences were significant (Table 3). This response was obtained from all but one subject who showed no difference between the experimental conditions 2 and 3 (Fig. 4).

With Ca tablets there were no differences between availability from whole or ground tablets; when taken with food, however, absorption was significantly higher than from a fasting stomach, and this pattern was found with all subjects. The large standard deviation is mainly because of interindividual variations; between subjects, the availabilities for the experimental condition 1 varied between 20 and 35 per cent, and those for experimental condition 4 between 35 and 59 per cent, respectively. The administration of  $CaF_2$  tablets to a fasting

The administration of  $CaF_2$  tablets to a fasting stomach resulted in practically no increase in plasma F-level, but when given as a powder with food between 26 and 47 per cent of the F was absorbed.

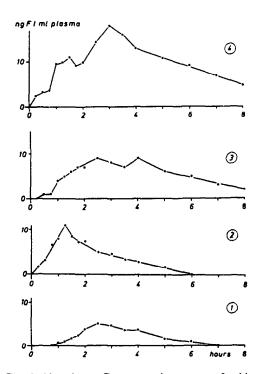


Fig. 2. Net plasma F-concentration curves of subject 4 following ingestion of bone-meal tablets (1.62 mg F). Whole tablets: fasting subject (1); with breakfast (2). Powdered tablets: fasting subject (3); with breakfast (4).

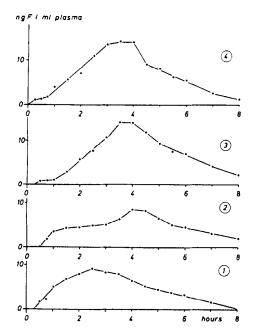


Fig. 3. Net plasma F-concentration curves of subject 4 following ingestion of Ca tablets (1.14 mg F). Whole tablets: fasting subject (1); with breakfast (2). Powdered tablets: fasting subject (3); with breakfast (4).

#### DISCUSSION

As the bioavailability of NaF in man is 100 per cent when ingested in water or tablets (Ekstrand *et* al., 1978) it is generally used as a standard for assessing relative F bioavailabilities from foods and other products (Rao, 1984). We have found identical F availabilities from NaF solution and NaF tablets (Trautner and Siebert, 1986), and so the same brand of tablets was used as a reference in this study.

The flattened plasma F curves after intake of NaF with food are similar to those observed before (Trautner and Siebert, 1986). The slow increase in plasma F after intake of the Ca-rich test substances might be because of slow liberation of F by digestive processes. The differences between subjects with respect to the AUC data might be caused by individual factors, e.g. speed and extent of F absorption is influenced by the pH of the stomach fluid (Whitford and Pashley, 1984), and urinary F clearance is also pH-dependent (Whitford, Pashley and Stringer, 1976). The relation between individual F availabilities within each experiment was fairly constant.

Several investigations of F availability from CaF, and Ca-rich substances have been made, but under different experimental conditions and with different analytical methods. Machle and Largent (1943) reported F availabilities of 62 and 37 per cent for solid CaF<sub>2</sub> or bone meal, respectively, when added to the diet of a human volunteer. Calculation of the extent of absorption was by measuring the F output in urine and faeces, and each experiment lasted for several weeks. McClure et al. (1945) found F-absorption rates from solid CaF<sub>2</sub> and bone meal of 69 and 54 per cent, respectively, when these substances were taken as part of the diet of five young men. Bone-meal and Ca-tablets were also taken unsupervised at home with meals by our subjects: the F availabilities were 54 and 65 per cent, respectively, as measured by urinary F output (Trautner and Einwag, 1986a).

Several investigations have, however, shown low or negligible F availabilities after intake of Ca-rich

percentage of fluoride available

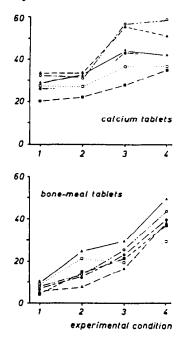


Fig. 4. Availability of F from Ca tablets and bone-meal tablets relative to that of NaF. Experimental condition: fasting stomach, whole tablet (1), powder (2), with break-fast, whole tablet (3), powder (4). Subjects:  $(\blacksquare \triangle \bigcirc)$  female;  $(\Box \triangle \bigcirc)$  male.

Table 3	. Relative bioa	vailability of F	, calculated for	r a 100 per cen	t availability	of F	from NaF tablets	

	•	-		
	Bone-meal tablets (per cent available.sign.)	Ca tablets (per cent available.sign.)	CaF <sub>2</sub> tablets (per cent available.sign.)	NaF tablets (per cent available.sign.)
Empty stomach Whole tablets Powdered tablets	$7.2 \pm 1.7$ $15.7 \pm 6.3$	27.9 ± 4.9 ]- n.s. 29.3 ± 4.8 ]	2.8 ± 2.17 n.d.	100—(reference) n.d.
With breakfast Whole tablets Powdered tablets	22.6 ± 4.5 39.6 ± 6.8 + †	42.6 ± 11.4 44.5 ± 10.1 <sup>⊥</sup> n.s.	n.d. 33.5 $\pm$ 9.6	$99 \pm 11.5$ n.s. n.d.

sign. = significance; \*p < 0.05; †p < 0.01; n.s. = not significant.

n.d. = experiment not done; data given as  $\vec{x} \pm SD$ .

substances or CaF<sub>2</sub> by fasting human subjects or animals and calculation of F absorption by measuring the plasma F profile versus time (AUC method). Patz and Fickenscher (1977) reported only slight or no increase in plasma F concentration after intake of tablets containing a mixture of F salts (0.25-1.3 mg F) and Ca salts (50-82 mg Ca). Administration of CaF<sub>2</sub> tablets to dogs (2 mg F) by Patz, Henschler and Fickenscher (1977) and to man (4 mg F) by Afseth et al. (1985) resulted in almost no increase of plasma F concentration; they concluded that: "in spite of a modest solubility (of these tablets) at pH values similar to that of the stomach fluid the degree of F-absorption after intake of CaF<sub>2</sub> tablets is poor". We too found low F availability of only 6 per cent from bone-meal tablets taken by fasting human subjects (Trautner and Siebert, 1986); we determined F availability by two different methods, the AUC and the net urinary F output. The discrepancies between these various studies might be because of differences in analytical and experimental procedures.

We obtained identical results from both the AUC and urinary-F methods after ingestion of NaF or F-containing substances by fasting subjects (Einwag and Trautner, 1984; Trautner and Einwag, 1986b; Trautner and Siebert, 1986) or when taken with food, except for substances like canned sardines or seaweed flour which gave a flattened plasma-F curve. With such substances F availability evaluated by urinary F output was 10–15 per cent higher than by the AUC method (Trautner and Siebert, 1986). This difference, however, is not sufficient to explain the discrepancies noted above.

A more important factor must be the mode of administration, i.e. whether chewed together with food or taken unchewed on a fasting stomach. When taken with food, the substance stays longer in the upper gastro-intestinal tract, when compared to the fasting stomach, and thus F might be liberated by digestion. Chewing increases the surface area and thus intensifies the action of digestive enzymes and facilitates solution of F which otherwise would be mechanically trapped in the whole tablet.

This hypothesis is strongly supported by our findings. For all products tested, F availability was lowest when the tablets were swallowed unchewed on a fasting stomach and highest when the substances were taken as a powder together with food. Thus our results correspond with those cited above and provide some explanation of their apparently contradictory findings. In some investigations (Patz and Fickenscher, 1977; Patz et al., 1977; Afseth et al., 1985; Trautner and Siebert, 1986), the substances were given to fasting subjects, similar to our experimental condition 1 or 2, and low bioavailability was observed. In others (Machle and Largent, 1943; McClure et al., 1945) and in our previous study (Trautner and Einwag, 1986a), the substances were taken together with food, similar to our experimental condition 3 or 4, and a high F availability was found.

From all available data, therefore, it must be concluded that the mode of administration of a given substance is of greater importance for F availability than is its chemical composition, especially in the presence of elements like Ca, Mg, Al etc which influence F absorption. Furthermore, our experiments show that the availability of F is best determined under conditions which simulate the normal mode of intake of the test products. Most human bioavailability studies have been carried out on fasting individuals. However, as concomitant intake of food may influence the absorption of F, the applicability of such data is questionable where substances are ingested more or less randomly in relation to food intake (Ritschel, 1984). Furthermore, our findings allow better evaluation of the contribution of food items rich in Ca and F like fish with bones (e.g. canned sardines) and meat products with bone particles (from mechanical deboning) to the dietary F provision of man. The possible high availability of F from Ca-rich products should be considered when recommending F supplements for caries prevention, at least to individuals who regularly ingest such products.

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Pedobiologia **36**, 129–135 (1992) Gustav Fischer Verlag Jena

Institute for Microbiology and Land Improvement, Justus-Liebig-Universität [Justus Liebig University], Giessen

# Ecotoxicity evaluation of different fluorides on *Eisenia fetida* (Oligochaeta, Lumbricidae)

#### JÜRGEN VOGEL and Johannes C. G. OTTOW

with 2 illustrations

#### Synopsis: Original scientific paper

[Ecotoxicity evaluation of different fluorides on Eisenia fetida (Oligochaeta, Lumbricidae)].

The toxicity of three fluorides (NaF, KF and FCH<sub>2</sub>COONa, respectively) on the earthworm *Eisenia fetida* andrei was examined using a contact filter paper test and an artificial soil test. In the contact test, NaF was found to be the most toxic compound. Toxicity decreased in the order: NaF > KF  $\ge$  FCH<sub>2</sub>COONa. In the artificial soil test however, the toxicity from most to least toxic fluoride decreased in the order: FCH<sub>2</sub>COONa  $\ge$  KF  $\ge$  NaF. Apparently, both methods evaluate different mechanisms of toxification.

In the artificial soil test, the earthworms showed a decreasing body weight with increasing contents of NaF or FCH<sub>2</sub>COONa in the substrate. Fluoride accumulation increased significantly with increasing fluoride content in substrate with all fluorides tested.

Key words: fluoride, acute toxicity, contact test, artificial soil test, earthworm, Eisenia fetida.

#### 1. Introduction

The emissions of potentially toxic fluorides in the vicinity of certain emitters (chemical and metal working industries, brickyards, etc.) can lead to a considerable burden on the environment, the effects of which on livestock, such as cattle, and plants have been known and investigated for quite some time (DRURY *et al.*, 1980). However, hardly any systematic *in-situ* experiments on loading on invertebrate soil fauna exist (BUSE, 1986; VOGEL *et al.*, 1989; VOGEL & OTTOW, 1991). Through their activity, soil fauna contribute considerably to the rapid mineralization of organic material (litter) in the soil. Over and above this, since they are links in complex food chains, fluoride accumulation in certain soil fauna such as earthworms or insect larvae could lead to the general spread of fluorides and to a possible endangering of the end units of food chains. Investigations on a longstanding (about 70 years) contaminated industrial location in southern Germany have shown that earthworms, depending on the type, were affected to varying degrees and could contain up to 700  $\mu$ g F • g<sup>-1</sup> in worms with intestinal content and up to 400  $\mu$ g F • g<sup>-1</sup> in worms without intestinal content (in dry matter) (VOGEL & OTTOW, 1991).

Basic knowledge on accumulation mechanisms and acute toxicity of various substances is indispensible for an estimation of the dangers of fluoride loading. In contrast to some heavy metals and certain environmental chemicals (HEIMBACH, 1984; NEUHAUSER *et al.*, 1985, 1986; VAN GESTEL & VAN DIS, 1988) no data on the toxic effects of fluorides on Lumbricidae can be found.

## 2. Materials and Methods

# 2.1. General

The experiments were conducted on the compost worm *Eisenia fetida andrei* BOUCHÉ, 1972, since it is well suited as a laboratory animal and additionally is recommended for toxicological tests in, among others, OECD guidelines and the Biologische Bundesanstalt [Federal Biological Center] (anonymous, 1982 and 1984). The three soluble fluorides, NaF, KF and FCH<sub>2</sub>COONa, were tested.

The worms were bred at approximately 25  $^{\circ}$ C in the dark in plastic containers in a substrate of 2.7 kg quartz sand, 300 g finely ground turf, 15 g CaCO<sub>3</sub> (Merck) and 1,700 ml distilled water. Dried and finely ground cattle dung was used *ad lib* as feed. It was ensured that the cattle had received no medication.

The  $LC_{50}$  concentrations were calculated according to the Probit Analysis and adaptation according to the Maximum Likelihood Method (WEBER, 1986). Other mathematical evaluations were done with the help of the statistical program STATGRAPHICS.

## 2.2 Contact Test

Skin contact toxicity was calculated according to OECD guidelines (anonymous, 1984; HEIMBACH, 1984). One ml of the dissolved test substance was put on a filter paper (Whatman grade 1; 9.5 cm x 6.1 cm) and introduced into 35 ml plastic tubes. Subsequently, all samples were dried at 60 °C for 24 hours and the filter paper was then moistened again with 1 ml distilled water. One worm (weight over 300 mg) was inserted per tube. The test worms were earlier kept on moist filter paper for 2 h. The tubes were closed and kept in the dark at  $20 \pm 1$  °C for 48 h and subsequently examined, whereby surviving worms were counted. Twenty repetitions were carried out per test stage.

## 2.3 Substrate Test

The acute toxicity in an artificial substrate was calculated according to OECD guidelines (anonymous, 1984; HEIMBACH, 1984). The substrate consisted of 830 g quartz sand, 100 g finely ground turf, 50 g acidic bentonite (Tonsil Standard, Süd-Chemie, Munich), 5 g finely ground cattle dung, 10 g CaCO<sub>3</sub> and 350 ml distilled water. This thoroughly mixed base substrate was added to the test substance, which had been dissolved in 200 ml distilled water and mixed again. 1,000 g of this test mixture were weighed into 21 polyethylene pots and 10 worms (weight over 400 g) were placed in each pot. The containers were then closed with a perforated lid and kept in the dark at  $20 \pm 1$  °C. The surviving worms were counted after 7, 14, 21 and 28 days, weighed and 5 g cattle dung was mixed into each sample. At least five repetitions were carried out per test stage.

## 2.4 Determination of Fluoride

For the determination of fluoride, the intestinal content of some of the worms was removed by dissection, the internals cleaned carefully with 0.25% CDTA solution (Titriplex IV, Merck) and distilled water. The worms were also cleaned externally with the same materials. Subsequently, the worms were dried at 60 °C for 72 h and finely ground to dust in an agate mortar. The samples were pyrolized and digested according to LEVAGGI *et al.* (1971) and the fluoride content measured with an F-sensitive electrode (WTW F 500) (BREIMER *et al.*, 1989; VOGEL & OTTOW, 1991a).

### 3. Results

#### 3.1 Comparison of Contact and Substrate Tests

Acute toxicity of fluorides in the contact test with *Eisenia fetida* is described in Table 1. The LC<sub>50</sub> values distinctly show the following order: NaF > KF >> FCH<sub>2</sub>COONa. The LC<sub>50</sub> values of NaF and KF show a significant difference

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 $(t = 2.80^{\circ})$ , those of FCH<sub>2</sub>COONa and NaF/KF even more significantly  $(t = 158.4^{***} \text{ for NaF}; t = 137.6^{***} \text{ for KF})$ . Apparently, Na as a cation increases the acute toxicity of fluoride, whereas the combination with acetate reduces F toxicity. The acute toxicities of the investigated fluorides in the substrate test were fundamentally different from that of the contact test. In the substrate test, FCH<sub>2</sub>COONa was the most poisonous substance, and the following order of acute toxicities was noted: FCH<sub>2</sub>COONa >> KF >> NaF (Table 1). The LC<sub>50</sub> values of all three compounds differed from each other very significantly (t =  $32.0^{***}$  for NaF/KF; t =  $48.6^{***}$  for NaF/FCH<sub>2</sub>COONa; t =  $22.1^{***}$  for KF/FCH<sub>2</sub>COONa). A pre-test with  $CaF_2$  showed no toxic effects on *E. fetida* even at concentrations of 20,000 mg F • kg<sup>-1</sup> in the substrate.

Table 1. LC50 values and 95% confidence levels of acute toxicity of NaF, KF and FCH2COONa in contact test and substrate test with E. fetida.

Substance	Contact test			Substrate test	
	mg $F \bullet 1^{-1}$	µg F • cm⁻²	95%	mg F•kg-1	95%
NaF	626	10.8	557 - 676	4,278	4,103 - 4,452
KF	693	12.0	633 - 754	1,861	1,772 - 1,951
FCH <sub>2</sub> COONa	3,568	61.6	3,529 - 3,606	978	936 - 1,020

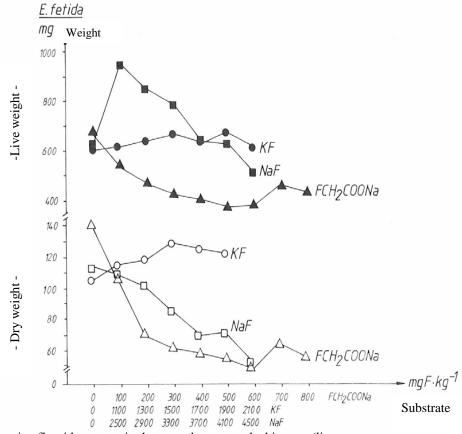


Illustration 1. Influence of increasing fluoride content in the test substrate on the biomass (live weights and dry mass) of the test worms E. fetida.

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9\*

#### 3.2. Influence of fluorides on the biomass of E. fetida

In all the variants (incl. control) of the substrate test, a weight loss in the worms was noted in the first week, subsequently a varying gain in weight.

After 28 days of the test, only the worms from the variant with KF had a homogenous live weight. The final weights of the worms from the FCH<sub>2</sub>COONa variant were very significantly lower than the control ( $F = 26.99^{**}$ ; Illustration 1). In the test with NaF, the final weights of the worms from the lower concentrations were higher than the control; those from the higher concentrations, on the other hand, were lower than the control (Illustration 1). As with the live weights, in the dry mass of *E. fetida* from the KF variant, there was also no difference between the control and load to be noted (Illustration 1). This applied to worms with and without intestinal content. On the other hand, NaF and FCH<sub>2</sub>COONa caused a very significant reduction of the dry weights of the test specimens (Illustration 1). This applied to worms with intestinal content ( $F = 15.39^{***}$  for FCH<sub>2</sub>COONa;  $F = 13.14^{***}$  for NaF; Illustration 1) as well as for those without intestinal content ( $F = 16.79^{***}$  for FCH<sub>2</sub>COONa;  $F = 10.82^{***}$  for NaF).

#### 3.3 Fluoride Accumulation in E. fetida

The fluorine content of the control worms was always significantly lower than that of the worms under experiment (Illustration 2). The accumulation factors (F content in tissue/F concentration in substrate), however, remained low (Table 2). Highly significant correlations exist between the fluoride content in the worms and the fluoride concentrations of the test substrate (Table 2). The values in the worms without intestinal content additionally show that an accumulation can take place in the tissue (Illustration 2, Table 2).

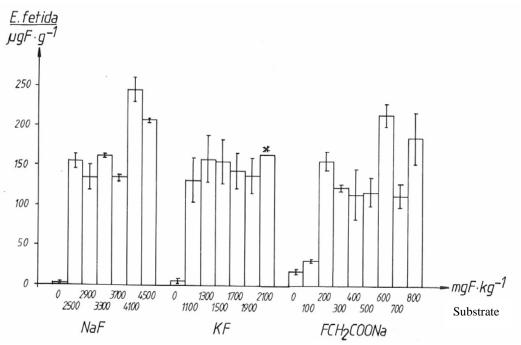


Illustration 2. Influence of increasing fluoride concentrations in the substrate on the fluoride content in the bodies (without intestinal content) of worms *E. fetida*. Per 15 worms, \* = 2 worms.

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Table 2. Correlation coefficients r and coefficient of determination B (in %) between the fluoride contents of <i>E. fetida</i> and the
fluoride contents of the test substrate and the concentration factors.

Substance	with intestinal	vith intestinal content without intestinal co		with intestinal content without intestinal content		al content	without intest. content
	r	В	r	В	Concentration factor <sup>1</sup>		
NaF	0.865***	75	0.938***	88	0.05		
KF	0.951***	90	0.619***	38	0.10		
FCH <sub>2</sub> COONa	0.935***	87	0.700***	49	0.35		

<sup>1</sup> F in tissue/F in substrate.

# 4. Discussion

In the present paper, the large differences in the LC<sub>50</sub> values between contact test and substrate test are noteworthy. In the case of NaF, the LC<sub>50</sub> values differ by a factor of 6.8. The poor conformity of the two methods has been repeatedly made known (HEIMBACH, 1984; CALLAHAN *et al.*, 1985; NEUHAUSER *et al.*, 1986; VAN GESTEL & VAN DIS, 1988). While assessing the results one has to basically keep in mind that the contact test only shows topical toxicity and additionally, the toxicity is dependent on the solubility of the test substance. Furthermore, the exposure time of 48 h is relatively short. On the other hand, in the substrate test, beside the topical toxicity, the toxicity *per os* is also determined due to the longer exposure time. Flouroacetate, which is described as extremely toxic (DRURY *et al.*, 1980), was effective only at unexpectedly high concentrations in the contact test. This could be due to the dissociation of the fluoride in FCH<sub>2</sub>COO<sup>-</sup> and Na<sup>+</sup> ions. Since the integument is less permeable for larger molecules than for smaller ones (LEE, 1985), the FCH<sub>2</sub>COO<sup>-</sup> ions would be absorbed relatively slowly by the skin. As it is, the absorption of acetate through the skin with Lumbricidae seems to be low (RICHARDS & ARME, 1980). However, the acute toxicity of the effective citrate cycle inhibitor FCH<sub>2</sub>COONa (DRURY *et al.*, 1980) shows clearly in the substrate test.

On the whole, the LC<sub>50</sub> values lie considerably higher than the values obtained in comparable experiments with various organic environmental chemicals (HEIMBACH, 1984; NEUHAUSER *et al.*, 1986). When compared to heavy metals, KF lies in the same order of magnitude as cadmium [as Cd(NO<sub>3</sub>)<sub>2</sub>], NaF is considerably more poisonous than lead [as Pb(NO<sub>3</sub>)<sub>2</sub>] (NEUHAUSER *et al.*, 1985).

A weight reduction caused by noxious substances in Lumbricidae has been repeatedly described (among others, by LOFS-HOLMIN, 1980; MA, 1984; NEUHAUSER *et al.*, 1984). In the present case, apart from the solubility, the effects seems to also be dependent on the type of fluoride compound, as can be seen in long-term experiments with sub-lethal fluoride concentrations and *E. fetida* (VOGEL & OTTOW, 1992). The weight gain found in NaF variants at low concentrations is presumably due to an increased absorption of water, as these effects did not manifest themselves with dry mass. Some fluorides, such as NaF and FCH<sub>2</sub>COONa, can also restrict the growth of Lumbricidae by accumulation in soils and organic substances and thus possibly have a negative effect on their populations.

The accumulation rate of pollutants is important for an ecotoxicity evaluation. The fluoride contents of the worms at the end of the substrate test show a distinct accumulation. "Accumulation" is interpreted here in the sense of the definition by HARTENSTEIN *et al.* (1980), according to which this is present if increased contents are found in the body after a given time or after a given enrichment in the environment. However, the concentration factors are small and appear to reduce in the substrate with increasing fluoride content. A similar reciprocal behavior in the case of

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heavy metals (HARTENSTEIN *et al.*, 1980) and the insecticide Dieldrin is also known (VENTER & REINECKE, 1987). At the maximum the fluoride concentration factors are comparable to those of lead in various types of Lumbricidae (IRELAND, 1983).

The present findings indicate that an acute danger to Lumbricidae due to fluorides can be expected only under extreme levels of pollution. Particularly in calcareous soil in which fluoride is quickly immobilized as CaF<sub>2</sub> in the topsoil (BREIMER *et al.*, 1989), acute danger would be minimal. Sub-acute fluoride effects with negative implications for growth and fertility with *E. fetida* are possible (VOGEL & OTTOW, 1992) and cannot be ruled out in the open countryside.

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# The influence of different fluorides in sub-lethal concentrations on growth, fertility and fluoride accumulation of *Eisenis fetida* (Oligochaeta, Lumbricidae)

#### JÜRGEN VOGEL and JOHANNES C. G. OTTOW

with 3 illustrations

#### Synopsis: Original scientific paper

[Influence of different fluorides in sublethal concentrations on growth, fertility and fluorideaccumulation of *Eisenia fetida* (Oligochaeta, Lumbricidae)]

The impact of four fluorides (NaF, KF,  $FCH_2COONa$  and  $CaF_2$ ) in sublethal concentrations on the earthworm E. fetida was investigated (model experiments) in relation to its growth, maturity (clitellum-development) and fertility (number of cocoons and number of hatchlings). Fluorideaccumulation was determined at the end of the 22 weeks' test period.

In higher concentrations NaF, KF and FCH<sub>2</sub>COONa reduced growth of *E. fetida* significantly.  $CaF_2$  had no effect. The maturity was delayed through higher concentrations of NaF and KF in the substrate. In the case of CaF<sub>2</sub>, most worms had a fully developed clitellum. Most cocoons were found in the experiments with FCH<sub>2</sub>COONa. Small concentrations of NaF, KF and FCH<sub>2</sub>COONa obviously raised cocoon-numbers, whereas higher concentrations of NaF and KF reduced it. Only NaF reduced the number of hatchlings per cocoon significantly. At the end of the test, all worms from the variants with NaF, KF and CaF<sub>2</sub> had a significantly higher fluoride content then the control worms.

In heavily polluted soils, fluoride may deffect the earthworm fauna by reduction of growth and maturity. Such impacts may influence nutrient-cycling by diminishing mineralisation processes through reduced earthworm activity. Since toxicity highly depends on type of fluoride salt and soil properties, hazardous effects are difficult to assess, particularly in calcareous soils.

Key words: fluoride, sublethal concentration, growth, fertility, accumulation, earthworm, Eisenia fetida

## **1. Introduction**

The immissions of potentially toxic fluorides can lead to a significant burden on the environment in the vicinity of certain emitters (e.g. brickyards, chemical industry or aluminum foundries). Whereas the negative effects on livestock (e.g. cattle) and plants have been known and investigated for quite some time (DRURY *et al.*, 1980), there have been very few experiments on the burden and endangerment of invertebrate soil fauna (BUSE, 1986; VOGEL *et. al.*, 1989; VOGEL & OTTOW, 1991).

Basic knowledge of the mechanism of accumulation and acute and sub-acute toxicities of the relevant substances is indispensible for an estimation of the danger due to fluoride contamination. Particularly the sub-acute effects of noxious agents are important, since the knowledge of these makes the assessment of long-term entry and effects of potential pollutants into the environment possible.

Whereas perceptions on the sub-acute effects of heavy metals and some environment chemicals on Lumbricidae exist (LOS-HOLMIN, 1980; MALECKI *et al.*, 1982; MA, 1984; NEUHAUSER *et al.*, 1984a), there is absolutely no corresponding knowledge on fluorides. The acute toxicities of some fluorides have been investigated in model experiments, and the results indicate that one must also expect sub-acute effects (VOGEL & OTTOW, 1992).

## 2. Materials and Methods

### 2.1. General

The experiments were conducted on the compost worm *Eisenia fetida andrei* BOUCHÉ, 1972, since it is well suited as a laboratory animal and additionally is recommended for toxicological tests in, among others, OECD guidelines (anonymous, 1984). The soluble fluorides NaF, KF and FCH<sub>2</sub>COONa and the insoluble CaF<sub>2</sub> were tested. The first three fluorides had a relatively high acute toxicity compared to CaF<sub>2</sub> (VOGEL & OTTOW, 1992).

The worms were bred at approximately 25  $^{\circ}$ C in the dark in plastic tubes in a substrate of 2.7 kg quartz sand, 300 g finely ground turf, 15 g CaCO<sub>3</sub> (Merck) and 1,700 ml distilled water. Dried and finely ground cattle dung was used *ad lib* as feed. It was ensured that the cattle had received no medication.

#### 2.2 Sub-lethal Test

The sub-lethal toxic effects of the fluorides on *E. fetida* were determined based on MALECKI *et al.* (1982) and NEUHAUSER *et al.*,(1984a). Two-week old worms with a weight of less than 10 mg were used. The four fluorides were tested in five concentrations in three parallels with four worms each; five parallels with four worms each were used as control. The substrate per sample was 100 g dried, finely ground cattle dung, brought to the optimal moisture content of 75% for *E. fetida* with 260 ml distilled water (REINECKE & VENTER, 1985a). The soluble fluorides (Merck and Riedel de Haen) were already dissolved in this distilled water. The insoluble CaF<sub>2</sub> (Riedel de Haen) was directly worked homogenously into the cattle dung. This substrate was laid onto 100 g sieved soil (TG, < 2 mm) (50% MWK; *p*H 5.0;  $F_{HCI} = 20$  mg F • kg<sup>-1</sup>; under meadow brown earth).

The experiment samples were kept in the dark for 22 weeks in plastic containers (160 mm x 85 mm x 90 mm) with perforated lids at  $20 \pm 1$  °C and inspected every 14 days, during which the weight, the development of the clitellum and the number of cocoons was noted. During the first evaluation, the dead worms were replaced by those of the same age from the same breeding sample. The cocoons collected were further bred in the dark on moist filter paper at  $20 \pm 1$  °C and the freshly hatched young were collected and counted every 2-3 days.

### 2.3. Determination of Fluoride

For the determination of fluoride, at the end of the experiment the intestinal content of some of the worms was removed by dissection, the internals cleaned carefully with 0.25% CDTA solution (Titriplex IV, Merck) and distilled water. The worms were also cleaned externally with the same materials. Subsequently, the worms were dried at 60 °C for 72 h and finely ground to dust in an agate mortar. The samples were pyrolized and digested according to LEVAGGI *et al.* (1971) and the fluoride content measured with an F-sensitive electrode (WIW F 500) (BREIMER *et al.*, 1989; VOGEL & OTTOW, 1991).

## 2.4. Evaluation

The statistical evaluation was done on a PC with the statistics program STATGRAPHICS. Single factorial variance analyses (F-test) and multiple mean value comparisons were carried out for normally distributed data; for abnormally distributed data the non-parametric tests were conducted according to Kruskal-Wallis (H-test) and Mann and Whitney (U-test) (WEBER, 1986).

#### 3. Results

#### 3.1 Growth

There was an enormous weight gain in all variants of the worms in the first six weeks of the experiment. At higher concentrations of NaF, KF and FCH<sub>2</sub>COONa,

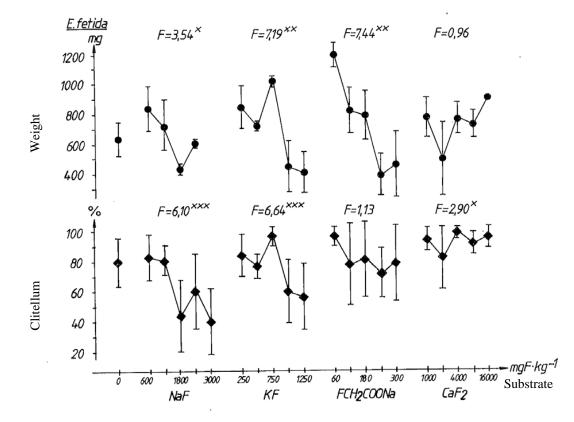


Illustration 1. Final weights (live weights) of *E. fetida* after 22 weeks of the test period and average percent of worms with clitellum. Mean values and standard deviations and F-values of the corresponding variance analyses.

the growth was, however, less than that of the control. The final weights of the worms that were subjected to higher concentrations were significantly below the final weights of the control worms, with the exception of the  $CaF_2$  variants (Table 1, Illustration 1).

	Substance			
	NaF	KF	FCH <sub>2</sub> COONa	$CaF_2$
Weight	neg. <sup>a)</sup>	neg.	neg.	none <sup>b)</sup>
	$1,800^{c}$	1,000	240	
Clitellum	neg	neg	none	pos. <sup>d)</sup>
	1,800	1,000		1,000
No. of	neg	neg	none	none
cocoons	1,800	1,000		
Hatchlings	neg	none	none	none
	600			
Fluoride	pos.	pos.	none	pos.
accumulation	600	250		1,000

Table 1. Influence of different fluorides on the growth, the clitellum development, number of cocoons and number of hatchlings of *E. fetida* as well as the fluoride accumulation at the end of the experiment.

(a) negative effect, (b) no effect, (c) fluoride concentration in mg F  $\cdot$  kg<sup>-1</sup> after which the effect is seen, (d) positive effect.

# 3.2. Sexual Maturity (Clitellum Development)

From the sixth week onwards, all variants showed *E. fetida* with sexual maturity with developed clitellum. After 10 weeks, there was minimal variation in the number of "clitellant" worms within the individual variants. In the test with CaF<sub>2</sub>, most of the worms had developed a clitellum and were just significantly higher than the control in all concentration stages (Illustration 1, Tab. 1). NaF and KF in higher concentrations (over 1,800 or 1,000 mg F • kg<sup>-1</sup>) led to a significant delay in sexual maturity (Tab. 1, Illustration 1). In the experiments with FCH<sub>2</sub>COONa, there were no differences between loading and control (Illustration 1). In the NaF variants there was a significant correlation between increasing fluoride concentration in the substrate and the decrease in the number of sexually mature worms ( $r = 0.836^*$ ).

## 3.3 Number of laid cocoons

The first cocoons were found starting the eighth week. The initially high cocoon numbers reduced with increasing experimental time. A maximum was found in the test with FCH<sub>2</sub>COONa (120 mg  $F \cdot kg^{-1}$ ) (Illustration 2). The variations in the number of cocoons within a single variant were very large, with the consequence that no statistical differences between load and control could be ensured (Illustration 2). Yet the average number of cocoons of the different test variants differ significantly (Illustration 2). Additionally, there were partially highly significant differences between the loading stages during the individual inspection times. There were no differences between control and the CaF<sub>2</sub> variants (Illustration 2, Tab. 1). Low doses of NaF (up to 1,200 mg  $F \cdot kg^{-1}$ ), KF (up to 750 mg  $F \cdot kg^{-1}$ ) and the loading with FCH<sub>2</sub>COONa apparently increase the number of laid cocoons. Higher concentrations of NaF and KF, on the other hand, lower the cocoon number (Illustration 2, Tab. 1).

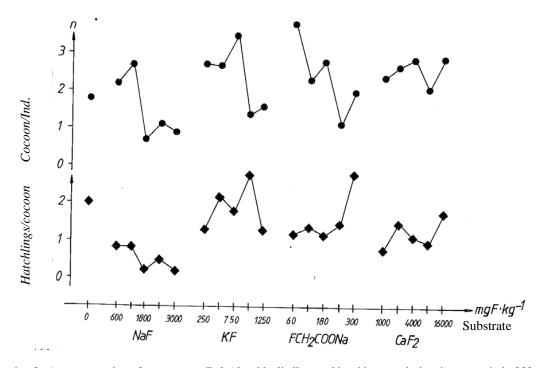


Illustration 2. Average number of cocoons per *E. fetida* with clitellum and hatching rate during the test period of 22 weeks in the four loading variants and the control.

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## 3.4. Number of hatched E. fetida per cocoon

On average, two worms hatched out of the cocoons in the control. The hatching rate was not negatively influenced by KF, FCH<sub>2</sub>COON[a] and CaF<sub>2</sub>, and no statistical difference could be noted between control and load variants (Illustration 2, Tab. 1). The loading with NaF resulted in significantly less hatched worms with increasing fluoride content in the substrate ( $r = -0.555^{***}$ ). Remarkably, many worms hatched in the variant with 300 mg F • kg<sup>-1</sup> FCH<sub>2</sub>COONa (Illustration 2).

# 3.5 Fluoride accumulation in E. fetida

The fluoride content due to NaF, KF and  $CaF_2$  in the test worms without intestinal content was significantly higher when compared to the control (Illustration 3, Tab 1). The fluoride contents in the worms increase proportionally with the fluoride

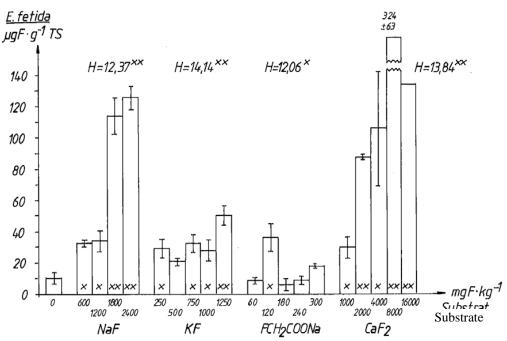


Illustration 3. Average fluoride content in *E. fetida* (without intestinal content) at the end of the experiment. Mean values and standard deviations and H values of the Krusal-Wallis test.

Table 2. Correlation coefficients r and coefficient of determination B (in %) between weight and clitellum development and between fluoride contents in *E. fetida* and the substrates as well as the concentration factors.

Substance	Weight/Clit. <sup>1)</sup> r	В	F weight/F Sub. <sup>2)</sup> r	В	Conc. Factor <sup>3)</sup>
NaF	0.976***	95.3	0.927***	85.9	0.05
KF	0.999***	99.8	0.766***	58.7	0.05
FCH <sub>2</sub> COONa	0.985***	97.0	0.026	0.1	0.11
$CaF_2$	0.871	75.9	0.653	42.6	0.03

1) Correlation weight/clitellum development, 2) correlation fluoride in worm tissue/fluoride in substrate, 3) fluoride in worm

tissue/fluoride in substrate

concentrations in the test substrate (Tab. 2). Except for FCH<sub>2</sub>COONa, the correlations between the fluoride content in the tissue of *E fetida* and in the test substrate are significant (Tab. 2). The concentration factors (concentration in tissue/concentration in substrate) remain low (Tab. 2). FCH<sub>2</sub>COONa did not influence the fluoride content of *E. fetida* in the deployed concentrations (Illustration 3).

#### 4. Discussion

The negative effect of noxious substances on the growth of Lumbricidae has been described many times (VAN RHEE, 1977; LOFS-HOLMIN, 1980; MALECKI *et al.*, 1982; HAQUE & EBING, 1983; MA, 1984; NEUHAUSER *et al.*, 1984 a & b). Thus, sub-acute effects seem to be well quantifiable from the biomass. From the very start of the experiments, differences in growth arose between the control and the higher loading stages of NaF, FCH<sub>2</sub>COONa and KF, which were sustained over the entire period of the experiment. They clearly showed the growth-restricting effect of the three soluble fluorides, the effects of which were particularly evident in the final weights. In spite of the high doses, CaF<sub>2</sub> had no influence on the biomass of *E. fetida*. These results indicate that sub-acute toxicity is dependent on the type and the solubility of the fluoride compounds, as has been already shown for heavy metals (MALECKI *et al.*, 1982; NEUHAUSER *et al.*, 1984a).

The time that the Lumbricidae take to reach sexual maturity, i.e. for development of a clitellum, is of ecological importance. A delayed clitellum development can lead to significant fluctuations in Lumbricidae populations and thus determine changes in the ecosystem (VAN RHEE, 1977; LOFS-HOLMIN, 1980; REINECKE & VENTER, 1985c). Under the present conditions NaF and KF slow down the formation of a clitellum. Both substances also lead to reduction of biomass. This finding shows that a lower growth can cause delayed sexual maturity. A similar correlation was found in the insecticide Dieldrin (REINECKE & VENTER, 1985c).

Each of the tested fluorides influenced the fertility of *E. fetida* differently. NaF and KF reduced the cocoon numbers significantly at higher doses. Why small doses of NaF, KF and FCH<sub>2</sub>COONa lead to an obvious enhancing influence on the cocoon number cannot be answered on the basis of the data available.

The hatching rate was significantly lowered only by NaF. A possible influence on the hatching rate due to exogenous factors such as temperature or type of feed (GRAFF, 1974; TSUKAMOTO & WATANABE, 1977) must be excluded in the present case. Even if the breeding on moist filter paper is not close to nature, it is still well suited for experiments in the laboratory (TSUKAMOTO & WATANABE, 1977; HARTENSTEIN *et al.*, 1979).

The fluoride contents in the worms at the end of the experiment furnish clear evidence of accumulation in *E. fetida* tissue. "Accumulation" is interpreted here in the sense of the definition by HARTENSTEIN *et al.* (1980), and is present if increased levels are found in the body after a particular time or a particular concentration in the environment. The concentration factors, however, remained low and reduced with increasing fluoride content in the substrate. Thus, for the lowest concentrations (FCH<sub>2</sub>COONa), the highest, and on the other hand, for the highest substrate concentrations (CaF<sub>2</sub>) the lowest enrichment factors were found. A similar reciprocal relationship has also been described for heavy metals, (HARTENSTEIN *et al.*, 1980), Dieldrin (VENTER & REINECKE, 1987) and for fluorides from tests for acute toxicity (VOGEL & OTTOW, 1992).

The present findings indicate that a threat to Lumbricidae in fluoride-contaminated locations can be expected only with high loadings. Since the effect of fluorides is heavily dependent on the type of compound and its solubility, the threat is minimal, particularly in calcareous soils in which the fluoride is quickly immobilized in the form of  $CaF_2$  (BREIMER *et al.*, 1989). However,  $CaF_2$  also caused histological changes in *E. fetida* (VOGEL & SEIFERT, 1992).

Since, in the present case, the sexual maturity and fertility of *E. fetida* was restricted at high loading, with high or prolonged fluoride loading, changes in the population cannot be ruled out. Since in the natural environment, the mortality of hatchlings is relatively high, fertility-reducing pollutants can eventually lead to a break-down of populations. Such influences would show their effects on the nutrient cycle, since a lowered or missing activity of earthworms can negatively influence the mineralization process. The experiments carried out can, however, only provide initial indications of processes in natural biotopes.

## 5. Acknowledgements

We wish to thank Mrs. G. WILL for her valuable technical assistance. We thank Prof. Dr. O. GRAFF (Braunschweig) for the bred samples of *E. fetida* and professional advice. The experiments were sponsored by the State of Baden-Württemberg through the Nuclear Research Center Karlsruhe, Project Water-Waste-Soil (PWAB 88066).

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# Appendix C

Illinois EPA Fluoride Water Quality Criteria Derivation (Not Confidential)

# BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:

UPDATED WATER QUALITY STANDARDS FOR BORON, FLUORIDE AND MANGANESE: PROPOSED AMENDMENTS TO 35 Ill. Adm. Code Part 302, Subparts B, C, E and F and Section 303.312 R11- 18 STATE OF ILLINOIS (Rulemaking - Water Pollution Control Board

RECE

#### NOTICE OF FILING

John Therriault, Clerk Illinois Pollution Control Board James R. Thompson Center 100 West Randolph Street, Suite 11-500-Chicago, Illinois 60601

Office of Legal Services Illinois Department of Natural Resources One Natural Resources Way Springfield, Illinois 62702-1271 Division Chief of Environmental Enforcement Office of the Attorney General 100 W. Randolph Street, Suite 1200 Chicago, Illinois 60601

PLEASE TAKE NOTICE that I have filed today with the Office of the Clerk of the

Illinois Pollution Control Board the Motion for Acceptance; Appearance; Certificate of

Origination; Statement of Reasons and Attachments; and Proposed Amendments to 35 Ill. Adm.

Code Part 302, Subparts B, C, E and F of the Illinois Environmental Protection Agency, a copy

of which is herewith served upon you.

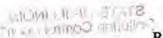
Dated:

1021 North Grand Avenue East P.O. Box 19276 Springfield, Illinois 62794-9276 (217) 782-5544 ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

Deborah iams

Assistant Counsel

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CLERK'S OFFICE

DEC 0 2 2010

STATE OF ILLINOIS

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:

UPDATED WATER QUALITY STANDARDS FOR BORON, FLUORIDE AND MANGANESE: PROPOSED AMENDMENTS TO 35 III. Adm. Code Part 302, Subparts B, C, E and F and Section 303.312 R11- 18 (Rulemaking - Water)

# MOTION FOR ACCEPTANCE

NOW COMES the Illinois Environmental Protection Agency ("Illinois EPA"), by and through its attorney, Deborah J. Williams, and pursuant to 35 Ill. Adm. Code 102.106, 102.200, and 102.202, moves that the Illinois Pollution Control Board ("Board") accept for hearing the Illinois EPA's proposal for the adoption of amendments to 35 Ill. Adm. Code Parts 301, 302, 303 and 304. This regulatory proposal includes:

- 1. Notice of Filing;
- 2. Appearance of Attorney for the Illinois Environmental Protection Agency;
- 3. Certification of Origination;
- 4. Statement of Reasons (including list of attachments and documents relied on);
- 5. Attachments to the Statement of Reasons;
- 6. Proposed Amendments;
- 7. Proof of Service;

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8. Computer disc containing Proposed Amendments.

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Respectfully Submitted,

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

Deborah J. Williams Assistant Counsel Division of Legal Counsel

Dated:

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STATE OF ILLINOIS Pollution Control Board

# BEFORE THE ILLINOIS POLLUTION CONTROL BOAREC 0 2 2010

IN THE MATTER OF:

UPDATED WATER QUALITY STANDARDS FOR BORON, FLUORIDE AND MANGANESE: PROPOSED AMENDMENTS TO 35 Ill. Adm. Code Part 302, Subparts B, C, E and F and Section 303.312 R11- (Rulemaking - Water)

# APPEARANCE

The undersigned, as one of its attorneys, hereby enters her appearance on behalf of the

Illinois Environmental Protection Agency.

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

By:

Deborah J. Williams Assistant Counsel Division of Legal Counsel

Dated:

1021 North Grand Avenue East P.O. Box 19276 Springfield, Illinois 62794-9276 (217) 782-5544

# BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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IN THE MATTER OF:

UPDATED WATER QUALITY STANDARDS FOR BORON, FLUORIDE AND MANGANESE: PROPOSED AMENDMENTS TO 35 Ill. Adm. Code Part 302, Subparts B, C, E and F and Section 303.312 R11- 18 (Rulemaking - Water)

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# CERTIFICATION OF ORIGINATION

NOW COMES the Illinois Environmental Protection Agency to certify in accordance with 35 Ill. Adm. Code. 102.202(i) that this proposal amends the most recent version of Part 302, Subparts B, C, E and F and Section 303.312 of the Pollution Control Board's regulations, as published on the Board's web site at

http://www.ipcb.state.il.us/SLR/IPCBandIEPAEnvironnlentalRegulations-Title35.asp.

Respectfully Submitted,

ILLINOIS ENVIRONMENTAL PROTECTION AGE BV

Deborah J. Williams Assistant Counsel Division of Legal Counsel

Dated:

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# BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:

UPDATED WATER QUALITY STANDARDS FOR BORON, FLUORIDE AND MANGANESE: PROPOSED AMENDMENTS TO 35 Ill. Adm. Code Part 302, Subparts B, C, E and F and Section 303.312

Pollution Control Board R11 (Rulentaking - Water)

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STATE OF ILLH

# STATEMENT OF REASONS

)

The Illinois Environmental Protection Agency ("Illinois EPA" or "Agency") hereby submits its Statement of Reasons for the above captioned rulemaking to the Illinois Pollution Control Board ("Board") pursuant to Section 27 of the Environmental Protection Act ("Act") [415 ILCS 5/27] and 35 Ill. Adm. Code 102.200 and 102.202,

#### I. INTRODUCTION AND STATUTORY AUTHORITY

Pursuant to the Federal Water Pollution Control Act (hereinafter "Clean Water Act"), it is the primary responsibility of the States to set water quality standards for intrastate waters and submit changes to those standards to U.S. EPA for approval. 33 U.S.C. §1313. Clean Water Act Section 303 provides that "the State water pollution control agency . . . shall from time to time (but at least once each three year period beginning with October 18, 1972) hold public hearings for the purpose of reviewing applicable water quality standards and, as appropriate, modifying and adopting standards." 33 U.S.C. 1313(c)(1). This requirement to periodically review and update standards is commonly referred to as the "triennial review" requirement. This proposal is a culmination of the Illinois EPA's obligation to conduct a triennial review and includes updated

In the provisions specific to protection of waters of the State, Section 13(a) of the Act

# provides that

The Board, pursuant to procedures prescribed in Title VII of this Act, may adopt regulations to promote the purposes and provisions of this Title. Without limiting the generality of this authority, such regulations may among other things prescribe: (1) Water quality standards specifying among other things, the maximum short-term and long-term concentrations of various contaminants in the waters, the minimum permissible concentrations of dissolved oxygen and other desirable matter in the waters, and the temperature of such waters; ...

# 415 ILCS 5/13(a).

The contents of this regulatory proposal are within the general substantive rulemaking authority conferred upon the Board under Sections 27 and 13(a) of the Act. This proposal is also one of general applicability pursuant to Sections 27 and 28 of the Act and Section 5-40 of the Illinois Administrative Procedure Act. 415 ILCS 5/27 and 28, 5 ILCS 100/5-40, 35 Ill. Adm. Code 102.106(a)(3) and (b)(1). In evaluating these proposed rules, the Board is required to take into account "the existing physical conditions, the character of the area involved, including the character of surrounding land uses, zoning classifications, the nature of the existing air quality, or receiving body of water, as the case may be, and the technical feasibility and economic reasonableness of measuring or reducing the particular type of pollution." 415 ILCS 5/27(a).

This Statement of Reasons will address the purpose and effect of this regulatory proposal and outline the specific amendatory language being proposed. A technical support document was prepared by the Bureau of Water in support of the proposed changes to the boron, fluoride and manganese water quality standards and is included as Attachment 1 to this Statement of Reasons.

# II. REGULATORY PROPOSAL: PURPOSE AND EFFECT

# A. History of the Existing Boron, Fluoride and Manganese water quality standards

The existing General Use and Lake Michigan Basin Standards for boron, fluoride, and manganese were adopted by the Board in its 1972 standards rulemaking establishing the initial Board water quality standards and have not been updated since that time. *See*, R71-14 (March 7, 1972). The existing General Use and non-open water Lake Michigan Basin standard for boron is 1.0 mg/L. The existing General Use and non-open water Lake Michigan Basin standard for fluoride is 1.4 mg/L. The existing General Use and non-open water Lake Michigan Basin standard for standard for manganese is 1.0 mg/L.

The Open Waters of Lake Michigan standards are based on background conditions of Lake Michigan rather than protection of human health or aquatic life. The existing manganese standard is 0.15 mg/L and will remain unchanged. Presently there are no boron or fluoride standards specifically adopted for the Open Waters of Lake Michigan, therefore the existing nonopen waters Lake Michigan Basin Standards for these substances are applicable in these waters. The Secondary Contact and Indigenous Aquatic Life standards for fluoride and manganese are 15 mg/L and 1 mg/L, respectively. No standard for this designated use currently exists for boron. At this time, the Agency intends to address all standards for Secondary Contact and Indigenous Aquatic Life Use waters in the "Use Attainability Analysis of the Des Plaines and Chicago Waterways" rulemaking. *See*, R08-09 (Sub-Docket D).

There are no existing Public and Food Processing Water Supply standards for boron or fluoride, therefore the General Use standards for these substances are applicable in these waters and are protective of Public and Food Processing Water Supply use. The existing Public and Food Processing Water Supply standard for manganese is 0.15 mg/L, which is based on aesthetics rather than human health.

## B. Purpose and Effect of the Proposal

## Boron, Fluoride and Manganese Water Quality Standards

The Agency's rulemaking proposal updates the water quality standards for boron, fluoride and manganese. Changes are proposed to the General Use standard itself as well as the to the Public and Food Processing Water Supply standards in Subpart C of Part 302 and the Lake Michigan standards in Subpart E of Part 302.

With no existing Public and Food Processing Water Supply water quality standards for boron or fluoride, the existing General Use standards for these substances are applied to these waters by default. As the Board stated in R71-14 "Since general criteria apply to all waters designated for public supply, the present regulation omits separate requirements for those parameters whose general standards are tight enough to protect public supplies; boron, chromium, copper, fluoride, mercury, silver and zinc." *See*, R71-14, March 7, 1972, Slip. Op. at 9. Since the proposed new General Use standards for boron and fluoride are higher than the

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existing standards of 1.0 mg/L and 1.4 mg/L, respectively, Illinois EPA is proposing to designate 1.0 mg/L boron and 1.4 mg/L fluoride as Public and Food Processing Water Supply standards. The proposed standards would be applied at the point of surface water intake and would be regulated as one-number, not to be exceeded standards. Because there are no specific Open Waters of Lake Michigan standards for boron and fluoride in Subtitle E, the Lake Michigan Basin standards for these substances are currently applicable. Relocating the existing Lake Michigan Basin standards of 1.0 mg/L boron and 1.4 mg/L fluoride into the Open Waters of Lake Michigan standards will provide a measure of protection against harmful loadings of these substances within these waters, and will continue to allow protection of these waters for Public and Food Processing Water Supply uses.

For manganese, the Public and Food Processing Water Supply and Open Waters of Lake Michigan standards are presently set at 0.15 mg/L. Open Waters of Lake Michigan standards are based on background conditions of Lake Michigan rather than protection of human health or aquatic life, therefore the existing manganese standard for these waters will remain unchanged.

Public and Food Processing Water Supply standards are intended to represent the maximum allowable concentration of a substance at the point of surface water intake that will allow for attainment of the finished drinking water maximum contaminant level ("MCL") for that substance following conventional treatment. As explained in the Agency's technical support document (Attachment 1, pages 9-12), the existing manganese Public and Food Processing Water Supply standard of 0.15 mg/L is overly protective of the finished manganese standard, as the finished MCL of 0.15 mg/L can easily be attained following conventional treatment of surface waters containing greater than 0.15 mg/L manganese. Because manganese often occurs in Illinois at concentrations above the existing water quality standards, the Public and Food

Processing Water Supply standard is exceeded in many surface waters with public water supply intakes and Illinois EPA has been forced to list these waters on the Clean Water Act Section 303(d) list and establish Total Maximum Daily Loads ("TMDL") unnecessarily for waters with naturally occurring sources of manganese that will be adequately addressed by conventional drinking water treatment. By conservatively estimating that 90% of manganese can be removed at conventional utilities in Illinois, and back-calculating the amount of manganese in surface waters that would still allow for attainment of the 0.15 mg/L finished MCL, it is apparent that a maximum surface water concentration of 1.5 mg/L would be sufficiently protective of the Public and Food Processing Water Supply use designation. However, in order to provide an additional measure of conservancy, the Agency is proposing to set the new manganese Public and Food Processing Water Supply standard at 1 mg/L (total manganese). The standard would be applied at the point of surface water intake and would be regulated as a one-number, not to be exceeded standard.

The proposed updates to the General Use and Lake Michigan Basin water quality standards for boron, fluoride and manganese were developed using U.S. EPA guidelines for deriving numerical water quality criteria. *See*, Attachment 1, Exhibit F. The U.S. EPA "1985 Guidelines" methodology is commonly used to derive state standards and U.S. EPA national criteria documents for substances that are toxic to aquatic life. This conventional methodology was used in deriving acute and chronic standards for boron, fluoride, and manganese. Given that fluoride and manganese toxicity is known to be influenced by the hardness of test water, standards for these substances were developed to account for hardness-dependent relationships. Literature reviews and additional laboratory tests studying the influence of water chemistry on boron toxicity had confounding results, therefore boron standards were developed independent of water chemistry.

The newly derived boron, fluoride and manganese standards were the result of collaborative work between the Agency, U.S. EPA and Dr. David Soucek of Illinois Natural History Survey (INHS). A literature review compiled by the Agency determined that insufficient data was available to derive Tier I acute and chronic standards for each substance, therefore it was necessary to conduct toxicity tests to supplement the dataset for each parameter. The Agency consulted with U.S. EPA to determine which test organisms would best fill the data gaps in order to derive fully protective aquatic life standards. U.S. EPA then contracted Great Lakes Environmental Commission (GLEC) and INHS to conduct toxicity tests on boron (acute tests using the fathead minnow Pimephales promelas (variable pH), Ceriodaphnia dubia, and the freshwater mussels Lampsilis siliquoidea, Ligumia recta, and Megalonaias nervosa; chronic test using Pimephales promelas), fluoride (acute tests using the fingernail clam Sphaerium simile and the amphipod Hydlella azteca) and manganese (acute tests using Lampsilis siliquoidea and Megalonaias nervosa). See Attachment 6. The Agency additionally contracted INHS to conduct additional toxicity tests on boron (acute tests using the stonefly Allocapnia vivipara, Sphaerium simile, Pimephales promelas, the waterflea Ceriodaphnia dubia (variable hardness and pH) and Hyalella azteca (variable hardness and pH); chronic tests using Pimephales promelas and Hyalella azteca), fluoride (acute and chronic tests using Hyalella azteca), and manganese (acute and chronic tests using Hyalella azteca). See, Attachment 1, Exhibit U.

Standards for each substance were then developed in accordance with 1985 Guidelines methodology. The following is a general overview of the 1985 Guidelines procedures used in

deriving the proposed standards. Further detail regarding the additional procedures required for deriving the hardness-based fluoride and manganese standards is provided in Attachment 1.

Only data from toxicity tests conducted on appropriate organisms using valid test methods, appropriate laboratory waters, and proper endpoints were used in deriving the proposed standards. For each substance, acute data expressed as an LC50 (concentration lethal to 50 percent of the tested organisms) was compiled for each species and was used to develop a Genus Mean Acute Value (GMAV) for each genus. The GMAVs were ranked by sensitivity and were used to develop the Final Acute Value (FAV). The FAV is the value protective of at least 95% of species at the LC50 level of effect. The FAV was then divided by 2 in order to convert the acute value from an LC50 level of protection to a level that is protective at the no observable adverse effect level.

Chronic standards for boron and fluoride were developed using the Acute-Chronic Ratio (ACR) approach, which requires ACRs from animals in at least three different families of which one species is a fish, one species is an invertebrate, and one is an acutely sensitive freshwater species. An ACR is calculated by dividing the acute LC50 of a species by the Maximum Acceptable Toxicant Concentration (MATC) of the same species derived from a test conducted in the same laboratory under test conditions identical to the acute test. The Final Acute-Chronic Ratio (FACR) was then calculated by taking the geometric mean of all available ACRs for each species. Chronic standards were then obtained by dividing the FAV of each substance by the FACR. The chronic manganese standard was not developed using the ACR approach because the resulting standard was not protective of *Hyalella azteca*, the most sensitive species. Rather, the chronic manganese standard was based off the *Hyalella azteca* MATC to afford proper protection for this organism and other untested, closely related organisms.

The procedures used by Illinois EPA in deriving acute and chronic standards for all three parameters are described in more detail in Attachment 1.

#### 2. Other Proposed Changes to Part 302 and 303

In addition to the updated water quality standards, the Agency is proposing a handful of minor amendments to Part 302.

#### a. Derived Water Quality Criteria publication requirement

In R88-21(A) the procedures in Subpart F of Part 302 for deriving site-specific water quality criteria for toxic parameters were adopted by the Board. One important procedural component of this method for establishing criteria was to require periodic public notice of the criteria that have been developed. In R97-25, parallel procedures were included in Subpart E for publication of derived criteria developed for the Lake Michigan Basin.

The Agency is required to and does publish notice of derived water quality criteria in the Illinois Register every quarter pursuant to 302.595 for Lake Michigan Basin criteria for bioaccumulative chemicals of concern and pursuant to 302.669 for all other toxicity criteria derived pursuant to Subpart F. The Agency has also maintained a list of derived criteria on its website. The Agency is proposing to simply change the required method of public notice to updating the list on its website not less frequently than quarterly, rather than requiring publication in the Illinois Register.

#### b. Correction to Error in Zinc General Use water quality standard derivation

The existing General Use chronic water quality standard for zinc is hardness-based and was adopted by the Board in the R02-11 rulemaking. See, In the Matter of Water Quality Triennial Review: Amendments to 35 Ill. Adm. Code 302.105, 302.208(e)-(g), 302.504(a),

302.575(d), 309.141(h); and Proposed 35 Ill. Adm. Code 301.267, 301.313, 301.413, 304.120, and 309.157, R02-11 (December 19, 2002). During the R02-11 proceeding, the Agency identified a number of mathematical and clerical errors in its proposal to the Board by submittal of three different Errata Sheets. See, Attachment 8. In Errata Sheet Number 3, the Agency addressed corrections to the zinc values in its original proposal that were eventually adopted by the Board. The Agency has discovered an additional error in the chronic water quality standard for zinc that was not identified in the R02-11 proceeding.

An error was made in regards to the chronic toxicity value reported by the Agency for *Hyalella azteca*. This value was taken from Table 2 of Borgmann et al. 1993 which is included as Attachment 1, Exhibit W to this Statement of Reasons. A transcription error resulted in the Agency using an incorrect value from that Table in its derivation of the chronic zinc water quality standard. An explanation of the error is provided on page 22 of Attachment 1 and both the incorrect and corrected values and equations are provided in Attachment 1, Exhibit X. Due to this change, the intercept value in the equation representing the chronic zinc standard must be modified from A = -0.8165 to A = -0.4456. The adopted chronic value for *Hyalella azteca* was erroneously calculated and resulted in a chronic zinc standard that was not representative of the true dataset and the Agency is proposing that the Board correct this error.

#### c. Elimination of STORET references

STORET is defined in Section 301.405 as "the national water quality data system of the federal Environmental Protection Agency." STORET codes, as they appear in current Board water quality standards, are no longer maintained and updated by U.S. EPA, therefore they are of little use in instructing the reader on what form of the substance is regulated. Because the STORET database is no longer being supported by U.S. EPA, the Agency is proposing to drop

10 580 STORET codes from throughout the regulations when those regulations are opened for other amendments.

#### d. Corrected cross-references

In developing these amendments, the Agency discovered a handful of typographical errors in cross references. Those incorrect or outdated cross-references were found in Sections 302.303, 302.553, 302.648, 302.657.

#### e. Language Clarification in 302.208

In addition to changes to the water quality standards in 302.208, the Agency is proposing to reorganize the language in each paragraph to more clearly identify how the acute, chronic, human health and single-value standards are interpreted. These changes generally involve splitting up the language in existing subsection (d) into the applicable language in subsections (a) through (c). In addition, language is added to subsection (d) to clarify the interpretation of the single-value standards in subsections (g) and (h). See below for the specific changes proposed.

#### f. Clarifications of references to Cyanide, Mercury, Chloride and Toluene in Tables

The Agency is proposing a handful of amendments to clarify the applicability of the water quality standards for toxic parameters. In 302.208, the Agency has proposed changing the term "metal" to "chemical constituent" to make clear that not all of the parameters regulated in that Section are metals.

For mercury and chloride, the Agency has proposed adding the phrase "(total)" following the parameter in the tables to clarify that the substance is regulated in its total form, rather than dissolved forms. For chloride, this is done to create consistency throughout the Board's water quality standard regulations. For mercury, it is done to clarify that, unlike the aquatic life standards which are based on dissolved mercury, the human health standard for mercury relies on total mercury given the potential for total mercury to become methylated and subsequently bioaccumulate in aquatic life.

The current General Use standard does not specify the form of cyanide, but it is interpreted as allowing either of two test methods for cyanide: the weak acid dissociable (WAD) form or the available form. Currently, the Lake Michigan Basin standards in Subpart E of Part 302 refer to the weak acid dissociable (WAD) form, while the total form is used in the existing Secondary Contact and Indigenous Aquatic Life standard and the effluent standard of 0.10 mg/L. Total Cyanide refers to all of the CN groups in cyanide compounds that can be determined as the cyanide ion (CN). Available cyanide consists of cyanide ion (CN), hydrogen cyanide in water (HCNao) and the cyano-complexes of zinc, copper, cadmium, mercury, nickel, and silver. Cyanide (WAD) is the hydrogen cyanide (HCN) that is liberated from a slightly acidified (pH 4.5 to 6.0) sample under the prescribed distillation conditions. Total cyanide and cyanide (WAD) are determined using standard methods, while available cyanide methods are taken from EPA-821-R-99-013 (August 1999). The Agency is proposing clarifications in both the Lake Michigan and General Use standards that clarify that the WAD and available cyanide are the two forms of cyanide tests that may be used in assessing attainment with the General Use cyanide water quality standard.

Two minor changes are proposed to the toluene standards in Part 302.Subpart E. In 302.504(a), the table mistakenly identifies the toluene standard in milligrams per liter, rather than micrograms per liter. In addition, the toluene standard in 302.504(d) is proposed for deletion because it is less stringent than the acute standard in 302.504(a) and therefore unnecessary. In R02-11, the Board updated the toluene standard in 302.504(a) to include the acute and chronic standards of 2,000 and 610 respectively. This standard was published and adopted in error in

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milligrams per liter units instead of micrograms per liter. To demonstrate that this was merely a typographical error, the Agency directs the Board to the transcript of the March 6, 2002 hearing in R02-11 where the Board questions for the Agency witnesses correctly identified the toluene standard proposed as being measured in micrograms per liter. *See*, R02-11, Hearing Transcript, March 6, 2002, pp. 104-105.

#### g. Repeal of Section 303.312

As explained in more detail below, the Agency has proposed repeal of a site-specific fluoride standard in 303.312 as obsolete and inconsistent with the new water quality standards.

#### III. REGULATORY PROPOSAL: REGULATORY LANGUAGE

The Agency is proposing additions and changes to 35 Ill. Adm. Code Part 302 and one change to Part 303. The specific Sections affected are Sections 302.208, 302.303, 302.304, 302.504, 302.553, 302.595, 302.648, 302.657, 302.669 and 303.312.

## SUBPART B: GENERAL USE WATER QUALITY STANDARDS

All of the proposed language changes in Part 302, Subpart B are contained in Section 302.208. The relevant amendments are included below for reference with the exception of the deletion of STORET numbers in the Tables.

#### Section 302.208 Numeric Standards for Chemical Constituents

- a) The acute standard (AS) for the chemical constituents listed in subsection (e) shall not be exceeded at any time except for those waters for which a zone of initial dilution (ZID) applies pursuant to Section 302.102as provided in subsection (d).
- b) The chronic standard (CS) for the chemical constituents listed in subsection (e) shall not be exceeded by the arithmetic average of at least four consecutive samples collected over any period of at least four days, except for those waters

in which the Agency has approved a mixing zone or allowed mixing pursuant to Section 302.102as provided in subsection (d). The samples used to demonstrate attainment or lack of attainment with a CS must be collected in a manner that assures an average representative of the sampling period. For the <u>chemical</u> <u>constituents metals</u> that have water quality based standards dependent upon hardness, the chronic water quality standard will be calculated according to subsection (e) using the hardness of the water body at the time the metals sample was collected. To calculate attainment status of chronic metals standards, the concentration of the <u>chemical constituent metal</u> in each sample is divided by the calculated water quality standard for the sample to determine a quotient. The water quality standard is attained if the mean of the sample quotients is less than or equal to one for the duration of the averaging period.

c) The human health standard (HHS) for the chemical constituents listed in subsection (f) shall not be exceeded when the stream flow is at or above the harmonic mean flow pursuant to Section 302.658 nor shall an annual average, based on at least eight samples, collected in a manner representative of the sampling period, exceed the HHS except for those waters in which the Agency has approved a mixing zone or allowed mixing pursuant to Section 302.102as provided in subsection (d).

- d) The standard for the chemical constituents of subsections (g) and (h) shall not be exceeded at any time except for those waters in which the Agency has approved a mixing zone or allowed mixing pursuant to Section 302.102. In waters where mixing is allowed pursuant to Section 302.102, the following apply:
  - The AS shall not be exceeded in any waters except for those waters for which the Agency has approved a zone of initial dilutions (ZID) pursuant to Section 302.102.
  - The CS shall not be exceeded outside of waters in which mixing is allowed pursuant to Section 302.102.
  - The HHS shall not be exceeded outside of waters in which mixing is allowed pursuant to Section 302.102.
- e) Numeric Water Quality Standards for the Protection of Aquatic Organisms

Constituent	STORET Number	AS (µg/L)	CS (μg/L)	
***				
Boron (total)		40,100	7,600	

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*	÷	æ	

Cyanide <u>(Weak acid</u> <u>dissociable</u> <u>or available)</u>	<del>00718</del>	22	5.2
Fluoride		eA+Bin(H)	et and the shall not exceed
(total)		where $A = 6.7319$	4.0 mg/L
		and $B = 0.5394$	where $A = 6.0445$
***			and $B = 0.5394$
Manganese		entra K 0.9812*	ex+Bln(H) X_0.9812*_
(dissolved)		where $A = 4.9187$	where $A = 4.0635$
		and $B = 0.7467$	and $B = 0.7467$
***	01090	$e^{A+Bln(H)} \ge 0.978^*,$	A+B(n(H) at a cost
Zinc	01090	е ло.970,	$e^{A+Bin(H)} \ge 0.986^*$ ,
(dissolved)		where $A = 0.9035$ and $B = 0.8473$	where $\frac{A = -0.8165}{A = -0.4456}$
			and $B = 0.8473$
***			
wh	ere: µg/L	= micrograms per li	ter
	er	= base of natural log	garithms raised to the x- power
	In (H	) = natural logarithm	of Hardness <del>(STORET 00900)</del>
	*	= conversion factor	multiplier for dissolved metals

f) Numeric Water Quality Standard for the Protection of Human Health

Constituent Number		t.	(µg/L)
Mercury (total)	71900		0.012

\*\*\*\*

g) <u>Single-value standards apply at the following concentrations for these</u> <u>substances:</u>Concentrations of the following chemical constituents shall not be exceeded except in waters for which mixing is allowed pursuant to Section 302.102.

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Constituent	Unit	STORET Number	Standard
Barium (total)	mg/L	01007	5.0
Boron (total)	mg/L	01022	-1.0
Chloride (total)	mg/L	00940	500
Fluoride	mg/L	<del>00951</del>	-1.4
Iron (dissolved)	mg/L	01046	1.0
Manganese (total)	mg/L	01055	-1.0
***			
where:	mg/L	= milligrams per	liter and
	$\mu g/L$	= micrograms pe	er liter

 Mater quality standards for sulfate are as follows: The following concentrations for sulfate must not be exceeded except in receiving waters for which mixing is allowed pursuant to Section 302.102:

\*\*\*

As explained above, the Agency is proposing to amend the language in Subsection 302.208(a), (b) and (c) to include the language from existing subsection 302.208(d) that addresses how each type of standard is applied. Subsection (d) is replaced with language from subsections (g) and (h) describing how the single-value standards are applied. This change is intended to assist the reader in understanding how each type of standard (acute, chronic, human health and single-value) will be applied.

Also in Section 302.208, the Agency is proposing to delete references to STORET numbers and to change the term "metal" to "chemical constituent" in subsection (b) for accuracy and for consistency with the other subsections. The Agency is proposing to add an "s" to milligram and microgram in the equation keys in subsections (e) and (g) and adding "of" between base and natural in the key in subsection (e). In subsection (e) the phrase "(Weak acid dissociable or available)" to the table after cyanide and "(total)" is added to mercury in subsection (f).

The Agency's proposal in Section 302.208 also corrects the error to the derivation of the chronic zinc water quality standard that was explained above. This correction of the error in the existing formula for the General Use chronic water quality standard for zinc results in a change in the equation in the Table in Section 302.208(e) from A = -0.8165 to A = -0.4456.

Finally, the outdated boron, fluoride and manganese standards are deleted from

subsection (g) and the new proposed standards are added to subsection (e).

# SUBPART C: PUBLIC AND FOOD PROCESSING WATER SUPPLY STANDARDS

The following amendments (in addition to the deletion of all STORET numbers in the

Table) are proposed for 35 Ill. Adm. Code Part 302, Subpart C, Sections 302.303 and 302.304:

# Section 302.303 Finished Water Standards

Water shall be of such quality that with treatment consisting of coagulation, sedimentation, filtration, storage and chlorination, or other equivalent treatment processes, the treated water shall meet in all respects the requirements of Part  $\underline{611604}$ . (Note: Prior to codification, Table I, Rule 304 of Ch 6: Public Water Supplies.)

#### Section 302.304 Chemical Constituents

The following levels of chemical constituents shall not be exceeded:

CONSTITUENT	STORET NUMBER	CONCENTRATION (mg/l)
***	1	100 C
Boron (total) ***		<u>1.0</u>
Chloride (total)	00940	250 <del>.</del> 1.4
Fluoride (total)		
Manganese (total)	01055	<u>1.0</u> 0.15

Nitrate-Nitrogen ***	00620	10 <del>.</del>
Sulfates	00945	250-
Total Dissolved Solids	70300	500-

In Section 303.303 the Agency is deleting a cross-reference to Part 604, which has been repealed, and replacing it with the appropriate cross-reference to the drinking water standards in Part 611. In Section 303.304, the Agency is proposing to delete all STORET numbers (even those not repeated above) and a handful of misplaced periods or decimal points. The term "(total)" is added after chloride in the table and the current General Use water quality standards for boron and fluoride are moved to this Section applicable at Public Water Supply intakes. The amended Public and Food Processing Water Supply standard for manganese of 1 mg/liter is also included.

## SUBPART E: LAKE MICHIGAN BASIN WATER QUALITY STANDARDS

The proposed changes to Subpart E are being made to 35 Ill. Adm. Code 302.504,

302.553 and 302.595. In addition to the deletion of all STORET numbers from the Tables, in

Section 302.504 the Agency proposal contains the following language:

#### Section 302.504 Chemical Constituents

The following concentrations of chemical constituents must not be exceeded, except as provided in Sections 302.102 and 302.530:

a) The following standards must be met in all waters of the Lake Michigan Basin. Acute aquatic life standards (AS) must not be exceeded at any time except for those waters for which the Agency has approved a zone of initial dilution (ZID) pursuant to Sections 302.102 and 302.530. Chronic aquatic life standards (CS) and human health standards (HHS) must not be exceeded outside of waters in which mixing is allowed pursuant to Section 302.102 and 302.530 by the arithmetic average of at least four consecutive samples collected over a period of at least four days. The samples used to demonstrate compliance with the CS or HHS must be collected in a manner which assures an average representation of the sampling period.

Constituent	STORET Number	Unit	AS	CS	HHS
***					
Boron (total)		mg/L	<u>40.1</u>	<u>7.6</u>	NA
***					
Cyanide (Weak acid dissociable <u>or</u> <u>available)</u>	<del>00718</del>	μg/L	22	5.2	NA
Fluoride (total)		µg/L	$\frac{\exp[A]}{+B\ln(H)}$	$\frac{\exp[A]}{+\operatorname{Bln}(H)]_{x}}$	NA
			$\frac{\text{where } A =}{6.7319}$	but shall not exceed 4.0	
			and $B =$	mg/L	
			0.5394	$\frac{\text{where } A =}{6.0445}$	
14				$\frac{\text{and } B}{0.5394}$	
*** <u>Manganese</u> (dissolved)		μ <u>g/</u> L	<u>exp[A</u> +Bln(H)] X	<u>exp[A</u> +Bln(H)] X	NA
			0.9812*	0.9812*	
			$\frac{\text{where } A =}{4.9187}$	$\frac{\text{where } A =}{4.0635}$	
			<u>and B =</u> 0.7467	<u>and B =</u> 0.7467	
***				200	124
Toluene	<del>78131</del>	<u>µg/Lmg</u> /L	2000	610	51.0
***					

Where:

NA = Not AppliedExp[x] = base of natural logarithms raised to the x-power ln(H) = natural logarithm of Hardness
(STORET 00900)
\* = conversion factor multiplier for dissolved metals

b) The following water quality standards must not be exceeded at any time in any waters of the Lake Michigan Basin, unless a different standard is specified under subsection (c) of this Section.

Constituent	STORET Number	Unit	Water Quality Standard
*** <del>Boron (total)</del> ***	<del>01022</del>	mg/L	1.0
Fluoride ***	<del>00951</del>	mg/L	1:4
<del>Manganese (total)</del> ***	01055	mg/L	<del>1.0</del>

c) In addition to the standards specified in subsections (a) and (b) of this Section, the following standards must not be exceeded at any time in the Open Waters of Lake Michigan as defined in Section 302.501.

Constituent	STORET Number	Unit	Water Quality Standard
*** <u>Boron (total)</u> ***		<u>mg/L</u>	<u>1.0</u>
Chloride (total)	00940	mg/L	12.0
Fluoride (total)		mg/L	<u>1.4</u>
***			
Manganese (total)	01055	mg/L	0.15

\*\*\*

d) In addition to the standards specified in subsections (a), (b) and (c) of this Section, the following human health standards (HHS) must not be exceeded in the Open Waters of Lake Michigan as defined in Section 302.501 by the arithmetic average of at least four consecutive samples collected over a period of at least four days. The samples used to demonstrate compliance with the HHS must be collected in a manner which assures an average representation of the sampling period.

Constituent	STORET Number	Unit	Water Quality Standard	
***				
Toluene	78131	mg/Ł	<del>5.60</del>	

The Agency has proposed elimination of STORET numbers throughout this Section. Subsection (a) contains the new boron, fluoride and manganese water quality standards which are in line with those proposed for General Use waters. The phrase "or available" is added after "weak acid dissociable" following the cyanide standard in subsection (a). An error in the toluene units is corrected from milligrams to micrograms in subsection (a). The outdated boron, fluoride and manganese standards are deleted from subsections (b), while the same standards for boron and fluoride are added to the Open Waters of Lake Michigan language in subsection (c). The term "(total)" is added after "chloride" in subsection (c). Finally, the duplicative and unnecessary toluene standard is deleted from subsection (d). No changes are proposed to subsection (e).

The following amendments are proposed for Section 302.553(d) and 302.595(a):

## Section 302.553 Determining the Lake Michigan Aquatic Toxicity Criteria or Values -General Procedures

The Lake Michigan Aquatic Life Criteria and Values are those concentrations or levels of a substance at which aquatic life is protected from adverse effects resulting from short or long term exposure in water.

\*\*\*\*

d) If data for acute effects are not available for all the eight families listed above, but are available for the family Daphnidae, a Tier II value shall be derived according to procedures in Section 302.563. If data for chronic effects are not available for all the eight families, but there are acute and chronic data available according to Section 302.565(b) so that three acute to chronic ratios (ACRs) can be calculated, then a Tier I chronic criterion can be derived according to procedures in Section 302.565. If three ACRs are not available, then a Tier II chronic value can be derived according to procedures in Section 302.565(be).

The cross-reference to Section 302.565(e) found in Section 302.553(d) is incorrect,

because that subsection does not exist in the Board's rules. It is being replaced with the correct

cross-reference to Section 302.565(b).

## Section 302.595 Listing of Bioaccumulative Chemicals of Concern, Derived Criteria and Values

a) The Agency shall maintain a listing of toxicity criteria and values derived pursuant to this Subpart. This list shall be made available to the public and updated periodically but no less frequently than quarterly, and <u>when updated</u> shall be published <u>on the Agency's website</u> when updated in the Illinois Register.

\*\*\*\*

The amendment to this subsection is designed to replace the duplicative effort of making

the list of derived water quality criteria available on both the Illinois EPA website and in the

Illinois Register as discussed above.

# SUBPART F: PROCEDURES FOR DETERMINING WATER QUALITY CRITERIA

In Subpart F of Part 302, the Agency is proposing changes to Sections 302.648, 302.657

and 302.669. The following changes are proposed to Section 302.648 and 302.657:

#### Section 302.648 Determining the Human Threshold Criterion

The HTC is calculated according to the equation:

\*\*\*

W = Per capita daily water consumption equal to 2 liters per day (L/d) forsurface waters at the point of intake of a public or food processing water supply,or equal to 0.01 liters per day (L/d) which represents incidental exposurethrough contact or ingestion of small volumes of water while swimming orduring other recreational activities for areas which are determined to be public access areas pursuant to Section 302.102302.201(b)(3), or 0.001 liters per day (L/d) for other General Use waters;

\*\*\*

#### Section 302.657 Determining the Human Nonthreshold Criterion

The HNC is calculated according to the equation:

\*\*\*

W = Per capita daily water consumption equal to 2 liters per day (L/d) forsurface waters at the point of intake of a public or food processing water supply,or equal to 0.01 liters per day (L/d) which represents incidental exposurethrough contact or ingestion of small volumes of water while swimming orduring other recreational activities for areas which are determined to be publicaccess areas pursuant to Section <u>302.102</u><del>302.201</del>(b)(3), or 0.001 liters per day(L/d) for other General Use waters;

\*\*\*

Both of these Sections contain a cross-reference to Section 302.201(b)(3). That referenced

provision does not exist and is being amended to the reference the correct and existing Section

302.102(b)(3). This was likely simply a typographical error in the existing rules.

The following language is proposed for Section 302.669:

#### Section 302.669 Listing of Derived Criteria

a) The Agency shall develop and maintain a listing of toxicity criteria pursuant to this Subpart. This list shall be made available to the public and updated periodically but no less frequently than quarterly, and <u>when updated shall be</u> published <u>on the Agency's website</u> when updated in the Illinois Register.

The Agency is proposing one final amendment to Part 302, which is to eliminate the

requirement in Section 302.669 to publish derived criteria quarterly in the Illinois Register and

to instead publish quarterly updates on the Illinois EPA website.

# PART 303, SUBPART C: SPECIFIC USE DESIGNATIONS AND SITE SPECIFIC WATER QUALITY STANDARDS

The Agency is also proposing one change at this time to 35 Ill. Adm. Code Part 303. This change is a repeal of Section 303.312:

#### Section 303.312 Waters Receiving Fluorspar Mine Drainage (Repealed)

- a) The fluoride standard of Section 302.208 shall not apply to waters which:
  - receive effluent from the mines and mills of the fluorspar mining and concentrating industry, and
  - 2) have been designated by the Illinois State Water Survey as streams which once in ten years have an average minimum seven day low flow of zero.

b) Such waters shall meet the following standard with regard to fluoride:

CONSTITUENT	STORET NUMBER	CONCENTRATION mg/	
Fluoride	00950	5	

This provision provided site-specific relief from the fluoride standard to two companies: Ozark-Mahoning and Minerva Oil who performed Fluorspar mining in Pope and Hardin Counties in southern Illinois. See, In the Matter of: Proposed Amendments to Rules 203 and 408 of the Illinois Water Pollution Control Regulations, R73-15 (March 6, 1975) (Attachment 4). The receiving streams impacted by discharges from these two companies are outlined in pages 3 and 4 of the Board's March 6, 1975 Opinion and Order. Both companies have ceased production and terminated their discharge permits. In fact, according to the Illinois State Geologic Survey there are currently no companies conducting fluorspar in Illinois or anywhere in the United States. See, Attachment 5. If fluorspar mining were to resume in Illinois, it is likely that such activity could comply with the new, less stringent, General Use fluoride water quality standards. If additional relief would be necessary, the Agency believes that the affected party should justify such future relief to the Board under the current science and the new, updated fluoride water quality standards.

#### IV. FACTS IN SUPPORT

The proposal before the Board relies on the technical support document prepared by Bureau of Water staff at the Illinois EPA and a variety of studies and papers cited in that report. The facts in support of this proposal are outlined in detail in Attachment 1. In particular, the Agency relied extensively on the results of tests conducted by Dr. Soucek of the Illinois Natural History Survey. Dr. Soucek's Report of the studies conducted is included this rulemaking submittal as Exhibit U to Attachment 1. The documents relied on and methods for obtaining underlying data are explained below and a comprehensive list of Exhibits and documents relied upon in developing this rulemaking proposal is provided at the end of this Statement of Reasons.

#### V. TECHNICAL FEASIBILITY AND ECONOMIC JUSTIFICATION

Section 27 of the Act requires the Board to consider the technical feasibility and economic reasonableness of all rulemaking proposals.

#### A. Technical Feasibility

Illinois EPA has investigated the treatment options for boron and fluoride as a result of the Agency's obligation to provide recommendations to the Board in response to petitions for site specific regulatory relief from these water quality standards. Both substances are highly soluble and this characteristic generally confounds attempts at treatment. Boron does not respond to the usual method of treating metals by raising pH and precipitating the metal to sludge. Fluoride likewise does not respond to this manner of treatment. The only methods of treatment identified have been reverse osmosis, which is seldom acceptable as it results in a high concentration wastewater that still must be disposed of, and various non-conventional treatment processes that are very expensive and have not seen routine use. In every case for site-specific water quality standards or adjusted standards brought before the Board, Illinois EPA has

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concluded that no reasonable treatment exists for boron and fluoride to reduce effluent concentrations. See, Attachment 1, Exhibit D.

Unlike boron and fluoride, manganese does respond to treatment by raising pH and thereby forcing precipitation. A chemical is added to a basin which raises effluent pH causing manganese to precipitate. The proposed change in the manganese water quality standard may relieve future mine outfalls from manganese treatment, however, manganese permit limits may still be dictated by 35 Ill. Adm. Code Subtitle D: Mine Related Water Pollution. Other than some coal mines, the only facilities known to treat for manganese are public water supply treatment plants that remove manganese from surface water to meet drinking water standards and then must filter or settle suspended manganese particles from the wastewater. The Agency does believe this rulemaking will result in the need to implement additional treatment technologies beyond those required by the existing regulations.

#### B. Economic Justification

In addition to technical feasibility, the Board is required to examine the economic impacts of any new technology required by this rulemaking proposal. The Agency does not expect that any of these water quality standards changes will require any new technology upgrades to achieve compliance. Although the proposal makes a number of changes to the boron, fluoride, and manganese standards applicable to the Lake Michigan Basin, Public and Food Processing and General Use water quality standards, these standards should not become more stringent than the existing standards in any waters of the State of Illinois. The only water quality standard that could become more stringent than the existing standard is in General Use waters where the ambient hardness is less than 45 milligrams per liter which would result in a chronic manganese standard of less than 1 milligram per liter. The Agency is not aware of any facilities that will be required to install upgrades to achieve compliance with this proposal. The only foreseeable exception to this will be if any of the facilities currently granted regulatory relief that is not moot as a result of this standard are unable to demonstrate that they can either meet the new standard or are no longer able to meet the standards for the grant of regulatory relief by the Board. As explained below, this is expected to be a small group of sources and the Agency hopes these sources will come forward and address their concerns as part of the rulemaking proceeding. For these reasons, the Agency's proposed changes are clearly technically feasible and economically reasonable.

## VI. AFFECTED FACILITIES AND OUTREACH

#### A. Affected Facilities

This rulemaking proposal would establish revised ambient water quality standards and does not seek to establish any specific effluent standards or other requirements targeted at specific facilities or classes of facilities. However, if a discharger in the State of Illinois has permit limits driven by water quality standards rather than or in addition to technology based limits, they could potentially be affected by one or more of the various standards being proposed.

In the case of dischargers who are currently in compliance with the existing water quality standards for boron, fluoride and manganese, there should be no impact. Illinois EPA expects that for those facilities, the applicable water quality standard is either staying the same or becoming less stringent, so there will be no impact. The only classes of facilities the Agency considers to be potentially impacted negatively by this proposal are those facilities with existing regulatory relief from the current standard or facilities that discharge to receiving waters with less than 45 mg/L hardness and have a reasonable potential to discharge greater than 1.0 milligrams per liter of manganese as a long term average. As further detailed on page 19 of

27 597 Attachment 1, critical hardness concentrations in Illinois waters are rarely less than 90 milligrams per liter and no ambient water quality monitoring network stations are known to possess a critical hardness of less than 45 milligrams per liter. *See also*, Attachment 1, Exhibit S.

A complete list of potentially affected facilities with existing regulatory relief from the current water quality standards is provided as Exhibit D to Attachment 1. This list of affected facilities and stream segments includes four facilities with fluoride relief and eight facilities with boron relief. There is also currently a site-specific rule that sets a water quality standard of 5 mg/L in waters receiving discharges from fluorspar mining activities in 303.312. That relief was originally adopted to impact two companies - Ozark-Mahoning and Minerva Oil. *See*, R73-15 (March 6, 1975). Since there is no longer any fluorspar mining in the United States and since this relief was granted thirty-five years ago, the Agency is proposing to repeal that provision at this time.

In the Board Opinion in In the Matter of: City of Galva Site Specific Water Quality Standard for Boron Discharges to Edwards River and Mud Run Creek: 35 Ill. Adm. Code

303.447 and 303.448 the Board found:

The Board notes that the record indicates the Agency is cooperating with the Illinois Natural History Survey (INHS) to generate additional boron toxicity studies to supplement the current database. Such data would help to ensure that boron general use standards proposed in the future would be protective of aquatic life. The results of the Agency/INHS study is expected to bolster the scientific justification for the revision of the general use boron water quality standard. If the Agency/INHS study results in new boron toxicity information that raises any concerns with the site specific standards or renders such standards as moot, the Board expects the Agency to address those concerns as part of its proposal to revise the general use standards. The Board notes that in the past, the Board has revised existing site specific rules to make them consistent with the adopted revisions to the rule of general applicability. See Proposed New and Updated Rules for Measurement and Numerical Sound Emissions Standards Amendments to 35 Ill, Adm. Code 901 and 910, (R03-9) March 2, 2006.

See, R09-11 (August 6, 2009). See also, In the Matter of: Proposed Site Specific Rule for City of Springfield, Illinois, Office of Public Utilities, City, Water, Light and Power and Springfield Metro Sanitary District from 35 Ill. Adm. Code 302.208(g): New 35 Ill. Adm. Code 303.446, -R09-8 (May 21, 2009).

Of the facilities with fluoride regulatory relief granted by the Board, there are none that have relief that would exceed the proposed acute standard. However, the Agency also had to consider whether any of the affected facilities would exceed the proposed chronic standard.

The relief granted to Granite City Steel in *In the Matter of: Granite City Division of National Steel Petition for Adjusted Standard from 35 III. Adm. Code 302.208: Numeric Standard for Fluoride*, AS 90-4 (April 8, 1993) should become moot because the chronic fluoride standard will be the same as the never to be exceeded standard granted in Horseshoe Lake. Based information contained in Discharge Monitoring Reports, it appears that the fluoride relief granted to Modine Manufacturing in *In the Matter of: Site-Specific Limitation for the Modine Manufacturing Company Facility, Ringwood, Illinois*, R87-36 (May 24, 1990) and to the City of Effingham in *In the Matter of Site Specific Rule for City of Effingham Treatment Plant Fluoride Discharge, 35 III. Adm. Code 304.233*, R03-11 (December 18, 2003) should no longer be necessary.<sup>1</sup> For Modine Manufacturing, the company's Discharge Monitoring Reports show that the facility no longer has elevated fluoride levels in its discharge, so the relief granted by the Board in R87-36 may no longer be necessary. For the City of Effingham, the Discharge Monitoring Reports show that the bighest fluoride value reported since July of 2005 is 4.0 mg/L.

<sup>&</sup>lt;sup>1</sup> The fluoride relief granted to the City of Effingham required compliance with a 2.0 mg/L water quality standard at the City of Flora's public water supply intake. That relief, as written, would have caused the Agency's proposed Public and Food Processing Water Supply standard to be exceeded. However, since the Board opinion was issued in R03-11, the City of Flora has connected to the Gateway Regional Water Supply System and no longer has a surface water intake in the Little Wabash River so compliance with the proposed new Public and Food Processing Water Supply fluoride water quality standard of 1.4 mg/L will not be a problem.

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Based on this information, it appears that Effingham would not need regulatory relief in order to comply with the proposed chronic fluoride standard of 4.0 mg/L as a monthly average.

General Motors is the only facility granted regulatory relief by the Board from the fluoride water quality standard that the Agency has identified will still need the Board relief upon adoption of the Agency's fluoride proposal. See, In the Matter of: Petition of General Motors Corporation to Amend 35 Ill. Adm. Code 303.222 (Site Specific Regulation for Fluoride), R93-13 (January 11, 1995) and Attachment I, Exhibit D.

For the site-specific regulatory relief from the boron water quality standards, none of the dischargers would cause an exceedance of the proposed acute boron standard of 40.1 mg/L. As with fluoride, the Agency investigated whether the chronic standard of 7.6 mg/L would be met in all cases.

The following three facilities have relief from the boron standard that will clearly become moot upon adoption of the Agency's proposal: City of Galva (Northeast STP)(*In the Matter of: City of Galva Site Specific Water Quality Standard for Boron Discharges to Edwards River and Mud Run Creek: 35 III. Adm. Code 303.447 and 303.448*, R09-11 (August 6, 2009)), Akzo Nobel (*In the Matter of: Petition of Akzo Chemicals, Inc. for an Adjusted Standard from 35 III. Adm. Code 304.105 and 302.208*, AS93-8 (September 1, 1994)) and CILCO (Duck Creek)(*In the Matter of: Petition of Central Illinois Light Company (Duck Creek Station) for Adjusted Standard from 35 III. Adm. Code 302.208 and 35 III. Adm. Code 304.105 Regarding the Parameter Boron*, AS96-8 (June 20, 1996)). These standards will become moot because the never-to-be-exceeded relief granted by the Board in these proceedings is lower than the new chronic standards proposed by the Agency.

Review of the relief granted and the Discharge Monitoring Reports and discussions with interested parties has led the Agency to conclude that the chronic standard will be consistently met and therefore the boron relief granted by the Board should no longer be needed for four of the remaining five facilities. These facilities are City of Springfield, Spring Creek STP; Dynegy Baldwin Station (Illinois Power); Southern Illinois Power Cooperative (SIPC); and Dynegy Midwest Generation - Wood River Station (Illinois Power). See, In the Matter of: Proposed Site Specific Rule for City of Springfield, Illinois, Office of Public Utilities, City, Water, Light and Power and Springfield Metro Sanitary District from 35 Ill. Adm. Code 302.208(g): New 35 Ill. Adm. Code 303.446, R09-8 (May 21, 2009); In the Matter of: Petition of Illinois Power Company (Baldwin Power Plant) for Adjusted Standard from 35 Ill. Adm. Code 302.208 and 35 Ill. Adm. Code 304.105 Regarding the Parameter Boron, AS96-1 (Mary 2, 1996); In the Matter of: Petition of South Illinois Power Cooperative (Marion Power) for Adjusted Standard from 35 Ill. Adm. Code 302.208(e), AS92-10 (July 1, 1993); and In the Matter of: The Proposed Amendment to Rule 203 of the Water Pollution Regulations (R76-18)(May 25, 1978). While there was initially a potential that relief granted to these facilities could have resulted in exceedance of the chronic boron water quality standard in one of the impacted stream segments, further investigation revealed that Board relief from the new chronic standard would no longer be necessary for these facilities.

Based on the Agency's initial investigations, it appears that the boron relief granted by the Board will still be necessary for at least one of the identified segments for one of the affected facilities. This facility is Springfield City Water Light and Power and the impacted segment is Sugar Creek from Spaulding Dam to Sewage Treatment Plant only. See, *In the Matter of*: Petition of the City of Springfield, Office of Public Utilities for an Adjusted Standard from 35 Ill. Adm. Code 302.208(e), AS94-9 (December 1, 1994).

In addition, there are several classes of facilities that have the potential to benefit from this proposal. Dischargers to streams with Public and Food Processing Water Supply intakes may benefit from removal of some streams from the 303(d) List for manganese. It is also possible that coal mines and other industrial or municipal dischargers with water quality based effluent limits may benefit from the new General Use standards for boron, fluoride and manganese. With regard to the proposed correction to the zinc water quality standard, it is possible that correction of this error will benefit some facilities that are currently having difficulty meeting their permit limits. The Agency has identified all facilities in the State with permit limits for zinc and has included that list of potentially impacted facilities at Attachment 7 to this Statement of Reasons.

#### B. Outreach

Illinois EPA shared a draft rulemaking proposal with approximately 120 stakeholders on September 17, 2009. These stakeholders included representatives of state and federal government agencies, universities, environmental groups, industrial dischargers, municipal dischargers, trade associations and consulting engineers.

A meeting was held on October 19, 2009 at the Illinois EPA Headquarters in Springfield to explain the draft proposal and respond to any questions or comments. Approximately 25 stakeholder representatives attended. The Agency made presentations on the different components of the draft proposal and answered questions on the presentations. The Agency also distributed copies of the various presentations following the meeting. The Agenda and Sign In list from the stakeholder meeting are included as Attachments 2 and 3 to this Statement of Reasons.

The Agency accepted written comments from the stakeholders following the meeting. Comments were received from the Springfield Metropolitan Sanitary District and the Illinois Environmental Regulatory Group.

Follow-up emails were sent to the stakeholders on July 8, 2010 and November 10, 2010. These emails updated the stakeholders on changes to the proposal as a result of additional tests and information becoming available and the Agency's progress and timeline towards filing this proposal with the Board.

#### VU. SYNOPSIS OF TESTIMONY

Pre-filed Testimony will be submitted by two Illinois EPA witnesses, Bob Mosher and Brian Koch.

#### A. Bob Mosher, Manager, Water Quality Standards Unit, Division of Water Pollution Control, Bureau of Water, Illinois EPA

Mr. Mosher will present testimony on the background and history of the current General Use, Lake Michigan Basin and Public and Food Processing Water Supply water quality standards for boron, fluoride and manganese. He will also present testimony on the proposed change to the derived water quality criteria publication provision and the additional nonsubstantive updates to the regulatory language in Part 302. Mr. Mosher will also be available to answer general questions on the water quality standards program and the triennial review process.

#### B. Brian Koch, Environmental Protection Specialist, Water Quality Standards Unit, Division of Water Pollution Control, Bureau of Water, Illinois EPA

Mr. Koch will present technical testimony regarding the development of the proposed changes to the boron, fluoride and manganese General Use, Lake Michigan Basin and Public and Food Processing Water Supply water quality standards. He will testify about the literature surveyed and new toxicity tests performed in support of this water quality standard proposal to the Board. He will be available to answer technical questions regarding the toxicity of boron, fluoride and manganese to aquatic life and the water quality standard derivation process for these parameters. Mr. Koch will also explain and answer questions related to the error discovered by the Agency in the derivation of the zinc water quality standard and the correction of that error in this proceeding.

#### C. Testimony in Support of the Agency's proposal

At this time, Mr. Mosher and Mr. Koch are the only anticipated witnesses in support of this rulemaking proposal that Illinois EPA intends to call to provide testimony. Both witnesses are expected to submit Pre-filed Testimony to the Board as directed by the Hearing Officer. The Agency also reserves the right to submit testimony from additional witnesses if necessary to address any questions or concerns raised by the public or the Board with respect to this proposal and to have additional Agency staff present at the Board hearings on this proposal to answer unforeseen questions that may arise.

#### VIII. SUPPORTING DOCUMENTATION

#### A. Statement Regarding Compliance with 5 ILCS 100/5-40(3.5)

Pursuant to the Illinois Administrative Procedure Act, the Board's procedural rules provide that rulemaking proponents must submit to the Board "A descriptive title or other description of any published study or research report used in developing the rule, the identity of the person who performed such study, and a description of where the public may obtain a copy

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of any such study or research report. If the study was performed by an agency or by a person or entity that contracted with the agency for the performance of the study, the agency shall also make copies of the underlying data available to members of the public upon request if the data are not protected from disclosure under the Freedom of Information Act [5ILCS 140]. [5 ILCS 100/5-40(3.5)]." 35 Ill. Adm. Code 102.202(e).

To assist the Board in compliance with these requirements, the Agency has attempted to file as Attachments to this proposal the bulk of the information relied on in developing this proposal to the Board. See Section B below for the List of Attachments that provides the relevant identifying information for these Attachments. In addition, the Agency has provided a second list in Section C below of documents relied upon, but not submitted to the Board as Attachments to this rulemaking proposal. Many of these documents are U.S. EPA guidance documents and Board opinions that are readily accessible by the Board and the public.

With regard to studies conducted by the Agency or by an entity that contracted with the Agency for performance of the study, the Agency has provided summaries of the underlying data from those studies as Attachments to the Statement of Reasons and Technical Support Document. To the extent that the Agency relied on studies with voluminous amounts of raw data or documents that are subject to copyright protection, the Agency will make such underlying data and supporting documents available to members of the public at the Illinois EPA Library which is located at the Agency Headquarters at the following address:

> Illinois Environmental Protection Agency 1021 North Grand Avenue East P.O. Box 19276 Springfield, Illinois 62794-9276

The studies relied on in developing these proposals which are summarized, but not attached are identified both in the list of references in Attachment 1 and in Subsection C below.

#### B. List of Attachments

Attachment 1 – Facts in Support of Changing Water Quality Standards for Boron, Fluoride, and Manganese (Illinois EPA, Bureau of Water, 2010)

Exhibit A - Water Quality Criteria (Boron), McKee and Wolf (1963)

Exhibit B – Water Quality Criteria (Fluoride) McKee and Wolf (1963)

Exhibit C - Water Quality Criteria (Manganese) McKee and Wolf (1963)

Exhibit D - Site-specific relief granted by the IPCB for boron and fluoride to date

- Exhibit E Manganese removal estimations at conventional utilities located on impaired Public and Food Processing water Supply waters with Mn exceeding 150 ug/L
- Exhibit F Guidelines for deriving numerical National Water Quality Criteria for the protection of aquatic organisms and their uses

Exhibit G - Acute Toxicity Data used in Boron Standard Derivation

Exhibit H - Chronic Toxicity in Boron Standard Derivation

Exhibit I - Boron Standard Derivation using 1985 Guidelines Methodology

Exhibit J – Influence of hardness and pH on boron toxicity

Exhibit K - Fluoride Standard Derivation Using 1985 Guidelines Methodology

- Exhibit L Manganese Standard Derivation Using 1985 Guidelines Methodology
- Exhibit M Acute and chronic fluoride standards at variable hardness using 1985 Guidelines Methodology
- Exhibit N Acute and chronic manganese standards at variable hardness using 1985 Guidelines Methodology
- Exhibit O Acute toxicity data used in fluoride Standard Derivation
- Exhibit P Chronic toxicity data used in fluoride Standard Derivation
- Exhibit Q Acute toxicity used in manganese Standards Derivation
- Exhibit R Chronic toxicity data used in manganese Standard Derivation
- Exhibit S Ambient Water Quality Monitoring Network (AWQMN)
- Exhibit T Calculation of the conversion factor multiplier for manganese standards derived from total and dissolved manganese data collected during the chronic *Hyalella azteca* test. For each treatment, the filtered (dissolved) results were divided by the unfiltered (total) results to calculate the percent of dissolved manganese
- Exhibit U Final Report, Acute and Chronic Toxicity of Boron, Fluoride, and Manganese to Freshwater Organisms, by David J. Soucek and Amy Dickinson, Illinois Natural History Survey, University of Illinois, October 14, 2010

Exhibit V - Excerpts from Exhibit S to Agency Rulemaking Proposal in R02-11

Exhibit W – Accumulation, regulation and toxicity of copper, zinc, lead and mercury in Hyalella azteca, U. Borgmann, W.P. Norwood & C. Clarke, Hydrobiologia, 259: 79 – 89 (1993)

Exhibit X: Revised chronic zinc standard using the corrected Hyalella azteca MATC

Attachment 2 - Water Quality Standards Stakeholders Meeting Agenda, dated October 19, 2009

Attachment 3 - Water Quality Standards Stakeholders Meeting Sign in list, dated October 19, 2009

- Attachment 4 Opinion and Order of the Illinois Pollution Control Board, In the Matter of: Proposed Amendments to Rules 203 and 408 of the Illinois Water Pollution Control Regulations, R73-15 (March 6, 1975)
- Attachment 5 Information from the Illinois State Geological Survey
- Attachment 6 Great Lakes Environmental Commission Final Report (October 22, 2010) (excerpts pertaining to boron, manganese and fluoride tests only)
- Attachment 7 Facilities with NPDES Permit Limits Based on the Incorrect Chronic Standard for Zinc

Attachment 8 - Agency Errata Sheets 1, 2 and 3 from R02-11

# C. List of Documents Relied Upon But Not Attached

#### **Guidance** Documents

Method OIA-1677 Available Cyanide by Flow Injection, Ligand Exchange, and Amperometry, 821-R-99-013, United States Environmental Protection Agency (August, 1999).

Standard Methods for the Examination of Water and Wastewater: Centennial Edition. 21st Edition. Eaton, AD, LS Clesceri, EW Rice, AE Greenberg, and MAH Franson (editors). ISBN: 0875530478. American Public Health Association. 2005. Washington, D.C.

#### Pollution Control Board Opinions: Rulemakings of General Applicability

In the Matter of: Water Quality Triennial Review: Amendments to 35 Adm. Code 302.105, 302.208(e)-(g), 302.504(a), 302.575(d), 309.141(h); and Proposed 35 Ill. Adm. Code 301.267, 301.313, 301.413, 304.120, and 309.157, R02-11 (December 19, 2002).

In the Matter of: Conforming Amendments for the Great Lakes Initiative: 35 Ill. Adm. Code Part 302,101; 302.105; 302.Subpart E; 303.443, and 304.222, R97-25 (

In the Matter of: Proposed Amendments to Title 35, Subtitle C (Toxins Control), R88-21 – Docket A (January 25, 1990).

In the Matter of: Water Quality Standards Revisions, R71-14 (Consolidated with R70-8 and R71-20) (March 7, 1972).

Pollution Control Board Opinions: Site Specific Rulemakings and Adjusted Standards

#### Boron

In the Matter of: City of Galva Site Specific Water Quality Standard for Boron Discharges to Edwards River and Mud Run Creek: 35 Ill. Adm. Code 303.447 and 303.448, R09-11 (August 6, 2009).

In the Matter of: Proposed Site Specific Rule for City of Springfield, Illinois, Office of Public Utilities, City, Water, Light and Power and Springfield Metro Sanitary District from 35 Ill. Adm. Code 302.208(g): New 35 Ill. Adm. Code 303.446, R09-8 (May 21, 2009).

In the Matter of: Petition of Central Illinois Light Company (Duck Creek Station) for Adjusted Standard from 35 Ill. Adm. Code 302,208 and 35 Ill. Adm. Code 304.105 Regarding the Parameter Boron, AS96-8 (June 20, 1996).

In the Matter of: Petition of Illinois Power Company (Baldwin Power Plant) for Adjusted Standard from 35 Ill. Adm. Code 302.208 and 35 Ill. Adm. Code 304.105 Regarding the Parameter Boron, AS96-1 (May 2, 1996)).

In the Matter of: Petition of the City of Springfield, Office of Public Utilities for an Adjusted Standard from 35 III. Adm. Code 302.208(e), AS94-9 (December 1, 1994).

In the Matter of: Petition of Akzo Chemicals, Inc. for an Adjusted Standard from 35 Ill. Adm. Code 304.105 and 302.208, AS93-8 (September 1, 1994).

In the Matter of: Petition of South Illinois Power Cooperative (Marion Power) for Adjusted Standard from 35 Ill. Adm. Code 302.208(e), AS92-10 (July 1, 1993).

In the Matter of: The Proposed Amendment to Rule 203 of the Water Pollution Regulations, R76-18 (May 25, 1978)(Illinois Power Wood River Station).

#### Fluoride

In the Matter of: Granite City Division of National Steel Petition for Adjusted Standard from 35 Ill. Adm. Code 302.208: Numeric Standard for Fluoride, AS 90-4 (April 8, 1993).

In the Matter of: Petition of General Motors Corporation to Amend 35 Ill. Adm. Code 303.222 (Site Specific Regulation for Fluoride), R93-13 (January 11, 1995).

In the Matter of: Site-Specific Limitation for the Modine Manufacturing Company Facility, Ringwood, Illinois, R87-36 (May 24, 1990)

In the Matter of Site Specific Rule for City of Effingham Treatment Plant Fluoride Discharge, 35 Ill. Adm. Code 304.233, R03-11 (December 18, 2003).

# Toxicity Studies and Data used in Derivation of Proposed Water Quality standards and summarized in Attachment 1, Exhibits G, H, O, P, Q and R:

Beleau, MH and JA Bartosz. 1982. Acute toxicity of selected chemicals: data base. U.S. Fish and Wildlife Service, Colorado River Fishery Project, Report No. 6. Salt Lake City, Utah. 3:242-254.

Biesinger, KE and GM Christensen. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. Journal of the Fisheries Research Board of Canada 29:1691-1700.

Buikema, AL, CL See, and J Cairns, Jr. 1977. Rotifer sensitivity to combinations of inorganic water pollutants. OWRT Project A-071-VA. Virginia Water Resources Research Center Bulletin No. 92. Blackburg, VA; 42 p.

Calleja, MC, G Persoone, and P Geladi. 1994. Comparative acute toxicity of the first 50 multicentre evaluation of *in vitro* cytotoxicity chemicals to aquatic non-vertebrates. Archives of Environmental Contamination and Toxicology 26:69-78.

Camargo, JA and JV Tarazona. 1990. Acute toxicity to freshwater benthic macroinvertebrates of fluoride ion (F-) in soft water. Bulletin of Environmental Contamination and Toxicology 45:883-887.

Camargo, JA and JV Tarazona. 1991. Short-term toxicity of fluoride ion (F-) in soft water to rainbow trout (Salmo gairdneri) and brown trout (Salmo trutta fario). Fluoride 24(2):76-83.

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Respectfully Submitted,

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## ATTACHMENT 1

Facts in Support of Changing Water Quality Standards for Boron, Fluoride, and Manganese

### Facts in Support of Changing Water Quality Standards for Boron, Fluoride, and Manganese

#### I. Background

#### Boron.

Boron is a naturally occurring metalloid element that is only found in the environment in a combined form, usually as borax or boric acid. The 21<sup>st</sup> Edition of <u>Standard Methods</u> for the Examination of Water and Wastewater (2005) gives the following account for boron:

Boron (B) is the first element in Group IIIA of the periodic table; it has an atomic number of 5, and atomic weight of 10.81, and a valence of 3. The average abundance of B in the earth's crust is 9 ppm; in soils it is 18-63 ppm; in streams it is 10  $\mu$ g/L; and in groundwater it is 0.01 to 10 mg/L. The most important mineral is borax, which is used in the preparation of heat-resistant glasses, detergents, porcelain enamels, fertilizers, and fiberglass.

The most common form of boron in natural water is H<sub>3</sub>BO<sub>3</sub>. Although boron is an element essential for plant growth, in excess of 2.0 mg/L in irrigation water, it is deleterious to certain plants and some plants may be affected adversely by concentrations as low as 1.0 mg/L (or even less in commercial greenhouses). Drinking waters rarely contain more than 1 mg B/L and generally less than 0.1 mg/L, concentrations considered innocuous for human consumption. Seawater contains approximately 5 mg B/L and this element is found in saline estuaries in association with other seawater salts.

Boron is naturally present in fruits and vegetables and is nutritionally important in the human diet (Murray 1995). It has been well established that boron is an essential micronutrient for plants, and there is also a growing body of evidence that suggests boron may be essential for early development of frogs and fish. The dose-response curve for boron exposure to rainbow trout, zebrafish, and African clawed frogs has been characterized as U-shaped (Eckhert 1998, Rowe et al. 1998, Fort et al. 1999), consistent with the distinguishing shape of an essential micronutrient. A U-shaped dose-response curve is characterized by adverse effects at extremely low concentrations, stimulated growth and/or survival at intermediate concentrations, and adverse effects at higher concentrations. Adverse effects at extremely low concentrations result from deficiencies of the substance, while at higher concentrations a toxic threshold is eventually reached. For example, Eckhert (1998) found that growth of rainbow trout embryo-larvae chronically exposed to <0.11 mg/L boron, with greatest growth occurring at the 10.1 mg/L boron treatment. In a similar chronic study by Rowe et al. (1998), embryo-larval rainbow

trout were exposed to higher boron concentrations, with only the highest treatment (108.1 mg/L boron) resulting in adverse effects on survival.

Sources of boron in Illinois waters include domestic wastewaters that contain boron from detergent boosters. Treated municipal sewage typically contains about 0.5 mg/L boron. Coal ash is another important source of boron. Coal ash ponds may contain boron concentrations approaching 20 mg/L. Some effluents from air emission control systems at coal-fired power plants in Illinois have boron concentrations in the hundreds of mg/L. Another minor source of boron is from certain discharges from nuclear power plants where boron is used in reactivity control in nuclear reactors. Given the high solubility of boron and its resistance to treatment technologies that are employed for metals, treatment to remove boron in any of these sources is non-existent.

Boron is naturally occurring in soils and is an essential micronutrient for plants. Boron can also be toxic to plants and a fairly narrow range of concentration exists between the required amount and detrimental amounts. Some groundwaters in Illinois have significant boron concentrations that approach the current surface water standard. These are believed to be natural sources.

The Illinois EPA's Ambient Water Quality Monitoring Network (AWQMN) historically has gathered chemical and physical water quality data from over 200 established stream stations across the State. Nine collections are made per year going back in many cases over a thirty year period. While this monitoring network has been cut back in recent years, a good understanding of the distribution of boron in Illinois waters exists. Waters that have no point sources of boron, such as sewage treatment plant effluents, generally have boron concentrations of between 0.01 and 0.05 mg/L boron. Both total and dissolved boron are measured in the network, but nowhere is there a large difference in the values given the high solubility of boron. The Illinois River, which carries the vast majority of treated sewage effluent in the State, as well as some of the coal ash pond discharges, has an average concentration of almost 0.2 mg/L at low river flows when the boron contributions from point sources are most prevalent. The highest boron concentrations are found in streams that receive coal-fired power plant effluents. Sugar Creek at Springfield, a stream with a natural 7Q10 flow of zero, has boron concentrations up to 17 mg/L. Little Saline Creek in southern Illinois at times will have a concentration of 9 mg/L. Highly urbanized streams in NE Illinois receiving most of their flow from sewage treatment plants have the highest boron concentrations apart from the receiving streams for coal ash ponds. Addison Creek in Cook County averages about 0.5 mg/L with high values up to 0.9 mg/L (http://www.epa.gov/storet/dbtop.html).

#### Fluoride.

The Second Edition of <u>Water Quality Criteria</u> by McKee and Wolf (1963) gives the following account for fluoride:

As the most reactive non-metal, fluorine is never found free in nature but it is a constituent of fluorite or fluorspar, calcium fluoride, in sedimentary rocks and also of cryolite, sodium aluminum fluoride, in igneous rocks. Owing to their origin only in certain types of rocks and only in a few regions, fluorides in high concentrations are not a common constituent of natural surface waters, but they may occur in detrimental concentrations in ground waters.

Fluorides are used as insecticides, for disinfecting brewery apparatus, as a flux in the manufacture of steel, for preserving wood and mucilages, for the manufacture of glass and enamels, in chemical industries, for water treatment, and for other minor uses. While not normally found in industrial wastes, they may be present in traces or in higher concentrations resulting from spillage.

Additionally, the 21<sup>st</sup> Edition of <u>Standard Methods for the Examination of Water and</u> <u>Wastewater</u> (2005) gives the following account regarding the benefits of fluoridated drinking water:

A fluoride concentration of approximately 1.0 mg/L in drinking water effectively reduces dental caries without harmful effects on health. Fluoride may occur naturally in water or it may be added in controlled amounts. Some fluorosis may occur when the fluoride level exceeds the recommended limits. In rare instances the naturally occurring fluoride concentration may approach 10 mg/L; such waters should be defluoridated.

Accurate determination of fluoride has increased importance with the growth of the practice of fluoridation of water supplies as a public health measure. Maintenance of an optimal fluoride concentration is essential in maintaining effectiveness and safety of the fluoridation procedure.

In Illinois, public water utilities are required to fluoridate between 0.9 and 1.2 mg/L for human health benefits. Sewage treatment plants discharge fluoridated water, and this is the largest source of human-sourced fluoride in Illinois. Other sources include steel manufacturers due to the use of fluoride in their process. Fluoride can also enter surface waters in higher concentrations through the discharge of cooling tower blowdown in which fluoridated city water which has been recycled and subsequently evaporated, resulting in increased fluoride concentrations. Although more localized, high fluoride concentrations may be found in sewage treatment plant effluents due to the use of fluoride compounds as brighteners in the truck washing industry.

The AWQMN does not measure fluoride routinely, but rather only at selected sampling stations. The Illinois River averages about 0.35 mg/L. Streams with no sewage treatment plant effluents typically range from <0.1 to 0.3 mg/L. A NE Illinois stream receiving many sewage treatment plant effluents, Salt Creek in Cook County, averages about 0.6 mg/L with high values sometimes exceeding 1.0 mg/L (http://www.epa.gov/storet/dbtop.html). The receiving streams (unnamed tributary to

Salt Creek and Salt Creek) for the City of Effingham's sewage treatment plant may have concentrations of fluoride up to 5 mg/L due to the presence of two truck wash facilities in that relatively small community.

#### Manganese.

The 21<sup>st</sup> Edition of <u>Standard Methods for the Examination of Water and Wastewater</u> (2005) gives the following account for manganese:

Manganese (Mn) is the first element in Group VIIB in the periodic table; it has an atomic number of 25, an atomic weight of 54.94, and common valences of 2, 4, and 7 (and more rarely, valences of 1, 3, 5, and 6). The average abundance of Mn in the earth's crust is 1060 ppm; in soils it is 61 to 1010 ppm; in streams it is 7  $\mu$ g/L, and in groundwaters it is <0.1 mg/L. Manganese is associated with iron minerals, and occurs in nodules in ocean, fresh waters, and soils. The common ores are pyrolusite (MnO<sub>2</sub>) and psilomelane. Manganese is used in steel alloys, batteries, and food additives.

The common aqueous species are the reduced  $Mn^{2+}$  and the oxidized  $Mn^{4+}$ . The aqueous chemistry of manganese is similar to that of iron. Since groundwater is often anoxic, any soluble manganese in groundwater is usually in the reduced state  $(Mn^{2+})$ . Upon exposure to air or other oxidants, groundwater containing manganese usually will precipitate black  $MnO_2$ . Elevated manganese levels therefore can cause stains in plumbing/laundry, and cooking utensils. It is considered an essential trace element for plants and animals. The United Nations Food and Agriculture Organization recommended maximum level for manganese in irrigation waters is 0.2 mg/L. The U.S. EPA secondary drinking water standard MCL is 50  $\mu$ g/L.

Manganese is an essential nutrient for microorganisms, plant, and animals (WHO 2004). WHO (2004) lists the major anthropogenic sources of environmental manganese as including municipal wastewater discharges, sewage sludge, mining and mineral processing, emissions from alloy, steel, and iron production, combustion of fossil fuels, and, to a much lesser extent, emissions from the combustion of fuel additives.

Unlike boron and fluoride, manganese often occurs in Illinois at concentrations above the existing General Use water quality standard. A more stringent manganese standard applies to waters designated for Public and Food Processing Water Supply use and, as later discussed in this document, this standard is exceeded in the majority of waters designated for this use. Although manganese is sometimes elevated in coal mine effluents, the high manganese concentrations in most Illinois streams and lakes are believed to be naturally occurring from the weathering of soils and the decomposition of plant material, as evidenced by the lack of coal mines or other point source contributions of manganese in these watersheds. There is a north to south increase in background

manganese concentrations. The Illinois River in central Illinois has average manganese concentrations of about 0.1 mg/L with high levels at about 0.2 mg/L. Lusk Creek in far southern Illinois lies entirely within the Shawnee National Forest and has no mine or other effluent sources. Manganese averages about 0.2 mg/L with high values occasionally over 1.0 mg/L. A high percentage of this manganese is dissolved whereas in the Illinois River a greater proportion of the manganese is suspended rather than dissolved. Groundwater is known to be high in manganese and this may account for some of the dissolved manganese in southern Illinois streams. The Little Muddy River in Jackson County is typical of many southern Illinois streams in that manganese averages about 1.0 mg/L with many samples up to 4.0 mg/L. Almost all this manganese is in the dissolved form (http://www.epa.gov/storet/dbtop.html).

#### II. Existing Water Quality Standards for Boron, Fluoride, and Manganese

General Use and Lake Michigan Basin Water Quality Standards. The existing General Use and Lake Michigan Basin Standards for boron, fluoride, and manganese were adopted by the Board in the March 7, 1972 standards rulemaking, "Water Quality Standards Revisions", R71-14. The standards were largely based on the opinions of McKee and Wolf (1963), a water quality criteria document published for the California State Water Quality Control Board. The reviews provided by McKee and Wolf (1963) for boron, fluoride, and manganese are presented in Exhibits A, B, and C, respectively. Below is a summary of the reasoning behind the Board's adoption of the existing boron, fluoride, and manganese standards.

The existing General Use and non-open water Lake Michigan Basin standard for boron is 1.0 mg/L. The Board's adopting opinion gives this description (slip opinion at page 6):

<u>Boron</u>. The May 12 and today adopted level of 1.0 mg/l is based on evidence that higher levels can harm irrigated crops. While 100% irrigation is unlikely in Illinois, the uncontrolled discharge of large quantities of boron is clearly undesirable. We have proposed no effluent standard because of the lack of evidence as to treatment methods. The testimony suggests that compliance with the stream standard should not be very difficult.

The existing General Use and non-open water Lake Michigan Basin standard for fluoride is 1.4 mg/L. The Board's adopting opinion gives this description (slip opinion at page 7):

<u>Fluoride</u>. Fluoride can delay the hatching of fish eggs and has been reported by McKee and Wolf to kill trout at concentrations ranging from 2.3 to 7.2 mg/l. They recommend a standard of 1.5 mg/l. The figure of 1.4, here repeated from the May 12 draft, is in line with that recommendation and also should assure a potable supply.

The existing General Use and non-open water Lake Michigan Basin standard for manganese is 1.0 mg/L. The Board's adopting opinion gives this description (slip opinion at page 7):

<u>Manganese</u>. There is no existing aquatic standard. The standard of 1.0 (May 12 and today) is based upon McKee and Wolf's report as to fish toxicity and should be easy to meet.

#### Open Waters of Lake Michigan Standards.

The Open Waters of Lake Michigan standards are based on background conditions of Lake Michigan rather than protection of human health or aquatic life. The existing manganese standard is 0.15 mg/L and will remain unchanged. Presently there are no particular boron or fluoride standards for the Open Waters of Lake Michigan, therefore the existing Lake Michigan Basin Standards for these substances are applicable in these waters.

**Secondary Contact and Indigenous Aquatic Life Standards.** The existing Secondary Contact and Indigenous Aquatic Life standards for fluoride and manganese are 15 mg/L and 1 mg/L, respectively. No standard for this designated use currently exists for boron. At this time, the Agency intends to address all standards for Secondary Contact and Indigenous Aquatic Life Use waters in the "Use Attainability Analysis of the Des Plaines and Chicago Waterways" rulemaking (R08-09).

**Public and Food Processing Water Supply Standards.** There are no existing Public and Food Processing Water Supply standards for boron or fluoride, therefore the General Use standards for these substances are applicable in these waters and are protective of Public and Food Processing Water Supply use. The existing Public and Food Processing Water Supply use. The existing Public and Food Processing Water Supply standard for manganese is 0.15 mg/L, which is based on aesthetics rather than human health. The standard is in place to assure that finished drinking water does not contain manganese at concentrations greater than the maximum contaminant level (MCL) of 0.15 mg/L (35 Ill. Adm. Code 611.300(b)). The finished drinking water MCL is set at 0.15 mg/L due to the potential of manganese to stain laundry and plumbing. Pursuant to 35 Ill. Adm. Code 611.300e, the following supplementary conditions apply to the MCL for manganese:

- 1) CWS [Community Water System] suppliers that serve a population of 1000 or fewer, or 300 service connections or fewer, are exempt from the standards for iron and manganese.
- 2) The Agency may, by a SEP [Special Exemption Permit] issued pursuant to Section 611.110, allow iron and manganese in excess of the MCL if sequestration tried on an experimental basis proves to be effective. If sequestration is not effective, positive iron or manganese reduction treatment as applicable must be provided. Experimental use of a sequestering agent may be tried only if approved by a SEP issued pursuant to Section 611.110.

Public and Food Processing Water Supply standards are intended to represent the maximum allowable concentration of a substance at the point of surface water intake that will allow for attainment of the finished drinking water MCL for that substance following conventional treatment. Conventional treatment is defined in 35 Ill. Adm. Code 302.303 as consisting of coagulation, sedimentation, filtration, storage and chlorination, or other equivalent treatment processes. Because the Public and Food Processing Water Supply standard and finished drinking water MCL are both set at 0.15 mg/L, the existing regulations do not account for any removal of manganese from surface waters that may occur during conventional treatment. The March 7, 1972 Board opinion (R71-14, slip opinion at page 9) provides the following justification for setting the manganese Public and Food Processing Water Supply standard equivalent to that of the finished water standard:

The remaining standards are based largely upon the Public Health Service standards, as amplified by the Green Book and by McKee and Wolf. While the PHS explicitly states that its standards are intended to prescribe the quality of finished rather than of raw water, it is clear from the evidence that many of the metals and other contaminants here listed are not substantially affected by ordinary water supply treatment, and therefore, as the Green Book recommends, the raw water must itself meet the standard to assure satisfactory finished water.

#### III. Site-Specific and Adjusted Standards for Boron and Fluoride.

The Board has granted special relief from boron and fluoride on several occasions upon request by permitted facilities, special relief for manganese has not been granted by the Board to date. Exhibit D summarizes the IPCB granted relief from boron and fluoride water quality standards. In addition to the adjusted standards and site-specific relief in Exhibit D, the Board has also established a fluoride standard of 5 mg/L for waters with zero 7Q10 flow that receive effluent from the mines and mills of the fluorspar mining and concentrating industry (35 Ill. Adm. Code 303.312). The Agency intends on repealing this standard.

#### IV. Treatment to Reduce Concentrations of Boron, Fluoride, and Manganese.

Due to several petitions for relief that have come to the IPCB in recent years for both boron and fluoride water quality standards downstream of wastewater discharges, Illinois EPA, under its obligation to address the merits of these petitions, has investigated treatment options for these substances. Both these substances are highly soluble and this characteristic generally confounds attempts at treatment. Boron does not respond to the usual method of treating metals by raising pH and precipitating the metal to sludge. Fluoride likewise does not respond to this manner of treatment. The only methods of treatment identified have been reverse osmosis, which is seldom acceptable as it results in a high concentration wastewater that still must be disposed of, and various nonconventional treatment processes that are very expensive and have not seen routine use. In every case for site-specific water quality standards or adjusted standards brought before the IPCB, Illinois EPA has concluded that no reasonable treatment exists for boron and fluoride to reduce effluent concentrations.

Unlike boron and fluoride, manganese does respond to treatment by raising pH and thereby forcing precipitation. A few coal mines use this technology periodically to meet permit limits for manganese. A chemical is added to a basin which raises effluent pH causing manganese to precipitate. The proposed change in the manganese water quality standard may relieve future mine outfalls from manganese treatment, however, manganese permit limits may still be dictated by 35 Ill. Adm. Code Subtitle D: Mine Related Water Pollution effluent standards. The Agency is not aware of other industries that treat for manganese other than public water supply treatment plants that remove manganese from surface water to meet drinking water standards and then must filter or settle suspended manganese particles from the wastewater. Issues of these facilities having problems meeting permit limits have not arisen.

#### V. Proposed Revisions to Boron, Fluoride, and Manganese Standards

#### A. Public and Food Processing Water Supply and Open Waters of Lake Michigan

**Boron and Fluoride** - There are no existing Public and Food Processing Water Supply Standards for boron or fluoride, therefore the existing General Use standards for these substances are applied to these waters by default. As later discussed, the newly proposed General Use standards for boron and fluoride are higher than the existing standards of 1.0 mg/L and 1.4 mg/L, respectively. Given that the existing General Use standards are currently protective of Public and Food Processing Water Supply use, we are proposing to designate 1.0 mg/L boron and 1.4 mg/L fluoride as Public and Food Processing Water Supply standards. The standards would be applied at the point of surface water intake and would be regulated as one-number, not to be exceeded standards. Because there are no specific Open Waters of Lake Michigan standards for boron and fluoride, the Lake Michigan Basin standards for these substances are currently applicable. Relocating the existing Lake Michigan Basin standards of 1.0 mg/L boron and 1.4 mg/L fluoride into the Open Waters of Lake Michigan standards will provide a measure of protection against harmful loadings of these substances within these waters, and will continue to allow these waters to be utilized for Public and Food Processing Water Supply use.

There is no evidence to suggest that boron and fluoride can be removed by conventional treatment such as coagulation/flocculation, sedimentation, filtration, or chlorination, therefore the Public and Food Processing Water Supply standards for these substances must be set at concentrations lower than the thresholds believed to adversely affect human health or other parameters (e.g., aesthetics). Finished drinking water containing boron or fluoride at or below the proposed standards will have no adverse effects on human health, nor will it lead to aesthetic or organoleptic (taste, color, or odor) problems. According to the U.S. EPA document *Drinking Water Health Advisory for Boron* (USEPA 2008) the lowest boron human health advisory is 2 mg/L, which is based on the Longer Term Health Advisory for children. Setting the Public and Food Processing Water Supply standard for boron at 1.0 mg/L is not a change from the existing applicable

standard and will be protective of human health and the irrigative uses of domestic waters (watering of house plants, greenhouses, etc.).

Although Illinois public water utilities are required to fluoridate drinking water to achieve 0.9-1.2 mg/L fluoride, adverse effects to human health may occur at higher fluoride concentrations. EPA currently has a fluoride drinking water standard of 4 mg/L (protection against bone disease) and also has a secondary fluoride standard of 2 mg/L for protection against dental fluorosis (staining or pitting of teeth in children). Illinois has adopted both of these federal drinking water standards for fluoride, which are located in 35 Ill. Adm. Code 611.301 and 611.908, respectively. Finished drinking water is not to exceed 4 mg/L fluoride, and utilities are required to notify the public in instances when the secondary fluoride standard of 2 mg/L is exceeded in drinking water, as mandated in 35 Ill. Adm. Code 611.908. Setting the Public and Food Processing Water Supply standard for fluoride at 1.4 mg/L is not a change from the existing applicable standard and will assure that finished drinking water standards will not be exceeded due to fluoride in surface waters withdrawn for public water supply use.

**Manganese** – The manganese Public and Food Processing Water Supply and Open Waters of Lake Michigan standards are presently set at 0.15 mg/L. Open Waters of Lake Michigan rather than protection of human health or aquatic life, therefore the existing manganese standard for these waters will remain unchanged. According to the Illinois Integrated Water Quality Report and Section 303d List - 2008, 85 of 121 General Use waters designated for Public and Food Processing Water Supply use were found to be impaired due to manganese. Fifty-five of these impaired waters were lakes, and 30 were rivers/streams. Thirteen additional sites located in the Open Waters of Lake Michigan were assessed and were not found to be impaired due to manganese. Total Maximum Daily Load (TMDL) studies on these impaired waters have concluded that the majority of manganese loadings to these waters are from natural sources rather than point source dischargers. The East Fork LaMoine River Watershed TMDL Report (2007) provides the following information regarding manganese:

For manganese, the primary sources are natural sources, including soils and groundwater. Manganese reductions are needed during mid- to low flow conditions. Soils naturally enriched in manganese can settle in the river and contribute to manganese exceedances during low flow, when colloidal manganese and, if anoxia develops, dissolved manganese, are in the water column. The extent to which these forms of manganese and chemical release mechanisms contribute to the exceedances of manganese is not known; however, controls targeted at reducing wet weather loads of sediment and manganese may also reduce sedimentation and subsequent release of soluble manganese during low flow periods.

Due to past uncertainty of the effectiveness of manganese removal through conventional treatment, the existing Public and Food Processing Water Supply manganese standard has been set equivalent to the finished drinking water MCL. However, recent publications

suggest that manganese can be effectively removed from surface waters via conventional treatment. The conventional process of chemical oxidation followed by sedimentation and filtration is estimated to remove as much as 90-100% of manganese from waters withdrawn for public water supply use (Hamann et al. 1990, Casale et al. 2002). In areas where hard water must be treated prior to distribution, lime softening is often employed and provides a secondary benefit by enhancing manganese removal. However, due to increased operating expenses, this treatment is only deemed practical in instances where water softening is required (Casale et al. 2002). Treatment consisting of chemical oxidation, sedimentation, and filtration is commonplace in Illinois. This degree of treatment is economically reasonable and technically feasible for any utility that requires treatment to reduce common raw water constituents, including naturally elevated concentrations of manganese in their water supply.

It is difficult to quantify the amount of manganese removal presently occurring at conventional treatment plants in Illinois. Public water utilities are required to report the amount of manganese in their finished water to the Agency at least once per year, but are not required to report the amount of manganese in their raw water intake prior to treatment. Manganese removal in Illinois can best be estimated by compiling finished manganese data from utilities withdrawing from waters impaired due to manganese, and comparing this data to raw surface water data collected in these waters as part of the Agency's surface water monitoring programs. Finished water data is available in electronic format from 1993-2009, but a significant amount of the surface water data from these impaired waters is not electronically available or is of little use in regards to this analysis. For example, some of these lakes and streams are not part of the Agency's ambient monitoring programs, therefore the amount of surface water data from these water bodies is limited and is unsuitable for this type of analysis. Furthermore, several of the impaired lakes and streams are backup public water supplies that are used sparingly, if ever, therefore surface water quality from these waters has no correlation to the finished water quality reported by these utilities. An additional limitation to this analysis is that several utilities use non-conventional treatment technologies such as lime softening, ion exchange, or reverse osmosis. Although not used specifically for manganese removal at these utilities, these advanced treatments are effective at removing manganese and may lead to greater manganese removal efficiencies compared to conventional treatment. Since Public and Food Processing Water Supply waters are intended to assure that finished water MCLs are attained following conventional treatment, more advanced technologies such as lime softening, ion exchange, or reverse osmosis were not considered in this analysis. And lastly, utilities that serve a population of 1000 or fewer, or 300 service connections or fewer, are exempt from meeting the finished drinking water MCL for manganese and therefore were not included in this analysis.

The amount and form (soluble or particulate) of manganese in surface waters can be highly variable throughout the year due to fluctuations in dissolved oxygen levels resulting from environmental factors such as lake stratification, lake turnover, and rainfall. Given the high seasonal variability of manganese in the environment, it is impractical to compare finished manganese data annually collected from one specific month (e.g., February) to raw surface water manganese data collected in other months (e.g., July, August, September). This is especially important given that the vast majority of surface water data is collected by the Agency during summer months, whereas finished water data from public water utilities is collected during all months. To limit potential discrepancies between raw and finished manganese data, the available data was further minimized to meet the following criterion: Manganese must be  $\geq 0.15$  mg/L in surface water samples and must have a corresponding finished water sample taken within  $\pm 7$  days from the local public water utility. The results from this analysis suggest that approximately 96% of manganese is being removed by conventional treatment in Illinois. When expanding the dataset to include finished water samples taken within  $\pm 30$  days of surface water samples containing  $\geq 0.15$  mg/L manganese, removal of manganese was estimated at 94%. When compiling all paired data, the average concentration of manganese in the surface waters was 0.34 mg/L, whereas the average finished water concentration was 0.019 mg/L. Exhibit E provides a summary of this data (presented in  $\mu$ g/L for ease of review).

Based on removal estimates within published literature, as well as data collected from conventional treatment plants in Illinois, it is apparent that >90% of manganese can be removed through conventional treatment. The highest surface water manganese concentration used in the analysis of Illinois data was 0.9 mg/L. Four days prior to collection of the surface water sample, the utility withdrawing from this water body reported a finished sample containing 0.032 mg/L manganese. Consistent with these findings, Kohl and Medlar (2006) performed detailed manganese surveys on 52 utilities and concluded that high influent concentrations of manganese are not problematic to properly equipped utilities. For example, one utility within the survey utilizing conventional gravity settling (rapid mix, flocculation, settling, and granular media filtration) reported a maximum and average influent concentration of 4.5 mg/L and 2.1 mg/L manganese, respectively, and a maximum and average finished water concentration of 0.025 and 0.019 mg/L, respectively. The authors further explained that utilities with influent that contains intermediate, markedly variable manganese loadings may be more susceptible to manganese removal problems than utilities with high manganese, as these utilities may be unaware that manganese is occasionally present at elevated concentrations as a result of naturally occurring fluctuations, especially in lakes.

The existing manganese Public and Food Processing Water Supply standard of 0.15 mg/L is overly protective of the finished manganese standard, as the finished MCL of 0.15 mg/L can easily be attained following conventional treatment of surface waters containing >0.15 mg/L manganese. By conservatively estimating that 90% of manganese can be removed at conventional utilities in Illinois, and back-calculating the amount of manganese in surface waters that would still allow for attainment of the 0.15 mg/L finished MCL, it is apparent that a maximum surface water concentration of 1.5 mg/L would not be problematic to Illinois utilities withdrawing this water. However, in order to provide an additional measure of conservancy, the Agency is proposing to set the new manganese). The standard would be applied at the point of surface water intake and would be regulated as a one-number, not to be exceeded standard. As concluded in

Agency TMDLs, manganese is naturally high in Illinois ground water and surface water primarily due to the weathering and deposition of manganese-enriched soils and plant matter. Other than the intake and subsequent discharge of manganese from their water supply, very few point source dischargers in Illinois are known to contribute significant loadings of manganese to surface waters as a byproduct of their operation. Modification of the existing standard should not result in an increase in manganese loadings to waters currently meeting the existing manganese standard of 0.15 mg/L, as NPDES facilities are not a significant source of manganese loadings to these waters. This is especially true given that the majority of impaired Public and Food Processing Water Supply waters (due to manganese) are lakes which do not receive discharges from NPDES facilities.

#### B. General Use and Lake Michigan Basin Aquatic Life-Based Standards

The existing General Use and Lake Michigan Basin standards for boron, fluoride and manganese are remnants from the Board's first standards rulemaking in 1972 entitled "Water Quality Standards Revisions", R71-14. Including these substances, the majority of standards adopted in this rulemaking were based on the opinions of McKee and Wolf (1963), a water quality criteria document published for the California State Water Quality Control Board. Although the publication provided water quality criteria recommendations for numerous substances, the authors emphasized in the foreword that the publication merely served as a survey and evaluation of the existing literature and that it should not be used to establish specific standards for the State of California or the Public Health Service. The water quality criteria recommendations within the publication were often rudimentary estimates based on the limited data available to the authors at that time. In the years since this publication, the amount and quality of literature available for water quality standards development has substantially increased, and USEPA methods are now available to develop standardized, scientifically valid water quality standards. It is now well known that environmental factors such as pH and hardness can substantially mitigate or increase the toxicity of many substances, therefore most new standards are developed dependent of specific water chemistry parameters. Consequently, many standards adopted in the R71-14 rulemaking have since been revised due to more recent. detailed information regarding the threshold of toxicity for these substances in the presence of a variable water quality parameter (usually hardness). Similarly, the proposed revisions to the existing boron, fluoride, and manganese standards are the result of new findings regarding the toxicity of these substances and the influence (or lack thereof) of water chemistry on toxicity.

The newly proposed standards for General Use and Lake Michigan Basin waters were developed using USEPA guidelines for deriving numerical water quality criteria. The U.S. EPA "1985 Guidelines" methodology (USEPA 1985, Exhibit F) is commonly used to derive standards (or USEPA "national criteria") for substances that display a classical dose-response relationship whereupon mortality is the endpoint of concern. This conventional methodology was used in deriving acute and chronic standards for boron, fluoride, and manganese. Given that fluoride and manganese toxicity is known to be influenced by the hardness of test water, standards for these substances were developed to account for hardness-dependent relationships. Literature reviews and additional laboratory tests studying the influence of water chemistry on boron toxicity had

confounding results, therefore boron standards were developed independent of water chemistry. The following paragraph provides a brief overview of the 1985 Guidelines procedures used in deriving the proposed standards. Further detail regarding the additional procedures required for deriving the hardness-based fluoride and manganese standards will be provided in a later section.

Only data from toxicity tests conducted on appropriate organisms using valid test methods, appropriate laboratory waters, and proper endpoints were used in deriving the proposed standards. For each substance, acute data expressed as an LC50 (concentration lethal to 50 percent of the tested organisms) was compiled for each species and was used to develop a Genus Mean Acute Value (GMAV) for each genus. Geometric means, rather than arithmetic means, were used to calculate GMAVs because the distributions of sensitivities of species within a genus are typically lognormal. The GMAVs were ranked by sensitivity and were used to develop the Final Acute Value (FAV), which was derived by calculating the 0.05 cumulative probability of each dataset using the four lowest GMAVs and the total number of GMAVs (see formula in Section IV. O of Exhibit F). The FAV is the value protective of at least 95% of species at the LC50 level of effect. The FAV was then divided by 2 in order to convert the acute value from an LC50 level of protection to a level that is protective at the No Observable Adverse Effect Level (NOAEL, 35 Ill. Adm. Code 302.603). Chronic standards for boron and fluoride were developed using the Acute-Chronic Ratio (ACR) approach, which requires ACRs from animals in at least three different families of which one species is a fish, one species is an invertebrate, and one is an acutely sensitive freshwater species. An ACR is calculated by dividing the acute LC50 of a species by the Maximum Acceptable Toxicant Concentration (MATC, 35 Ill. Adm. Code 302.603) of the same species derived from a chronic test conducted in the same laboratory under test conditions identical to the acute test. The Final Acute-Chronic Ratio (FACR) was then calculated by taking the geometric mean of all available ACRs for each species. Chronic standards were then obtained by dividing the FAV of each substance by the FACR. As later discussed, the chronic manganese standard was not developed using the ACR approach because the resulting standard was not protective of Hyalella azteca, the most sensitive species. Rather, the chronic manganese standard was based off the Hyalella azteca MATC to afford proper protection for this organism and other untested, closely related organisms.

Organisms used in standards derivation were restricted to those meeting Illinois data requirements, as specified in 35 Ill. Adm. Code 302.612 (General Use waters) and 302.553 (Lake Michigan Basin waters). In Illinois, family Salmonidae only naturally exists in Lake Michigan Basin waters, therefore these organisms are included in Lake Michigan Basin standards derivation but are excluded from General Use standards derivation. Given that family Salmonidae organisms are typically more sensitive to pollutants than other Illinois organisms, the resulting Lake Michigan Basin standards are typically more stringent than the corresponding General Use standards calculated without these organisms. However, in regards to boron, manganese, and fluoride toxicity, family Salmonidae genera are no more sensitive than other Illinois organisms and are not one of the four lowest GMAVs within the datasets. Thus, inclusion of these organisms in the database results in Lake Michigan Basin standards that are less stringent than General Use standards, as the inclusion of additional GMAVs into each dataset increases the

confidence of the cumulative probability estimate of the FAV. It is impractical to regulate Lake Michigan Basin waters with standards that are relaxed in comparison to General Use standards, therefore we are proposing that the proposed General Use standards be applied to both categories of waters.

Use of Hyalella azteca data – Hyalella azteca, a freshwater amphipod (order Amphipoda) native to Illinois, is considered a valuable species for standards derivation due to its standing as both an important component of the state's stream ecosystems and a pollutant sensitive species. Along with the two other orders of organisms in Class Malacostraca (Decapoda and Isopoda), organisms within this class are common in Illinois waters (predominately in rivers/streams) and represent a niche of organisms that until recently, were not commonly represented in the toxicity database for most substances. For acute standards derivation a benthic macroinvertebrate is required to meet Tier I data requirements (35 Ill. Adm. Code 302.612). Other benthic macroinvertebrates commonly used in toxicity testing (e.g. Lumbriculus sp., Chironomus sp., Physella sp., etc.) are acceptable for meeting data requirements but are typically recognized as tolerant species. As previously discussed, the FAV determination is highly dependent on the distribution of the four lowest GMAVs, therefore it is pertinent that species suspected as being most sensitive to a given toxicant be tested so as to determine an accurate FAV. Given that Hyalella azteca is a recognized as a sensitive species, and in the case of boron, fluoride and manganese this sensitivity has been documented in acute tests, it is appropriate to conduct chronic tests on this organism rather than a more tolerant benthic macroinvertebrate. For chronic standards derivation, ACRs are required from animals in at least three different families of which one species is a fish, one species is an invertebrate, and one is an acutely sensitive freshwater species. For each substance, exclusion of chronic *Hyalella azteca* data would result in only two families being represented in each database, therefore Tier II chronic procedures (35 Ill. Adm. Code 302.565(b)) would be required which would result in a default ACR of 18 being used in place of Hyalella azteca ACRs. Given that all chronic Hyalella azteca data was the result of EPA-funded research and was conducted specifically to meet Tier I chronic data requirements (35 Ill. Adm. Code 302.565(a)), it is appropriate to use this data in standards derivation.

Although toxicity testing with *Hyalella azteca* has been standardized with ASTM methods and has been used in past EPA national criteria recommendations as well as Illinois EPA standards, test methods for *Hyalella azteca* are currently being refined due to recent findings regarding the importance of chloride (and possibly bromide) to *Hyalella azteca* survival. For toxicity testing EPA typically recommends using moderately hard reconstituted water (MHRW) which has a very low chloride content (1.9 mg/L chloride). However, several laboratories have reported difficulty in obtaining acceptable survival and growth of *Hyalella azteca* during not only toxicity testing, but during culturing with MHRW. In fact, it is not uncommon for cultures to fail in MHRW within one week, without any toxicant added. Consequently, several researchers are currently developing specific culture waters and foods to improve survival, growth, and reproduction of *Hyalella azteca*. Dr. David Soucek of the Illinois Natural History Survey is at the forefront of this research and was contracted by Illinois EPA to conduct *Hyalella* 

*azteca* tests using these refined methods. Rather than using MHRW, Smith water (34 mg/L chloride) or Borgmann water (72 mg/L chloride) was used in acute and chronic *Hyalella azteca* toxicity testing. Ambient waters in Illinois contain chloride at concentrations higher than those found within MHRW (1.9 mg/L). A review of data from all Illinois AWQMN stream stations from January, 1999, to February, 2004, found the average chloride concentration to be 87.5 mg/L, and the median concentration to be 40.4 mg/L. The average concentration of chloride is much higher due to the seasonal impacts of road salting. Ambient conditions (in terms of chloride) in Illinois are much more similar to that of Smith or Borgmann water compared to MHRW. Given that *Hyalella azteca* survival, growth, and reproduction is maximized in these dilution waters, the results of Dr. Soucek's testing are much more reflective of the true tolerance of this organism to boron, fluoride, and manganese.

**Boron** – Acute and chronic toxicity data used in deriving the proposed boron standards are summarized in Exhibits G and H, respectively. Data that were initially considered potentially useful for standards derivation but were later discarded are marked with strikethrough. A brief explanation of the shortcomings of each study is highlighted in bold within the "Notes" column. Exhibit I provides a ranked summary and illustration of the GMAVs used in developing the FAV and acute standard for boron. A summary of the valid ACRs and the resulting FACR used in determining the chronic standard for boron is also provided.

The relationship between water chemistry and boron toxicity to aquatic life has previously been studied with varied results. Maier and Knight (1991) found that variable hardness and sulfate concentrations did not significantly affect mortality of *Daphnia magna* when exposed to boron. Dethloff et al. (2009) studied the effects of several water quality parameters on boron toxicity to *Ceriodaphnia dubia* and observed some positive correlations, as waters with high hardness (>500 mg/L CaCO<sub>3</sub>) and dissolved organic carbon (2.6-11.4 mg/L) significantly diminished the toxicity of boron. However, it should be noted that the magnitude of these influences on boron toxicity. Additional tests conducted by Dethloff et al. (2009) at variable chloride, sulfate, alkalinity, and pH had insignificant or inconclusive results; these individual test results are included in Exhibit G.

Due to limited substantiation of whether boron toxicity is strongly correlated to water chemistry, it was decided to conduct additional boron toxicity tests at two variable parameters commonly known to influence the toxicity of metals, hardness and pH. Tests were conducted at various water chemistries by Dr. Soucek and by Great Lakes Environmental Commission (GLEC). Three boron-sensitive species were chosen as the test organisms, *Pimephales promelas*, *Ceriodaphnia dubia*, and *Hyalella azteca*. All individual boron toxicity tests conducted on these species at variable hardness and pH are included in Exhibit G, and graphical representations of these relationships are provided in Exhibit J. In addition to hardness and pH-dependent toxicity tests, Dr. Soucek and GLEC conducted boron toxicity tests on additional organisms to fulfill Tier I data requirements for acute and chronic standards development. A summary of this data is provided in Exhibits G and H.

In contrast to the hardness relationship found with fluoride and manganese toxicity data, no consistent, significant relationship between boron toxicity and hardness or pH was observed. Hardness-dependent testing with *Ceriodaphnia dubia* resulted in small, contrasting slopes when comparing data from the Dethloff and Soucek laboratories. Hardness-dependent testing with *Hyalella azteca* from the Soucek laboratory resulted in slightly larger slopes, but the slopes were contrasting dependent on the dilution water used. In tests using Smith water, higher hardness concentrations appeared to mitigate boron toxicity. However, given that *Hyalella azteca* prefer waters with higher chloride, and that the higher hardness treatments in Smith water tests had increased chloride concentrations, the mitigating effect observed may be more so attributed to increased chloride rather than hardness. When tested at variable hardness concentrations in Borgmann water, chloride concentrations remained consistent across treatments and a small, negative relationship between boron toxicity and hardness was observed. This confirms that chloride was the ameliorating factor for *Hyalella azteca* in the Smith water tests.

Similar to hardness-dependent tests, confounding results were also observed amongst species exposed to boron in pH-dependent toxicity tests. *Ceriodaphnia dubia* and *Pimephales promelas* survival was positively correlated with increased pH, whereas *Hyalella azteca* survival was negatively affected at high pH. Developing a pH based standard using slopes derived from *Ceriodaphnia dubia* and *Pimephales promelas* testing would result in less stringent boron standards at high pH, but the standards would be non-protective of *Hyalella azteca* which are more sensitive to boron under these conditions. Similarly, when considering the contrasting relationships seen with hardness-based tests on *Ceriodaphnia dubia* and *Hyalella azteca*, it is also impractical to develop hardness-based boron standards. Given that a clear, consistent relationship between water chemistry and boron toxicity does not exist, aquatic life standards for boron were developed independent of water chemistry.

Given that the existing General Use standard for boron is based on the sensitivity of irrigated crops, it was appropriate to research the effects of boron to aquatic plants. Although an essential nutrient for plant growth, chronic exposures of boron can be toxic to aquatic plants at elevated concentrations. A literature search for valid aquatic plant data was conducted with little success, as all data was deemed inappropriate due to improper test conditions, durations, and/or endpoints. Plant data that were initially considered useful for standards derivation but were later discarded are marked with strikethrough in Exhibit H. A brief explanation of the shortcomings of each study is highlighted in bold within the "Notes" column. Upon consultation with U.S. EPA, Illinois EPA concluded that plant data will not be of use in deriving the boron standards. Excluding criteria for herbicides (e.g., atrazine), most national criteria documents do not use aquatic plant data in the derivation of criteria. For example, the recently proposed EPA draft ammonia criteria do not incorporate plant data, as aquatic animals are more sensitive to ammonia toxicity and therefore drive the criteria. The 1985 Guidelines

(Exhibit F) provides the following guidance in regards to the acknowledgment of aquatic plant data when deriving aquatic animal-based criteria.

Appropriate measures of the toxicity of the material to aquatic plants are used to compare the relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting the results of toxicity tests with plants are not well developed, results of tests with plants usually indicate that criteria which adequately protect aquatic animals and their uses will probably also protect aquatic plants and their uses.

No aquatic plant toxicity tests on boron with valid methods, endpoints, and test conditions that would be applicable for standards derivation in Illinois were found in literature searches. Nonetheless, by evaluating the relative sensitivity of aquatic plants to chronic exposures of boron, it is apparent that the proposed chronic boron standard would adequately protect aquatic plants and their uses.

Fluoride and Manganese – Many substances can adversely affect aquatic organisms by interfering with osmoregulation, whereupon the substances can bind with gill membranes and impair the ability of the gills to properly regulate ions. Waters with high hardness are known to mitigate the toxic effects of these substances by competitively binding with gill membranes and promoting osmoregulation. Similarly, upon review of the available literature it is apparent that the toxicity of fluoride and manganese to aquatic life is diminished in response to increased water hardness. Given this finding, it is necessary to develop water quality standards for these substances that account for this hardness-dependent relationship. The 1985 Guidelines (Exhibit F) explains this methodology in great detail in "Section V. Final Acute Equation". A brief summary of this procedure and the resulting fluoride and manganese standards are provided below.

The relationship between hardness and acute toxicity is typically non-linear, therefore the relationship must be linearized by logarithmically transforming the data and performing a least squares regression to obtain the pooled slope ("V") of the line describing the relationship. Because toxicity tests are conducted at different hardness concentrations, data for each species must be normalized to an arbitrary hardness denoted as "Z" (50 mg/L in this case) with an equation utilizing the pooled acute slope ("V"), the natural log of the geometric mean of LC50s ("W") and hardness ("X") for each species, and the natural log of the selected hardness concentration ("Z") to be used in normalization. The result of this equation ( $e^{Y} = \ln W - (V^{*}(\ln X - \ln Z)))$  is the Species Mean Acute Value (SMAV) at the selected hardness concentration (Z). The GMAV for each genera is then compiled and sorted in order to rank the sensitivities of each genera. It is important to note that the hardness concentration selected for data normalization has no affect on the resulting standards, as it is merely used to normalize the data so that organism sensitivities can be ranked. Exhibits K and L summarize the results of the GMAV calculations for fluoride and manganese, respectively. The FAV at a hardness of 50 mg/L is then calculated by applying the four lowest GMAVs and the total number of GMAVs into the FAV formula. The FAV is then divided by two in order to convert the acute standard from an LC50 level of protection to a level that is protective at the

NOAEL. The resulting value is the acute standard at a hardness of 50 mg/L, and this value is used in deriving the intercept that is incorporated into the equation which expresses the acute standards at variable hardness. Exhibits M and N summarize the acute standards developed upon completion of these calculations for fluoride and manganese, respectively. Example calculations at various hardness concentrations are provided to illustrate the effect of hardness on the resulting standards. Acute and chronic toxicity data used in deriving the proposed fluoride standards are summarized in Exhibits O and P, respectively, and acute and chronic toxicity data used in deriving the proposed fluoride toxicity data used in fulfillment of Tier I data requirements, Dr. Soucek and GLEC conducted manganese and fluoride toxicity tests on additional organisms. A summary of this data is provided in Exhibits O, P, Q, and R.

Similar to boron, the chronic standard for fluoride was developed using the ACR approach. The FACR was calculated by taking the geometric mean of all available ACRs for each species. The hardness-dependent chronic standard was then obtained by dividing the FAV (normalized at 50 mg/L hardness) by the FACR, which gives the chronic fluoride standard at a hardness of 50 mg/L. The chronic equation used to calculate fluoride standards at variable hardness is similar to the acute equations used for each substance, with the one exception being that the chronic intercept (derived using the chronic standard calculated at 50 mg/L hardness) replaces the acute intercept within the equations. The chronic equation and example calculations of chronic standards for fluoride at various hardness concentrations are provided in Exhibit M.

The chronic standard for manganese was not developed using the ACR approach because the resulting standard was not protective of *Hyalella azteca*, the most sensitive species in the database. The *Hyalella azteca* ACR (5.48) is the highest in the database and when combined with lower ACRs from the five other species the resulting FACR is 3.34. By dividing the FAV by the FACR the resulting chronic manganese standard at 50 mg/L hardness would be 1.52 mg/L, whereas the chronic MATC for *Hyalella azteca* at 50 mg/L hardness is estimated at 1.08 mg/L (Exhibit L). As stated in 35 Ill. Adm. Code 302.627(d), if a resident species whose presence is necessary for sustainment of a waterbody's ecosystem will not be protected by the calculated chronic standards then the MATC for that species should be used in developing the chronic standard. Given that this organism represents a class of benthic macroinvertebrates common in Illinois waters and is considered ecologically important, the chronic manganese standard was developed to protect at a concentration equivalent to the *Hyalella azteca* chronic MATC. This was done by replacing the FACR-based chronic intercept of 1.52 mg/L with the *Hyalella azteca* chronic MATC of 1.08 mg/L (Exhibit N).

**Chronic Fluoride Standard for Protection of Wildlife and Livestock** - Waters designated for General Use or Lake Michigan Basin Use are required to have standards that are protective of aquatic life, as well as human health through physical contact with water and consumption of fish. In the case of boron, fluoride, and manganese, aquatic life are sensitive to these substances at concentrations far lower than standards that would be calculated for human health based on incidental ingestion of water and consumption of

fish. Given that aquatic life-based standards for these substances are protective of aquatic life use, human health will not be adversely impacted through these exposure routes. However, another use to be protected by General Use standards is the consumption of surface waters by wildlife and livestock that could potentially depend on ambient waters for drinking water. When calculated for water bodies with higher hardness concentrations (Exhibit M), the resulting chronic fluoride standards far exceed the 4 mg/L drinking water standard for fluoride. The skeletal effects of fluoride in drinking water on wildlife and livestock are similar to those exhibited in humans and are believed to occur at equivalent exposure levels (McKee and Wolf, 1963). The Integrated Risk Information System (IRIS) safe exposure level for fluoride has been determined to be 0.12 mg fluoride/kg/day for human adults, which was derived from a NOAEL of 2 liters/day of water containing 4 ppm fluoride in addition to dietary fluoride contributions. Given that chronic fluoride standards calculated for protection of aquatic life in high hardness waters would exceed the 4 mg/L drinking water standard, it is appropriate to cap the chronic fluoride standards at 4 mg/L for protection of wildlife and livestock.

Because hardness is variable amongst Illinois watersheds, the resulting fluoride and manganese standards will be site-specific based on ambient hardness. Hardness is defined by Standard Methods as "the sum of calcium and magnesium concentrations, both expressed as calcium carbonate, in milligrams per liter". For aquatic toxicity testing, USEPA typically recommends the use of MHRW which has a hardness of 90 mg/L. In Illinois, most waters are generally classified as hard or very hard waters. As can be seen in Exhibit S, only about 2.5% of Illinois waters are expected to have hardness values below 90 mg/L during low flow events based on the findings of the Ambient Water Quality Monitoring Network. To produce the "Critical" hardness values in the document, data from a 15-year period from all stations in the network (approximately 135 samples per each of over 200 stations) were analyzed. Samples from the 10th percentile low stream flows were segregated and, of this data, the 10th percentile hardness value was determined. Therefore, the hardness values given in Exhibit S represent the lowest hardness expected in streams when they are at vulnerable low flows. There is a northsouth pattern to hardness in Illinois. Northern Illinois streams and lakes typically have hardness values in the 200-300 mg/L range. This is due to the limestone bedrock that underlies most of the northern 90% of the state. In contrast, several Southern Illinois streams are in areas where bedrock is comprised of sandstone or a limestone and sandstone mix that results in low hardness. However, where mining occurs in Southern Illinois, hardness is often elevated due to exposure of mine overburden to rainwater.

**Conversion Factor Multiplier for Manganese** – Toxicity results are typically reported as the total amount of toxicant present in a test, yet for metals, it is the dissolved fraction that is bioavailable for uptake across gill membranes and is the toxic component. Factors such as precipitation and sorption with suspended solids can reduce the dissolved fraction of a metal and reduce bioavailability, therefore it is necessary to measure total and dissolved metal concentrations when developing toxicity-based water quality standards. Aquatic life water quality standards for metals are expressed in the dissolved form. However, because permit limits for metals are expressed in the total form, water quality standards for metals are written with a conversion factor multiplier to convert from total to dissolved standards. A conversion factor multiplier is based on the total and dissolved metal concentrations that exist in test chambers throughout toxicity testing. Given that manganese is a metal and is known to exist in ambient waters at a dissolved fraction less than 100%, a conversion factor multiplier is necessary to properly regulate manganese in permitting and water quality standards attainment. A conversion factor for boron (a semi metal) and fluoride (a halogen) are not needed given that these substances are not true metals and are found in nature in dissolved form. However, for convenience in setting permit limitations these standards will be expressed in the total form.

The conversion factor multiplier for manganese was derived from total and dissolved manganese data collected during the chronic Hyalella azteca test conducted by Dr. Soucek. Total and dissolved manganese was measured for each treatment six separate times throughout the length of the static-renewal test. Total manganese was determined by measuring each sample without filtration, and dissolved manganese was determined by filtering each sample to remove suspended manganese. Three sets of samples were collected immediately after sample renewal ("in" samples), and three sets of samples were collected prior to sample renewal after four days of exposure ("out" samples). For each treatment, the filtered results were divided by the unfiltered results to calculate the percent of dissolved manganese. Exhibit T summarizes the results of these calculations. By observing the geometric means of "in" and "out" samples it is apparent that the amount of dissolved manganese is lower in "out" water, likely due to sorption with increased amounts of suspended solids resulting from feeding of the test organisms. The geometric mean of all "in" and "out" conversion factors is 0.9812, which is the multiplier which will be used to convert total manganese test results to dissolved manganese standards. A comprehensive summary of this data, as well as all other data acquired through boron, fluoride, and manganese toxicity tests conducted by Dr. Soucek is included in Exhibit U. A detailed summary of additional boron, fluoride, and manganese toxicity data conducted by GLEC is included as Attachment 6 to the Agency's Statement of Reasons.

#### VI. Conclusions and Recommended Standards

Protection of aquatic life in General Use and Lake Michigan Basin waters will be fully achieved through implementation of the numerical standards for boron at 35 Ill. Adm. Code 302.208(g) and 302.504(a), respectively, and the hardness dependent equations for fluoride and manganese specified in 35 Ill. Adm. Code 302.208(e) and 302.504(a), respectively. Protection of Public and Food Processing Water Supply use and Open Waters of Lake Michigan use will be achieved by inclusion of the applicable standards specified in 35 Ill. Adm. Code 302.304 and 302.504(c), respectively.

Along with the proposed changes to boron, fluoride, and manganese standards, various housekeeping changes are proposed in order to modify/eliminate outdated regulations, improve comprehension of regulations, and to fix typographical errors. An overview of some of the more noteworthy changes is as follows:

<u>Mixing zones</u>: Small changes are proposed within this section in order to improve comprehension of mixing zone language. No changes to mixing zone policies are proposed. However, language within 35 Ill. Adm. Code 302.208(d) has been replaced and other language has been removed from this section to eliminate redundant references to 35 Ill. Adm. Code 302.102.

<u>Cyanide standards</u>: No changes will be made to the cyanide standards. However, the existing regulations are silent on the type of cyanide that must meet the water quality standards of 35 Ill. Adm. Code 302.208(e) and 302.504(a). The correct form of cyanide to be assessed against the existing acute and chronic standards is either weak acid dissociable cyanide (as in Standard Methods) or available cyanide as in USEPA's Method OIA-1677 Available Cyanide by Flow Injection, Ligand Exchange, and Amperometry (USEPA 1999). Appropriately, cyanide is now listed as weak acid dissociable or available cyanide.

STORET Codes: STORET is no longer a viable data system at USEPA, therefore we are proposing to drop STORET codes from the regulations that are open for amendment in this proposal. STORET codes, as they appear in current IPCB water quality standards, are no longer maintained and updated, therefore they are of little use in instructing the reader on what form of the substance is regulated.

Listing of Derived Water Quality Criteria: Pursuant to 35 Ill. Adm. Code 302.595 and 302.669, water quality criteria derived by Illinois EPA following regulations within 35 Ill. Adm. Code 302.210 and 302.540 are required to be published quarterly in the Illinois Register. Derived water quality criteria are currently published and updated on the Agency's website, therefore publishing this list in the Illinois Register results in a duplication of effort. We are proposing to make it a requirement for Illinois EPA to publish criteria on our website rather than in the Illinois Register.

<u>Toluene standards</u>: A typographical error was identified in the Lake Michigan Basin toluene standards, as the toluene standards contained within 35 Ill. Adm. Code 302.504(a) were adopted in  $\mu$ g/L (adopted in R02-11), yet were incorrectly entered into the regulatory language as being expressed in mg/L. Additionally, the Open Waters of Lake Michigan human health standard for toluene (35 Ill. Adm. Code 302.504(d)) is no longer needed and will be removed from this subsection (adopted in R97-25), as this value is superseded by the more stringent Lake Michigan Standards and is no longer applicable.

<u>Mercury</u>: Most metals standards in 35 Ill. Adm. Code 302.208 are specifically designated as applicable in the dissolved form because this is the form that is toxic to aquatic life. Exceptions are designated as applicable in the total metal form. The existing General Use human health standard for mercury (35 Ill. Adm. Code 302.208(f)) has no designation and to avoid confusion, it is desirable to clarify that for human health purposes, mercury in subsection (f) should be designated as total mercury. Total mercury was the form intended by the adopted standard due to the potential for total mercury to become methylated and subsequently bioaccumulate in aquatic life.

Zinc: The existing chronic aquatic life standard for zinc is hardness-based (See 35 III. Adm. Code 302.208(e)) and was adopted in the R02-11 rulemaking. The data initially filed with the IPCB and used in deriving the existing chronic zinc standard is provided in Exhibit V. Exhibit V is an excerpt from the original water quality standard derivation worksheet (labeled as Exhibit S in the documentation for the R02-11 rulemaking). The standard was developed using Tier I methodology and therefore, similar to the acute procedures detailed for the proposed fluoride and manganese standards, is highly dependent on the distribution of the four lowest chronic values in the database. An error was made in regards to the chronic toxicity value reported for Hyalella azteca, which was at that time considered the most sensitive organism within the chronic dataset. In Table 2 of Borgmann et al. 1993 (Exhibit W), a significant effect (% survival at week 10) was noted as occurring in the 180 µg/L nominal zinc treatment, whereas no effect was noted in the 100 µg/L nominal zinc treatment. The measured zinc concentrations that were to be used in the MATC calculation were 108 µg/L and 42.3 µg/L, however the percent survival values that resulted at these concentrations (35% and 51%) were mistakenly used to develop the MATC of 42.25. The correct MATC from the Borgmann et al. study should be 67.59  $\mu$ g/L, which is derived by taking the geometric mean of the measured concentrations that resulted in no observable adverse effect (42.3  $\mu$ g/L) and the lowest observable adverse effect (108  $\mu$ g/L). The test was conducted at hardness 130 mg/L and, when normalized to a hardness of 50 mg/L (as were all data in the zinc rulemaking), the resulting genus mean chronic value for Hyalella azteca is 30.08 µg/L (normalization calculation is given in Exhibit X), which is markedly different from the existing GMCV of 18.8 µg/L. The adopted chronic value for *Hvalella azteca* was erroneously calculated and resulted in a chronic zinc standard that was not representative of the true dataset. A summary of the four lowest mean chronic values and the resulting final chronic value (FCV) at 50 mg/L hardness for the existing zinc standard, as well as the revised standard with the corrected Hyalella azteca data, is included in Exhibit X. The revised FCV at 50 mg/L hardness is 17.62 µg/L and replaces the errant FCV of 12.16 µg/L (at a more typical Illinois hardness of 200 mg/L, the corresponding values are 57 and 39  $\mu$ g/L). Due to this change, the equation representing the chronic zinc standard must be modified to include the appropriate intercept (the slope remains unchanged). The revised equation is included in Exhibit X.

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## Attachment 1 – Exhibit A

Water Quality Criteria (Boron)

# WATER QUALITY CRITERIA

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DIVISION OF SANITARY ENGINEERING ILLINOIS DEPT. OF PUBLIC NEALTI Second Edition by McKEE and WOLF

> ELEPTAILS ENVIRONMENTE TOTECHON ACTIVES STATECT PLANOIS SPECIALIZIO, ILLINOIS

THE RESOURCES AGENCY OF CALIFORNIA STATE WATER QUALITY CONTROL BOARD SACRAMENTO, CALIFORNIA

**Publication No. 3-A** 

It is easier for calcium to replace sodium in the exchange complex than for sodium to replace calcium, and unless the sodium in the soil solution is considerably in excess of the calcium, no calcium will be replaced. It must be borne in mind that the soil solution is always more concentrated than the irrigation water. If magnesium constitutes a high proportion of the total replaceable cations of the soil, more sodium will be absorbed than if calcium is the only divalent cation present (281). It has been widely recommended that the percentage of sodium  $\left(\frac{Na \times 100}{Na + Ca + Mg + K}\right)$ in irrigation water should not exceed 50-60, in order to avoid the deleterious effects on soil which have been described above. Where the soil has a high cation exchange capacity and where the irrigation water is very dilute, values above 50 may be within safe limits (2386).

Aluminum, as well as calcium, in soluble form and in appreciable quantities, has been found to counteract the injurious effects of sodium on clay; and hence applications of these cations may be used to remedy such injury (283, 348).

In 1954 the staff of the U.S. Salinity Laboratory proposed that the sodium (or alkali) hazard of irrigation water can best be expressed in terms of the Sodium Adsorption Ratio, or SAR (1642). This ratio expresses the relative activity of sodium ions in the exchange reactions with soil. It is defined as follows:

SAR = 
$$\frac{Na}{[\frac{1}{2}(Ca + Mg)]^{1/2}}$$

where Na, Ca. and Mg are concentrations of the respective ions in milliequivalents per liter of water. If sodium percentage is defined as

$$Na \% = \frac{100Na}{Na + Ca + Mg}$$

then SAR can be expressed in terms of the milliequivalents per liter of sodium and the sodium percentage as follows:

SAR = 
$$Na^{1/2} \left[ \frac{2Na \%}{100 - Na \%} \right]^{1/2}$$

A thorough description of the SAR and its use is contained in Agricultural Handbook No. 60, U.S. Department of Agriculture (1642). Chapter 5 of this handbook is an excellent treatise on the entire subject of the quality of irrigation water.

Based on a SAR scale from 0 to 30 and conductivity values of 100 to 5000 micromhos per em at 25° C a diagram has been prepared for classifying irrigation waters with respect to sodium and salinity hazards, taking into account that a given SAR represents a greater hazard when the total concentration of ions is high than when it is low. This diagram appears as Figure 25 of U.S.D.A. Handbook No. 60 and it is reproduced herewith as Figure 5-1.

Water in the Cl-Sl area of the diagram can be used on almost all soils and for almost all crops without detri-

mental effects. With increasing salinity, less exchanged able sodium can be tolerated and more leaching will required to prevent salinity damage. Waters with SAR value greater than 10 will present an approximate sodium hazard in fine-textured soil having high cation exchange capacity, especially as the salinity increase Water in the S2 range may be used on coarse-textun or organic soils with good permeability (1642, 2387) further analysis of this diagram, the reader should con sult U.S.D.A. Handbook No. 60.

Doneen (2385, 2388) uses the term "sodium inder or "permeability index" to combine the effects of sodium and bicarbonate ions and the total concentration of cations (c) in the irrigation water, all measured milliequivalents per liter, thus:

For a water having 5 meq/l of sodium, 4 of bicarbonate and 8 of total cations, the index would be  $\frac{5+2}{8} \times 100$ 

or 87.5. Doneen (2388) presents curves to show the relation of the permeability index and the total ionic concentration for three types of soil and three classes of irrigation water

#### **BICARBONATE EFFECTS**

The sodium hazard is also increased if the water con tains a high concentration of bicarbonate ions, for as the soil solution becomes more concentrated there is a tend ency for calcium and magnesium to precipitate as car bonates and for the relative proportion of sodium to be increased as a consequence. Therefore the bicarbonate concentration of the water has been suggested as an additional criterion for irrigation water. It has been found convenient to express the bicarbonate value of the water in terms of the "residual sodium carbonate" (RSC) concentration, a concept devised by Eaton (2406) and defined as follows:

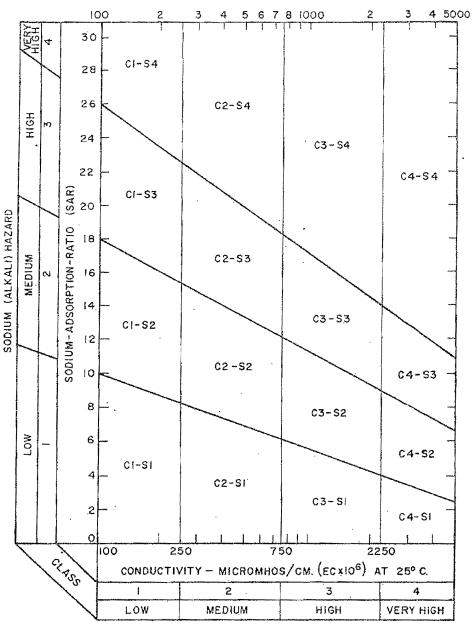
$$RSC = (CO_3^{--} + HCO_3^{-}) - (Ca^{++} + Mg^{++})$$

when the ionic constituents are expressed as milliequivalents (meq.) per liter.

Analyses of irrigation water and soil samples at the Salinity Laboratory have led to the conclusion that waters containing less than 1.25 meq. per liter of resid ual sodium carbonate are probably safe; those containing 1.25-2.5 meq. per liter are marginal; and those with more than 2.5 meq. per liter are not suitable. Marginal waters might be used successfully where good management practices are followed (1642, 2389).

#### BORON IN IRRIGATION WATERS

Boron is found in almost all waters used for irrigation in the U.S.A., in concentrations from a trace to over 100 mg/l. It occurs naturally in the form of boras borates, boric acid, and various borosilicates, such as tourmaline, which are of magmatic origin. It can also be found in fertilizers and certain waste-waters, such as those from citrus washing. In most natural waters, borol probably occurs as almost completely undissociated borid acid (2379, 2390). Although traces of boron are essential for all plant growth, it is doubtful whether more than 0.5 mg/l can be applied continuously to soils without ultimately producing some plant injury (265, 275).



SALINITY HAZARD

FIGURE 5-1. DIAGRAM FOR THE CLASSIFICATION OF IRRIGATION WATERS (from USDA handbook No. 60)

Agricultural authorities agree that for irrigation water the critical concentration is 0.4 to 0.5 mg/l; but because plants vary in their sensitivity to boron, waters may be classified not only according to their boron content, but also according to the tolerance of the crops to which they are applied. Tables grouping plants in the order to their sensitivity to boron will be found in several papers, including the following references (246, 263, 264, 269, 274, 1642, 2391). The most sensitive crops are citrus, nuts, and deciduous fruits; semitolerant are truck crops, cereals, and cotton; most tolerant are lettuce, al-falfa, beets, asparagus, and date palms.

While some crops such as alfalfa and date palms are stated to be uninjured by as much as 20 to 100 mg/l of boron, it is considered that the maximum concentration safe for even the least sensitive plants is about 4.0 mg/l (276).

Symptoms of boron injury can be distinguished easily from those of most other types of injury, although occasionally they are confused with those of sulfate poisoning. Among trees, advanced damage will result in leafyellowing and burning, premature leaf drop, and reduced yield (276, 277). The quality of soil, drainage, and climatic and other environmental factors, such as the amount of rainfall and total amount of irrigation water applied, can modify the safe concentration limits. However, symptoms of boron injury may not become apparent for as long as several years. They develop more rapidly in light than heavy soils. Concentration of the soil solution owing to evaporation and transpiration tends to accelerate their apearance, but the absorptive capacity of the soil may delay it. Parenthetically, it is essential to remember that when boron in the irrigation water is 0.5 mg/l, its concentration in the soil solution may be more than 4 mg/l (265).

It has been suggested that where the boron concentration in irrigation water is high and cannot be reduced economically, an effort should be made to grow moreresistant crops in the area affected. A widely used classification of water according to its boron concentration is shown in Table 5-10.

#### STOCK AND WILDLIFE WATERING

Paradoxically, data with respect to the water-quality requirements of animals are both abundant and sparse. There is a wealth of information about the  $LD_{50}$  values of thousands of compounds fed to laboratory animals, mostly rats, mice, and guinea pigs, either in their diet or in their drinking water. Yet, there are very few quantitative data concerning the water-quality tolerances of livestock and poultry. Veterinarians and animal-husbandry personnel in this country do not appear to be particularly concerned over water quality; but in Australia and South Africa, where water for livestock is frequently highly mineralized, considerable attention has been directed to this problem.

Since the total quantities of substances ingested daily are the critical values for animal metabolism, the permissible concentrations of such substances in water will depend, to some extent, on the daily water consumption of the animals. The daily water requirements of animals vary with a number of factors, such as the temperature and humidity of the atmosphere, the water content of the diet, the degree of exertion by the individual with a resulting loss of water as sweat, and the salinity of the available supply (284, 286).

The quantity of water required for livestock and poultry has been estimated as follows (284, 286, 2392):

Animal	Water consumption in gpd per head, except as noted		
Beef cattle	7-12		
Dairy cattle	10-16		
Horses	8-12		
Swine	3-5		
Sheep and goats	1-4		
Chickens	8-10 (per 100 birds)		
Turkeys	10-15 (per 100 birds)		

TABLE 5-10 PERMISSIBLE LIMITS FOR CONCENTRATION OF BORON IN SEVERAL CLASSES OF WATER FOR IRRIGATION (After Scofield) (263)

	Concen	tration of Boron	in mg/l
	F	or Crops That A	re
Class of Water	Sensitive	Semitolerant	Tolerant
Excellent	Less than 0.33	Less than 0.67	Less than 1.0
Good	0.33-0.67	0.67-1.33	1.0-2.0
Permissible	0.67-1.0	1.33-2.0	2.0-3.0
Doubtful	1.0-1.25	2.0-2.5	3.0-3.75
Unsuitable	Over 1.25	Over 2.50	Over 3.75

It has been assumed that water safe for human consumption may be used safely by stock; indeed, it has been recommended that stock, for their highest production, should have such water (284, 285). On the other hand, it appears that animals can tolerate higher saling ities than men, and it is conceivable also that they differ in their tolerance of specific substances.

The use of highly mineralized waters can cause among animals, as well as among men, physiological disturb ances of varying degrees of severity, such as gastrointes tinal symptoms, wasting disease, and death. Among the functions of animals, lactation and reproduction are generally the first to be disturbed by continuous use of waters with unfavorable mineral concentrations, so that milk and egg production are reduced, if not terminated

It has been stated that no animal will choose to drink saline water if better water is available. Within limits however, animals can adjust to the use of saline water that at first were impossible to consume. On the other hand, sudden changes from slightly mineralized to highly mineralized water may cause acute salt poisoning and rapid death (282). The tolerance of animals to salts in water depends also on other independent factors, including their species, age, and physiological condition, the season of year, and the salt content of the diet, as well as the quality and quantity of salts present.

The officers of the Department of Agriculture and the government chemical laboratories of Western Australia (282, 2393) have listed the threshold concentrations of salinity tolerated by livestock in that region. The total salts include the chlorides, sulfates, and bicarbonates of sodium, calcium, and magnesium, with sodium chloride constituting as much as 75 percent of the total salinity. In general, it is stated that waters containing less than 300 grains per Imperial gallon (about 5000 mg/l can be used continuously by all livestock. Sheep are more tolerant than cattle, and cattle are more tolerant than horses or pigs. The standards in use in Western Australia as the safe upper limits for stock are reported as follows:

	Threshold Salinity Concentra	tions in
Animal	grains per Imperial gallon	1497 E
Poultry	200	2860
Pigs	300	4290
Horses	450	6435 7150
Cattle, dairy	500	10,000
Cattle, beef	700	12,900
Adult dry sheep	900 .	12,000

When total salts exceed the above listed concentrations, practical tests are needed to show whether or not the water is safe. When green feed is available, animals can tolerate more saline water than when "bush or scrub" is the only feed. Where feed is low in salt content, water of higher salinity is also tolerable. Sheep In U. S. waters that support a good fish fauna, 5 percent of such waters have less than 40 mg/l of bicarbonate, 50 percent have less than 90 mg/l, and 95 percent have less than 180 mg/l (310).

#### BIOCHEMICAL OXYGEN DEMAND

(see also Dissolved Oxygen, Oxygen Consumed)

As in tests for alkalinity, acidity, color, turbidity, and specific conductance the determination of biochemical oxygen demand (B.O.D.) does not reveal the concentration of a specific substance. Instead it measures the effect of a combination of substances and conditions. The rate at which B.O.D. is exerted generally follows the unimolecular pattern as shown by equations in Chapter II.

As a parameter of the detrimental effects of organic matter upon a surface water, the 5-day B.O.D. value alone means very little. In itself, B.O.D. is not a pollutant and exercises no direct harm. Only by depressing the dissolved-oxygen content to levels that are inimical to fish life and other beneficial uses does B.O.D. exert an indirect effect. Where reaeration, dilution, and/or photosynthetic action offset or minimize this depletion, B.O.D. does not interfere with the reasonable uses of the water.

B.O.D. is important only insofar as it produces septicity or decreased dissolved oxygen, or subsequent growth of saprophytic bacteria which increase the turbidity or other undesirable characteristics of the streams. In a glow, sluggish stream, a 5-day B.O.D. of 5 mg/l might be sufficient to produce deoxygenation resulting in anaerobic conditions, whereas a swift mountain stream can easily handle 50 mg/l of 5-day B.O.D. without appreciable depletion of dissolved oxygen. Each stream must be considered in its own right, and until the reacration characteristic of the stream is known the limiting values of B.O.D. cannot be set.

Many state and interstate agencies include B.O.D. limitations in stream standards while others specify that effluents shall not exceed a given concentration of B.O.D. or that B.O.D. reduction by treatment shall reach or exceed a stated efficiency. For details of these state and interstate standards, see Chapter III of this report and the appendices thereto.

#### BLAST

 $(\text{see Chapter } \mathbf{X})$ 

#### B.O.D.

(see Biochemical Oxygen Demand)

#### BORANES

(see Boron)

#### BORAX

(see Sodium Borate)

#### BORIC ACID

See Boron)

#### BORON

1. General. Never found in nature in its elemental form, boron occurs as sodium borate (borax) or as calcium borate (colemanite) in mineral deposits and natural waters of southern California and in Italy. Elemental boron is used in nuclear installations as a shielding material (neutron absorber). It is also used in metallurgy to harden other metals (364, 2121).

Boric acid and boron salts are used extensively in industry for weatherproofing wood, fireproofing fabrics, manufacturing glass and porcelain, production of leather and carpets, cosmetics, photography, artificial gems, and many other purposes. Boric acid is used as a bactericide and fungicide. Finally, boron in the form of boron hydrides or borates is used in high-energy fuels (354, 2121).

Boron may be substituted for carbon in many organic compounds, e.g., boron trichloride, boron tribromide. It may also be synthesized directly with hydrogen to form boranes, such or diborane,  $B_2H_6$ , a gas with a nauseating odor; pentaborane,  $B_5H_9$ , a volatile liquid with a sweetish odor; and a decaborane,  $B_{10}H_{14}$ , a crystalline solid with a bitter-chocolate odor. The boranes are used as rocket fuels and may be encountered in other situations where high-energy fuels are desired.

2. Cross References. Sodium Borate, Sodium Perborate, Chapter V—Irrigation Waters.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Although boron is essential in the nutrition of higher plants, there is no evidence that it performs any vital function in human or animal nutrition (2121). It is present in the ordinary human diet to the extent of 10 to 20 mg/day, with fruits and vegetables as the largest contributors. In food or in water it is rapidly and completely absorbed by the human system, but it is also promptly excreted in urine (2121).

The ingestion of excessive doses of borates may cause nausea, cramps, convulsions, coma, and other symptoms of distress. The fatal dose for adults has been reported as 5 to 20 grams (364) and as 20 to 45 grams (2121). Normal adults were fed 3 grams of boric acid daily for 11 to 16 days without apparent toxic effects (3265).

Boron in drinking water is not generally regarded as a hazard to human beings (633). Goudey and others have reported that boron concentrations up to 30 mg/l are not harmful in drinking water. Above this concentration, it may interfere with digestion because of its preservative action on foods (353, 1055, 1056). Quantities up to 0.5 grams per day of either borax or boric acid have no immediate effect of any kind on healthy individuals (997). Hoskins, however, has recommended a boron limit of 20 mg/l in drinking water (1057).

mg/l in drinking water (1057). b. Irrigation. The problem of boron in irrigation water is covered extensively in Chapter V under "Irrigation." Boron is an essential element in the nutrition of higher plants, yet concentrations of boron in irrigation waters in excess of 0.5 mg/l may be deleterious for certain crops. Crops such as asparagus, date palms, sugar beets, alfalfa, onions, turnips, cabbages, lettuce, and carrots can tolerate boron concentrations of 2.0 to 4.0 mg/l. Crops such as potatoes, tomatoes, peas, wheat, corn, oats, and lima beans can grow well at 1.0 to 2.0 mg/l of boron. Among the sensitive crops are pecans, artichokes, plums, pears, apples, cherries, grapes, peaches, oranges, avocados, grapefruits, and lemons, which can tolerate no more than 0.5 to 1.0 mg/l of boron (2391). Plant roots take up small quantities of dissolved boron from the soil solution Boron adsorbed on the soil is not utilized by plants (3352). The absorbed boron is moved to the leaves, where the water is lost by transpiration. Boron remains in the leaf and tends to accumulate in the tip and margin. As the process continues, the boron concentration becomes sufficiently high to be toxic to the leaf tissue. This type of injury is found only on mature leaves, thus differing from boron-deficiency symptoms that appear only on the new growth (3266).

c. Stock and Wildlife Watering. The lethal dose of boric acid for animals varies from 1.2 to 3.45 grams per kg of body weight, according to the species (2121). Concentrations of 2500 mg/l of boric acid in drinking water have been detrimental to animals only insofar as growth was inhibited (2121). A dairy cow received 16 to 20 grams of borax daily over a 40-day period without ill effects, but the concentration of boron in the milk rose from 0.7 to 3.0 mg/l. The synthetic boranes are far more toxic to animals than natural boron compounds, for example the LD<sub>50</sub> for decaborane administered orally to rats was reported as 64.3 mg/kg (3267, 3268).

d. Fish and Other Aquatic Life. LeClerc and Devlaminck (2942, 2943, 2944) reported the minimum lethal dose for minnows exposed to boric acid for six hours at 20°C to be 18,000 to 19,000 mg/l in distilled water and 19,000 to 19,500 mg/l in hard water. Wallen et al. (2940) tested the effect of boric acid and sodium borate in highly turbid water on the mosquito-fish (*Gambusia affinis*), with the following results:

	0		Concentration in mg/l		
Ť	emperatur	e	24-hour	48-hour	96-hour
Ohemical	Range	pH Range	$TL^m$	TLm	$TL^{m}$
Boric acid	20-23°C	5.4-7.3	18,000	10,500	5,600
Sodium borate	22-26°C	8.6-9.1	12,000	8,200	3,000

Wurtz (1054) has reported the results of a study of the effects of boric acid on one rainbow trout and one rudd. A solution of 2,000 mg/l of boric acid was harmless to both fish; 5,000 mg/l caused only a slight darkening of the skin of the trout. The trout became immobile and lost its balance in a few minutes in concentrations up to 80,000 mg/l but recovered rapidly when it was transferred to fresh water, even after immersion in the boric-acid solution for 30 minutes. The rudd appeared unharmed by concentrations up to 80,000 mg/l for short periods; however, it died after 18 hours in a 6,250 mg/l solution of boric acid. A roach in 6,250 mg/l solution also died, after 46 hours.

Boric acid can be toxie to fresh water fish without lowering the pH to 5.0. Thus, pH is not a reliable index of dangerous pollution by boric acid (361).

Turnbull et al. (2093) found the 24-hour  $TL_m$  of boron trifluoride toward the bluegill sunfish in Philadelphia tap water at 20 °C to be 15,000 mg/l.

To produce a 50-percent inhibition of the 5-day oxygen utilization of synthetic sewage, Herman (2923) found that over 1000 mg/l of boric acid was required.

#### BREWERY WASTES

(see also B.O.D., Sugars, Detergents, Soaps)

For a thorough discussion of the nature of brewery wastes, the reader is referred to a standard text on chemical processes and industrial wastes (189, 346). The principal deleterious effect of such wastes is their high B.O.D It has been reported (465) that yeast wort is harmless to fish in a dilution of 1:40.

#### BROMINE

A dark reddish-brown fuming liquid, elemental bromine is relatively soluble in water. It is used for medicinal compounds, dyestuffs, and antiknock compounds for gasoline motors. It has also been used for sterilization of swimming-pool water. Sources of molecular bromine in water are chemical industries and salt-works effluent Bromine, like other halogens, is antiseptic and disinfectant; hence it may possibly interfere with bacterial and other natural purification processes.

A concentration of 10 mg/l of bromine in soft water has killed *Daphnia magna* (313). Jones (2920) reported that 20 mg/l of bromine killed goldfish at 18-23°C. Hiaft (3350) indicated that 1.0 mg/l of bromine showed no irritant response from marine fish, but 10 mg/l caused violent irritant activity.

#### BSM-11, BUFFEN 30, BUTROL

(see Chapter IX)

#### BUTADIENE

CH2=CHCH=CH

CH<sub>3</sub>COOC<sub>4</sub>H

 $B_{r_2}$ 

A colorless gas, 1, 3-butadiene is insoluble in water. It is used as a polymer component in the synthesis of runber. According to Garrett (2959), the 24-hour  $TL_m$  for the marine pinperch (*Lagodon rhomebodies*) is 715 mg/l. No deaths occurred at 50 mg/l.

#### BUTANONE

(see Methyl Ethyl Ketone)

#### BUTYL ACETATE

Normal butyl acetate is a liquid highly soluble in water. It is used in the manufacture of plastics, lacque artificial leather, and photographic films (364). The ord  $LD_{50}$  for rats has been reported as 4.13 grams/kg 0 body weight and for mice 7.06 grams/kg (3242).

Bringmann and Kuhn (2158) found that the median threshold effect of n-butyl acetate toward *Daphnia* during a two-day exposure at 23°C occurred at a concentration of 44 mg/l. For *Scenedesmus* at 24°C for 4 days, the median effect occurred at 320 mg/l; but for *E. coli* at 27°C, no effect was apparent at concentrations less than 1000 mg/l.

#### BUTYL ALCOHOL

#### C'H'0I

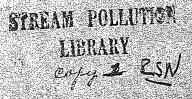
Normal butyl alcohol, a colorless liquid, is used extensively in industry, being prepared from cornstarch of from acetylene. It may occur in many types of wastes, including those from the paint, varnish, and chemical industries. The oral  $LD_{50}$  of n-butyl alcohol for rats has been reported as 4.36 mg/kg of body weight (364) and as 2.75 mg/kg (3248). According to Ettinger et al. (2172, 3269) the median response to the odor threshold of n-butyl alcohol occurred at a concentration of about 0.2 mg/l.

Attachment 1 – Exhibit B

Water Quality Criteria (Fluoride)

1

# WATER OUALITY CRITERIA



# RECEIVED

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DIVISION OF SANITARY ENGINEERING ILLINOIS DEPT. OF PUBLIC HEALTH Second Edition by

McKEE and WOLF

CLERY SEX ENVIENDMENDAL FROTEOLOGIACTNOV STATE OF HUBCHS SPRIESEED, LUNCIS

THE RESOURCES AGENCY OF CALIFORNIA STATE WATER QUALITY CONTROL BOARD SACRAMENTO, CALIFORNIA

Publication No. 3-A

year-old girl was caused by ingestion of 1500 mg of FeCl<sub>2</sub>.

#### FERROUS OXIDE

FeO

Using highly turbid water at 16-23 °C and the mosquitofish (*Gambusia affinis*) as the test organism. Wallen et al. (2940) found the 96-hour  $TL_m$  of ferrous oxide to be over 10,000 mg/l.

#### FERROUS SULFATE

FeSO<sub>4</sub> and FeSO<sub>4</sub>·7H<sub>2</sub>O

1. General. The anhydrous and crystalline forms of this substance are highly soluble in water, and the salts are used in many industrial operations. Sources of pollution by ferrous sulfate include canneries, tanneries, textile mills, mines containing pyrites, and metal-cleaning operations involving the use of pickling liquors. Ferrous sulfate is sometimes used as a coagulant in water and sewage treatment.

2. Cross References. Iron, Distilled Water, Sulfates, and other iron salts.

3. Effects Upon Beneficial Uses.

a. Fish and Other Aquatic Lafe. The threshold concentration of ferrous sulfate for immobilization of *Daphnia magna* in Lake Erie water was found to be less than 152 mg/1 (358).

The following concentrations of ferrous sulfate have been harmful or lethal to fish in the time specified:

Concentra Non in mg	- Type of 1 Water	Time of Exposure	Species of Fish	Reference
2.9	distilled	4-24 hours	shiners, suckers, carp_	313
6.4		24 hours	shiners, suckers, carp_	359
100		24 hours	minnows, goldfish,	
	····· .		trout	359
100		4.2-7 days	bass	1035, 1030
		2.5-3.5 days	sunfish	1035, 1030
133		24 hours	brook trout	359
315	distilled	3 hours	minnows	313
500 -		1.3-5 days	goldfish	1030
1,000		9-23 bours	bass	1030
1,000		48 hours	very young carp	1459
1.000		5-30 hours	goldfish	1030
		2.5-9 hours	bass	1030
1.000	hard	2-10 hours	goldfish	313
1.390		144 minutes	minnows	991
2.721	tap	31-66 minutes	trout. salmon	813
6.950		104 minutes	minnows	. 991
10.000		1 week	tench	1459
10.000		1 day	other fish	1459
13,900		68 minutes	minhows	991

The following concentrations of ferrous sulfate have been reported as not harmful to fish within the time specified:

Con con-

tration in mg/1	Type of Water	Time of Exposure	Species of Fish	Reference	
` <b>1</b> 5	·	24 hours	carp, shiners, suckers	359	
17.1		1 hour	minnows	362	٠
60		7 days	bass, bluegills	1459	
50	_	24 hours	trout	359	
			bass, sunfish	1035, 1030, 859	
100	hard	95 hours	goldfish	.313	
•		· 7 days	goldnish	1035, 1030	
100		7 days	goldfish	1459	
100			carp, tench	1459	
380		185 minutes	minnows	353	
1,000		over 1 week	mature fish	1459	

Ferrous sulfate has also been reported to be lethal to fish at the following concentrations of iron:

Concentration of iron, mg/l	Type of Water	Time of Exposure	Type of Fish	Reference
1.28	distilled	24 hours 2-10 hours	coldfinh	1459

On the other had, 37 mg/l of iron has not been harmful to goldfish in 100 hours (1466). The effects of disposal of as much as 3000 tons per day of acid ferrous sulfate solution at sea have been investigated independently by Arnold and Royce (1466) and Redfield and Walford (1467, 1561). They found no evidence of significant changes or harmful results among the aquatic life in the areas studied.

#### FERROUS SULFIDE

This black solid is highly insoluble in water. Wallen et al. (2940) reported its 96-hour  $TL_m$  toward mosquitofish in highly turbid water at 20-26°C to be over 10,000 mg/l. Undoubtedly the ferrous sulfide remained in suspension or settled out of suspension, for it would not be expected to go into solution.

FERROUS SULFITE

Using highly turbid water at 20-21°C, Wallen et al. (2940) found the 24-, 48-, and 96-hour  $TL_m$  concentrations toward the mosquito-fish (*Gambusia affinis*) to be 350 mg/l.

#### FERTILIZER MANUFACTURING PLANT WASTES

Ellis (611) reported that wastes from a fertilizer manufacturing plant in Mississippi constituted no hazard to fish.

#### FLUORIDES

F

1. General As the most reactive non-metal, fluorine is never found free in nature but it is a constituent of fluorite or fluorspar, calcium fluoride, in sedimentary rocks and also of cryolite, sodium aluminum fluoride, in igneous rocks. Owing to their origin only in certain types of rocks and only in a few regions, fluorides in high concentrations are not a common constituent of natural surface waters, but they may occur in detrimental concentrations in ground waters (152).

Fluorides are used as insecticides, for disinfecting brewery apparatus, as a flux in the manufacture of steel, for preserving wood and mucilages, for the manufacture of glass and enamels, in chemical industries, for water treatment, and for other minor uses (364). While not normally found in industrial wastes, they may be present in traces, or in higher concentrations resulting from spillage.

2. Cross References. Hydrogen Fluoride and various fluoride salts.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Fluorides in sufficient quantity are toxic to humans, with doses of 250 to 450 mg giving severe symptoms and 4.0 grams causing death (364). The fatal dose has also been reported (1161) as 0.5 gms per kg of body weight and as 2.5 grams (3481).

There are numerous articles describing the effects of fluoride-bearing waters on dental enamel of children and a few papers pertaining to skeletal damage. The effects reported in many of these references, summarized in Table 6-5, lead to the generalization that water containing less than 0.9 to 1.0 mg/l of fluoride will seldom cause mottled enamel in children, and for adults concentrations less than 3 or 4 mg/l are not likely to cause endemic cumulative fluorosis and skeletal effects.

Abundant literature is also available describing the advantages of maintaining 0.8 to 1.5 mg/l of fluoride

FeS

FeSO3·24H2O

ion in drinking water to aid in the reduction of dental decay, especially among children. A review of such treatment processes is not relevant to this report, but it is significant to note that the presence of about 1.0 mg/l of fluoride ion in natural waters may be more beneficial than detrimental.

There is evidence to support the contention that fluorides in excess of the threshold for mottling of teeth and up to 5 mg/l produce no harmful effects other than mottling (1463, 1564, 1566). Radiologic surveys of 114 persons who had lived for over 15 years at Bartlett, Texas where water had 8 mg/l of fluoride revealed minimal evidence of an increase in density of bones of only 12 percent of those examined, but in no case was there found any interference with the use of bones or joints. Comparisons of mortality rates from nephritis, heart disease, or cancer in high or low fluoride areas has failed to show an association of these diseases with the fluoride content of water (1563, 1564). It has been estimated that daily intakes of about 15-20 mg of fluoride over a period of several years are required to induce chronic. fluorosis in an adult man (1567).

The taste of sodium fluoride is salty, but less so than sodium chloride. A solution of sodium fluoride at a concentration of 2.4 mg/l of fluoride can be distinguished from distilled water (1568).

Shay (729, 730) used statistical evidence to show that the incidence of poliomyelitis is lower in districts where the surface waters contain over 1.0 mg/l of fluoride than in areas where the fluoride content is lower. Fellenberg (1163) investigated the correlation between goiter incidence and fluoride in the drinking water, but reached no definite conclusions.

The USPHS Drinking Water Standards (2036) of 1962 set a mandatory limit on fluorides that is based on the annual average of maximum daily air temperatures in accordance with the following table. It is reasoned that children drink more water in warm climates and hence the fluoride content of the water should be lower to prevent excessive total fluoride consumption (1563, 1564, 1565).

Annual Average of Maximum Daily Air Temperatures, °F		Recommended Control Limits of Fluoride Concentrations, mg/l		
		Lower	Optimum	Upper
50.0-53.7		0.9	1.2	1.7
53.8-58.3	· · · · · · · · · · · · · · · · · · ·	0.8	1.1	1.5
58.4-63.8		0.8	1.0	1.3
63.9-70.6		0.7	0.9	1.2
70.7-79.2		0.7	0.8	1.0
79.3-90.5		. 0.6	0.7	0.8

The WHO International Drinking Water Standards (2328) of 1958 do not set a limit on fluoride concentration, but the WHO European Drinking Water Standards (2329) of 1961 prescribe a recommended limit of 1.5 mg/l.

#### TABLE 6-5.

#### REPORTED EFFECTS OF FLUORIDES IN DRINKING WATER FOR HUMANS

Concentration of Fluorides, in mg/l	Reported Effect	Reference
0.2	Mottled teeth in 1 percent of children .	1164
0.6	No effects at this concentration, or low	er 555
0.7	Mild dental fluorosis in 8.5 percent	
	of children	3451
0.8	No effects at this concentration,	
·.	or lower 8	6, 1165
0.8 to 0.9	Mild mottling of teeth	1166
0.8 to 1.5	Threshold for mottling of teeth	

Concentration of Fluorides, in mg/l	Reported Effect Re	ference
0.9	Mild mottling of tooth 1165	1167
0.9	Mild mottling of teeth 1165, Mottling occurred as a result of	1101
	high water use	353
0.9	high water use Critical concentration for mottling 555,	1168
1.0	Threshold for mottling of teeth	741
1.0	10 percent of children had	
	mottled teeth	1164
1.0	10 percent of children had mottled teeth 1169, 90 percent of children had	
	mottled teeth	1170
1.0 to 2.0	Mild to moderate mottling	1165
1.2	No effect at this concentration	353
1.4	No skeletal schlerosis found	353
1.5	Limiting concentration for	
	drinking water	1171
1.7 to 1.8	drinking water 50 percent of children had	
	mottled teeth 1169, Gave mottling and weakening of	1194
2.0	Gave morthing and weakening of	1100
	tooth structure	1172
2.0 to 3.0	Retained in system Moderate to severe mottling	200
2.0 to 3.0	Moderate to severe mottling	1100
2.5	15 to 80 percent children	1104
2.5	75 to 80 percent children had mottled teeth 555; No evidence of skeletal fluorosis	1168
2.0	No evidence of skeletal muorosis	3492
3 to 4	Not likely to cause endemic cumulative	
<b>5</b> 4 5 A	toxie fluorosis in adults 1173,	1114
3 to 6	Gave severe motunny	1100
3.5 to 6.2	Gave severe mothing No adverse effect on carpol bones of children 90 percent of children had mothed teeth	3453
4.0	90 percent of children had mottled teeth	1164
4.0	No disorders other than dental mottling	3454
4.4 to 12	Caused chronic fluorosis and affected	
5.0	skeletal system 1175, This concentration had no effect on	
	height, weight or bone	1177
5.0	height, weight or bone Threshold for appreciable effect on bones	1168
6.0	100 percent of children had mottled teeth	1164
6.0	Gave nitting and chinning of	
8.0	tooth enamel No deleterious bone changes except dental mottling Some cases of skeletal fluorosis Gave chronic fluorine intoxication	1192
9.0	dantal mottling	2455
10	Some arges of stalets! francis	9155
11.8	Cano abrania finanina interioation	. 9499.
11.0	Affects deciduous teeth	1178
12	Affects deciduous teeth	1179
13.7	100 percent of children had	
	mottled teeth	353
115 .	mottled teeth Sub-lethal in drinking water	152.
180	Toxic to man in drinking water	555
2000	Toxic to man in drinking water Lethal dose in drinking water	152

b. Industrial Water Supplies. Excessive fluorides may be harmful in certain industries, particularly those involved in the production of food, beverages, pharmaceutical and medical items, according to Bratton (1569). If wet milling of corn is carried on with water containing one mg/l of fluoride, it is estimated that the concentrated steep water will contain more than 6 mg/l and the corn syrup more than 5 mg/l. Malt syrup made with similar water may contain up to 8 mg/l of fluoride. Weir (1570) points out that fluoride up to 10 mg/l in dough water has no effect on bread, that one mg/l stimulated the yeast fermentation of malt, that 10 mg/l may stimulate or depress yeast fermentation, and that 25 mg/l inhibits yeast activity.

In brewing, fluoride concentrations of 1 to 5 mg/l appear to stimulate yeast metabolism. Continued re-use of yeast in wort containing 10 mg/l of fluoride results in severe deterioration after six fermentations (2349). Concentrations of fluoride permissible in domestic water should have no deleterious effects on brewery processes (2348).

Fluoride concentrations of 1.0 mg/l caused no change in the amount or rate of corrosion of iron, copper, or lead (3482). Fluoride limits have been recommended for some industrial processes, as described in Chapter V and tabulated below:

Use	Recommended Threshold Values in mg/l
Brewing	
Carbonated beverages	
Food canning and freezing	
Food equipment washing	
Food processing, general	

c. Irrigation Water. Concentrations of fluoride likely to be found in natural waters or in polluted streams apparently will have no detrimental effects on plants. Moreover, fluoride added to soil or water has little or no effect on the fluoride content of plants grown in such soil (1049, 1182, 3457). At high concentrations, fluoride has been reported to produce the following effects:

Concentration Fluoride, mg,	of /l Effect I	<i>leterence</i>
10	No injury to peach, tomato, and buckwheat	3458
100	plants Peach and buckwheat plants severely injured in 3 days	
100-500	Inhibited sprouting of beans	1180
180	Did not injure buckwheat at pH over 5.5	3459
200	Killed peach, tomato, and buckwheat in short	3458
360	Injurious to peach and buckwheat even at pH	3459
1000	Stunted growth of large bean plants	1180

The use of fluoride-bearing insecticides appears to cause no harmful concentrations of fluoride in the soil moisture (1182).

d. Stock and Wildlife Watering. The effects of fluorides in drinking water for animals is analogous to those for humans. Table 6-6 lists the reported effects as reported in the survey literature, and indicates that 1.0 mg/l appears to be the threshold value below which no harm results. It is interesting to note that the addition of fluorides to a cow's ration or drinking water had no influence on fluorides in the milk (1181, 1188), and doses of 500 mg/l in the drinking water did not increase the milk fluoride above 0.5 mg/l (1186).

#### TABLE 6-6 REPORTED EFFECTS OF FLUORIDES IN DRINKING

WATER FOR LIVESTOCK

Fluoride	е на селот н Селот на селот на село			
Concentratio in mg/I	n Dose	Animal	Remarks	Reference
1.0		cattle	harmless	292
1.0		sheep	fluoride poisoning_:_	1183
1.4-4.5		mice	mottling of teeth	
A.T	0.4 mg per kg	cattle	no mottling	
·	1 mg per kg	rats	mottled teeth	
	1 mg per kg	cattle	mottled teeth	1190, 3462
	3 mg per kg	cattle	bone damage and	
			death	853
'	5 mg	dogs	gave hypotension	3461
4.0	·	sheep	mottled and pitted	·
		•	teeth	1184
5.0		cows	disliked water	
5.0	-	sheep	slight dental mot-	
		·	tling	1571
6 to 16			severe mottling	1005
11.78		cows	mottled teeth	. 1178
15		mice	affected thyroid and	3460
18		cows	kidney slowly increasing	2400
10		COWA	fluorosis	1190
28	•	sheep	5 percent reduction	
20		sneep	in weight	1571
AF # 64	•		III Weight	TOVT'
25-100		young cattle	teeth lesions	3464
				010%
44-61		sheep	chronic fluoride	1184
~~		<b>Te a sea set</b>	poisoning	
50		namsters	dental fluorosis in	1100
		•	10 weeks	1185
55		cows.	disliked such water	
	· · · ·	· ·	and drank less	1186
'	60 mg per day	sheep	affected teeth and	
			bones	1187
	65 mg per day	dogs	no effect on organs	
	120 mg per day	sheep	threshold for gen-	
		•	eral health	1187
	200 mg per kg	rabbits	lethal dose	353
100		cattle .	no economic harm_	3463

e. Fish and Other Aquatic Life. Fluoride ions appear to have direct toxic properties toward aquatic life, and in addition there seems to be a relationship between the fluorides in water and the condition of the teeth of the fish (1189). The following effects of fluorides on fish have been reported:

	-		·	
Concentration of Fluoride, mg/1	Salt used	Type of . fish	Effect	Referance
$\begin{array}{c} 1.5\\ 3.3-7.3\\ 2.6-6.0\\ 2.7-4.7\\ 5.9-7.5\\ 7.7\\ 64\\ 75-91\\ 100\\ 120\\ 358\\ 419\\ 578\\ 1000\\ 1000\\ 1000\\ \end{array}$	NaF NaF NaF NaF NaF NaF NaF NaF	eggs trout trout trout trout trout carp goldfish rainbow trout mosquito-fish <i>Tinca rulparis</i> goldfish goldfish	slower and poorer hatching TLm at 18°C, in soft water TLm at 13°C, in soft water TLm at 7.5°C, in soft water not harmed in one hour 10-day TLm TLm survived over 4 days hilled in 4 days toxio in soft water behour TLm in turhid water lethal dese killed in 12 to 29 hours in soft water killed in 0 to 102 hours in hard water	3467 353 3468 1756

For toxicities toward lower aquatic organisms, see Sodium fluoride.

4. Summary. On the basis of the foregoing information, it appears that the following concentrations of fluoride will not interfere with the specified beneficial uses:

a.	Domestic water supply	0.7 to 1.2 mg/l
	Industrial water supply	
c.	Irrigation water	10.0 mg/1
	Stock watering	
e.	Aquatic life	1.5 mg/l
		· .

#### FORMALDEHYDE

HCHO idation of

This simple aldehyde is formed by the oxidation of methyl alcohol by air in the presence of metallic silver or copper at high temperatures (300°C). It results also from the incomplete combustion of many organic substances and is found in the atmosphere over cities. It also occurs in some tannery wastes, penicillin wastes, and effluent from the manufacture of plastics and results. At ordinary temperatures it is a colorless, flammable gas with a pungent suffocating odor, and it is intensely irritating to inucous membranes. It is very soluble in water, and a 37-40 percent solution in water is sold as "formalin". Because of its toxicity to lower forms of life, formaldehyde is used for preserving biological specimens.

The odor of HCHO is reported to be detectable at 50 mg/l (2983) and also at 20 mg/l (3483). The oral  $LD_{50}$  for rats is given as 800 mg/kg of body weight (3484).

In a concentration of 10 mg/l, formaldehyde had no apparent effect on rainbow trout in three days but 50 mg/I killed them in one to three days of exposure (659). For killing shiners in 120 hours at 18°C, the minimum lethal concentration was also 50 mg/l (190, 344). In stabilized tap water saturated with oxygen, minnows were harmed by a short exposure to 146 mg/l (362). For rainbow trout, the critical level of formaldehyde was reported (2091) as less than 31.8 mg/l and for young chinook salmon less than 28.2 mg/l. Clemens and Sneed (2979, 2981) investigated the toxicity of formalin (37 percent formaldehyde by weight) toward fingerling channel catfish. They found the 24-hour TL<sub>m</sub> to be 32 mg/l as formaldehyde while the 48- and 96-hour  $TL_m$ concentrations were 25 mg/l. All fish survived at 18 mg/l as formaldehyde. If they are given a chance to do so, during short-term exposure, fish will avoid solutions Attachment 1 – Exhibit C Water Quality Criteria (Manganese)

# WATER OUALITY CRITERIA

STREAM POLLUTION LIERARY Copy 2 CSN

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DIVISION OF SANITARY ENGINEERING ILLINOIS DEPT. OF PUBLIC HEALTH Second Edition by McKEE and WQLF

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THE RESOURCES AGENCY OF CALIFORNIA STATE WATER QUALITY CONTROL BOARD SACRAMENTO, CALIFORNIA

Publication No. 3-A

MgO

concentrations of magnesium nitrate have been reported to kill fish:

Concentration in mg/l	Time of Exposure	Type of Fish	Reference
300	long-time	stickleback	1460
400		stickleback	2920
500	4 days	stickleback	1460
1500	2 days	stickleback	1460
1820	14-16 hours	stickleback	598
2000	one day	stickleback	1460
12500	Pri san	goldfish	313

#### MAGNESIUM OXIDE

(see also Magnesium)

Known in the dry state as "magnesia", this oxide combines with water to form magnesium hydroxide, which is sparingly soluble at high pH values. It is used medicinally as an antacid and laxative, in doses of 0.25 to 3.0 grams. One authority (1254) reports that drinking water should contain some magnesium and calcium oxides; the most satisfactory ratio of calcium oxide to magnesium oxide is said to be 7:1. In the soft-drink industry, magnesium oxide in the wash water gradually "clouds" the bottles, causing unsightliness (180).

#### MAGNESIUM SILICOFLUORIDE MgSiF<sub>6</sub>·6H<sub>2</sub>O

This highly soluble salt is used for mothproofing fabrics. The oral  $LD_{50}$  in guinea pigs is given as 200 mg/kg of body weight (364). A concentration of 50 mg/l is reported to kill tench (3271).

#### MAGNESIUM SULFATE

 $MgSO_4 \cdot 7H_2O$ 

1. General. Known also as Epsom salt, this compound is freely soluble in water. It occurs in natural deposits and soils, thereby contributing to the concentration in natural waters. It is used in weighting cotton and silk, in dyeing and printing calico, in tanning processes, and in fertilizers, explosives, and matches (364).

2. Cross References. Dissolved Solids, Magnesium, Sulfates.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of magnesium sulfate is 400 to 600 mg/l (621, 3241). A dose of 30 grams of magnesium sulfate is toxic and 120 grams fatal for man (284).

Magnesium sulfate in excessive concentrations in drinking water may have purgative effects (623). The most sensitive individuals are affected at about 400 mg/l and the average person at about 1000 mg/l (3392). Waters containing 1200 mg/l of magnesium sulfate and 500 mg/l of sodium sulfate have caused diarrhea in humans. Ordinarily, according to Taylor (36) waters containing half this quantity would be regarded as unsuitable for domestic use.

Dosages of 1 to 2 grams of magnesium sulfate have a purgative effect; therefore, in drinking-water standards magnesium sulfate should be limited to 1000 to 2000 mg/l. Concentrations below this limit are physiologically harmless (621).

b. Industrial Water Supplies. The following concentrations of magnesium sulfate have been recommended for industrial waters:

· ·	Concentration, mg/l			
Process	Optimum	Maximum	Reference	
Brewing, pale ales, I	60-90		170	
pale ales, II	60 - 120	****	170	
mild ales	60		170	
stout	60	~s	170	
Brewing	100		170	
Brewing, light or dark		200	173	
Ice, raw water		130	173	

c. Irrigation. See Calcium, Hardness, and Chapter V-Irrigation.

d. Stock and Wildlife Watering. High concentrations of magnesium sulfate in the drinking water of rats and other small animals have retarded growth, caused emaciation, rough coat, diarrhea, and increased mortality among the young (284, 287, 640). Concentrations from 10,000 to 25,000 mg/l have been harmful to rats. A combination of 5000 mg/l of magnesium sulfate and 20,000 mg/l of sodium chloride has inhibited the growth of rats (640) (see also Dissolved Solids). On the other hand, 5000 mg/l in drinking water has not been harmful to rats (287). Livestock will tolerate 2050 mg/l of magnesium sulfate without laxative effects (2394). In drinking water, 12,000 mg/l had no effect on the water and food consumption of male rats (2398).

e. Fish and Other Aquatic Life. The following concentrations of magnesium sulfate have been reported to have killed fish:

Concentration	Type of	Time of	Type of Reference	
in mg/l	Water	Exposure	Fish	
15,500	turbid	96-hour TL <sub>m</sub>	mosquito-fish 2940	
20,900-28,400	cistern	14 days	perch 644	
24,500-27,500	well	78 days	perch 644	

The maximum concentration of magnesium sulfate tolerated by young eels for over 25 hours was reported to be about 12,000 mg/l (1459).

#### MALATHION

(see Chapter IX)

#### MALEIC ANHYDRIDE

 $C_4H_2O_3$ 

This solid dissolves readily in water, forming maleie acid, HOOCHC = CHCOOH. It is used in the manufacture of alkyd-type resins, dye intermediates, and pharmaceuticals (364). Wallen et al. (2940) exposed mosquito-fish (*Gambusia affinis*) to maleie anhydride in turbid water at 20-23°C. They found the 24- and 48heur TL<sub>m</sub> values to be 240 mg/l and the 96-hour TL<sub>m</sub> was 230 mg/l. The pH value was lowered from 8.0 to 5.8 and the 128 mg/l of turbidity was coagulated and removed by this compound. Using bluegill sunfish (*Lepomis macrochirus*) in Philadelphia tap water at 20°C, Turnbull et al. (2093) found the 24-hour TL<sub>m</sub> to be 150 mg/l and the 48-hour TL<sub>m</sub> to be 138 mg/l. They estimated a safe concentration to be 35 mg/l.

#### MANGANESE

1. General. Manganese metal is not found pure in nature, but its ores are very common and widely distributed. The metal or its salts are used extensively in steel alloys, for dry-cell batteries, in glass and ceramics, in the manufacture of paints and varnishes, in inks and dyes, in matches and fireworks, and in agriculture to

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Mn

enrich manganese-deficient soils (2121). Like iron, it occurs in the divalent and trivalent form. The chlorides, nitrates, and sulfates are highly soluble in water; but the oxides, carbonates, and hydroxides are only sparingly soluble. For this reason, manganic or manganous ions are seldom present in natural surface waters in concentrations above 1.0 mg/l. In ground water subject to reducing conditions, manganese can be leached from the soil and occur in high concentrations. Manganese frequently accompanies iron in such ground waters and in the literature the two are often linked together.

2. Cross References. Iron, Manganese Salts, Potassium Permanganate, Turbidity, Tastes.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 Drinking Water Standards of the USPHS (2036) set a recommended limit for manganese of 0.05 mg/l. The 1958 WHO International Standards (2328) prescribe a "permissible limit" of 0.1 mg/l and an "excessive limit" of 0.5 mg/l, but no maximum allowable limit is given. The 1961 WHO European Standards have a recommended limit of 0.1 mg/l.

These limits have been established on the basis of esthetic and economic considerations rather than physiological hazards. Manganese is essential for the nutrition of both plants and animals (2121, 2129). Diets deficient in manganese result in impaired or abnormal growth, symptoms of central nervous system disturbance, anemia, and possibly interference with reproductive functions (2121, 2129). The daily intake from a normal human diet is about 10 mg (2129). It is absorbed very slightly and deposits mainly in the liver and kidneys (2129).

In concentrations not causing unpleasant tastes, manganese is regarded by most investigators to be of no toxicological significance in drinking water (633, 1077). However, some cases of manganese poisoning have been reported in the literature. A small outbreak of an encephalitis-like disease, with early symptoms of lethargy and edema, was traced to manganese in the drinking water in a village outside of Tokyo; three persons died as a result of poisoning by well water contaminated by manganese derived from dry-cell batteries buried nearby (36, 1225). Excess manganese in the drinking water is also believed to be the cause of a rare disease endemic in Manchukuo. That manganese may be toxic is also indicated by the reports that 0.5 to 6.0 grams of manganese per kilogram of body weight administered daily to rabbits had stunted growth and interfered with bone development (921).

Despite the possible toxic effects of manganese under unusual circumstances, it cannot be considered a physiological hazard because the normal dietary intake is far higher than the amount that would be tolerated esthetically in drinking water.

Manganese is undesirable in domestic water supplies because it causes unpleasant tastes, deposits on food during cooking, stains and discolors laundry and plumbing fixtures, and fosters the growth of some micro-organisms in reservoirs, filters, and distribution systems (1593, 3539, 3540, 3541, 3542) (see Fish and Other Aquatic Life, below). It has been reported by one observer that manganese salts impart a metallic taste to water at concentrations above 0.5 mg/l (945); and by another reference at above 20 mg/l (759). Cohen et al. (3301) found the taste threshold for manganous ion in spring water to occur at about 180 mg/l for the median of a large panel, but at 32 mg/l for the most sensitive members. In distilled water the taste thresholds were much lower, about 35 mg/l for the median and about 0.9 mg/l for the most sensitive panel members (3301). Manganese in excess of 0.15 mg/l has also been reported to cause turbidity in water (1594).

For domestic water supplies a maximum concentration of manganese, or of iron and manganese together, as low as 0.017 mg/l has been recommended (1256). Concentrations as low as 0.1 mg/l are reported to cause laundry trouble (219, 284); concentrations of 0.2 to 0.4 mg/l are likely to cause complaints (36); and, in general, limiting concentrations from 0.02 to 0.5 mg/l have been recommended (499, 555, 628, 1257, 3541).

b. Industrial Water Supplies. Excessive manganese is undesirable in water for use in many industries, including textiles (255, 256, 257); dyeing (261); food processing, distilling, and brewing (240, 224, 284); ice (234); paper (212, 879); and many others (see Chapter V). The following tabulation summarizes the recommendations as to maximum permissible concentrations of manganese in industrial waters:

, , , , , , , , , , , , , , , , , , ,	Maximum Permissible Concentration		
	<u>Uanganese</u>	Iron + Manganese	Reference
Industrial Use	in mg/I	in mg/l	,
Air conditioning	0.5	0.5	162
	.0.5		152
Baking	0.2	0.2	162, 152
Brewing, light and da	rk 0.1	0.1	162, 152
Canning	0.2	0.2	162, 152
Carbonated beverages	0.2	0.2	162, 152, 184
		0.1	179
Confectionary	0.2	0.2	162, 152
Cooling water	0.2	0.2	152
	0.5.	0.5	162
Dyeing	0	0	. 36
Food processing	0.2	0.2	162, 152
Ice	0.2	0.2	182, 152, 234
Milk industry	0.03-0.1	4	2344
Paper and pulp	0	0	36
Groundwood	0.5	1.0	162,152
Groundwood	0.1	1.0	244
Kraft pulp	0.1	0.2	162, 152
Soda and sulfate	0.05	01	162, 152
· boda and barrace	0.05		245
Highgrade paper	0.05	0.1	162, 152
Fine paper	0.05	0.1	350
Kraft paper		-	
blenched	0.1	· · ·	351
unbleached	0.5		351
Photography	0	0	36
Plastics (clear)	0.02	0.02	162, 152
Rayon and viscose		010	2000, 200
Pulp production	0.03	0.05	162, 152
Manufacture	0	0	162, 152
	0.02		550, 405
Tanning	0.2	0.2	162, 152
Textiles, general	0.25	0.25	162, 152
Textnes, general	U.20	0.1	852
· ·	0.1	V.1	256
dyeing	0.25	0.25	162, 152
wool scouring	1.0	1.0	162, 152
bandages	0.2	0.2	162, 152
Dationageo	V.4		1941 - 192

c. Irrigation. Manganese is essential for plant growth, apparently as an enzyme activator (3543). It is especially abundant in the reproductive parts of plants, seeds being highest while woody sections contain the least manganese (3544). Nuts contain the highest concentrations (22.7 mg/kg) and sea foods the lowest (0.25 mg/kg). Tea diffuses enough so that the normal liquid has 1 to 7 mg/l (2121). Manganese has been used to enrich soil, yet in some concentrations it may be phytotoxic (219, 277, 563).

Manganese in the nutrient solutions has been reported to be toxic to many plants, as grown in solution cultures. The sensitivity and response of the plants to the presence of manganese varies both with the species of plant and the composition of the nutrient solution. Symptoms of manganese injury have been intensified in the presence of molybdenum, vanadium (1595), or nitrate (1596). Symptoms of manganese injury have been diminished in the presence of cobalt (1499), iron, molybdenum, aluminum, phosphorus deficiency (1458), ammonium or ammonium nitrate (1596). The following concentrations of manganese have been reported to be harmful to plants in solution culture:

Concentration of Manganese in mg/l	Type of Plant	Reference
0.5	Various plants	1597
1-10	Various legumes	1597
3.5	Various plants	1597
5	Orange and mandarin seedlings	1524
5-10	Tomatoes	1499
10-25	Soybean, flax	1595
25-100	Flax	1458
50	Flax	1596
62.5	Various plants	1597
150-500	Oats	1462

It has also been reported that 0.25 mg/l of manganese has permitted good growth of tomatoes, and that up to 5.0 mg/l of manganese has reduced the severity of cobalt poisoning in tomatoes (1499). In the presence of ammonium or of ammonium nitrate, 50 mg/l of manganese was not harmful to flax, although this concentration was harmful in the presence of nitrate without ammonium (1596). Manganese sulfate, at a concentration of 100 mg/l as manganese caused no apparent injury to oat plants (1462).

d. Stock and Wildlife Watering. A deficiency of manganese in animals produces ovarian disfunction, testicular degeneration, poor lactation, lack of growth, bone abnormalties, and symptoms of central nervous disturbance (2121). Cattle are reported to have received dosages of 50 to 600 mg/kg in the diet for 20 to 45 days without serious effects. Birds have received single oral dosages of up to 600 mg/kg without adverse effects, but the continuous excess of manganese in fodder was suspected as an etiological factor in the occurrence of infectious anemia in horses. Manganese appears to oxidize vitamin B in the horse body, producing avitaminosis (1049).

The metabolism of manganese is closely related to that of calcium, phosphorus, iron, copper, and possibly other minerals, and the proper balance must be maintained. The manganese requirement for chicks has been reported to be 30-50 mg/kg (dry ration); for hens. 40-50 mg/kg. However, 1000 mg/kg in the dry ration was not toxic (1551).

e. Fish and Other Aquatic Life. The toxicity of manganese toward fish is dependent upon many factors. Jones (2941) gives the lethal concentration for the stickleback as 40 mg/l; however, the toxic action is slow and manganese does not appear to precipitate the gill secretions. According to Oshima (3545) and Iwao (3546) the toxicities of manganous chloride and manganous sulfate are slight, being about 2400 and 1240 mg/l of manganese respectively. Manganese appears to be somewhat antagonistic to the toxic action of nickel toward fish (1468).

The following concentrations of manganese have been tolerated by fish under the stated conditions:

Concentration in mg/l	n Time of Exposure	Type of Fish	Reference
1	475.104	river crayfish	2977
15	7 days	tench, carp, trout	2151
40*	4 days	fingerling catfish	2981
50**	3 days	stickleback	1459
2700	50 hours	eels -	1459
from manushese	directivm versanate		

\* from manganese cisodium verser

Manganese and iron in concentrations above 0.1 mg/l stimulate the growth of certain organisms, such as *Cren*othrix, Gallionella, and other related forms in reservoirs, filters, and distribution systems (152, 921, 945, 1258). The addition of as little as 0.0005 mg/l of manganese resulted in increased growth and multiplication of various microbiota in sea water (1259). Guseva (584, 1260), on the other hand, found that concentrations of manganese above 0.005 mg/l had a toxic effect on some algae.

The threshold concentration of manganese for the flatworm *Polycelis nigra* has been reported to be 700 mg/l as manganese chloride and 660 mg/l as manganese nitrate (608). Crustacea, worms, and insect larvae were not harmed by 15 mg/l of manganese during a 7-day exposure (2151).

The permanganates are much more toxic to fish than the manganous salts. Permanganates killed fish in 8 to 18 hours at concentrations of 2.2 to 4.1 mg/l of manganese (3545, 3546). However, permanganates are not stable for long in water.

4. Summary. On the basis of the literature surveyed, it appears that the following concentrations of manganese will not be deleterious to the stated beneficial uses:

a.	Domestic water suppl	Y	$0.05 \mathrm{mg/l}$
b.	Industrial water supp	ly	0.05 mg/l
e.	Irrigation		0.50  mg/l
đ.	Stock watering		10.0 mg/1
e.	Fish and aquatic life		1.0  mg/l

#### MANGANESE CHLORIDE

MnCl<sub>2</sub> and MnCl<sub>3</sub>

(see also Manganese, Chlorides)

This highly soluble salt, occurring generally in the manganous form, is used in dyeing operations, in disinfecting, in linseed oil driers, and in electric batteries (364). In fresh water, 12 mg/l has been reported as fatal to minnows (*Fundulus*) within six days (1459), but other fish have been found to be much more tolerant of MnCl<sub>2</sub>. For the small fresh-water fish (*Orizias*), the 24hour lethal concentration was about 7850 mg/l (1459) and for other fish 5500 mg/l (3545, 3546). The highest concentration tolerated by young eels for 50 hours was 6300 mg/l (1459). The first toxic effects of  $MnCl_2$  for fish were observed at 330 mg/l but the lethal concentration did not occur until 800 mg/l (2977).

Toward lower organisms there is similarly a wide variation in reported toxicity. For immobilization of Daphnia magna in Lake Erie water, the threshold concentration was found (598) to be 50 mg/l of MnCl<sub>2</sub>. In River Havel water at 23°C, the threshold effect of MnCl<sub>s</sub> occurred at 50 mg/l of manganese (2158). For the flatworm, Polycelis nigra, the threshold concentration of MnCl<sub>2</sub> was reported to be 700 mg/l (608).

#### MANGANESE DIFLUORIDE MnF<sub>2</sub>

(see also Manganese, Fluorides)

This highly soluble manganous salt is reported to be lethal to tench in 48 hours at a concentration of 500 mg/l (3271).

#### MANGANESE NITRATE

 $Mn(NO_3)_2 \cdot 4H_2O$ This manganous salt is very soluble in water. For sticklebacks in tap water, the minimum lethal concentration of manganese nitrate has been reported to be 40 mg/l as manganese (598, 1460). The average survival times of the fish in different concentrations was as follows: one week at 50 mg/l, four days at 100 mg/l, two days at 150 mg/l and only one day at 300 mg/l, all measured as managanese (1460). For the flatworm, Polycelis nigra, the threshold concentration has been reported to be 660 mg/l as manganese nitrate (608).

#### MANGANESE SULFATE

 $MnSO_4 \cdot H_2O$ 

This pale-pink manganous salt, highly soluble in water, is used in dyeing, porcelain glazing, varnishes, and specialized fertilizers (364). In culture solution, 100 mg/l as manganese caused no apparent injury to oat plants, 150-200 mg/l caused chlorosis, and 500 mg/l produced injury (1462).

Toward fish, the toxicity of manganous sulfate is slight. In tap water, 50 mg/l as manganese did not kill sticklebacks within three days (1459). Young eels tolerated 1500 mg/l as manganese sulfate for more than 25 hours. The first influence of this salt toward fish is reported to occur at 500 mg/l as Mn, and at 1000 mg/l as Mn the salt is lethal (2977). Japanese investigators (3545, 3546) report the toxicity of this salt at 3400 mg/L

#### MANOXOL OT

(see Chapter X)

#### MASONITE MANUFACTURING WASTES

Ellis (611) investigated wastes from a Masonite plant in Mississippi, containing chemical compounds, fibers, pigments, and an unidentified substance with a high B.O.D. that was toxic to fish in one to three days at 1:100,000 dilution. Loose fibers menaced fish for 12 miles below the plant.

#### MERCAPTANS, GENERAL

(see also Methanethiol)

Mercaptans (RSH) are the sulfur analogs of the alcohols (ROH) and phenols (R'OH). They are generally

odoriferous and can be detected in very small concentrations. They occur in coal tar and in the wastes from Kraft-process pulp mills.

The threshold concentration for taste and odor of mercaptans from Kraft mill wastes has been reported at less than 0.02 mg/l (686). The untreated waste from the mill, containing 12 mg/l of mercaptans, required a dilution of 1:50,000 to render it odorless, i.e., down to a concentration of 0.00024 mg/l; but after chlorination to a residual of 1.5 mg/l, the required dilution was only 1:40, i.e. down to a concentration of 0.3 mg/l.

Gersdorff (695) shows that phenyl mercaptan, C<sub>6</sub>H<sub>5</sub>SH (thiophenol), a liquid with a repulsive, penetrating, garlic-odor, and tolyl mercaptan CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SH (thiocresol) have a similar toxic effect on goldfish, but the toxic action differs from that of phenol. Metatolyl mercaptan is about four times as toxic, o-tolyl mercaptan about five times, and p-tolyl mercaptan about 8.5 times as toxic as phenol (see Phenols). The relative toxicities of m-o-, p-tolyl mercaptans are in the ratios 1 to 1.19 to 2.19, a relationship nearly the same as that found for the corresponding cresols. The replacement of the oxygenation of the cresol molecule by sulfur appears to cause a fourfold increase in the toxicity of the compound to goldfish (695).

#### MERCURIC ACETATE

(see Mercuro-Organic Compounds)

#### MERCURIC CHLORIDE

1. General. This salt is soluble in water at 20°C to the extent of 61,000 mg/l (911). It is used in embalming, disinfecting, preserving, printing of fabrics, tanning, electroplating, manufacturing ink, and numerous other processes (364). It may occur in wastes from any of these industries, or in lead mining and chemical wastes (313).

2. Cross References. Mercury, Other Mercury Salts, Mercuro-Organic Compounds, Chlorides, and Chapter V. -Fish and Other Aquatic and Marine Life.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The ingestion of 1.0 to 2.0 grams of mercuric chloride is frequently fatal to human beings.

b. Stock and Wildlife Watering. The lethal dose for dogs has been reported as 10 to 15 mg per kg of body weight (353). The LD<sub>50</sub> value of mercuric chloride for rats was given as 37 mg/kg while that for mercurous chloride (Calomel) was 210 mg/kg (3009, 3067).

c. Fish and Other Aquatic Life. From a study of the relation between concentration of the salt and period of survival, it appears that mercuric chloride is infinitely toxic to fish, i.e. that infinitesimal traces of the compound will be toxic if exposure continues long enough (3547). The following concentrations of mercuric ion from chloride have been shown to injure or kill fish in the time indicated :

Concentration of	Time of	Species of	Reference
Mercury, in mg/l	Exposure	Fish	
0.008		sticklebacks	1460, 2941
0.01		sticklebacks	2962, 2920
0.01	80-92 days	minnows	1459
0.011 *		sticklebacks	598
0.02	· · · ·	guppies	2921

HgCl<sub>2</sub>

## Attachment 1 – Exhibit D

Site-specific relief granted by the IPCB for boron and fluoride to date

Stream or Lake Name	Discharger	Parameter	Relief (mg/L)
Stream of Lake Hame	Discharger	<u>1 arameter</u>	<u>Renet (mg/L)</u>
Horseshoe Lake	Granite City Steel	Fluoride	4.0
Unnamed tributary of Vermilion River downstream to confluence with Vermilion River, relief ending 0.9 miles downstream of the Norfolk and Western Railroad bridge crossing.	General Motors Corporation	Fluoride	10.0
Unnamed tributary of Salt Creek downstream to confluence with Salt Creek; Salt Creek downstream to confluence with Little Wabash River	Effingham POTW	Fluoride	5.0
Confluence of Salt Creek with Little Wabash River, downstream to monitoring station C-19 on Little Wabash River (2.8 miles downstream of Louisville, Illinois)	Effingham POTW	Fluoride	3.2
Monitoring station C-19 on Little Wabash River downstream to confluence of Buck Creek and Little Wabash River (9.8 miles downstream of Louisville, Illinois)	Effingham POTW	Fluoride	2.0
Unnamed tributary of Dutch Creek extending 1,200 yards downstream of facility discharge	Modine Manufacturing	Fluoride	5.6
Unnamed tributary of Wood River Creek to confluence with Wood River Creek; Wood River Creek downstream to confluence with Mississippi River	Dynegy Midwest Generation – Wood River	Boron	15
Sangamon River downstream of Spring Creek STP Outfall 007 and extending until 182 yards downstream of confluence with Spring Creek	Springfield – Spring Creek STP	Boron	11.0
Sangamon River 182 yards downstream of confluence with Spring Creek, downstream to confluence with Salt Creek (39 river miles)	Springfield – Spring Creek STP	Boron	4.5
Sangamon River at confluence with Salt Creek and extending to	Springfield – Spring Creek	Boron	1.6

**Exhibit D**: Site-specific relief granted by the IPCB for boron and fluoride to date.

confluence with Illinois River	STP		
Sangamon River at confluence with Illinois River and extending 100 yards downstream	Springfield – Spring Creek STP	Boron	1.3
Unnamed tributary of South Branch Edwards River to confluence with South Branch Edwards River; South Branch Edwards River downstream to confluence with Edwards River	Galva Northeast Sewage Treatment Plant	Boron	3.0
Mud Run Creek to confluence with Walnut Creek	Galva Southwest Sewage Treatment Plant	Boron	3.0
Little Saline Creek to confluence with South Fork Saline River, downstream to where South Fork Saline River leaves the SE quarter of Section 6 T10SR4E	So. IL power Coop (SIPC)	Boron	9.0
South Fork Saline River from the downstream edge of SE quarter of Section 6 T10SR4E to confluence with Middle Fork Saline River	SIPC	Boron	3.0
Aux Sable Creek to confluence with Illinois River	Akzo Nobel	Boron	2.0
Sugar Creek from Spaulding Dam to the confluence with Springfield S.D. discharge 008	Springfield City Water Light and Power (CWLP)	Boron	11.0
Sugar Creek from Springfield S.D. discharge to confluence with South Fork Sangamon River	CWLP	Boron	5.5
Confluence of South Fork Sangamon River with Sugar Creek, downstream to confluence with Sangamon River; South Fork Sangamon River confluence with Sangamon River, to 100 yards downstream of Sangamon River confluence with Spring Creek	CWLP	Boron	2.0
Kaskaskia River from 310 feet upstream of Baldwin Station 001 discharge to the Plant intake structure	Dynegy Baldwin Station (Illinois Power)	Boron	2.7
Kaskaskia River from 310 feet upstream of Outfall 001 of the Baldwin Station to 300 feet downstream	Dynegy Baldwin	Boron	9.9
Kaskaskia River from 300 feet downstream of the Baldwin Station discharge to 2,000 feet downstream	Dynegy Baldwin	Boron	2.7

Kaskaskia River from 2,000 downstream of the Baldwin Station 001 discharge, downstream to confluence with Mississippi River	Dynegy Baldwin	Boron	1.2
Duck Creek from the 002 outfall to the confluence with Illinois River	CILCO	Boron	4.5
Illinois River from the confluence with Duck Creek downstream for 100 yards	CILCO	Boron	4.4

## Attachment 1 – Exhibit E

Manganese removal estimations at conventional utilities located on impaired Public and food Processing water Supply waters with Mn exceeding 150 ug/L <u>Exhibit E</u>: Manganese removal estimations at conventional utilities located on impaired Public and Food Processing Water Supply waters with Mn exceeding 150  $\mu$ g/L.

Facility	Finished Collection Date	Finished Total Mn (µg/L) <sup>1</sup>	Finished Detection Level (µg/L)	Surface Water Intake <u>Site</u>	Intake ⊺otalMn <u>(µg/L)</u>	Intake Collection <u>Date</u>	<u>% Removal</u>	Difference of Sample <u>Dates (+)</u>
BREESE	1/7/1998	0	5	OI-08	310	1/7/1998	0.98	0
CLAY CITY	4/13/1999	0 .	15	C-19	200	4/13/1999	0.93	0
CLAY CITY	4/25/2000	0	15	C-19	220	4/25/2000	0.93	0
BREESE	2/7/1994	0	15	OI-08	270	2/8/1994	0.94	1
BREESE	1/7/1997	0	5	01-08	250	1/8/1997	0.98	1
VANDALIA	7/24/2007	0	1	O-08	300	7/23/2007	1.00	1
FLORA	4/12/1999	0	5	C-19	200	4/13/1999	0.98	1
IL AMERICAN-PONTIAC	10/31/2000	0	10	DS-06	230	11/3/2000	0.96	3
MARION	10/29/2004	5	5	RNL	240	10/26/2004	0.98	3
MOUNT OLIVE	7/15/2003	32	15	RJG-1	900	7/11/2003	0.96	4
SLM WATER COMMISSION	10/21/2002	0	15	0-20	250	10/25/2002	0.94	4
BREESE	1/22/1996	10	15	OI-08	300	1/17/1996	0,95	5
HILLSBORO	5/1/2006	24	1	ROL-1	340	4/26/2006	0.93	5
VANDALIA	7/13/2004	0	1	O-08	170	7/19/2004	0.99	6
HILLSBORO	4/24/2000	18	15	ROL-1	150	4/18/2000	0.88	6
SLM WATER COMMISSION	10/21/2003	0	15	O-20	580	10/27/2003	0.97	6
BREESE	2/7/1995	0	15	OI-08	340	2/14/1995	0,96	7
BREESE	1/24/2007	7	1	OI-08	220	1/17/2007	0.97	7
HILLSBORO	5/14/2008	12	15	ROL-1	280	5/6/2008	0,95	8
CLAY CITY	5/21/2003	0	15	C-19	171	5/13/2003	0.91	8
BREESE	1/12/1999	0	5	OI-08	300	1/21/1999	0,98	9
NASHVILLE	4/23/2007	10	1	R00-1	360	5/2/2007	0.97	9
MOUNT OLIVE	10/10/2006	81	15	RJG-1	840	10/20/2006	0.90	10
OAKWOOD	4/18/1994	0	15	BPJ-03	290	4/28/1994	0.95	10
SLM WATER COMMISSION	10/18/1994	0	15	O-20	270	10/28/1994	0.94	10
BREESE	5/8/2000	21	15	01-08	300	4/27/2000	0.93	11
MOUNT OLIVE	10/15/2003	140	15	RJG-1	530	10/3/2003	0,74	12
VIENNA	10/15/2003	15	15	RAW-1	300	10/2/2003	0,95	13
SLM WATER COMMISSION	10/29/1996	0	15	O-20	470	10/16/1996	0.97	13
SLM WATER COMMISSION	10/22/2001	õ	15	0-20	520	10/9/2001	0.97	13
MARION	5/22/2007	8	1	RNL	250	6/5/2007	0,97	14
NASHVILLE	5/3/2004	11	5	R00-1	190	4/19/2004	0.94	14
CLAY CITY	4/7/1998	0	15	C-19	200	4/21/1998	0.93	14
SLM WATER COMMISSION	10/24/1995	0	15	0-20	560	11/7/1995	0.97	14
SLM WATER COMMISSION	10/21/1998	0	15	O-20	230	10/7/1998	0.93	14
BREESE	2/10/2004	1,9	1	01-08	430	2/25/2004	1,00	15
MOUNT OLIVE	4/1/2003	78	15	RJG-1	190	4/16/2003	0.59	15
BREESE	3/23/1993	0	15	01-08	260	4/8/1993	0.94	16
NASHVILLE	4/14/1999	32	15	R00-1	210	4/30/1999	0.85	16
BREESE	1/9/2002	2	15	OI-08	260	1/30/2002	0.94	21
IL AMERICAN-ALTON	7/18/2001	ō	10	J-36	450	6/27/2001	0.98	21
SLM WATER COMMISSION	10/12/1999	ů.	15	O-20	780	11/2/1999	0.98	21
CLAY CITY	4/23/2001	0	15	C-19	430	5/15/2001	0.97	22
IL AMERICAN-GRANITE CITY		18	15	J-36	280	8/15/2007	0.94	30

<sup>1</sup>Where finished Mn results were lower than the detection level, the detection level was used in calculating the removal estimates.

Attachment 1 – Exhibit G Acute Toxicity Data used in Boron Standard Derivation

### Acute Toxicity Data Used in Boron Standard Derivation

\* Results marked with strikethrough are considered invalid and have been excluded from standards derivation. Reason for exclusion is highlighted in bold within Notes column.

		<b>T</b>	D	1.050				
Species	Chemical	Test Type	Duration (days)	LC50 (mg/L)	CHAN	GMAV	Reference	Notes
Water flea	Sodium tetraborate	SR.M	2	141			Maler and Knight 1991	
Daphnia magna	Boric acid	S,U	2	226	104.8	104.5	Lewis and Valentine 1981	Done at 5 different hardnesses (10.6-170 mg/L, no differences, geomean = 60.8), pH was 9.1 in treatments pH and hardness not reported, carbon-filtered well water used for dilution water
Daprinia magria	Boric acid	5.U	2	133			Gersich 1984	pH and hardness hor reported, carbonnikered weil water used for dilution water pH 6.7-8.1, hardness 148
	Boric acid	S.U	1	319.8			Hickey 1989	24 hour test (D. carinata also available), not used because 48 hour tests are available
	Boric acid	UNK	2	52.4			MELP 1996 (unpublished)	Study is unattainable, hardness = 100
	Boric acid	UNK	2	439.2			MELP 1996 (unpublished)	Study is unattainable, hardness = 250
	Boric acid	UNK	2	24,3			MELP 1996 (unpublished)	Study is unattainable, hardness = 25
	Boric acid	S,U	2	135.8			OPP 2000 (TN 2750)	777 ppm LC50 w/ 99.9% H3B03, crystal form, pH 7.5, hardness = 180 mg/L in dilution water
Water flea	Boric acid	S,M	2	45.5	84.8	84.8	Dethloff et al. 2009	Hardness/alkalinity ≍ 96/94 mg/L
Ceriodaphnia dubia	Boric acid	S,M	2	50.8	04.0	04.0	Dethloff et al. 2009	Hardness/alkalinity = 290/95 mg/L.
oonooapinna oobia	Boric acid	S,M	2	50.6			Dethloff et al. 2009	Hardness/alkalinity = 168/167 mg/L
	Boric acid	S.M	2	62.8			Dethloff et al. 2009	Hardness/alkalinity = 508/167 mg/L
	Boric acid	S.M	2	99.4			Dethloff et al. 2009	Hardness/alkalinity = 100/94 mg/L, pH = 8.1
	Boric acid	S,M	2	60.3			Dethloff et al. 2009	Hardness/alkalinity = 100/94 mg/L, pH = 7.1, CO <sub>2</sub> introduced to lower pH, elevated control mortality
	Boric acid	S.M	2	134.1			Dethloff et al. 2009	Hardness/alkalinity = 90/188, pH = 8,4
	Boric acid	S.M	2	79.7			Dethioff et al. 2009	Hardness/alkalinity = 90/186 mg/L, pH = 7,4, CO <sub>2</sub> introduced to lower pH, elevated control mortality
	Boric acid	S.M	2	83.8			Dethloff et al. 2009	Low CI (5.7 mg/L)
	Boric acid	S,M	2	60.9			Dethioff et al. 2009	High CI (105 mg/L)
	Boric acid	S.M	2	72.1			Dethloff et al. 2009	Low Na (26.8 mg/L)
	Boric acid	S,M	2	78.8			Dethloff et al. 2009	High Na (91.0 mg/L)
	Boric acid	S.M	2	62.4			Dethloff et al. 2009	0.7 mg/L DOC
	Boric acid	S,M	2	75.5			Dethloff et al. 2009	1.6 mg/L DOC
	Boric acid	S.M	2	85.2			Dethloff et al. 2009	2.6 mg/L DOC
	Boric acid	S,M	2	90.8			Dethloff et al. 2009	6.1 mg/L DOC
	Boric acid	S,M	2	89.9			Dethloff et al. 2009	11.4 mg/L DOC
	Boric acid	S.U	1	180.6			Hickey 1989	24 hour test (C. pulchella also available), not used because 48 hour tests are available
	Boric acid	SR.M	2	85.2			Sanders and Associates 2007	Hardness = 80-84 mg/L (geomean 82), pH = 7.44 (geomean of pH from treatments on page 61,62)
	Boric acid	S.M	2	102			Soucek and Dickinson 2010	Hardness = 90 mg/L, pH = 7.4
	82% boric acid:18% borax	S.M	2	165			Soucek and Dickinson 2010	Hardness = 91 mg/L, pH = 8.0
	82% boric acid: 18% borax	S,M	2	109			Soucek and Dickinson 2010	Hardness = 89 mg/L, pH = 8.1
	82% boric acid:18% borax	S.M	2	104			Soucek and Dickinson 2010	Hardness = 282 mg/L, pH = 8.1
	82% boric acid: 18% borax	S,M	2	93			Soucek and Dickinson 2010	Hardness ≈ 469 mg/L, pH ≈ 8.1
	99.56% boric acid:0.44% borax	S,M	2	91			Soucek and Dickinson 2010	Hardness = 85 mg/L, pH = 6.7
	96.5% boric acid:3.5% borax	S,M	2	115			Soucek and Dickinson 2010	Hardness = 87 mg/L, pH = 7.6
	86.15% boric acid:13.85% borax	S,M	2	142			Soucek and Dickinson 2010	Hardness = 84 mg/L, pH = 8.4
	Boric acid	F,M	2	76.9			GLEC 2010	Hardness = 134.7 mg/L, pH = 7.75
Water flea	Boric acid	S.U	1	123.4	423.4	123.4	Hickey 1989	24 hour test
Simocephalus vetulus		-,-						
56	Contraction and	<b>6</b> M	4	- 201 1	> 001 1	- 201 1		20 Mula Terra Dada Asid 1000 Dada asid 1000 1010 mall basis sold basisana 50 mall atta 7.07.0
Bluegill Lepomis macrochirus	Boric acid	S,M	4	>201.1	>201.1	>201.1	OPP 2000 (40594602)	20 Mule Team Boric Acid, 100% Boric acid, LC50≥1049 mg/L boric acid, hardness ≈ 52 mg/L, pH ≈ 7,0-7.9
Laponia matroomaa								
Midge	Boric acid	UNK	4	118	1663.7	1513.0	MELP 1996 (unpublished)	Study is unattainable, hardness = 100 mg/L
Chironomus tentans	Boric acid	UNK	4	137.7			MELP 1996 (unpublished)	Study is unattainable, hardness ≈ 250 mg/L
	Boric acid	UNK	4	467.3			MELP 1996 (unpublished)	Study is unattainable, hardness = 25 mg/L
	Boric acid	SR,M	2	1597			Sanders 1998	Geomean hardness ≍ 130 mg/L, geomean pH ≍ 6.6, highest treatment (pH 5.5) buffered with NaOH
	Boric acid	SR,M	2	2990			Sanders 1998	Geomean hardness = 117 mg/L, geomean pH = 6.7, highest treatment (pH 6.1) buffered with NaOH
	Boric acid	S,U	2	964,3			Sanders 1999	Hardness = 115-130 mg/L (geomean 122), pH = 5,7 in highest treatment (geomean 6,7)
Chironomus decorus	Sodium tetraborate	SR,M	2	1376	1376.0		Maier and Knight 1991	Done at 5 different hardnesses (10.6-170 mg/L, no differences, geoMean = 60.8), pH was 9.1 in treatments
Amphipod	Boric acid	UNK	4	291.3	136.6	136,8	MELP 1996 (unpublished)	Study is unattainable, hardness = 100 mg/L
Hyalella azleca	Boric acid	UNK	4	333.6			MELP 1996 (unpublished)	Study is unattainable, hardness = 250 mg/L
	Boric acid	UNK	4	28.9			MELP 1996 (unpublished)	Study is unattainable, hardness = 25 mg/L
	Boric acid	SR,M	4	94.9			Sanders and Associates 2007	Hardness = 80-84 mg/L (geomean 82), geomean pH = 7.4, MHRW used (chloride = 1.9 mg/L)
	82% boric acid: 18% borax	S,M	4	107			Soucek and Dickinson 2010	Hardness = 106 mg/L, pH was 8.1, Smith water used, chloride was 34 mg/L.
	82% boric acid:18% borax	S,M	4	151			Soucek and Dickinson 2010	Hardness = 302 mg/L, pH 8.1, Smith water used, chloride increased to 98 mg/L

	82% boric acid:18% borax 82% boric acid:18% borax 82% boric acid:18% borax 82% boric acid:18% borax	S,M S,M S,M S,M	4 4 4	170 104 127 64			Soucek and Dickinson 2010 Soucek and Dickinson 2010 Soucek and Dickinson 2010 Soucek and Dickinson 2010	Hardness = 507 mg/L, Geomean pH 8.1, Smith water used, chloride increased to 161 mg/L Hardness = 102 mg/L, pH 6.6, Smith water used (chloride = 34 mg/L) Hardness = 102 mg/L, pH 7.6, Smith water used (chloride = 34 mg/L) Hardness = 103 mg/L, pH 8.4, Smith water used (chloride = 34 mg/L)
	82% boric acid:18% borax	S,M	4	269			Soucek and Dickinson 2010	Hardness = 111 mg/L, pH 8.1, Borgmann water used (chloride = 72 mg/L)
	82% boric acid:18% borax 82% boric acid:18% borax	S,M S,M	4 4	203 188			Soucek and Dickinson 2010 Soucek and Dickinson 2010	Hardness = 291 mg/L, pH 8.1, Borgmann water used (chloride ≈ 72 mg/L)
	62% burle aciu. 16% burax	3,M	4	100			Soucek and Dickinson 2010	Hardness = 475 mg/L, pH 8.1, Borgmann water used (chloride = 72 mg/L)
Annelid	Boric acid	SR,M	4	260	267.4	267.4	Sanders 1998	Hardness = 125-135 mg/L (geomean 130), pH 5.8 in highest treatment (geomean 7.5 from treatments)
Lumbriculus variegatus	Boric acid	SR,M	4	275			Sanders 1998	Hardness = 110-125 mg/L (geomean 117), pH 5.7 in highest treatment (geomean 6.9)
Coho salmon	Boric acid	S,U	4	447	447.0	>360.8	B Hamilton and Buhl 1990	Freshwater (hardness = 211 mg/L in dilution water, pH = 6.5-8.1 measured at beginning and end of tests)
Oncoryhnchus kisutch	Boric acid Boric acid	UNK UNK	4	304.1 477.1			MELP 1996 (unpublished)	Study is unattainable, hardness = 100 mg/L
	Boric acid	UNK	4	357.4			MELP 1996 (unpublished) MELP 1996 (unpublished)	Study is unattainable, hardness ≈ 250 mg/L
Chinook salmon	Boric acid	S,U	4	<del>357.4</del> 566	640,6		Hamilton and Suhi 1990	Study is unattainable, hardness = 25 mg/L
O. tsha wytscha	Boric acid	3,0 S.U	4	725	040.0		Hamilton and Suhl 1990	Soft water (hardness ≈ 41.7 mg/L, pH ≈ 7,57 in dilution water) Freshwater (hardness ≈ 211 mg/L in dilution water, pH ≈ 6,5-8.1 measured at beginning and end of tests)
Rainbow trout	Boric acid	UNK	4	37 <del>9.6</del>			MELP 1996 (unpublished)	Study is unattainable, hardness = 100 mg/L
O. mykiss	Boric acid	UNK	4	336			MELP 1996 (unpublished)	Study is unattainable, hardness ≈ 250 mg/L
C. Hykob	Boric acid	UNK	4	436.2			MELP 1996 (unpublished)	Study is unattainable, hardness = 25 mg/L
	Boric acid	S,M	4	> 192.5			OPP 2000 (40594601)	20 Mule Team Boric Acid, granular, 100% Boric acid, LC50≥1100, hardness ≈ 52 mg/L, GeoMean pH ≈ 7,16
	Boric acid	S	4	>140			OPP 2000 (TN 2751)	>800 ppm LC50 w/ 99.9% HB03, crystal form, no pH or hardness reported
	Boric acid	s	4	50-100			OPP 2000 (TN 2749)	Formulation (perma-dust pt 240), contains 20% boric acid plus proprietaries
Fathead minnow	Boric acid	SR,M	4	75.9	96.1	96.1	Sanders and Associates 2007	Hardness = 84 mg/L in control, 60 mg/L in 250 mg/L treatment (geomean = 71), geomean pH = 7,43
Pimephales promelas	90% boric acid:10% borax	S,M	4	79.7			Soucek and Dickinson 2010	Hardness = 91 mg/L, pH = 8.0
	Boric acid	S,M	4	101			GLEC 2010	Hardness = 151.6 mg/L, pH = 8.15
	Boric acid	F,M	4	70.6			GLEC 2010	pH 6.75, hardness = 128 mg/L
	Boric acid Boric acid	F,M F,M	4 4	137 133			GLEC 2010 GLEC 2010	pH 7.75, hardness ≈ 134.2 mg/L
	Bolic acid	F,MI	**	135			GLEC 2010	pH 8.75, hardness ≈ 113.9 mg/L
Brown planarian Dugesia tigrina	Boric acid	SR,M	4	1358	1358.0	1358.0	Sanders and Associates 2007	Geomean dilution and final hardness = 51.8 mg/L, pH Geomean = 6.75
Colorado Squawfish Ptychocheilus /uclus	Boric acid	S,U	4	279	279.0	279.0	Hamilton 1995	Youngest, most sensitive life stage used (swimup fry, 17-31 days) Hardness ≈ 196 mg/L, pH≃ 7.8 in dillution water; results during tests not reported
Razorback Sucker Xyrauchen texanus	Boric acid	S,U	4	233	233.0	233.0	Hamilton 1995	Youngest, most sensifive life stage used (swimup fry, 10-17 days) Hardness ≃ 196 mg/L, pH≃ 7.8 in dilution water; results during tests not reported
Bonytail Gila elegans	Boric acid	S,U	4	280	280.0	280.0	Hamilton 1995	Youngest, most sensitive life stage used (swimup fry, 11-18 days) Hardness = 196 mg/L, pH= 7.8 in dilution water; results during tests not reported
Flannelmouth Sucker Catostomus latipinnis	Boric acid	S,U	4	125	125.0	125.0	Hamilion and Buhl 1997	13 day old larvae used Geomean pH in treatments 7.72 (range of 6.7-8.9), hardness was 144 mg/L in dilution water
Mosquitofish Ga <i>mbusi</i> a affinis	Sodium tetraborate Boric acid	s,u s,u	4 4	408 980	632.3	632.3	Wallen et al. 1957 Wallen et al. 1957	Turbid water used, potential organics problem, 3600 Na tetraborate, 11.3% B Turbid water used, potential organics problem, 5600 boric acid, 17.5% B
Grooved Fingernail Clam Sphaerium simile	90% boric acid:10% borax	S,M	4	>447	>447	>447	Soucek and Dickinson 2010	Hardness = 102 mg/L, pH = 7.9
Winter stonefly Allocapnia vivipara	90% boric acid:10% borax	S,M	4	476	476	476	Soucek and Dickinson 2010	Hardness = 98 mg/L, pH = 7.9
Fatmucket Lampsilis siliquolodea	90% boric acid:10% borax	S,M	4	137	137	137	GLEC 2010	Hardness = 90 mg/L, pH = 8.0
Black sandshell <i>Ligumia rect</i> a	82% boric acid:18% borax	S.M	4	147	147	147	GLEC 2010	Hardness = 91 mg/L, pH = 8.1
Washboard Megalonaias nervosa	82% boric acid:18% borax	S,M	4	>544	>544	>544	GLEC 2010	Hardness = 88 mg/L, pH = 8.0

Attachment 1 – Exhibit H Chronic Toxicity in Boron Standard Derivation

#### **Chronic Toxicity Data Used in Boron Standard Derivation**

\* Results marked with strikethrough are considered invalid and have been excluded from standards derivation. Reason for exclusion is highlighted in bold within Notes column.

\*\* Tests using Birge's test methods (Birge and Black 1977, Black et al. 1993) were deemed unacceptable for the following reasons: Extremely low test concentrations led to boron deficiency; flat dose-response at very low concentrations (<1 ppm) led to perceived significant differences at very low test responses (e.g., Birge et al. 1993 found a 3.4% response of organisms in a 0.01 mg/L B treatment and deemed it significant form controls, whereas EPA recommends an EC20 (concentration affecting 20% of organisms) as a suitable chronic endpoint); dead embryos were not removed in chronic tests; and different trout strains, test durations, and dilution, waters were used in Black et al. 1993 which resulted in highly variable results. The longest chronic test (87 days) from the Birge et al. 1993 study is listed below. See Rowe et al 1998, Loewengart 2001, and Eckhert 1998 for explanation of boron deficiency in rainbow trout and resulting U shaped dose-response curve, as well as additional information regarding the analamous Birge/Black results. For example, in contrast to the Birge/Black studies, Eckhert (1998) found significantly greater resultions >0.123 mg/L, with greatest growth at 10 mg/L B (growth was significantly lower in treatments with <0.123 mg/L coron).

Species	Chemical	Test Type	Duration (days)	t <u>Endpoint</u>	NOEC (mg/L)			SMAV	ACR	Reference	Notes
Water flea 'Daphnia magna	Boric acid Boric acid Boric acid Boric acid Boric acid	SR,M SR,M SR,UNK SR,UNK SR,M	21 21 UNK UNK 21	Brood size Brood size UNK UNK Brood size <u>GeoMe</u> :	6 6,4 <del>13,1</del> 12,4 10 ans (va	13 13.6 <del>25.4</del> <del>26.4</del> 46 lid data):	8.83 9.33 <del>18.2</del> <del>18.1</del> <del>12.6</del> 9.1	226 133 <del>52,4</del> <del>139,2</del> NA 173,4	14.3 2.9 7.7	Lewis and Valentine 1981 Gersich 1984 MELP 1996 (unpublished) MELP 1996 (unpublished) Hoofiman et al 2000	pH was 7.1-8.7, hardness averaged 166 mg/L, no pH/hardness reported in acute test pH and hardness not reported in chronic test, same dilution water used in acute test Study is unattainable, 100 mg/L hardness Study is unattainable, 250 mg/L hardness No corresponding acute test available for ACR determination
Rainbow trout Oncorhynchus mykiss	Boric acid Boric acid Borax Borax Boric acid	F,M F,M F,M F,M	28 28 28 28 87	Survival Survival Survival Survival Grow(h/survival	23.5 0.5 8.7 0.63 48	45.5 + <del>22.6</del> 49.7 >18	32.7 0.71 14.81 21.88 ≻18.0	-		Birge and Black 1977 Birge and Black 1977 Birge and Black 1977 Birge and Black 1977 Black et al. 1993	50 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable 200 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable 50 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable 200 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable 200 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable Well water used (hardness = 38.5 mg/L), no corresponding acute test
Fathead minnow <i>Pimephales promel</i> as	Boric acid 90% boric acid;10% borax Boric acid	SR,M SR,M F,M	7 32 32	Growth Growth/survival Survival <u>GeoMe</u>	12.9	48 23 27.4 lid data):	<del>14.1</del> 16.0 18.8 17.4	75.9 79.7 101 89.7	5,4 5.0 5.4 5.2	Sanders and Associates 2007 Soucek and Dickinson 2010 GLEC 2010	7 day ELS test not sufficient for ACR determination pH = 8.2, hardness = 129.8 mg/L, GLEC acute test at pH 8.15 used for ACR determination
Amphipod Hyalella azteca	90% boric acid:10% borax	SR,M	42	Reproduction	6.6	13	9.3	107	11.6	Soucek and Dickinson 2010	Growth was less sensitive, reproduction was the lowest endpoint
Protozoan Spirostomum ambiguum	Boric acid Boric acid	S,U S,U	2 2	EC50 LC50	-	63.7 852	-	-	•	Nalecz-Jawecki and Sawicki 1998 Nalecz-Jawecki and Sawicki 1998	Unicellular organism, chronic test (1985 guidelines), no acute test for ACR determination Unicellular organism, chronic test (1985 guidelines), no acute test for ACR determination

#### **Aquatic Plant Data**

\* Vascular plants: Chuck Stephan's (USEPA-Duluth) opinion during atrazine criteria review is that exposures ≥9 days are chronic

\*\* 96 hour algal tests are considered chronic due to the rapid life cycle of these organisms

Species	Chemical	Test <u>Type</u>	Duration (days)	Endpoint	NOEC (mg/L)	LOEC (mg/L)		EC/IC50 (mg/L)	ACR	Reference	Notes
Greater duckweed	Boric acid	SR,M	10 10	Growth rate	6.1 0.0	48.9 3.6	40.7	<del>11.7</del> <del>14.3</del>	-	Davis et al. 2002 Davis et al. 2002	pH ranged from 5.2-5.8 pH ranged from 5.2-5.8, LOEC is misleading, EC20 ≂ ~9 mg/L
Spirodella polyrhiza	Boric acid Boric acid	SR,M SR,M	10	Frond number Abnormalities		22.4	4.8 20.6	47,7	-	Davis et al, 2002	pH ranged from 5,2-5,8
Common duckweed	Boron (unknown form)	S,U	4	Growth	60	>60	-	>60	-	Wang 1986	No mortality or growth effect occurred at highest treatment, invalid endpoint for acute test
Lemna minor	Boric acid Boric acid	S,M S.M	7	Weight Weight	5-10 20	40-20 50	UNK 31.6	-		Frick 1985 Frick 1985	Unclear methods, results not quantified, pH was 5 Unclear methods, results not quantified, pH was 5
Lemna gibba	Boric acid	S,M	12	Weight	<b>≻</b> 40	<b>≫</b> 40	<del>&gt;10</del>	-	-	Marin and Oron, 2007	Greatest growth in 10 mg/L treatment, pH ranged from 6.0 - 7.0
Blue-Green Algae	Boric acid	s,u	4	Growth	50	75	64,3	-	-	Martinez et al. 1986	No pH or hardness measurements, no acute tests for ACR development
Anocystis nidulans	Boric acid	S,U	4	Protein	50	75	61.3	-	-	Martinez et al. 1986	No pH or hardness measurements, no acute tests for ACR development
	Boric acid	S,U	4	Chlorophyll	60	76	61,3	-	-	Martinez et al. 1986	No pH or hardness measurements, no acute tests for ACR development
Green Algae Chlorella pyrenoidosa	Boric acid	S,U	4	Growth	40	>10	<del>&gt;1</del> 0	40حد	-	Fernandez et al. 1984	B unmeasured, no pH or hardness reported, no effect on cell composition at 10 mg/L
Eurasian watermilfoil	Sodium tetraborate	S,UNK	32	Root Weight	-	-	-	39,9	-	Stanley 1974	IC50 not an appropriate chronic endpoint
Myriophyllum spicatum	Sodium tetraborate	S,UNK	32	Shoot Weight	-	-	-	59.7	-	Stanley 1974	IC50 not an appropriate chronic endpoint
	Sodium tetraborate	S,UNK	32	Root Length	-	-	-	42.4	-	Stanley 1974	IC50 not an appropriate chronic endpoint
	Sodium tetraborate	S,UNK	32	Shoot Length	-	-	-	47.7	-	Stanley 1974	IC50 not an appropriate chronic endpoint

## Attachment 1 – Exhibit I

Boron standard Derivation using 1985 Guidelines Methodology

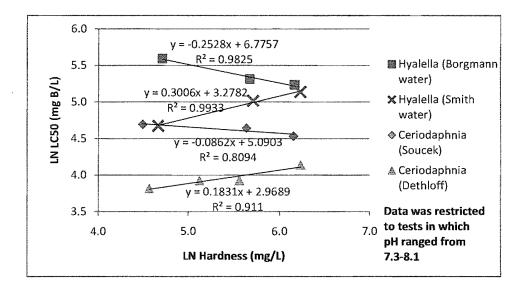
Number of MAV's in data set         17         Chironomus         1358           List of lowest MAV's         1         84.8         Megalonaias         >544           List of lowest MAV's         1         84.8         Allocapnia         476           2         96.1         Sphaerium         >447           3         125         Oncoryhnchus         >360.8           4         136.6         Gila         280           Ptychocheilus         279         279           Number of MAV's entered         4         267.4           Kaute Standard =         80.1         Lepomis         2201.1           Acute Standard =         40.1 mg/L         154.9         147           Ligumia         154.9         147         154.9           Acute Standard =         40.1 mg/L         154.9         154.9           1355         Ligumia         154.9         154.9           147         Lampsilis         137         147           154.9         Ligumia         147         147           156.6         Catostomus         125         136.6           1585         Guidelines General Use Boron CATC Calculation         Pimephales         96.1	This spreadsheet calcu	his spreadsheet calculates the FAV when there are less than 59 GMAV's							
List of lowest MAV's       1       84.8       Allocapnia       >544         List of lowest MAV's       1       84.8       Allocapnia       476         2       96.1       Sphaerium       >447         3       125       Oncoryhnchus       >360.8         4       136.6       Gila       280         Ptychocheilus       279         Number of MAV's entered       4       267.4         Acute Standard =       40.1 mg/L       Lepomis       >201.1         Acute Standard =       40.1 mg/L       Ligumia       147         Lampsilis       137       Hyalella       136.6         Catostomus       136.6       Catostomus       125         1985 Guidelines General Use Boron CATC Calculation       Pimephales       96.1				Chironomus	1513				
List of lowest MAV's       1       84.8       Alocapnia       476         2       96.1       Sphaerium       >447         3       125       Oncoryhnchus       >360.8         4       136.6       Gila       280         Ptychocheilus       279         Number of MAV's entered       4       1       267.4         Karle       Lumbriculus       267.4       233         FAV=       80.1       Lepomis       >201.1         Acute Standard =       40.1 mg/L       Ligumia       154.9         Ligumia       137       136.6       137         Hyalelines General Use Boron CATC Calculation       Pimephales       96.1	Number of MAV's in da	ta set	17	Dugesia	1358				
2       96.1       Sphaerium       >447         3       125       Oncoryhnchus       >360.8         4       136.6       Gila       280         Ptychocheilus       279         Number of MAV's entered       4       Lumbriculus       267.4         Xyrauchen       233         FAV=       80.1       Lepomis       >201.1         Acute Standard =       40.1 mg/L       Ligumia       154.9         Lampsilis       137       147       136.6         Catostomus       136.6       136.6       136.6         1985 Guidelines General Use Boron CATC Calculation       Pimephales       96.1				Megalonaias	>544				
3       125       Oncoryhnchus       >360.8         4       136.6       Gila       280         Ptychocheilus       279         Number of MAV's entered       4       Lumbriculus       267.4         FAV=       80.1       Lepomis       >201.1         Acute Standard =       40.1 mg/L       Ligumia       154.9         Image: Acute Standard =       40.1 mg/L       Ligumia       147         Lampsilis       137       Hyalella       136.6         Catostomus       125       1985 Guidelines General Use Boron CATC Calculation       Pimephales       96.1	List of lowest MAV's	1	84.8	Allocapnia	476				
4       136.6       Gi/a       280         Ptychocheilus       279         Number of MAV's entered       4       Lumbriculus       267.4         Xyrauchen       233         FAV=       80.1       Lepomis       >201.1         Acute Standard =       40.1 mg/L       Ligumia       154.9         Acute Standard =       40.1 mg/L       Ligumia       147         Lampsilis       137       Hyalella       136.6         Catostomus       125       1985 Guidelines General Use Boron CATC Calculation       Pimephales       96.1		2	96.1	Sphaerium	>447				
Number of MAV's entered4Ptychocheil/us279Number of MAV's entered4Lumbriculus267.4Yrauchen233233FAV=80.1Lepomis>201.1Acute Standard =40.1 mg/LDaphnia154.9Acute Standard =40.1 mg/LLigumia147Lampsilis137Hyalella136.6Catostomus1251985 Guidelines General Use Boron CATC CalculationPimephales96.1		3	125	Oncoryhnchus	>360.8				
Number of MAV's entered         4         Lumbriculus         267.4           Kyrauchen         233           FAV=         80.1         Lepomis         >201.1           Acute Standard =         40.1 mg/L         Daphnia         154.9           Acute Standard =         40.1 mg/L         Ligumia         147           Lampsilis         137         136.6         261.0           1985 Guidelines General Use Boron CATC Calculation         Pimephales         96.1		4	136.6	Gila	280				
FAV=80.1Xyrauchen233FAV=80.1Lepomis>201.1Daphnia154.9Daphnia154.9Acute Standard =40.1 mg/LLigumia147Lampsilis137136.6Catostomus1251985 Guidelines General Use Boron CATC CalculationPimephales96.1				Ptychocheilus	279				
FAV=       80.1       Lepomis       >201.1         Acute Standard =       40.1 mg/L       Daphnia       154.9         Acute Standard =       40.1 mg/L       Ligumia       147         Lampsilis       137       136.6       136.6         Catostomus       125       1985 Guidelines General Use Boron CATC Calculation       Pimephales       96.1	Number of MAV's enter	red	4	Lumbriculus	267.4				
Acute Standard =40.1 mg/LDaphnia154.9Acute Standard =40.1 mg/L147Ligumia147Lampsilis137Hyalella136.6Catostomus1251985 Guidelines General Use Boron CATC CalculationPimephales96.1				Xyrauchen	233				
Acute Standard =       40.1 mg/L       Ligunia       147         Lampsilis       137         Hyalella       136.6         Catostomus       125         1985 Guidelines General Use Boron CATC Calculation       Pimephales       96.1	FAV= 80	).1		Lepomis	>201.1				
Lampsilis 137 Hyalella 136.6 Catostomus 125 <u>1985 Guidelines General Use Boron CATC Calculation</u> Pimephales 96.1				Daphnia	154.9				
Hyalella136.6Catostomus1251985 Guidelines General Use Boron CATC CalculationPimephales96.1	Acute Standard = 40	).1 mg/L		Ligumia	147				
1985 Guidelines General Use Boron CATC Calculation       Catostomus       125         Pimephales       96.1				Lampsilis	137				
1985 Guidelines General Use Boron CATC Calculation         Pimephales         96.1				Hyalella	136.6				
				Catostomus	125				
Chronic Standard = FAV / Geomean of all ACRs (FACR) Ceriodaphnia 84.8	1985 Guidelines Gene	eral Use Boron C/	ATC Calculation	Pimephales	96.1				
	Chronic Standard = FA	V / Geomean of al	Ceriodaphnia	84.8					

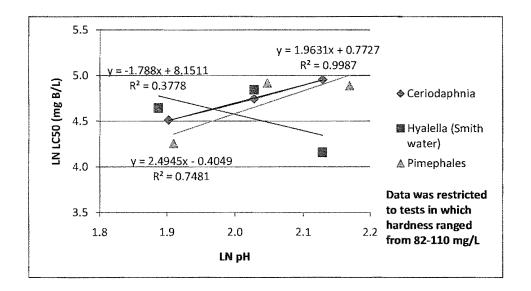
Chronic Standard = 80.1 / Geomean of 19.1, 5.2, and 11.6

Chronic Standard = 80.1 / 10.5 = **7.6 mg/L** 

Attachment 1 – Exhibit J Influence of hardness and pH on boron toxicity

### Exhibit J: Influence of hardness and pH on boron toxicity





## Attachment 1 – Exhibit K

Fluoride Standard Derivation Using 1985 Guidelines Methodology

## Fluoride Standard Derivation Using 1985 Guidelines Methodology

Species	<u>LC50 (mg/L)</u>	Hardness (mg/L)	Geometric <u>Mean LC50</u> W	GeoMean <u>Hardness</u> X	LN (LC50 / GeoMean <u>LC50)</u>	LN (Hardness / GeoMean <u>Hardness)</u>	Slope	<u>R squared</u>	
Daphnia magna	342	266	206.8	136.4	0.502918052	0.667903651	0.809962436	0.984271775	
	251	169			0.193560254	0.214306057			
	187	110			-0.100784069	-0.215112292			
Dimension and the second second	114	70		440 7	-0.595694237	-0,667097416	0.000007000	0.00004000	
Pimephales promelas	112.2 190	67 260	144.9	118.7	-0.255886289 0.27085479	-0.572208025 0.783780987	0.388007883	0.936661966	
	179	168			0.211216524	0.347063335			
	134	112			-0.078329482	-0.058401773			
	125	72			-0.147855544	-0.500234525			
Ceriodaphnia dubia	248	288	177.4	147.7	0.334825414	0.667556873	0.47229169	0.876197806	
	180 182	186 117			0.014353519 0.025403355	0.230343066			
	122	76			-0.374582287	-0.664670267			
Gasterosteus aculeatus	340	78	390.2	150.6	-0.137835502	-0.657990481	0.225405768	0.987652284	*Low stope, precipitation
	380	146			-0.026609867	-0.031092686			may have occurred w/
	460	300			0.16444537	0.689083167			increased hardness
Actinonaias pectorosa	259	28	277.8	48.5	-0.070132712	-0.549306144	0.127675092	1	*Low slope, poor
Livellate externa	298	84 112	35.9	112.0	0.070132712 0	0.549306144			relationship w/ hardness
Hyallela azteca Lepomis macrochirus	25.8 375.6	40	25.8 375.6	112.0 40.0	0	0			across all four tests
Ceratopsyche bronta	17	40.2	17.0	40.2	ŏ	ŏ			
Hydropsyche occidentalis	34.7	40.2	34.7	40.2	0	0			
Hydropsyche bulbifera	26.3	16,9	26.3	16.9	0	0			
Hydropsyche exocellata	26.5	12.6	26.5	12.6	0	0			
Hydropsyche lobata	48.2 38.5	17.5 18.2	48.2 38.5	17.5	0	0			
Hydropsyche pellucidula Chimarra marginata	38.5 44,9	12.6	38.5 44.9	18.2 12.6	0	0			
Cheumatopsyche pettiti	42.5	40.2	42.5	40.2	ŏ	õ			
Hexagenia limbata	32.3	145	32.3	145.0	0	0			
Lampsilis fasciola	172	32	172.0	32.0	0	0			
Utterbackia imbecillis	234	34	234.0	34.0	0	0			
Chironomus tentans	124.1 183.33	145 90	124.1 183.3	145.0 90.0	0	0			
Brachionus calyciflorus Physa sp.	163.1	36.1	163.1	36.1	0	0			
Lumbriculus variegatus	93.5	49.5	93.5	49.5	ŏ	õ			
Simocephalus vetulus	201.5	250	201.5	250.0	0	0			
Philodina acuticomis	212	40	212.0	40.0	0	0			
Alasmidonta raveneliana	303	28	303.0	28.0	0	0			
Sphaerium simile	62.2	96	62.2	96.0	0 led Slope (V) =	0	*Coloulated fre		in Donksin and
				Pot	R squared =		Pimephales da		iia, Daphnia, and
							<ul> <li>mophatoo ac</li> </ul>		
	<u>In W</u>	Σ	<u>In X</u>	<u>Z</u>	LN Z	Y	<u>SMAV</u>	<u>GMAV</u>	GMAV Rank
Hyallela azteca	3.250374492	0.539423	4.718498871	50	LN Z 3.912023005	<u>Y</u> 2.815342549	16.70	16,70	1
Hexagenia limbata	3.250374492 3.47506723	0.539423 0.539423	4.718498871 4.976733742	50 50	<u>LN Z</u> 3.912023005 3.912023005	<u>¥</u> 2.815342549 2.900737359	16.70 18.19	16,70 18.19	1 2
Hexagenia limbata Ceratopsyche bronta	3.250374492 3.47506723 2.833213344	0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996	50 50 50	LN Z 3.912023005 3.912023005 3.912023005	<u>¥</u> 2.815342549 2.900737359 2.950891798	16.70 18,19 19,12	16.70 18.19 19.12	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile	3.250374492 3.47506723	0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742	50 50	<u>LN Z</u> 3.912023005 3.912023005	<u>¥</u> 2.815342549 2.900737359	16.70 18.19	16,70 18.19	1 2
Hexagenia limbata Ceratopsyche bronta	3.250374492 3.47506723 2.833213344 4.130355	0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191	50 50 50 50	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	<u>¥</u> 2.815342549 2.900737359 2.950891798 3.778475539	16.70 18.19 19.12 43.75	16.70 18.19 19.12 43.75	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petfili Hydropsyche occidentalis Hydropsyche bulbifera	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 3.693866996 2.827313622	50 50 50 50 50 50 50	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.950891798 3.778475539 3.867182529 3.6644181 <b>4</b> 3.854686548	16.70 18.19 19.12 43.75 47.81 39.03 47.21	16.70 18.19 19.12 43.75 47.81 56.57 56.57	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petfili Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche exocellata	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 3.693866996 2.827313622 2.533696814	50 50 50 50 50 50 50 50	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.667182529 3.66441814 3.854686548 4.020646115	16.70 18.19 19.12 43.75 47.81 39.03 47.21 55.74	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche exocellata Hydropsyche lobata	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 3.693866996 2.827313622 2.533696814 2.862200881	50 50 50 50 50 50 50 50 50	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.66441814 3.854686548 4.020646115 4.441657626	16.70 18.19 19.12 43.75 47.81 39.03 47.21 55.74 84.92	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche kozellata Hydropsyche pellucidula	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594	50 50 50 50 50 50 50 50 50 50	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.66441814 3.854686548 4.020646115 4.441657626 4.195800277	16.70 18.19 19.12 43.75 47.81 39.03 47.21 55.74 84.92 66.41	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche pettiti Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche kobata Hydropsyche lobata Hydropsyche pellucidula Chironomus tentans	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 3.693866996 2.827313622 2.533696814 2.862200881	50 50 50 50 50 50 50 50 50	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.66441814 3.854686548 4.020646115 4.441657626	16.70 18.19 19.12 43.75 47.81 39.03 47.21 55.74 84.92	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche kozellata Hydropsyche pellucidula	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742	50 50 50 50 50 50 50 50 50 50 50 50	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.66441814 3.854686548 4.020646115 4.441657624 4.195800277 4.246757821	16.70 18.19 19.12 43.75 47.81 39.03 47.21 55.74 84.92 66.41 69.88	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 56.57 56.57	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche bulbifera Hydropsyche kocellata Hydropsyche kobata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.867182529 3.8641814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.543382822	16.70 18.19 19.12 43.75 47.81 39.03 47.21 55.74 84.92 66.41 69.88 84.57 90.89 94.01	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 56.57 56.57 69.88 84.57 90.89 94.01	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche kobata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chima ra marginata	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437795	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 3.693866996 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267 2.533696814	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.8641814 3.854686548 4.020646115 4.441657624 4.195800277 4.246757821 4.437620933 4.509634057 4.543382822 4.547939176	16.70 18.19 19.12 43.75 47.81 39.03 47.21 55.74 84.92 66.41 69.88 84.57 90.89 94.01 94.44	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 69.88 84.57 90.89 94.01 94.44	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche pettiti Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche lobata Hydropsyche lobata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chimar marginata Ceriodaphnia dubia	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437795 5.178603332	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267 2.533696814	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.86441814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.54382822 4.547939176 4.594202499	16.70 18,19 19,12 43,75 47,81 39,03 47,21 55,74 84,92 66,41 69,88 84,57 90,89 94,01 94,44 98,91	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 56.57 69.88 84.57 90.89 94.01 94.44 98.91	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche exocellata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chimarra marginata Ceriodaphnia dubia Daphnia magna	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437792 5.178603332 5.331892685	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267 2.533696814 4.995592658	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.66441814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.543382822 4.547939176 4.594202499 4.790543745	16.70 18,19 19,12 43,75 47,81 39,03 47,21 55,74 84,92 66,41 69,88 84,57 90,89 94,01 94,44 98,91 120,37	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 56.57 56.57 90.89 94.01 94.44 98.91 120.37	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche pettiti Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche lobata Hydropsyche lobata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chimar marginata Ceriodaphnia dubia	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437795 5.178603332	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267 2.533696814	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.86441814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.54382822 4.547939176 4.594202499	16.70 18,19 19,12 43,75 47,81 39,03 47,21 55,74 84,92 66,41 69,88 84,57 90,89 94,01 94,44 98,91	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 56.57 69.88 84.57 90.89 94.01 94.44 98.91	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche vocellata Hydropsyche ekotellata Hydropsyche ellucidula Chironomus tentans Simocephalus vetulius Pimephaies promelas Lumbriculus variegatus Chima rra marginata Ceriodaphnia dubia Daphnia magna Brachionus calyciflorus Physa sp. Gasterosteus aculeatus	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437795 5.178603332 5.331892685 5.211287808 5.09436351 5.96678112	0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423 0.539423	$\begin{array}{r} 4.71\overline{8498871}\\ 4.976733742\\ 3.693866996\\ 4.564348191\\ 3.693866996\\ 2.827313622\\ 2.533696814\\ 2.862200881\\ 2.901421594\\ 4.976733742\\ 5.521460918\\ 4.776900644\\ 3.90197267\\ 2.533696814\\ 4.995403607\\ 4.915592658\\ 4.49980967\\ 3.586292865\\ 5.014699308\\ \end{array}$	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005 3.91202305 3.9120235 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.6641814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.543382822 4.547939176 4.594202499 4.790543745 4.894221934 5.270069965 5.371971735	16.70 18,19 19,12 43,75 47,81 39,03 47,21 55,74 84,92 66,41 69,88 84,57 90,89 94,01 94,44 98,91 120,37 133,52 194,43 215,29	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 56.57 90.89 94.01 94.44 98.91 120.37 133.52 194.43 215.29	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche exocellata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chimarra marginata Ceriodaphnia dubia Daphnia magna Brachionus calyciflorus Physa sp. Gasterosteus aculeatus Lampsilis fasciola	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437792 5.31892685 5.211287808 5.09436351 5.96678112 5.147494477	0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267 2.533696814 4.995403607 4.915592658 4.49980967 3.586292865 5.014699308 3.465735903	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.66441814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.5433828222 4.547939176 4.594202499 4.790543745 4.894221934 4.894221934 5.370069965 5.371971735 5.388232177	16.70 18,19 19,12 43,75 47,81 39,03 47,21 55,74 84,92 66,41 69,88 84,57 90,89 94,01 94,44 98,91 120,37 133,52 194,43 215,29 218,82	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 56.57 90.89 94.01 94.44 98.91 120.37 133.52 194.43 215.29 218.82	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche exocellata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chima rra marginata Ceriodaphnia dubia Daphnia magna Brachionus calyciflorus Physa sp. Gasterosteus aculeatus Lampsilis fasciola Philodina acuticornis	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437795 5.178603322 5.31892685 5.211287808 5.09436351 5.90436351 5.947494477 5.356586275	0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 4.976733742 4.976733742 4.99690644 3.90197267 2.533696814 4.995403607 4.915592658 4.49980967 3.568292865 5.014699308 3.465735903 3.688879454	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.8641814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.543382822 4.547939176 4.594202499 4.790543745 4.894221934 5.270069965 5.371971735 5.388232177 5.476955125	16.70 18,19 19,12 43,75 47,81 39,03 47,21 55,74 84,92 66,41 69,88 84,57 90,89 94,01 94,44 98,91 120,37 133,52 194,43 215,29 218,82 239,12	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 69.88 84.57 90.89 94.01 94.44 98.91 120.37 133.52 194.43 215.29 218.82 239.12	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche exocellata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chima rra marginata Ceriodaphnia dubia Daphnia magna Brachionus calyciflorus Physa sp. Gasterosteus aculeatus Lampsilis fasciola Philodina acuticornis Actinonaias pectorosa	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.5467396873 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437795 5.178603332 5.331892685 5.211287808 5.09436351 5.96678112 5.147494477 5.356586275 5.626960774	0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267 2.533696814 4.995403607 4.915592658 4.49980967 3.586292865 5.014699308 3.465735903 3.688879454 3.881510655	50 50	LN Z 3.912023005 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.8641814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.543382822 4.547939176 4.594202499 4.790543745 4.894221934 5.270069965 5.371971735 5.388232177 5.476655125 5.64341985	16.70 18,19 19,12 43,75 47,81 39,03 47,21 55,74 84,92 66,41 69,88 84,57 90,89 94,01 94,44 98,91 120,37 133,52 194,43 215,29 218,82 239,12 282,43	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 69.88 84.57 90.89 94.01 94.44 98.91 120.37 133.52 194.43 215.29 218.82 239.12 282.43	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche exocellata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chima rra marginata Ceriodaphnia dubia Daphnia magna Brachionus calyciflorus Physa sp. Gasterosteus aculeatus Lampsilis fasciola Philodina acuticornis	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.546739687 3.269568939 3.277144733 3.875359021 3.850658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437795 5.178603332 5.331892685 5.211287808 5.09436351 5.96678112 5.147494477 5.356586274 5.626960774 5.455321115	0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267 2.533696814 4.995403607 4.915592658 4.49980967 3.586292865 5.014699308 3.465735903 3.688879454 3.881510655 3.526360525	50 50 50 50 50 50 50 50 50 50 50 50 50 5	LN Z 3.912023005 3.91202305 3.91205	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.66414814 3.854686548 4.020646115 4.441657624 4.195800277 4.246757821 4.437620933 4.509634057 4.547382822 4.547399176 4.594202499 4.790543745 4.894221934 5.270069965 5.371971735 5.3882322177 5.476955125 5.64341985 5.663356477	$\begin{array}{c} 16.70\\ 18.19\\ 19.12\\ 43.75\\ 47.81\\ 39.03\\ 47.21\\ 55.74\\ 84.92\\ 66.41\\ 69.88\\ 84.57\\ 90.89\\ 94.01\\ 94.44\\ 98.91\\ 120.37\\ 133.52\\ 194.43\\ 215.29\\ 218.82\\ 239.12\\ 282.43\\ 288.11\\ \end{array}$	$\begin{array}{c} 16.70\\ 18.19\\ 19.12\\ 43.75\\ 47.81\\ 56.57\\ 56.57\\ 56.57\\ 56.57\\ 56.57\\ 69.88\\ 84.57\\ 90.89\\ 94.01\\ 94.44\\ 98.91\\ 120.37\\ 133.52\\ 194.43\\ 215.29\\ 218.82\\ 239.12\\ 282.43\\ 288.11\\ \end{array}$	1 2 3
Hexagenia limbata Ceratopsyche bronta Sphaerium simile Cheumatopsyche petitii Hydropsyche occidentalis Hydropsyche bulbifera Hydropsyche exocellata Hydropsyche pellucidula Chironomus tentans Simocephalus vetulus Pimephales promelas Lumbriculus variegatus Chima rra marginata Ceriodaphnia dubia Daphnia magna Brachionus calycifforus Physa sp. Gasterosteus aculeatus Lampsilis fasciola Philodina aculicomis Actinonais pectorosa Utterbackia imbecillis	3.250374492 3.47506723 2.833213344 4.130355 3.749504076 3.5467396873 3.269568939 3.277144733 3.875359021 3.650658241 4.821087692 5.305789381 4.976169282 4.537961436 3.804437795 5.178603332 5.331892685 5.211287808 5.09436351 5.96678112 5.147494477 5.356586275 5.626960774	0.539423 0.539423	4.718498871 4.976733742 3.693866996 4.564348191 3.693866996 2.827313622 2.533696814 2.862200881 2.901421594 4.976733742 5.521460918 4.776900644 3.90197267 2.533696814 4.995403607 4.915592658 4.49980967 3.586292865 5.014699308 3.465735903 3.688879454 3.881510655	50 50	LN Z 3.912023005 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91202305 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91205 3.91	¥ 2.815342549 2.900737359 2.950891798 3.778475539 3.867182529 3.8641814 3.854686548 4.020646115 4.441657626 4.195800277 4.246757821 4.437620933 4.509634057 4.543382822 4.547939176 4.594202499 4.790543745 4.894221934 5.270069965 5.371971735 5.388232177 5.476655125 5.64341985	16.70 18,19 19,12 43,75 47,81 39,03 47,21 55,74 84,92 66,41 69,88 84,57 90,89 94,01 94,44 98,91 120,37 133,52 194,43 215,29 218,82 239,12 282,43	16.70 18.19 19.12 43.75 47.81 56.57 56.57 56.57 56.57 56.57 69.88 84.57 90.89 94.01 94.44 98.91 120.37 133.52 194.43 215.29 218.82 239.12 282.43	1 2 3

## Attachment 1 – Exhibit L

Manganese Standard Derivation Using 1985 Guidelines methodology

### Manganese Standard Derivation Using 1985 Guidelines Methodology

		Hardness	Geometric	GeoMean Hardness	LN (LC50 /	LN (Hardness /			
Species	LC50 (mg/L)	(ma/L)	Mean LC50 W	<u>(ma/L)</u> X	GeoMean LC50)	GeoMean Hardness)	Slope	<u>R squared</u>	
Pimephales promelas	3.54	26	10.396265	69.83676907	-1.076755068	-0.988064111	0.675466164	0.677839398	
	6,23	50			-0.511749298	-0.334137643			
	9.35	100			-0.106498163	0,359009537			
	15.83	200			0.42020755	1.052156718			
	10.30	48			-0.009108556	-0.374959638			
	17,28 27,44	92 176			0.508045284 0.970555196	0.275627928 0.924323346			
	27.44 8.56	28			-0.194696946	-0.913956138			
Hvalella azteca	3,00	26	10.3708414	100.5484771	-1.240385869	-1.352543432	0.948608781	0.977275646	
	8.56	80			-0.191897968	-0.228613336			
	13.70	164			0.278397675	0.489226458			
	31.00	269			1.094989047	0.984071409			
	11.00	112			0.058897115	0.107858901			
Ceriodaphnia dubia	5.70	26	13.2927304	72.95820557	-0.846751127	-1.031790213	0.520161431	0.734117436	
	14,50	92			0.086931347	0.231901826			
	14.50 9,44	184 25			0.086931347	0.925049007			
	9.44 11.20	25 50			-0.342261322 -0.171303524	-1.071010926 -0.377863745			
	21.20	100			0.466783879	0.315283435			
	27.30	200			0.7196694	1.008430616			
Daphnia magna	28.70	100	27.7892934	106,5458376	0.032246307	-0.063405106	1.150551122	0.992093377	
	76,30	267			1.010022123	0.918673366			
	9.80	45.3			-1.04226843	-0.85526826			
Tubifex tubifex	26.80	12	95.2496886	93,06388048	-1.268099857	-2.048379494	0.746925146	0.812047797	
	42.70	45			-0.802302825	-0.726623654			
	85.90	173			-0.103317916	0.62000545			
	464.75	305			1.584997882	1.187025632			
	171.61	245		404 004000	0.588722716	0.967972066	0.000.000.00		
Chironomus tentans	42.20 94.30	100 272	63.0829612	164,924225	-0.402030484	-0.50031594	0.803553219	1	
Pytchocheilus oregonensis	94,30 189,48	347	189.482	347	0.402030484 0	0.50031594 0			
Anodonta imbecillus	36,20	80	36.2	347	0	0			
Agosia chrysogaster	130.00	224	130	224	0	ō			
Bulo boreus	42.30	52.6	42,3	52.6	0	0			
Physa inlegra	147.12	162	147.12	162	0	0			
Brachionus calyciflorus	38.70	36.2	38.7	36.2	0	0			
Megalonaias nervosa	31.50	91	31.5	91	0	0			
Lampsilis siliquoidea	43,30	91	43,3	91	0	0			
					Pooled Slope (V) =	0.746723791			
					R squared =	0.80			
	<u>In W</u>	¥	<u>In X</u>	Z	LN Z	¥	SMAV	GMAV	GMAV Rank
Hyalella azteca	2,338998158	0.746724	4,61063997	50	3.912023005	1.817324249	6.155366169	6,155366169	1
Pimephales promelas	2.341446607	0.746724	4.24616065	50	3.912023005	2.091938079	8.100599565	8.100599565	2
Ceriodaphnia dubia	2.587217302	0.746724	4.28988675	50	3.912023005	2.305057454	10.0247542	10.0247542	3
Daphnia magna	3.324650816	0.746724	4.66857529	50	3,912023005	2.759715224	15.79534417	15.79534417	4
Megalonaías nervosa	3,449987546	0.746724	4.51085951	50	3,912023005	3,002822083	20.14230004	20.14230004	
Lampsilis siliquoidea		0.746724	4.51085951	50	3.912023005	3.320987173	27.68766958	27.68766958	
Anodonta imbecillus Chimnomus lenlans		0.746724 0.746724	4,38202663 5,10548613	50 50	3.912023005 3.912023005	3.238096227	25.48515758	25.48515758 25.87464157	
Chironomus ienians Bulo boreus	3,744787086	0.746724	3,96271612	50	3.912023005	3.253263399 3.706933332	25.87464157 40.72871335	40,72871335	
Agosia chrysogaster	4,86753445	0.746724	5,41164605	50	3,912023005	3.747730244	42,42467896	42.42467896	
Pytchocheilus oregonensis	5.244294033	0.746724	5.84932478	50	3.912023005	3,797664707	44.59691594	44.59691594	
Brachionus calyciflorus	3.6558396	0.746724	3.58905912	50	3.912023005	3.897004418	49.25468145	49.25468145	
Tubifex tubifex	4.556501745	0.746724	4.53328614	50	3,912023005	4.092589778	59,89480529	59.89480529	
Physa integra	4,99124858		5.08759634	50	3.912023005	4,113420007	61.15551258	61.15551258	
Calculation of Chronic Intercer				rdness) = 115 m	g/L				
Hyalella azteca (chronic)	<u>In W</u> 0.698134722	<u>⊻</u> 0.746724	<u>In X</u> 4.74493213	<u>Z</u> 50	<u>LN Z</u> 3.912023005	<u>¥</u> 0.076181664	MATC 1.079158601		

3.912023005 <u>1</u> 0.076181664

## Attachment 1 – Exhibit M

Acute and chronic fluoride standards at variable hardness using 1985 Guidelines Methodology

### Exhibit M: Acute and chronic fluoride standards at variable hardness using 1985 Guidelines methodology

This spreadsheet calculates the FAV when there are less than 59 MAV's

Number of MAV's	in data set	22		
List of lowest MAV	's 1	16. <b>7</b>		
	2	<b>1</b> 8.19		
	3	19.12		
	- 4	43.75		
Number of MAV's	entered	4		
FAV	at 50 mg/L hardness =	13.85 n	ng/L	
	FAV / 2 =	6.92 n	ng/L	
		Acute standards at	variable hard	iness = e <sup>(V(LN(hardness)) + LN(acule standard at 50 hardness) - (V(LNZ))</sup>
	Acute standard at 100		10.06	Equation = EXP((0.539423386280342*(LN(100)))+(LN(6.92)-(0.539423386280342*(3.912023005))))
	Acute standard at 200	mg/L hardness =	14.62	Equation = EXP((0.539423386280342*(LN(200)))+(LN(6.92)-(0.539423386280342*(3.912023005))))
	Acute standard at 300	mg/L hardness =	18.19	Equation = EXP((0.539423386280342*(LN(300)))+(LN(6.92)-(0.539423386280342*(3.912023005))))
	Chronic standard at 50	) mg/L hardness = F	AV (at 50 mg	g/L hardness) / FACR (geometric mean of 7.36, 9.88, 1.19, and 2.9 from available ACRs)
		= 1	3.85 / 3.98	
		= 3	.48 mg/L	
		Chronic standards a	at variable ha	ardness = e <sup>(V(LN(hardness)) + LN(chronic standard at 50 hardness) - (V(LNZ))</sup>
(	Chronic standard at 100		5.06	Equation = EXP((0.539423386280342*(LN(100)))+(LN(3.48)-(0.539423386280342*(3.912023005))))
(	Chronic standard at 200	mg/L hardness =	7.35	Equation = EXP((0.539423386280342*(LN(200)))+(LN(3.48)-(0.539423386280342*(3.912023005))))
(	Chronic standard at 300	mg/L hardness =	9.15	Equation = EXP((0.539423386280342*(LN(300)))+(LN(3.48)-(0.539423386280342*(3.912023005))))

\*Chronic will be capped at 4 mg/L for protection of wildlife and domesticated animals

## Attachment 1 – Exhibit N

Acute and chronic manganese standards at variable hardness using 1985 Guidelines Methodology

#### Exhibit N: Acute and chronic manganese standards at variable hardness using 1985 Guidelines methodology.

This spreadsheet calculates the FAV when there are less than 59 MAV's

Number of MAV's in data set		14		
List of lowest MAV's	1	6.16		
	2	8.1		
	3	10.02		
	4	15.8		
Number of MAV's entered		4		
FAV at 50 mg/L	hardness =	<b>5.0</b> 7 n	ng/L	
	FAV / 2 =	<b>2.54</b> n	ng/L	
	Acute :	standards a	t variable ha	rdness = e <sup>(V(LN(hardness))</sup> + LN(acule standard at 50 hardness) - (V(LNZ))
Acute stands	ard at 100 mg/L ha	rdness =	4.26	Equation = EXP((0.746723791250639*(LN(100)))+(LN(2.54)-(0.746723791250639*(3.912023005))))
Acute stands	ard at 200 mg/L ha	rdness =	7.15	Equation = EXP((0.746723791250639*(LN(200)))+(LN(2.54)-(0.746723791250639*(3.912023005))))
Acute standa	ard at 300 mg/L ha	rdness =	9.68	Equation = EXP((0.746723791250639*(LN(300)))+(LN(2.54)-(0.746723791250639*(3.912023005))))

<u>35 III. Adm. Code 302.627(d)</u>: If a resident or indigenous species whose presence is necessary to sustain commercial or recreational activities, or prevent disruptions of the waterbody's ecosystem, including but not limited to loss of species diversity or a shift to a biotic community dominated by pollution-tolerant species, will not be protected by the calculated CATC, then the MATC for that species is used as the CATC.

Chronic standard at 50 m/L hardness = FAV (at 50 mg/L hardness) / FACR (geometric mean of 3.22, 5.32, 1.4, 4.74, 2.24 and 5.48 from available ACRs)

Note: Hyalella chronic MATC at 50 mg/L hardness = 1.08 mg/L, use of all availble

ACRs results in chronic standards that are not protective of Hyalella

Recalculation of chronic standards using Hyalella MATC as basis:

Hyalella chronic MATC = 1.08 mg/L at 50 mg/L hardness, so

Chronic standards at variable hardness = e<sup>(V(LN(hardness)) + LN(Hyalella MATC at 50 hardness) - (V(LNZ))</sup>

Chronic standard at 100 mg/L hardness =	1.81	Equation = EXP((0.746723791250639*(LN(100)))+(LN(1.08)-(0.746723791250639*(3.912023005))))
Chronic standard at 200 mg/L hardness =	3.04	Equation = EXP((0.746723791250639*(LN(200)))+(LN(1.08)-(0.746723791250639*(3.912023005))))
Chronic standard at 300 mg/L hardness =	4,12	Equation = EXP((0.746723791250639*(LN(300)))+(LN(1.08)-(0.746723791250639*(3.912023005))))

# Attachment 1 – Exhibit O

Acute Toxicity Data Used in Fluoride Standard Derivation

### Acute Toxicity Data Used in Fluoride Standard Derivation

\* Results marked with strikethrough are considered invalid and have been excluded from standards derivation. Reasons for exclusion is highlighted in bold within Notes column. \*\* GMAVs are not listed because values must be hardness-normalized. See associated derivation worksheet.

<u>Species</u>	Chemical	Test <u>Type</u>	Duration (hours)	LC50 <u>(mq/L)</u>	Hardness (mg/L)	Reference	Notes
Water flea	Na F	s	48	420	UNK	OPP 2000	Can't access the data, no hardness
Daphnia magna	Na F	S,U	48	88	250	Dave 1984	Precipitate and pH problems
, u	Na F	s,u	48	464	173	LeBlanc 1980	Unmeasured, poor relationship with other data
	Na F	S,U	48	282.8	145	Metcalffe-Smith et al. 2003	Unmeasured, poor relationship with other data
	Na F	S,M	48	342	266	Fieser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
	Na F	S,M	48	251	169	Fieser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
	Na F	S,M	48	187	110	Fieser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
	Na F	S,M	48	114	70	Fieser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
	Na F Na F	S,U S,U	48 24	284 352	169 UNK	Feiser et al. 1986 Kuhn et al. 1989	Publication reports the total (nominal) fluoride in the modhard water (table 3), data listed for comparison onty 24 hour test, hardness and fluoride not measured, not used hereine 40 hour tests are sublible.
	NaF	3,0 S.U	24	310.8	250	Hickey 1989	24 hour test, hardness and fluoride not measured, not used because 48 hour tests are available 24 hour test (D. carinata also available), not used because 48 hour tests are available
	NaF	3,0 S,U	24	202.3	90	Calleja et al. 1994	24 hour test, not used because 48 hour tests are available
	Na F	5.U	24	137.4	UNK	Lilius et al. 1995	24 hour test, not used because 48 hour tests are available
D. pulex	Na F	S,U	24	<del>220.8</del>	UNK	Lillus et al. 1995	24 hour test, not used because 48 hour tests are available
Water flea	Na F	S,M	48	248	288	Fieser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
Ceriodaphnia dubia	Na F	S,M	48	180	186	Fieser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
	Na F	S,M	48	182	117	Fieser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
	Na F	S,M	48	122	76	Fleser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
	Na F	s,U	24	<del>157.0</del>	250	Hickey 1989	24 hour test, not used because 48 hour tests are available
Water flea Simocephalus vetulus	Na F	S,U	24	201.5	250	Hickey 1989	Native species, similar tolerance to other Daphnidae
Mosquitofish Gambusia affinis	Na F	S,U	96	<del>418</del>	UNK	Wallen et al. 1957	Turbid water, no hardness, turbidity decreased through test, unmeasured, possible precipitate
Threespine stickleback	Na F	S,M	96	340	78	Smith et al. 1985	Tests were ok but other tests had severe hardness/precipitate problems, not used for slope derivation
Gasierosteus aculeatus	Na F	S,M	96	380	146	Smith et al. 1985	Tests were ok but other tests had severe hardness/precipitate problems, not used for slope derivation
	Na F	S,M	96	460	300	Smith et al. 1985	Tests were ok but other tests had severe hardness/precipitale problems, not used for slope derivation
Bluegill Lepomis macrochirus	Na F	SR,M	96	375.6	40	OPP 2000 (43648201)	
Rainbow trout	Na F	S.M	96	107.5	22.4	Camargo and Tarazona 1991	
Oncorhynchus mykiss	Na F	S,U	96	18	12	Herbert and Shurben 1964	
	Na F	S,M	96	140	182	Pimentel and Buikley 1983	temp=12
	Na F	S,M	96	193	385	Pimentel and Bulkley 1983	temp=12
	Na F	S,M	96	51	17	Pimentel and Bulkley 1983	lemp=12
	Na F	S,M	96	128	49	Pimentel and Bulkley 1983	temp=12
	Na F	S,M	96	200	23-62	Smith et al. 1985	Major precipitation likely occurred, see hardness data
	Na F	S	96	347	UNK	OPP 2000	Can't access the data, no hardness
Brown trout Salmo trutta	Na F	S,M	96	164.5	21,2	Camargo and Tarazona 1991	
Fathead minnow	Na F	S,M	96	345	20-48	Smith et al. 1985	Major calcium fluoride precipitation occurred, see hardness data
Pimephales prometas	Na F	SR.U	96	262.4	145	Metcalfe-Smith et al. 2003	Unmeasured, organisms were fed at 48 hours
	Na F	R,M	96	UNK	UNK	Smith et al. 1985	Major precipitation likely occurred
	Na F	R,M	96	UNK	UNK	Smith et al. 1985	Major precipitation likely occurred
	Na F	R,M	96	225.4	67	The Advent Group, Inc., 2000	14 day old organisms used, hardness not reported but test coincided with chronic test (hardness of 64 mg/L)

		Na F	R.M	96	112.2	67	The Advent Group, Inc., 2000	8 day old organisms more sensitive, hardness from other test used, test done at 25C, ion measured
		Na F	S.M	96	190	260	Feiser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
		Na F	S.M	96	179	168	Feiser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
		Na F	S.M	96	134	112	Feiser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
		Na F	S.M	96	125	72	Feiser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20 Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
		ING F	0,141	30	120	12	Feiser 1900	Results are in tonic hourde, total (nominal) huonde was added but only ionic was measured, temp=20
Midg	e	Na F	S.U	96	124.1	145	Metcattle-Smith et al. 2003	Organisms were fed on the day of the test
	nomus tentans	Na F	R,M	48	93.1	~50	The Advent Group, Inc., 2000	Hardness not reported, 48 hour test, use 96 hour test instead, ion measured
01.10	nionito (citaira	Na F	R.M	48	110.0	~50	The Advent Group, Inc., 2000	Hardness not reported, 48 hour test, use 96 hour test instead, ion measured
			13,00	40	+10.0	-50	The Advent Group, the, 2000	nal anesa nor reported, 49 noti rear, use so nou resi insteau, ion measured
Ampi	hìnari	Na F	S,U	48	14.6	145	Metcalffe-Smith et al. 2003	Test was only 48 hours, supplemental tests are needed
	ella azteca	Na F	S.M	96	43.4	82.4	GLEC 2010	Improper lab water used, chloride too low
. iyon		Na F	S,M	96	25.8	112	Soucek and Dickinson 2010	Correct lab water used for Hyalella testing, higher chloride, bromide present
		140.1	0,11	30	2.0.0	114	Soucer and Diokinson 2010	Contest las water used for hydraila testing, higher chiphice, biomise present
	yrayed lampmussel	Na F	S,M	96	172	32	Keller and Augspurger 2005	Only juveniles were tested, data reported as ionic fluoride
Lamp	osilis fasciola							
Dopo	r pondshell	Na F	S,M	96	234	34	Keller and Augspurger 2005	Deputy are from inspected, starbidic wars every taleaset, data separated as incide
	backla imbecillis	Na F	5,M	90	234	-34	Kellel and Augspurger 2005	Results are from juveniles, glochidia were more tolerant, data reported as ionic fluoride
Utter	oackia impeciuis							
Anna	lachian elktoe	Na F	S.M	96	303	28	Keller and Augspurger 2005	Juvenile test, glochidia were equally tolerant, data reported as ionic fluoride
	midonta raveneliana	146 1	0,14	30	000	20	Keller and Augspurger 2003	Suverine rest, globinula were equally folerant, uata reported as follo indunde
Masi	niuunta taveneilana							
Dhee	santshell	Na F	S.M	96	259	28	Keller and Augspurger 2005	Only interplan was tasted, data several disc incident
								Only juveniles were tested, data reported as ionic fluoride
ACUN	onaias pectorosa	Na F	S,M	96	478	30	Keller and Augspurger 2005	Only juveniles were tested 95% confidence limits uncalculable
		Na F	S,M	96	347	68	Keller and Augspurger 2005	Only juveniles were tested 95% confidence Ilmits uncalculable
		Na F	S,M	96	298	84	Keller and Augspurger 2005	Only juveniles were tested, data reported as ionic fluoride
klaf a	pinning caddisfly	Na F	S.M	96	17	40.2	Camargo et al, 1992 (#3882)	Author states no preciptate formed, species native to Illinois
		Na F	3,W	90	11	40,Z	Canalgo et al. 1992 (#3662)	Author states to precipitate formed, species harve to autors
Cera	topsyche bronta							
	to a factor of all the Maria							
	spinning caddisfly	N- F	<u>.</u>	00	247	40.0	Company at al. (000 /#2200)	
	opsyche occidentalis	Na F	S,M	96 96	34.7	40.2	Camargo et al. 1992 (#3882)	Native genera
	Ibilera	Na F	S,M		26.3	16.9	Camargo and Tarazona 1990	Chloride = 7.4 mg/L, native genera
	rocellala	Na F	S,M	96	26.5	12.6	Camargo and Tarazona 1990	Chloride = 6.3 mg/L, native genera
H. Ioi		Na F	S,M	96	48.2	17.5	Camargo and Tarazona 1990	Chloride = 10.7 mg/L, nalive genera
H, pe	ellucidula	Na F	S,M	96	38.5	18.2	Camargo and Tarazona 1990	Chloride = 5.1 mg/L, native genera
N - 1 -	an ta actual a su alaba dha	N- F	<b></b>		44.0	40.0	C	
	spinning caddisfly	Na F	S,M	96	44,9	12.6	Camargo and Tarazona 1990	Chloride = 6.3 mg/L, native genera
Chim	arra marginata							
Mote	alasias and disfly	No E	C 14	96	42.5	40.2	Compres et al 4002 (#2892)	Native gapage
	spinning caddisfly	Na F	S,M	90	42.5	40.2	Camargo el al. 1992 (#3882)	Native genera
Cneu	ımatopsyche pettili							
D-66		N= 5	<b>C</b> 11	24	102.2	90	Colleia at at 1001	Notice senses the unital sense is different shufur.
Rotifi		Na F	S,U	24	183.3	90	Calleja et al. 1994	Native genera, shows tolerance in differnet phylum
Braci	hionus calyciflorus							
01		N= 5	<b></b>	~~	004 7	45.5	The Advert Creve last 2000	
Snail		Na F	R,M	96	234.7	45.5	The Advent Group, Inc., 2000	Dose response curve was irregular, hardness increased for unknown reason
Phys	a sp.	Na F	R,M	96	163.1	36.1	The Advent Group, Inc., 2000	good dose-response, ion measured
•	e (	N	<b></b>	96	<b>60 5</b>	10.5	1 ++ 0000	
Anne		Na F	R,M	90	93.5	49,5	Advent 2000	Other tests had problems with nominal vs. measured concentrations, ion measured
Lumt	briculus variegatus							
	n	N - 7	<b>C</b> 11		20.7	4.45	Materia Carlin at al 2002	Nation remains users for but channed and little
Mayf		Na F	S,U	96	32.3	145	Metcalffe-Smith et al. 2003	Native, organims were fed but showed sensitivity
неха	igenia limbata							
<b>D</b> -///		Mr. T		00	240	10	Bulkoma at at 1077	Native reports bordness not measured nominal way 40 mail
Rotif		Na F	S,M	96	212	40	Buikema et al. 1977	Native genera, hardness not measured, nominal was 40 mg/L
Philo	dina acuticornis							
<b>A</b>	und Ennera - I - I	No 7	c ••	00	00.0	00	CI 50 2010	
	ved fingernailclam	Na F	S,M	96	62.2	96	GLEC 2010	
spha	ierium simile							

## Attachment 1 – Exhibit P

Chronic toxicity data used in fluoride Standard Derivation

### Chronic Toxicity Data Used in Fluoride Standard Derivation

\* Results marked with strikethrough are considered invalid and have been excluded from standards derivation. Reasons for exclusion is highlighted in bold within Notes column.

		Test	Duration	NOEC	LOEC	MATC/IC25		Hardness				
Species	Chemical	Туре	(days)	(ug/L)	(ug/L)	(ug/L)	Endpoint	(mg/L)	LC50 @ H	ACR	Reference (ECOTOX#)	Notes
Water flea	Na FI	SR,U	21	3.7	7,4	6.23	MATC Rep	250	98	18.73	Dave 1984	Precipitate formed at ₂7.4 mg/L fluoride, acute test had precipate at ≥30 mg/L fluoride
Daphnia magna	Na FI	SR,M	21	34	48.1	40,44	MATC Hatch	283	342	8.46	Fieser 1985	LC50 at 266 mg/L hardness used
	Na Fl	SR,M	21	26.1	35.5	30,44	MATC Hatch	181	251	8,25	Fieser 1985	LC50 at 169 mg/L hardness used
	Na FI	SR,M	21	25,9	41.2	32,67	MATC Hatch	117	187	5,72	Fieser 1985	LC50 at 110 mg/L hardness used
	Na FI	SR,M	21			28.5	ENP	114	187	6.56	Fieser 1985	ENP = Equivalent neonate production (to controls), use MATC instead
	Na FI	SR,M	21			31.5	ENP	170	251	7.07	Fieser 1985	ENP = Equivalent neonate production (to controls), use MATC instead
	Na FI	SR,M	21			47	ENP	236	342	7-28	Fieser 1985	ENP = Equivalent neonate production (to controls), use MATC instead
	Na Fl	S,U	21	14	-	•	Reproduction	UNK	352	•	Kuhn et al. 1989	No hardness measured in tests, acute test was only 24 hours, no LOEC/MATC data
							Geomean Day	ohnia ACRs	(valid data):	7.36		
Water flea	Na Fi	SR,M	7	16,3	26.1	20.63		290	260	12.61	Fieser 1985	Chronic done at 25C, acute results at 25C presented
Ceriodaphnia dubia	Na FI	SR,M	7	16.2	26.2	20.60		189	174	8.45	Fieser 1985	Summary at table 43
	Na Fl	SR,M	7	¢.	6	~6		124	146	NA	Fieser 1985	All treatments were sig different from control, unusual given the hardness treatment, see table 34
	Na Fi	SR,M	7	10,4	17.4	13.45		77	122	9.07	Fieser 1985	Acute data from 20C test used, no valid results at 25C
						Ge	omean Ceriodar	ohnia ACRs	(valid data);	9,88		
Fathead minnow	Na Fl		-	475		476-78	Survival	280	145		Metcalife-Smith et al. 2003	Duration too short
Pimephales promelas	Na Fi Na Fi	R,U R,U	4	425 63	250 125	<del>470:78</del> 8 <del>8:74</del>	Survival	160	145		Metcallfe-Smith et al. 2003 Metcallfe-Smith et al. 2003	Duration too short
runephales prometas	Na Fl	F.M	28	66,6	134.3	94,57	Surv/Growth	67	112.2	1.19		
	Na Fl Na Fl	F,M F,M	28	66.6	134.3	94,57 84,1	IC25 Biomass		112.2	4.33		1 IC25 estimate used for chronic value
	Nari	r.M	20			04.1				1,19	The Auvent Group, Inc., 2000	EPA prefers MATC over IC25
							Geon	iean Pimep	hales ACRs:	1.18		
Rainbow frout	Na Fi	S.M	8			27.6	MATC Surviva	22,4			Camargo and Tarazona 1991	Duration too short
Oncorhynchus mykiss		-,	•									
Wavyrayed tampmussel	Na Fi	S,M	9			477	Survival	32			Keller and Augspurger 2005	Duration too short
Lampsliis fasciola												
Long fingemailclam	Na Fi	S,M	56			2,8	LC50	-			Sparks 1983	High control mortality (25%), no hardness, no acute test
Musculium transversum												
										• •	n	
Amphipod	Na FI	SR,M		6.7	11.7	8,9	Reproduction	114	25.8	2,9	Soucek and Dickinson 2010	
Hyajella azteca												

# Attachment 1 – Exhibit Q

Acute toxicity used in manganese Standards Derivation

### Acute Toxicity Data Used in Manganese Standards Derivation

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\* Results marked with strikethrough are considered invalid and have been excluded from standards derivation. Reasons for exclusion is highlighted in bold within Notes column.

\*\* GMAVs are not listed because values must be hardness-normalized. See associated derivation worksheet.

\*\*\* Soft water used in Reimer sludy was prepared by diluting well water (100 hardness) with deionized water, poor control survival or extremely low results occurred in all but one test (acule rainbow trou) study). (acute rainbow trout study). Soft-water data is being excluded from analysis.

		Test	Duration	LC50	Hardness		
Species	Chemical	Type	(hours)	(mg/L)	(mg/L)	Reference	Notes
Rainbow trout	MnSO <sub>4</sub>	F,M	96	3.17	27.6	Davies et al. 1998b	Fry
Oncorhynchus mykiss	MnSO4	F,M	96	16.20	147.8	Davies et al. 1998b	Fry
	MnCl <sub>2</sub>	s,u	96 96	2.10 20.70	47.6 100	Reimer 1999 Reimer 1999	Hardness was measured, Mn was not. Nominal hardness was 25 mg/L but was measured as 47.6 mg/L. Use Davies data
	MnCl₂ MnCl₂	S,U S,M	96	42.70	259	Reimer 1999 Reimer 1999	Mn and hardness were unmeasured, use Davies data Hardness and Mn measured on Day 0, nominal Mn was 19.1 and measured was 12,7 mg/L, not measured on day 4
	MnSO <sub>4</sub>	F,M	96	4.83	34	Davies and Brinkman 1994	42 mm fish, ELS/fingerling stage
			•••		•		
Brown trout	MnSO4	F,M	96	3.77	38	Davies and Brinkman 1994	Juveniles were tested
Salmo trutta	MnSO <sub>4</sub>	F,M	96	49,90	454	Davles and Brinkman 1995	Includes corrections to 1994 study, Table 22 hardnesses were wrong
Brook trout	MnSO4	F,M	96	5.12	31.3	Davies et al. 1998b	ELS, 37 mm organisms
Sa/velinus fontinalis	MnSO <sub>4</sub>	F,M	96	27.50	148.1	Davies et al. 1998b	ELS, 37 mm organisms
	MnSO4	F,M	96	269.00	5	Gonzalez et al. 1990	Yearlings were used, size unkown, results a factor higher than others
	MnSO <sub>4</sub>	F,M	96	291:00	25	Gonzalez et al. 1990	Yearlings were used, size unkown, results a factor higher than others
	MnSO <sub>4</sub>	F,M	96	3-19-00	100	Gonzalez et al. 1990	Yearlings were used, size unkown, results a factor higher than others
	MnSO <sub>4</sub>	F,M	96	560.00	250	Gonzalez et al. 1990	Yearlings were used, size unkown, results a factor higher than others
	UNK	UNK	UNK	73,30	28	ENSR 1996a	Used in Colorado standard, ENSR won't give us the report, 74 mm organisms used
	UNK	UNK	UNK	3.61	48	ENSR 1994	Unpublished in-house data used in Colorado standard, can't get the data for review, 36 mm organisms used
Coho salmon	MnCl <sub>2</sub>	S,M	96	2.40	25.2	Reimer 1999	Mn measured only on day 0, soft-water results not trustworthy
O. kisutch	MnCl <sub>2</sub>	S,M	96	13.10	100	Reimer 1999	Mn measured on Day 0 and 4, hardness was not measured
	MnClz	S,M	96	47,40	250	Reimer 1999	Mn measured only on day 0, data not needed for slope derivation (other valid Salmonid data exists). Hardness measured
Amphipod	MnCl <sub>2</sub>	S.M	96	3.00	26	Lasier et al. 2000	Chloride = 9 ma/L.
Hyalella azleca	MnClz	S.M	96	8.56	80	Lasier et al. 2000	Chloride = 2 mg/L, geomean of three replicates
rijelene uzloču	MnCl <sub>2</sub>	S.M	96	13.70	164	Lasier et al. 2000	Chloride = $2 \text{ mg/L}$
	MnClz	S,M	96	3.60	25	Reimer 1999	Hardness not measured, soft-water data not trustworthy due to control issues in other tests
	MnCl <sub>2</sub>	S,M	96	22.20	100	Reimer 1999	Hardness not measured, not used for slope derivation because other valid data exists
	MnCl <sub>2</sub>	S.M	96	31.00	269	Reimer 1999	Mn and hardness measured
	UNK	UNK	UNK	6,62	96	ENSR 1996b	Unpublished in-house data used in Colorado standard, can't get the data for review
	UNK	UNK	UNK	40.17	94	ENSR 1996b	Used in Colorado standard, unpublished, can't get the data for review
	44% MnSO /56% MnCl	S,M	96	11.00	112	Soucek and Dickinson 2010	Dilution water hardness = 112 mg/L, hardness increases with higher Mn concentrations
Crangonyx pseudogracilis	44 % MinSO #30 %MinCiz	S.U	96	694.00	50	Martin and Holdich 1986	Results seem artificially high, factor higher than other genera
Crangonyx pseudograciiis	WING IZ	3,0	30	004:00	20	Martin and Holdien 1900	Results seen annoinly nigh, ractor nigher than other general
Water flea	MnCl <sub>2</sub>	S,M	48	5,70	26	Lasier et al, 2000	Chloride = 9 mg/L
Ceriodaphnia dubia	MnClz	S,M	48	14,50	92	Lasier et al, 2000	Chloride = 2 mg/L
	MnCl <sub>2</sub>	S,M	48	14.50	184	Lasier et al, 2000	Chloride = 2 mg/L
	MnCl <sub>2</sub>	R,M	48	9,44	25	ENSR 1992b	total manganese
	MnCl <sub>2</sub>	R,M	48	11.20	50	ENSR 1992b	total manganese
	MnCl <sub>z</sub>	R,M	48	21.20	100	ENSR 1992b	total manganese
	MnCl <sub>2</sub>	R,M	48	27.30	200	ENSR 1992b	total manganese
	MnCl <sub>2</sub>	R.M	48	8.76	26	ENSR 1992b	acid-soluble result (nitric acid added), use total
	MnClz	R,M	48	12.61	50	ENSR 1992b	acid-soluble result (nitric acid added), use total
	MnClz	R,M	48	20.60	100	ENSR 1992b	acid-soluble result (nitric acid added), use total
	MnCl <sub>2</sub>	R.M	48	25,48	200	ENSR 1992b	acid-soluble result (nitric acid added), use total
	UNK	UNK	UNK	45,64	48	ENSR 1990	Unpublished in-house data used in Colorado standard, can't get the data for review
	UNK	UNK	UNK	23,45	92	ENSR 1990	Unpublished in-house data used in Colorado standard, can't get the data for review
	UNK	UNK	UNK	28,85	176	ENSR 1990	Unpublished in-house data used in Colorado standard, can't get the data for review
	UNK	UNK	UNK	<del>~45.0</del>	396	ENSR 1990	Unpublished in-house data used in Colorado standard, can't get the data for review
Water flea	MnCl <sub>2</sub>	S,M	48	0.80	26.3	Reimer 1999	Well water diluted with deionized water, chronic study had excess control mortality in soft water
Vvater tiea Daphnia magna	MnCl <sub>2</sub>	S.M	48	28,70	100	Reimer 1999	Mn measured, hardness was nominal
vapinka magna	14111012	3,141	40	20.10	100	(Control 1220	

	MnCl	S.M	48	76,30	267	Reimer 1999	Mn and hardness measured
	MnCl <sub>2</sub>	S,M	48	9,80	45.3	Blesinger and Christensen 1972	Lake Superior water used
	MnCl <sub>2</sub>	S,M	48	22.50	UNK	Baird et al. 1991	Reported as ionic, hard water used but concentration unknown
	MnSO4	S,M	48	10.40	UNK	Kimball 1978	Hardness unknown
		-,		10.70	0		
Midge	MnCl <sub>2</sub>	S,M	96	6.80	27.2	Reimer 1999	Soft-water results deemed unacceptable due to control problems in other tests
Chironomus tentans	MnCl <sub>z</sub>	S,M	96	42,20	100	Reimer 1999	Mn measured on day 0, hardness not measured
	MnGl <sub>2</sub>	S,M	96	94,30	272	Reimer 1999	Mn and hardness measured
	UNK	UNK	UNK	327.80	96	ENSR 1996c	Unpublished in-house data used in Colorado standard, can't get the data for review
	••						
Fathead minnow	MnCl <sub>2</sub>	SR,M	96	3,54	26	ENSR 1992a	Used in slope derivation by Colorado
Pimephales promelas	MnCi <sub>2</sub>	SR,M	96	6.23	50	ENSR 1992a	Used in slope derivation by Colorado
	MnCl <sub>2</sub>	SR,M	96	9,35	100	ENSR 1992a	Used in slope derivation by Colorado
	MnCl <sub>2</sub>	SR,M	96	15.83	200	ENSR 1992a	Used in slope derivation by Colorado
	MnCl <sub>2</sub>	SR.M	96	10.30	48	ENSR 1990	Used in slope derivation by Colorado
	MnCl <sub>2</sub>	SR,M	96	17.28	92	ENSR 1990	Used in slope derivation by Colorado
	MnCl <sub>2</sub>	SR,M	96	27.44	176	ENSR 1990	Used in slope derivation by Colorado
	MnCl <sub>z</sub>	SR M	96	>45 <del>.</del> 0	396	ENSR 1990	> data not suitable for slope
	MnCl <sub>2</sub>	F,M	96	8,56	28	ENSR 1996e	Used in slope derivation by Colorado
	MnSO <sub>4</sub>	F,M	96	33:60	UNK	Kimball 1978	Animals were fed, hardness not measured
Paper pond shell	MnSO₄	S,R	216	36,20	80	Wade et al. 1989	USEPA tentatively approved this study, tolerant species, fed daily
Anodonta imbecillus							
							/······
Rotifer	MnClz	s,u	24	38.70	36.2	Couillard et al. 1989	Native, shows tolerance from different phylum
Brachionus calyciflorus							
Tubificid worm	MnSO <sub>4</sub>	s.u	96	26.80	12	Rathore and Khangarot 2003	
Tubifex tubifex	MnSO4	5,U	96	42.70	45	Rathore and Khangarot 2003	
Tublex (ublex	MnSO <sub>4</sub>	3,U S,U	96	85,90	173	Rathore and Khangarot 2003	
	MnSO <sub>4</sub>	3,0 S,U	96 96	464,75	305	Rathore and Khangarot 2003	
	-					•	
	MnSO <sub>4</sub>	S,U	96	171.61	245	Khangarot 1991	
Western toad	MnSO₄	SR,M	96	42.30	52.6	Davies et al. 1998a	Tadpoles were tested, native genera, LC50 unable to be calculated in flowthrough tests
Bufo boreus	UNK	UNK	UNK	339.80	95	ENSR 1996d	Unpublished in-house data used in Colorado standard, can't get the data for review
	•••••						
Isopod	MnCl <sub>2</sub>	s,u	96	333.00	50	Martin and Holdich 1986	Results seem artificially high, factor higher than other genera, Genus is now Caecidolea
Asellus aquaticus							
Nematode	MnCl <sub>2</sub>	S,M	24	5379-90	0.1	Tatara et al. 1997	Hardness not reported, test performed in K-agar
Caenorhabdilis elegans							
Longfin dace	MnSO <sub>4</sub>	SR,M	96	130,00	224	Lewis 1978	Native genus, juveniles tested
Agosia chrysogasler							
Martha en en carto Cab	MnSO4	S,U	96	130.47	316	Beleau and Barlosz 1982	Poor confidence limits, older organisms (juveniles)
Northern squawlish							
Ptychocheilus oregonensis	MnSO <sub>4</sub>	s,u	96	189,48	347	Seleau and Barlosz 1982	post-larvai
Snail	MnCl	s.u	96	147.12	162	Harding ESE, Inc. 2001	Unpublished data from Michigan
Shall Physa integra	10111012	3,0	90	141.12	102	Herong Coc, 116, 2001	onpublished data nontrindingan
rnysanneyia							
Washboard mussel	44% MnSO /56% MnCl <sub>2</sub>	S,M	96	31.50	91	GLEC 2010	
Megalonaias nervosa		-,	20		2,		
magaronana nervoa							
Fatmucket	44% MnSOJ/56%MnCl	S.M	96	43.30	91	GLEC 2010	
Lampsilis siliquoidea		-,					

# Attachment 1 – Exhibit R

Chronic toxicity data used in manganese Standard Derivation

#### Chronic Toxicity Data Used in Manganese Standards Derivation

Results marked with strikethrough are considered invalid and have been excluded from standards derivation. Reasons for exclusion is highlighted in bold within Notes column.
 Soft water used in Reimer study was prepared by diluting well water (100 hardness) with deionized water, poor control survival or extremely low results occurred in all but one test (acute rainbow trout study). Soft-water data is being excluded from analysis for these reasons.

		Test	Duration		Hardness	NOEC	LOEC	MATC or other endpoint	LC50 at			
Species	Chemical	Type	(days)	Endpoint	(mg/L)	(mg/L)	(mg/L)	(mg/L)	Hardness -	ACR	Reference	Notgs
Rainbow trout	MASO4	F,M	65	LW	29,2	0.76	1.47	1.06	3.17	3.00	Davies et al. 1998b	ELS, fry had very similar results
Oncorhynchus mykiss	MnSO <sub>4</sub>	F,M	65	L,W	t51,2	3,39	5.48	4.69	16.2	3,46	Davies et al, 1998b	ELS, LC50s are from fry, no ELS acute tests conducted
	MnSO <sub>4</sub>	F.M	31	L,W	27.6	0.74	4.42	4,03	3.17	3.00	Davies et al. 1998b	Fry, use longer ELS tests. LC50 is from 1/y test, no acute ELS tests
	MnSO,	F.M	44	L,W	147,6	3,34	6.46	4.55	16.2	3,40	Davies et al, 1998b	Fry, use longer ELS tests. LC50 is from fry test, no acute ELS tests
	MnSO <sub>4</sub>	F,M	65	IC25 L,W	29.2	-	-	1.01	3.17		Davies et al. 1998b	ELS, MATC or EC20 preferred over IC25
	MnSO <sub>2</sub>	F,M	65	IC25 L,W	151.2	-	-	4.80	16.2	<del>3.38</del>	Davies et al. 1998b	ELS, MATC or EC20 preferred over IC25
	MnSO₄	F.M	65	EC20 L.W	29.2	-	-	4,40	3,17	2:27	Davies et al. 1998b	ELS, MATC is preferred method
	MnSO <sub>4</sub>	F,M	65	EC 10 L,W	29.2	-	-	1.30	3,17		Davies et al. 1998b	ELS, MATC is preferred method
	MnSO <sub>4</sub>	F,M	65	EC20 L,W	151,2	-	-	4.26	16.2	3.80	Davies et al. 1998b	ELS, MATC is preferred method
	MnSO,	F,M	65	EC10 LW	151.2	-	-	3,48	16,2	4,66	Davies et al. 1998b	ELS, MATC is preferred method
	MnCl <sub>2</sub>	SR,M	28	LC50	104	•	-	2.91	-	•	Birge et al. 1978	ELS, NOEC/LOEC not reported, no corresponding acute, longer tests available for ACR development
	MnSO4	F,M	120	Survival	36.6	<del>1.0</del> 4	1.06	4.43	4,83	8.38	Davies and Brinkman 1994	Used growth instead (Davles 98), used diff N/LOEC because mort was 10% + acclimated tish were more tolerani (table 24)
	MnCl <sub>2</sub>	S,M	7	Rep EC50	25,7	-	-	14.60	-	•	Reimer 1999	Duration too short, 37.5-45.8% mortality in two initial replicates, third replicate was ok
	MnCl <sub>2</sub>	S.M	7	Rep EC 50	100	-	-	20.00	-	•	Reimer 1999	Duration too short
	MnCl <sub>2</sub>	S.M	7	Rep EC50	252	-	-	22.70		-	Reimer 1999	Duration too short
						Geor	nean Onci	orhynchus ACR	(valid dala);	3.22		
Brook trout	MnSO4	F,M	65	Length	3 t.2	0.55	0.85	0.68	5. t2	7.49	Davies et al. 1998b	ELS, control length=37.5mm, 0.85 mg/L treatment length=35.2mm, significant? Fry test used for ACR, no acute ELS test
Satvetinus tonlinatis	MnSO4	F,M	65	Weight	31.2	0,85	2,18	1.36	5.12	3,76	Davies et al. 1998b	ELS, acute fry last used for ACR, no acute lest on ELS
	MnSO4	F,M	65	L,W	155,5	3,53	7.53	5.t6	27.5	5,33	Davies et al. 1998b	ELS
	MnSO4	F.M	31	L,W	31.3	2.53	5.35	2.68	5.12	4,30	Davies et al. 1998b	Fry (60 days old at start of test), use ELS tests
	MnSO₄	F,M	44	w	148,1	1.71	3,59	2.48	27.5		Davies et al. 1998b	Fry (60 days old at start of test), use ELS tests, length was affected at higher concentrations
	MnSO <sub>4</sub>	F,M	44	L	t48.1	3,50	7.39	5-15	27.5	6-34	Davies et al. 1998b	Fry (60 days old at start of test), use ELS tests
	MnSO₄	F,M	65	IC25 weight	31.2	-	-	1.53	5.12	3-36	Davies el al, 1998b	ELS, MATC or EC20 preferred over IC25
	MnSO₄	F,M	31	IC25 weight	31,3	-	-	3.01	5.12	1.70	Davies et al. 1993b	Fry (60 days old at start of test), ELS test preferred, MATC or EC20 preferred
	MnSO₄	F,M	65	IC25 weight	155.5	-	-	4.23	27.5	6.60	Davies el al. 1998b	ELS, MATC or EC20 preferred over iC25
	MnSO <sub>4</sub>	F,M	44	IC25 weight	155.5	-	-	3,00	27.5	5,89	Davies et al. 1998b	Fry (60 days old at start of test), ELS test preferred, MATC or EC20 preferred
	MnSO4	F,M	65	EC20 weight	31.2	-	-	2.10	5,12		Davies et al. 1998b	ELS, MATC is preferred method
	MnSO4	F,M	65	EC t0 weight	31,2	-	-	1.70	5.12	3.04	Davies et al. 1996b	ELS, MATC is preferred method
	MnSO4	F,M	65	EC20 weight	155,5	-	-	3,79	27.5	7.44	Davies et al. 1993b	ELS, MATC is preferred method
	MnSO <sub>4</sub>	F,M	65	EC t0 weight	155,5	- ,	- Seomean :	2,83 Salvelinus ACR:	27.5 (valid data):	0.73 5.32	Davies et al. 1993b	ELS, MATC is preferred method
						-			11010.0000	0,01		
Brown trout	MnCh	F.M	62	ELS survival	30.9	3,94	7,38	5.39	-	-	Stubblefield et al. 1997	No corresponding acute test, based on survival, no effect was found on growth
Salmo trutte	MnCl <sub>2</sub>	F,M	62	ELS growth	151.9	2.76	4.41	3,50	•	•	Stubblefield et al. 1997	No corresponding acute test, LOEC for survival was 8.8, NOEC was 4.4 mg/L
	MnCl <sub>2</sub>	F,M	62	ELS growth	449,6	4.66	8.68	6.28	-	-	Stubblefield et al. 1997	No corresponding acute test, LOEC for survival was 15.2, NOEC was 8.7 mg/L
	MnCl <sub>2</sub>	F,M	62	IC25 survival	30.9	-	-	4.67	•	-	Stubblefield et al. 1997	No corresponding acute test
	MnCl	F,M	62	IC25 growth	151,8	-	-	6,59	-	-	Stubblefield et al. 1997	No corresponding acute test
	MnCL	F,M	52	IC25 growth	449.6	-	~ ~~	8.66	3.77	-	Stubblefield et al. 1997 Davies and Brinkman 1994	No corresponding acute test Survival was only endpoint measured
	MnSO₄	F,M	t20	Survival	37.5	2.03	3.59 Geolán	2.70 an Salmo ACR:		1.40 1.40	Davies and Brinkman 1994	Survival was only endpoint measured
							GEOMO		i train norol.	1,40		
Fathead minnow	MnSO <sub>4</sub>	F,M	36	Lengtivweight	?	4,27	2.48	<del>1.78</del>	-	-	Kimbali 1978	Hardness not measured, acute lest results was 33,6 mg/L but organisms were fed
Pimepheles prometas				• •								•
Amphipod	MaCh	SR.M	28	LC50	alk = 85	-	4,62	-	-	-	Norwood et al. 2007	No hardness, chlotide was 23.9 mg/L, temp = 25 C, renewed weekly, extremely high variability, duration too short
Hysiella aztoca	MnC(2	SR,M	28	LC25	alk = 85	-	0.53	-		-	Norwood et al. 2007	No hardness, chloride was 23.9 mg/L, temp = 25 C, renewed weekly, extremely high variability, duration too short
	MnCl <sub>2</sub>	SR,M	28	IC25% growth	alk = 85	-	0,12	-	-	-	Norwood et al. 2007	No hardness, chloride was 23.9 mg/L, temp = 25 C, renewed weekly, extremely high variability, duration too abort
	MnSO <sub>4</sub>	S,U	7	LC50	124	-	-	5.05	-	-	Borgmann et al. 2005	Duration too short, control survival issues, not aerated
	MnSO₄	S M	7	LC 50	124	-	-	2,73	-	-	Borgmann et al. 2005	Duration too short, control survival issues, not aerated
	MnSO4	S,U	7	LC 50	18	-	-	<b>≈1.00</b>	-	-	Borgmann et al. 2005	Duration too short, control survival issues, not aerated
	MnSO4Cl2	SR,M	42	Survival	110	0.71	4,40	1,00	11.01		Soucek and Dickinson 2010	44% MnSO4/56%MnCl2, dissolved oxygen problems
	MnSO4/Cl2	SR,M	42	Survival	115	1.4	2.9	2.01	11.04	5.48	Soucek and Dickinson 2010	44% MnSO4/56%MnC12
Water ties	MnCb	S.M	21	Reproduction	25				-		Reimer 1999	Excess control deaths due to soft water, no results reported by author
Daphnia magna	MnCL	S.M	21	Reproduction	100	3.60	6.90	4.98	28.7	5.76	Reimer 1999	Hardness was unmeasured
South Living Hand Built	MnCip	S.M	21	IC25% rep	100	-	-	5.40	28,7	6.34	Reimer 1999	Mn measured, Hardness was nominal, MATC is preferred method
	MnCl	S.M	21	Reproduction	269	7.30	13.40	9,89	76.3	7,71	Reimer 1999	Mn and hardness meaured
	MnCh	S.M	21	1C25% rep	269	-	-	0.40	76.3	8.42	Reimer 1999	Mn and hardness measured, MATC is preferred method
	MnCL	S.M	21	IC 16% rep	45,3	-	-	4.10	9,8	2,39	Biesinger and Christensen 1972	16% rep impairment reflects value below which rep variability could not be delected from control variability

	MnSO <sub>4</sub>	S,M	26	Reproduction	UNK	< t. t0	<1.100	<del>~1,100</del>	-	-	Kimball 1978	Bardness not measured, hard well water used, treated for iron
							<u>GeoMean</u>	Daphnia ACRs	(valid date);	4.74		
Water flea	MnCl <sub>2</sub>	S,M	7	IC 50% rep	26			3-90	5.7	1,46	Lasier et al. 2000	MATC method is preferred over IC50
Ceriodaphnia dubla	MnCl <sub>2</sub>	S.M	7	IC50% rep	92	-		0.00	14.5	4.61	Lasier et al. 2000	MATC method is preferred over IC50
	MnCh	S.M	7	C50% rep	184	-		11,40	t4,5	4,27	Lasier et al. 2000	MATC method is preferred over IC50
	MnCl <sub>2</sub>	S.M	7	Reproduction	26	2.40	4.90	3.43	5.7	1.66	Lasier et al. 2000	NOEC/LOEC from Michigan database
	MnCL	S.M	7	Reproduction	92	4,90	9.60	6.93	t4,5	2.09	Lasier et al. 2000	NDEC/LOEC from Michigan database
	MnCh	S.M	7	Reproduction	184	10.00	19.80	14.07	14.5	1.03	Lasier et al. 2000	NOEC/LOEC from Michigan database
	MnCl <sub>2</sub>	S.M	7	IC25% (ep	26			3.10	5.7	<del>1,8</del> 4	Lasier et al. 2000	MATC method is preferred over IC25
	MnCi	S.M	7	IC25% rep	92			5,20	t4.5	2,70	Lasier et al, 2000	MATC method is preferred over IC25
	MnCl <sub>2</sub>	SR,M	7	Reproduction	46	2.29	3.59	2.81	11.2	<del>3.03</del>	ENSR 1969	No acute test done with chronic test, acute test from 1992 entered for comparison, use Lasier data instead
	MnCl <sub>2</sub>	SR,M	6	Reproduction	156	2,80	4.80	3,77			ENSR 1989	Dilution water taken from pond in Alaska, no acute tests performed with this water, ACR not practical
	MnCl <sub>2</sub>	SR,M	6-7	Reproduction	25	2.04	4.41	3.00	9.44	3.15	ENSR 1992c	Acute data used from ENSR June 1992, same lab, same researcher, same week
	MnCh	\$R,M	6-7	Reproduction	50	2.06	4.55	3,06	11.2	3.66	ENSR 1992c	Acute data used from ENSR June 1992, same lab, same researcher, same week
	MnCl <sub>2</sub>	SR,M	6-7	Reproduction	too	4.80	9,30	6,68	21.2	3. t7	ENSR 1992c	Acute data used from ENSR June 1992, same lab, same researcher, same week
	MnCl <sub>2</sub>	SR,M	6-7	Reproduction	200	7.82	20.40	12.63	27.3	2, t6	ENSR 1992c	Acute data used from ENSR June 1992, same lab, same researcher, same week
	MnCl <sub>2</sub>	SR,M	6-7	IC25% rep	25	-	-	3.37	9.44	2.80	ENSR 1992c	MATC method is preferred over IC25
	MnCl <sub>2</sub>	SR,M	6-7	IC25% rep	50	-		<del>5.1</del> 6	11.2	2.17	ENSR 1992c	MATC method to preferred over IC25
	MnCl <sub>2</sub>	SR,M	6-7	IC25% rep	100	-	-	6.60	21.2	3.08	ENSR 1992c	MATC method is preferred over IC25
	MnCl <sub>2</sub>	SR,M	6-7	IC25% rep	200	-	-	8.01	27.3	3.06	ENSR 1992c	MATC method is preferred over IC25
						Geo	Mean Ceric	odaphnia ACRs	(valid data);	2.24		
Protozoan Spiroslomum ambiguum	MnCl <sub>z</sub>	s.u	48	EC\$0	150		-	499.00		-	Nalecz-Jawecki and Sawicki 1998	Unicallular organism, lest is considered chronic (1985 guidelines; IV.E.4), no corresponding acute test for ACR
Goldfish <i>Carassius aura</i> tus	MnCl <sub>2</sub>	SR,M	7	LC50	195	-	-	8.22		•	Birge 1978	7 day ELS test (unfed?), not a sullable chronic test
Narrow-mouthed toad Gastrophryne carolinens/s	MnCl <sub>2</sub>	S,M	7	LC50	195	*	-	4 <del>,42</del>	-	-	Birge 1978	7 day ELS test (unfed?), not a suitable chronic test, non-native species to iL
Paper pond shell Anodonta imbecillus	MnSO₄	F,M	90	Shell Growth	80	-	•	20-30	-	•	Wade et al. 1989	Insufficient methods and results reported

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Attachment 1 – Exhibit S Ambient Water Quality Monitoring Network (AWQMN)

	and the state, adding	(4/30/97)	ing normo		
Station Code	Stream Name	Criticai Haroness	County	Latitude Longitude	Description
	OHIO RIVER BASIN			-	,
A 06	Ohio River	88	Pulaski	37 12 11 89 02 30	North End of Dam 53. East of Olmsted
AD 02	Cache River	125	Johnson	37 20 11 88 55 26	Co. Rd. 8r., 1.0 miles NE of Beiknap
AK 02	Lusk Creek	54	Pope	37 28 20 88 32 50	Co. Rd. Br., 2.8 miles SE of Edayville
AT 06	Saline River	233	Gallatin	37 38 53 88 14 30	Peabody Br., 1.3 miles E of Gibsonia
入TF 04	N. Fork Saline River	220	Saline	37 53 18 88 23 06	Rt. 45 Br., 5.1 miles NE of Eldorado
ATG 03	Middle Fork Saline Riv	579	Saline	37 42 28 88 29 31	Co. Rd Br., 2.7 miles SE of Harnsburg
ATGC D1	Bankston River	998	Saline	37 46 05 88 32 25	Rt. 34 Br., 2.5 miles N of Harrisburg
ATH 02	S. Fork Saline River	219	Williamson		5 Co. Rd. Br., 3.4 miles 5 Crab Orchard
ATH 05	S. Fork Saline River	317	Saline	37 38 16 88 40 40	Rt. 45 Br., 3.8 miles SW of CARner Mills
ATHG 01	Sugar Creex	:400	'Williamson	37 39 19 88 45 48	Co. Rd. Br., 5.1 miles NE of Creal Springs
	WABASH RIVER BASIN				
B 06	Wabash River	223	Crawford	39 06 37 87 39 18	Indiana Rt. 154 Br. at Hudsonville
B 07	Wabasn R:ver	190	White	38 0 <b>7</b> 55 87 56 25	Rt. 460 Br.; near New Harmony, IN
BC 02	Bonpas Creek	150	Edwards- Wabasn	38 23 11 87 58 32	Rt. 15 Br., 0.6 miles NE of Browns
8E 01	Embarras River	* 189	Lawrence	38 39 54 87 37 35	Co. Rd. Br., 1.3 miles E of Billet
BE 07	Embarras River	254	Jasper	38 56 10 88 01 10	Co. Rd. Br., at N edge of St. Marie
BE 09	Embarras River	250	Cumberland	39 20 43 88 10 15	Rvan Bridge, County Rd. 9 miles S of Charleston

1 of 13 indicates no flow data collected

note: critical hardness expressed as CaCC3 (mg/L)

		(4/30/97) Critical		Latitude	~,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
Station Code	Stream Name	Hardness	County		Description
3E 14	Embarras River	280	Dougias	39 47 59 88 10 13	Co. Rd. Br., west edge of Camargo
8EF 05	N Fork Embarras Riv	193	Crawiord	39 00 01 87 56 52	Rt. 33 Br., 2.8 miles W of Oblong
BF 01	Sugar Creek	294	Crawford	39 00 16 87 35 50	Twp. Rd. Br., NE of Palestine near ICRR
BM 02	Sugar Creek	260	Edgar	39 29 53 87 33 11	Co. Rd. Br., 1 mile from Indiana line
EN 01	Brouiletts Creek	250	Vermilion	39 40 53 87 31 16	Indiana Rt. 71 Br., 0.5 miles N of Blanford
80 07	Little Vermillion Riv	244	Vermillion	38 07 55 87 56 25	Co, Rd. Br., 4 miles SE of Georgetown
BP 01	Vermilion River	278	Vermillion	40 05 53 87 35 37	Grape Creek Rd., 3.5 miles SE of Danville
BPG 09	N. Fork Vermillion Riv	231	Vermillion	40 16 13 87 38 34	2 miles W of Bismark on Co. Rd.
BPJ 03	Salt Fork Vermilion Riv	239	Vermilion	40 04 56 87 46 53	Coi, Rd, Br., 3 miles S of Oakwood
BPJ 07	Salt Fork Vermilion Riv	277	Champaign	0 07 59 88 06 15	Co. Rd. Br., 2.5 miles N of St. Joseph
BPJC 03	Saline Br	172	Champaign	40 08 12 87 07 55	Co. Rd. Br., 1 mile N of Mayview
8PK 07	Middle Fork Vermillion Riv	334	Vermilion	40 08 12 87 44 45	Kickapoo St. Park Br., upstream of I-74 Br.
C 09	Little Wabash River	135	Edwaros	38 31 08 88 07 55	W Salem-Mt. Erie Rd Br SW of Blood
C 19	Little Wabash River	130	Clay	38 46 23 88 29 50	Co. Rd. Br., NE of Louiville
C 21	Little Wabash River	143	Effingham	39 06 13 88 35 33	US 40 Br., 2.2 miles SW of Effingham
C 22	Little Wabash River	141	Clay	38 38 05 88 17 50	Co. Rd. 8r., 5 miles SE of Clay City
C 23	Little Wabash River	136	White	38 05 31 88 09 20	Main St. Br. in Carmi

2 of 13 ' indicates no flow data collected

note; critical hardness expressed as CaCO3 (mg/L)

		(4/30/97)	ing tions		all of the second s
Station Code	Stream Name	Critical Hardness	County	Latitude	Description
	Gueannaane	i la chess	Soony	Longhuec	Cesenplion
CA 03	Skillet Fork	113	White	38 09 12 88 09 55	Winters Br. Co. Rd., 4.0 miles N of Carmi
CA 05	Skillet Fork	137	Wayne	38 21 25 88 35 00	Rt. 15 Br., 1.0 miles N of Wayne City
CA 06	Skillet Fork	160	Marion	38 31 10 88 43 39	Co. Rd. Br., 7.5 miles SE of luka
CD 01	Elm Creek	106	Wayne	38 26 28 88 15 33	Price Br. Co. Rd., 6 miles NE of Fairfield
	ILLINOIS RIVER BASIN				
D 01	Illinois Ríver	245	Calhoun / Greene	39 09 37 90 36 55	Rt. 100 Br. at Hardin
O 05	Illinois River	221	Peoria / Tazweil	40 34 23 89 39 17	Rt. 9 Br. at Pekin
D 09	Illinais River	• 251	Marsnall	41 01 30 89 25 02	Rt. 17 Br. at Lacon
D 16	Illinois River	214	Putman	41 15 2 <del>0</del> 89 20 45	Rt., 26 Br. at Hennepin
D 23	Illinois River	220	LaSalle	41 19 40 88 45 10	Marseilles downstream from Nabisco Blvd.
D 30	Illinois River	216	Peoria	40 43 30 89 32 58	Peoria PWS intake
D 31	Illinois River	242	Mason	40 16 40 90 04 53	Illinois Power Intake near Havana
D 32	Illinois River	252	Scott	39 42 10 90 38 40	Wagaxh RR 8r., 0.5 miles E of Valley City
DA 04	Масоцріл Стеек	169	Macoupin	39 12 05 89 58 41	Macoupin Station; Plainview Rd, Br.
DA 06	Macoupin Creek	227	Greene	39 14 03 90 23 40	Rt. 267 Br., 3,5 miles NW of Kane
DB 01	Apple Creek	- 233	Greene	39 22 11 90 32 46	Co. Rd. Br., 6 miles N of Eldred
DD 04	Mauvalse Creek	194	Scott	39 43 53 90 24 26	

3 of 13 indicates no flow data collected note: critical hardness expressed as CaCO3 (mg/L)

	Ambient redict Quanty	(4/30/97)					
Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description		
DE 01	МсКее Стеек	249	Pike	39 49 04 90 39 09	Rt. 104 Br., at Chambersburg		
DF 04	Ілбіал Слеек	272	Cass	39 52 40 90 22 38	Co. Rd. Br., SW of Arenzville		
DG 01	LaMoine River	154	Brown / Schuyler	40 01 31 90 37 55	US Rt. 24 Br. at Ripley		
DG 04	LaMoine River	188	McDonougn	40 19 45 90 53 55	Rt. 61 Br. at Colmar		
DH 01	Sugar Creek	245	Schuyler	40 05 49 90 24 16	Rt. 100 Br., 2 miles NE of Frederick		
DJ 02	Spoon River	370	Кпох	40 54 33 90 05 12	US 150 Br., 3.6 miles SW of Williamfield		
DJ 06	Spoon River	460	Stark	41 03 47 89 47 43	Rt. 17 Br., 2 miles W of Wyoming		
80 LO	Spoon River	306	Fulton	40 29 24 90 20 26	Rt. 95 ; 0.4 miles NE of Seville		
DJ 09	Spoon River	335	Fulton	40 42 51 90 16 00	Br. at noπh edge of London Mills		
DJB 19	Big Creek	455	Fulton	40 27 32 90 08 00	Co. Rd. Br., 2.0 miles SW of Bryant		
DJ82 01	Sług Run	1065	Fuiton	40 28 24 90 08 37	Private Rd., 2.5 miles NW of Bryant		
DJL 01	Indian Creek	244	Stark	41 01 06 89 50 07	Co. Rd. Br., 3 miles W of Wyoming		
DK 12	Mackinaw River	279	Tazeweil	40 26 51 89 41 28	Co. Rd. Br., 4 miles SSW of S. Pekin		
DK 13	Mackinaw River	282	Tazeweil	40 35 12 89 16 42	Co. Rd. Br., 4 miles SE of Deer Creek		
DL 01	Кіскароо Стеек	- 398	Реопа	40 39 18 89 39 19	US 24 Br., No of Banonville		
DQ 03	Від Вигеаи Сгеек	167	Bureau	41 21 55 89 29 55	Rt. 6 Br. near Princeton		
DQD 01	West Bureau Creek	267	Bureau	41 21 57 89 34 07	US 6-34 Br. at E edge of Wyanet		

4 of 13 \* indicates no flow data collected

note: critical hardness expressed as CaCO3 (mg/L)

	Anthient water Quanty	(4/30/97)					
Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description		
DR 01	Little Vermillion River	• 340	LaSalle	41 20 00 89 34 07	US 6 Br. in LaSalle		
DS 05	Vermillion River	312	Livingston	40 49 42 88 34 29	Co. Rd. Br., 0.5 miles E of McDowell		
DS 07	Vermillion River	282	LaSalle	41 17 10 88 55 51	Co. Rd. Br., 3 miles NE of Leonore		
DV 04	Mazon River	265	Grunay	41 17 10 88 21 35	Rt. 113 Br. 4 miles W of Coal City		
DW 01	Aux Sable Creek	• 335	Grundy	41 25 02 88 20 51	US 6 Br., 6 miles NE of Morris		
DZZP 03	Farm Creek	• 344	Tazeweil	40 40 16 89 34 48	Camp St. Br., NE of Peoria, 400 ft. from Br.		
	FOX RIVER BASIN						
DT 06	Fox River	299	McHenry	42 09 59 88 17 25	Rt. 62 Algonquin Rd. Br.		
DT 09	Fox River	249	Kane	41 59 40 88 17 40	State St. Br. in S. Elgin		
DT 22	Fox River	• 300	McHenry	42 16 44 88 13 31	Rt 176 Br., 5 miles ENE of Crystal Lake		
DT 35	Fox River	252	Lake	42 28 45 88 10 42			
OT 38	Fox River	275	Kane	41 43 46 88 20 19	Mill St. Br, in Montgomery		
DT 46	Fox River	241	LaSalle	41 23 14 88 47 21	Co, Hwy, 18 at Dayton		
DTB 01	Somonauk Creek	* 311	LaSalle	41 32 37 88 41 12	E-W Twp. Rd. Br. 1 mille N of Shendan		
DTD 02	Blackberry Creek	364	Kendall	41 40 18 88 26 29	US Rt. 47 Br., north of Yorkville		
DTG 02	Poplar Creek	329	Cook	42 01 35 88 15 20	US Rt. 20 Br., Villa St. in Elgin		
DTK 04	Nippersink Creek	335	McHenry	42 26 37 88 14 51			

5 of 13 \* indicates no flow data collected

note: critical hardness expressed as CaCOC (mg/L)

	Ambient Water Quality Monitoring Network (AWQMN) (4/30/97)						
Station Code	Stream Name	(4/30/97) Critical Hardness	County	Latitude Longitude	Description		
	SANGAMON RIVER BASIN						
E 05	Sangamon River	242	Macon	39 47 48 89 06 15	Lincoln Trail Br., 5 miles SE of Niantic		
E 06	Sangamon River	238	Macon	39 49 28 88 57 20	Decatur PWS intake, near dam		
E 09	Sangamon River	215	Macon	39 49 52 88 58 35	Rt. 48 Br. at Decatur		
E 16	Sangamon River	280	Christian / Sangamon	39 44 32 89 23 57	Co. Rd. Br., 4.5 miles S of Mechanicsburg		
E 24	Sangamon River	238	Menard	40 00 37 89 50 42	Rt. 123 Br., E of Petersburg		
E 25	Sangamon River	286	Menard / Mason	40 07 25 89 59 05	Rt. 97 Br. near Oakford		
E 26	Sangamon River	263	Sangamon	39 50 34 89 32 52	Old Rt. 36, W of Riverton		
E 28	Sangamon River	261	Piatt	40 04 08 88 38 07	Co. Rd. Br., 4.5 miles SW of Monticello		
E 29	Sangamon River	292	Champaign	40 18 40 88 19 20	Rt. 136 Br., 0.75 miles E of Fisher		
EI 02	Salt Creek	299	Mason	40 08 01 89 44 08	Rt. 29 Br., 4 miles N of Greenview		
EI 06	Salt Creek	254	DeWitt	4 006 54 89 02 57	Co. Rd. Br., 2 miles NE of Kenney		
EID 04	Sugar Creek	166	Logan	40 13 20 89 24 12	Twp. Rd., 2.6 miles SE of Hartsburg		
EIE 04	Kickapoo Creek	315	DeWitt	40 15 20 89 07 40	Co. Rd. Br., 0.75 miles N of Waynesville		
EIE 05	Kickapoo Creek	300	Logan	40 11 30 89 21 40	Co. Rd Br., 1.75 miles N of Lincoln		
EIG 01	Lake Fork	286	Logan	39 57 00 89 41 16	Rt. 54 Br., 2 miles NE of Comland		
EL 01	Spring Creek	197	Sangamon	39 49 16 89 41 16	Bruns Lane Br., NW edge of Springfield		

6 of 13 \* indicates no flow data collected

note: critical hardness expressed as CaCO3 (mg/L)

	(4/30/97)							
		Critical		Latitude				
Station Code	Stream Name	Hardness	County	Longitude	Description			
EO 01	South Fork	140	Sangamon	39 45 50 89 33 43	Rt. 29 Br., 1.5 miles NW of Rochester			
EO 02	South Fork	230	Christian	39 34 44 89 23 31	Rt 104 Br., 1 mile E of Kinkaid			
EOA 01	Sugar Creek	250	Sangamon	39 47 07 89 35 20	Rt., 29 Br., 1 mile SE of Springfield			
EOD 01	Clear Creek (Lake Sangchris)	210	Sangamon / Christian	39 39 05 89 29 07	New City Rd., Lake Sangchris Dam			
EOH 01	Flat Branch	180	Christian	39 <b>3</b> 3 14 89 15 12	Old Rt. 29 Br., 1 mile E of Taylorville			
	KANKAKEE RIVER BASIN							
F 01	Kankakee River	279	Will	41 20 48 88 11 11	Old Rt. 29 Br., 1 mile E of Wilmington			
F 02	Kankakee River	305	Kankakee	41 09 36 87 40 07	Hwy 1 Br., at Momence			
FL 02	Iroquois River	262	Kankakee	41 00 29 87 49 22	Co. Rd. Br., 5 miles W of Anne			
FL 04	Iroquois Ríver	312	lroquois	40 49 25 87 34 55	US 52 Br. at Iroquois			
FLI 02	Sugar Creek	277	Iroquois	40 37 50 87 43 25	Co. Rd. Br., 1 mile W of Millord			
	DES PLAINES RIVER / LAKE MI	CHIGAN BAS	SIN					
G 07	Des Plaines River	248	Lake	42 20 39 87 56 18	Rt. 120, Belvidere Rd. Br., E of Grayslake			
G 08	Des Plaines River	395	Lake	42 29 22 87 55 32	Russel Rd. Br., 1 mile downstream of Wisconsin			
G 11	Des Plaines River	246	Will	41 35 47 88 04 07	Division St. Br. at Lockport			
G 15	Des Plaines River	257	Cook	41 57 11 87 51 15	Irving Park Rd. Br. at Schiller Park			
G 22	Des Plaines River	286	Cook	42 04 55 87 53 25				

7 of 13 \* indicates no flow data collected

d note: critical hardness expressed as CaCO3 (mg/L)

	·····2·2·····	(4/30/97)		•	
Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description
G 23	Des Plaines River	205	Will	41 32 18 88 05 00	Rt. 53 (Ruby St. Br.) in Joliet
G39	Des Plaines River	275	Cook	41 49 20 88 09 58	Barry Point Rd. at Ríverside
G <b>B</b> 10	DuPage River	270	Will	41 41 24 88 09 58	Plainfield/Naperville Rd. Br.
G8 11	DuPage River	288	Will	41 31 20 88 11 35	Rt. 52 at Shorewood
GBK 05	West Branch DuPage River	372	DuPage	41 49 22 88 10 23	Rt. 56 Butterfield Rd Br. near Warrenville
GBK 09	West Branch DuPage River	204	DuPage	41 54 39 88 10 44	Rt. 64/St. Charles Rd. Br. N of W Chicago
GBL 10	East Branch DuPage River	218	DuPage	41 48 02 88 04 53	Rt. 34 Br. near Lisle
GG 02	Hickory Creek	191	Will	41 31 10 88 04 10	Washington St. Br. at Joliet
GI 01	Sanitary & Ship Canal	192	Will	41 38 27 88 03 36	135łh St. Br. at Romeoville
GI 02	Sanitary & Ship Canai	187	Will	41 34 11 88 04 42	Division St. Br. at Lockport
GL 09	Salt Creek	234	Cook	41 49 35 87 54 00	Wolf Road Br.
GLA 02	Addison Creek	286	Cook	41 52 48 87 52 07	Washington Blvd, Br. In Bellwood
H 01	Calumet-Sag Channei	• 218	Cook	41 41 45 87 56 11	Rt. 83 Br., 3 mile NE of Lemont
HB 42	Little Calumet R S.	343	Lake (W)	41 34 07 87 31 18	Hohman Ave. Br., N of Munster
HDB 04	Thom Creek	321	Cook	41 34 05 87 35 30	Thornton/Lansing Rd, Br, in Thornton
HCC 07	North Branch Chicago River	199	Cook	42 00 44 87 47 45	Touhy Ave. Br. in Niles
HCCC 02	Middle Fork North Brancn	234	Lake / Cook	42 09 10 87 49 07	Lake/Cook Co, Line Rd. Br, Chicago River

8 of 13 indicates no flow data collected

note: critical hardness expressed as CaCO3 (mg/L)

	Ambient Water Quality		Monitoring Network (AWQMN)				
Station Code	Siream Name	Ċ	Critical ardness	County	Latitude Longitude	Description	
	MISSISSIPPI RIVER SOUTH BA	SIN					
184	Mississippi River		226	Alexander	37 13 00 89 27 50	at Thebes, IL	
11 03	Marys River		773	Randolph	37 57 22 89 42 22	Co. Rd. Br., 0.3 miles E of Welge	
IX 04	Cache River	•	102	Alenxander / Pulaski	37 12 12 89 15 29	Co. Rd. Br., 0.7 miles E of Sandusky	
	MISSISSIPPI RIVER SOUTH CE	NTF	RAL BASIN	1			
J 05	Mississippi River	•	196	Jersey	38 57 07 90 22 12	near Elsah Rm. 214.6	
JMAC 02	Harding Ditch (Cahokia Canal #	•	311	St. Clair	38 35 42 90 05 18	Lake Drive at Frank Holten State Park	
JN 02	Cahokia Canal	-	313	Madison	38 40 01 90 03 56	Sand Prarie Ln. Br. SE of Horseshoe Lake	
JNA 01	Canteen Creek	-	344	Madison	38 39 58 90 03 56	Sand Prane Ln. Br. SE of Horseshoe Lake	
JQ 05	Cahokia Creex		130	Madison	38 49 28 89 58 29	Rt. 143 Br. NW of Edwardsville	
JR 02	Wood River	•	288	Madison	38 53 03 <sup>.</sup> 90 07 20	Rt, 3 Br. at Milton Rd. Junction in Alton	
	MISSISSIPPI RIVER NORTH CI	ENT	RAL BASI	N			
К 04	Mississippi River		167	Hancock	40 23 37 91 22 27	at Keokuk, Iowa	
KCA 01	Bay Creek		168	Pike	39 26 35 90 47 45	Twp. Rd. Br. at west edge of Nebo	
KI 02	Grady Boy Creek		157	Adams	40 08 34 91 20 14	Co. Rd. Br., 2.2 miles NE of Marcelline	
LD 02	Henderson River		222	Henderson	41 00 05 90 51 15	Rt. 94 Br., 1 mile S of Bald Bluff	
LF 01	Edwards River		251	Mercer	41 11 15 90 58 05	Rt. 17 Br., 2 miles NE of New Boston	

9 of 13 indicates no flow data collected note: critical hardness expressed as CaCO3 (mg/L)

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	Ambient Water Quality	/ Monitoring Network (AWQMN) (4/30/97)					
Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description		
	MISSISSIPPI RIVER NORTH BA	SIN					
M 04	Mississippi River	156	Whiteside	41 46 53 90 15 04	Rt. 136 Br. at Fulton		
MJ 01	Plum River	306	Carroll	42 05 50 90 07 38	US 52 Br. at E edge of Savanna		
MN 03	Apple River	345	Jo Daviess	42 19 07 90 15 18	US 20 Br., 2 miles W of Elisabeth		
MQ 01	Galena River	450	Jo Daviess	42 24 50 90 25 40	US 20 Br. at Galena		
	BIG MUDDY RIVER BASIN						
N 08	Big Muddy River	108	Jefferson	38 18 36 88 59 18	Rt. 15 Br., 3.0 miles W of Mt. Vernon		
N 10	8ig Muddy River	• 86	Franklin	38 02 30 88 57 30	Dam Access Rd. Br., 2.5 miles NW of Benton		
N 11	Big Muddy River	120	Franklin	37 54 05 89 00 50	Rt. 149 Br., 0.7 miles W of Plumfield		
N 12	Big Muddy River	250	Jackson	37 45 30 89 19 38	Rt. 127 Br. S of Murphysboro		
NA 01	Cedar Creek	• 58	Jackson	37 40 15 89 19 21	Rt. 127 Br., 6 miles NNE of Alto Pass		
NB 01	Kinkaid Creek	• 74	Jackson	37 46 38 89 27 14	dwnstrm fo Crissenberry Dam, Murphysboro		
NC 07	Beaucoup Creek	832	Jackson	37 54 12 89 22 36	Co. Rd. Br., 2.0 miles W of Vergennes		
ND 01	Crab Orchard Creek	128	Jackson	37 46 18 89 10 49	Dillinger Rd. Br., 3.2 miles NE of Carbondale		
ND 02	Crab Orchard Creek	100	Williamson	37 42 51 89 09 04	Crab Orchard Lake Spillway Road		
ND 04	Crab Orchard Creek	429	Williamson	37 43 52 88 53 21	Rt. 13 Br., 1.3 miles E of Marion		
NE 05	Little Muddy River	237	Jackson	37 54 03 89 12 31	Co. Rd. Br., 1.3 miles E of Elkville		

10 of 13 'indicates no flow data collected

note: critical hardness expressed as CaCO3 (mg/L)

		(4/30/97)			
Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description
NG 62	Pond Creek	98	Franklin	37 54 03 88 55 54	Co. Rd. Br., 0.5 miles S of W Frankfort
NH 06	Middle Fork Big Muddy River	132	Franklin	37 56 58 88 54 00	Co. Rd. Br., 2,7 miles SSE of Benton
NJ 07	Casey Fork	116	Jefferson	38 16 10 88 53 55	Rt. 37 Br., 3 miles S of Mt. Vernon
NK 01	Rayse Creek	93	Jefferson	38 15 14 89 02 23	Twp Rd, Br, 2,4 miles N of Waltonville
	KASKASKIA RIVER BASIN				
0 <b>02</b>	Kaskaskia River	218	Coles	39 34 59 88 24 50	Local Rd. Br. in Cook Mills
0 07	Kaskaskia River	150	Clinton	38 34 28 89 22 09	Rt. 127 Br., 2.3 miles S of Carlyle
0 08	Kaskaskia River	240	Fayette	38 57 35 89 05 20	US Rt. 51 Br. at SE edge of Vandalia
O 10	Kaskaskia River	245	Shelby	39 13 50 88 50 33	Rt. 128 Br., 2 miles SE of Cowden
011	Kaskaskia River	205	Shelby	39 24 25 88 46 50	RI. 16 Br. at Shelbyville near dam
O 15	Kaskaskia River	- 29 <b>8</b>	Moultrie	39 34 22 88 31 53	Rt 121 Br., 1 mile N of Allenville
O 20	Kaskaskia Ríver	180	Clinton / Washington	38 27 02 89 37 39	Rt 160-177 Br., 4.3 miles NW of Okawville
O 30	Kaskaskia River	190	Randolph	38 00 58 89 57 14	Co. Rd. Br., 2.7 miles W of Ellis Grove
O 31	Kaskaskia River	• 249	Dougias	39 51 53 88 21 52	Co. Rd. Br., 4 miles W of Hayes
OC 64	Richland Creek	• 291	St. Clair	38 19 26 89 58 15	Rt. 1565 Br., 1.6 miles NE of Hecker
OD 06	Silver Creek	183	Madison	38 43 00 89 49 45	
OD 07	Silver Creek	191	St. Clair	38 24 22 89 52 25	

11 of 13 I indicates no flow data collected

note: critical hardness expressed as CaCOS (mg/L)

	Amblent Water Quality	(4/30/97)	4/30/97)					
Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description			
OH 01	Sugar Creek	116	Clinton	38 32 29 89 37 36	Rt. 161 Br., 0.5 miles W of Albers			
OI 08	Shoal Creek	157	Clinton	38 36 35 89 29 40	Rt. 50 Br., 1,4 miles E of Breese			
OI 09	Shoal Creek	198	Montgomery	39 03 46 89 32 46	Co. Rd. Br. 523, 3 miles NW of Panama			
OJ 07	Croaked Creek	118	Marion	38 33 50 89 03 01	Co. Rd Br., 3.1 miles S of Odin			
OJ 08	Сгоокеа Сгеек	134	Washington	38 30 25 89 16 24	Hoyleton Rd. Br., 2.2 miles SW of Hoffman			
OK 01	East Fork Kaskaskia	92	Marion	38 41 20 89 05 55	RI. 51 Br., 5.2 miles N of Sandoval			
OKA 01	Nonth Fork Kaskaskia	• 106	Marion	38 46 25 89 09 15	Old Patoka Rd Bridge			
OL 02	Hurncane Creek	208	Fayette	38 55 21 89 14 14	Rt. 140 Br., 1.0 mile E of Mulberry Grove			
ON 01	Hickory Creek	175	Fayette	38 55 30 89 02 20	Co. Rd. Br., 2.7 miles S of Bluff City			
OQ 01	Beck Creek	182	Shelby / Fayette	39 12 04 89 01 53	Co. line Rd. Br., 2 miles W of Herrick			
OT 02	West Okaw River	262	Moultrie	39 42 15 88 39 51	Rt. 32 Br., NW of Lovington			
OU 01	Jonathon Creek	- 32 <b>9</b>	Moultrie	39 36 03 88 32 43	Rt. 121 Br., 2.5 miles E of Sullivan			
OZC 01	Plum Creek	300	Randolph	38 08 48 89 50 35	Co. Rd. Br., 2.5 miles S of Baldwin			
OZZT 01	Asa Creek	258	Moultrie	39 37 11 88 36 17				
	ROCK RIVER BASIN							
P 04	Rock River	250	Henry / Rock Island	41 33 35 90 10 55				
P 06	Rock River	235	Whiteside	41 47 00 89 44 58				

12 of 13 indicates no flow data collected

note: critical hardness expressed as CaCO3 (mg/L)

	Amplent Water Quanty	(4/30/97)	(/30/97)				
Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description		
P 14	Rock River	241	Ogle	42 07 18 89 15 09	Rt. 72 Br. at Byron		
P 15	Rock River	277	Winneoago	42 26 55 89 04 11	Rt. 75 Br. at Rockton		
P 20	Rock River	244	Ogle / Lee	41 53 23 89 25 10	Rt. 2 Br., near Grand Detour; county line		
PB 02	Green River	338	Whiteside	41 35 38 89 41 22	Rt. 88 Br., 1 mile S of Deer Grove		
PB 04	Green River	323	Henry	41 29 20 90 09 30	Rt. 82 Br., N of Genesea		
PE 05	Rock Creek	• 349	Whiteside	41 40 14 90 01 34	Rt. 2 Br., 3 miles NE of Erie		
PH 16	Elkhorn Creek	338	Whiteside	41 54 10 89 41 40	2 miles NW of Penrose Co. Rd. Br.		
PL 03	Kyte River	275	Ogle	41 59 50 89 17 30	Honey Crk Rd. Br. 1 mile E of Daysville		
PQ 02	Kishwaukee River	277	Winnebago	42 12 06 88 58 43	Perryville Rd. Br., ner S. Branch		
PQ 10	Kishwaukee River	323	Boone	42 15 40 88 43 00	Co. Rd Br., 0.5 miles N of Graden Prane		
PQ 12	Kishwa⊔kee River	279	Winnepago	42 11 45 88 59 55	Blackhawk Rd. Br.		
PQB 02	Kilbuck Creek	336	Winnebago	42 09 37 89 04 34	US 251 Br., 4 miles S of Rockford		
POC Cô	South Branch Kishwaukee River	281	DeKalb	42 06 40 88 54 00	Co. Rd. Br., 0.5 miles N of Rt, 72		
PQF 07	Coon Creek	336	McHenry	42 10 58 88 38 28	Riley-Harmon Rd. 0.8 miles SW of Riley		
PW 01	Pecatonica River	333	Winnebago	42 25 39 89 11 44	Rt. 75 Br. at Harrison		
PW 08	Pecatonica River	327	Stephenson	42 18 13 89 36 57	Rt. 75 Br., Westbound at Freeport		
PWN 01	Yellow Creek	- 336	Stephenson	42 16 56 90 01 34	Hollywood Road at SE edge of Freeport		

## Attachment 1 – Exhibit T

Calculation of the conversion factor multiplier for manganese standards derived from total and dissolved manganese data collected during the chronic *Hyalella azteca* test. For each treatment, the filtered (dissolved) results were divided by the unfiltered (total) results to calculate the percent of dissolved manganese

**<u>Exhibit T</u>**: Calculation of the conversion factor multiplier for manganese standards derived from total and dissolved manganese data collected during the chronic *Hyalella azteca* test. For each treatment, the filtered (dissolved) results were divided by the unfiltered (total) results to calculate the percent of dissolved manganese.

Nominal Mn	ln	In	ln	Out	Out	Out	Geometric	Geometric	Geometric
Concentration (mg/L)	Set #1	Set #2	Set #3	Set #1	Set #2	Set #3	Mean In	Mean Out	Mean All
0.3	1.0000	1.0000	0.9722	0.8947	1.0000	0.9565	0.9907	0.9494	0.9698
0.7	0.9870	0.9857	0.9861	0.9103	1.0735	0.9831	0.9863	0.9867	0.9865
1.4	0.9375	1.0000	1.0000	0.9333	1.0714	1.0000	0.9787	1.0000	0.9893
2.9	0.9677	1.0000	1.0000	0.9032	1.0357	0.9310	0.9891	0.9550	0.9719
5.7	1.0000	1.0000	1.0000	0.9016	1.0536	0.9825	1.0000	0.9772	0.9886
							0.9889	0.9735	0.9812

## Attachment 1 – Exhibit U

Final Report, Acute and Chronic Toxicity of Boron, Fluoride, and Manganese to Freshwater Organisms, by David J. Soucek and Amy Dickinson, Illinois Natural History Survey, dated October 14, 2010

#### --FINAL REPORT--

#### Acute and Chronic Toxicity of Boron, Fluoride, and Manganese to Freshwater Organisms

by

David J. Soucek and Amy Dickinson Illinois Natural History Survey Institute of Natural Resource Sustainability University of Illinois, Urbana-Champaign 1816 S. Oak St. Champaign, IL 61820

Submitted to: Brian Koch and Robert Mosher Illinois Environmental Protection Agency 1021 North Grand Avenue East Springfield, Illinois 62794-9276

October 14, 2010

#### A. BORON

#### PURPOSE

This study was designed to generate further data on acute and chronic boron toxicity in support of an effort by Illinois Environmental Protection Agency (IL EPA) to update their State general-use standard for boron. First, we conducted acute toxicity tests with boron on a variety of freshwater species, including a fingernail clam and a stonefly, as well as several commonly used standardized test organisms. Next, we sought to further clarify whether hardness or pH affect boron toxicity by conducting tests at three hardnesses and three pHs with two different test organisms, *C. dubia* and the amphipod *Hyalella azteca*. Finally, we conducted chronic boron toxicity tests with two species (*H. azteca* and *P. promelas*) in an effort to generate acute to chronic ratios (ACRs) for use in a chronic boron standard.

#### MATERIALS AND METHODS

#### Culture and holding of test organisms

Five species (four invertebrates and one vertebrate) were selected to generate acute toxicity data for boron based on data gaps in the literature, and the need for acute to chronic ratios (ACR) for use in chronic standard development. Useful data are available from the literature for a number of fish species, but we included fathead minnow, *Pimephales promelas*, because of the need to generate an ACR. There are relatively fewer data available on toxicity of boron to invertebrates. No published data exist for mollusks so we included a native fingernail clam, *Sphaerium simile*. The only insect data point available in the literature is for *Chironomus* (Maier and Knight, 1991), which is the least sensitive species tested, so we chose a winter stonefly, *Allocapnia vivipara*. Finally we tested the crustaceans *Ceriodaphnia dubia* and *Hyalella azteca* because of their greater availability and usefulness in testing under a variety of water quality conditions.

The cladoceran, *C. dubia*, and the amphipod *H. azteca* were cultured in-house (Soucek laboratory, Illinois Natural History Survey) according to U.S. EPA methods (USEPA 2000, 2002). *C. dubia* were cultured in moderately hard reconstituted water (USEPA 2002), which will also be referred to as our "hard 100a" water (Table 1), at 25 °C and a 16:8 (L:D) photoperiod. *C. dubia* were fed approximately 0.3 ml of a YTC/*Pseudokirchneriella subcapitata* (3.0 X 10<sup>7</sup> cells/ml) mixture (1:1, v.v) daily. Amphipods, *H. azteca*, were cultured in a "reformulated moderately hard reconstituted water, RMHRW" (Smith et al. 1997), which will be referred to as "hard 100b" (Table 1), at 22 °C and a 16:8 (L:D) photoperiod. *H. azteca* were fed *Pseudokirchneriella subcapitata* (3.0 X 10<sup>7</sup> cells/ml) and TetraMin® (TetraWerke, Melle, Germany) flake food. Other details of crustacean culturing followed recommendations of USEPA (2000, 2002). For use in tests with different hardnesses and pHs, *C. dubia* were cultured in test water for the different hardnesses, but for the different pH tests, organisms were acclimated to test water for three to four days prior to testing.

*Pimephales promelas* for use in both acute and chronic testing were obtained as embryos from Aquatic Bio Systems, Fort Collins, CO, and upon receipt, were transferred to aquaria containing our "hard 100a" water. Embryos were received <24 h after fertilization and chronic bioassays (see below) were initiated upon receipt. A separate cohort for acute testing was maintained in aquaria at 25 °C and a 16:8 (L:D) photoperiod, and upon hatching, larvae were fed brine shrimp (Brine Shrimp Direct, Ogden, UT) twice daily. Other details of fathead minnow holding followed recommendations of American Society of Testing and Materials (ASTM) method E 1241-05 (2005).

Sphaerium simile were field-collected from Spring Creek, near Loda, IL, in Iroquois County. Clams collected from this site were previously identified to species by Dr. Gerald Mackie of the University of Guelph, Department of Zoology, Guelph, Ontario, Canada. Clams were collected as adults, returned to the laboratory (at INHS, Champaign, IL) in site water, and they subsequently released juveniles from their brood chambers in the laboratory. Juveniles were used for testing. The juvenile clams were gradually acclimated to laboratory conditions for approximately two weeks. Twenty percent of the water was changed daily until holding water was 100% "hard 100a" water; afterward, 50% of the water was changed daily. The temperature of the clam holding water was gradually adjusted (1 °C/day) from the water temperature at the time of collection to a test temperature of  $22 \pm 1$  °C. The clams were held in aquaria containing 6 L with a photoperiod of 16:8 (L:D). Prior to testing, clams were fed daily a suspension of the green alga (*Ankistrodesmus falcatus*) at a rate of 1.25 mg (d.w.) per gram of clam (w.w.). Other details of clam holding conditions followed recommendations of ASTM E729 (2002).

Allocapnia vivipara were field-collected from Stoney Creek, near Muncie, IL, in Vermilion County, as later instar nymphs at 4 °C. Stoneflies were returned to the laboratory in site water, and were gradually acclimated to laboratory conditions for approximately two weeks; temperature was gradually adjusted (1 °C/day) to a test temperature of  $12 \pm 1$  °C, and 50% of the water was changed every third day until holding water was 100% "hard 100a" water. The stoneflies were held in 6 L aquaria with a photoperiod of 16:8 (L:D). Prior to testing, stoneflies were fed maple leaves that were collected from Stoney Creek and rinsed with deionized water. Other details of stonefly holding conditions followed recommendations of ASTM E729 (2002).

#### Test chemicals and dilution waters

The boron source for both acute and chronic toxicity tests was a combination of sodium tetraborate decahydrate or borax (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>•10H<sub>2</sub>O, 99.5+%, CAS # 1303-96-4) and boric acid (H<sub>3</sub>BO<sub>3</sub>, reagent grade, CAS# 10043-35-3). Previous studies investigating boron toxicity to invertebrates have used both boric acid and borax. In two studies that used boric acid as the boron source, pH of various treatments ranged from 6.7 to 8.1 (Gersich 1984), and 7.1 to 8.7 (Lewis and Valentine 1981). Maier and Knight (1991) used borax as their boron source, and the pH of their treatments was 9.1, while the pH of their controls ranged from 7.3 to 8.6. Because it was our intention to study the effect of pH on boron toxicity, having a range of pHs in treatments within a given test was undesirable.

Both boric acid and borax readily dissolve in water to form undissociated boric acid  $(H_3BO_3)$  and borate anion  $(B(OH)_4)$ , and different proportions of these two species are present depending on pH (Power and Woods 1997). Therefore, we decided to use boric acid and borax as a buffer system in which a given combination of the two salts would be used to match the desired pH of the dilution water, thereby allowing for a relatively constant pH for all treatments within a given test. In most cases 82% of the boron in solution was as boric acid and 18% was as borax, allowing for a test pH of ~8.0. Tests with different target pHs had different ratios of boric acid to borax (detailed in Table 1). We also conducted one acute test with *C. dubia* using only boric acid to determine if the boron source used affected its toxicity.

We used a variety of dilution waters depending on the species tested, the desired hardness, and the desired pH. Waters were formulated by adding a combination of four to five salts to distilled/deionized water (Table A.1). All tests with P. promelas, S. simile, and A. vivipara, were conducted using our "hard 100a" water, which is called Moderately Hard Reconstituted Water (MHRW) in U.S. EPA (2002). Tests with C. dubia and H. azteca were conducted at three different hardnesses (~100, 300, and 500 mg/L as CaCO<sub>3</sub>) and three different pHs (6.5, 7.5, and 8.5), but different recipes were used for the two species to achieve these water quality formulations because the formulations for H. azteca were based on a water recipe developed by Smith et al. (1997), and Borgmann (1996), which were both specifically developed for use with Hyalella. Different hardnesses were achieved by adding MgSO<sub>4</sub>, CaSO<sub>4</sub>, and in the case of *H. azteca*, CaCl<sub>2</sub> in the same ratios as found in the corresponding hardness = 100 recipe. All toxicity tests were conducted as static, non-renewal tests; therefore, pH could not be varied by the addition of acid because the alkalinity of the dilution water would change the pH too much by 48 hours after the start of the test (DJS personal observation). Instead we added different amounts of NaHCO<sub>3</sub> depending on the desired test pH (Table A.1). This resulted in relatively stable pH readings for the duration of the 96-h acute tests, and between changeovers in the chronic bioassays.

#### Acute test procedures

For *P. promelas, C. dubia, H. azteca, S. simile,* and *A. vivipara*, static, non-renewal, acute toxicity tests were conducted according to guidelines detailed in ASTM E729-96 (2002)., Treatments were comprised of a 50% dilution series. Five to six concentrations were tested using various dilution waters (as described above (Table A.1)) as both the diluent and control with four replicates tested per concentration. Tests with *C. dubia* were conducted for 48 h with a 16:8 (L:D) photoperiod with all others being 96 h in duration. Further details on test conditions for each species are provided in Table A.2. For *H. azteca* and *A. vivipara*, nitex mesh was added to each test chamber to provide substrate for these benthic invertebrates. Percent survival in each replicate was recorded every 24 h and at the end of the exposure period. A dissecting microscope was used to assess survival of all species. At the end of 96 h tests, fingernail clams were transferred to boron free dilution water with food for evaluation of survival. Individuals with undetectable foot movement or ciliary motion were considered dead.

Standard water chemistry parameters were measured at both the beginning and the end of each exposure period, including temperature, pH, conductivity, dissolved oxygen, alkalinity and hardness. The pH measurements were made using an Accumet<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet<sup>®</sup> gelfilled combination electrode (accuracy  $\leq \pm 0.05$  pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments (RDP, Dayton, OH, USA) model 55 meter. Conductivity measurements were made using a Mettler Toledo<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model MC226 conductivity/TDS meter. Alkalinity, and hardness were measured by titration as described in American Public Health Association (APHA) et al. (2005). At both the beginning and end of acute tests, water samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of boron concentrations by inductively couple plasma - atomic emission spectrometry according to U.S. EPA method 200.7 (Martin et al. 1994). To address the potential need to account for total versus dissolved boron, samples from the acute toxicity test with S. simile (selected at random), were analyzed for both total and dissolved boron at the beginning and at the end of the test. For measurement of dissolved boron, samples were filtered using 0.45 µm cellulose nitrate filters (Whatman<sup>®</sup>), Maidstone, England). Total boron was determined with unfiltered samples.

#### Chronic test procedures

Hyalella azteca -- A 42-d, water only, static-renewal, chronic reproduction bioassay was conducted with H. azteca using recommendations detailed in the U.S. EPA sediment toxicity testing guidelines (USEPA 2000), but with modifications. Treatments included five nominal boron concentrations (3.125, 6.25, 12.5, 25, and 50 mg B/L) and a control with no boron added. The control and dilution water was our "hard 100b" recipe (Table A.1). Test chambers were 300-ml, high form beakers and 200 ml of test solution was used per test chamber. Organisms were 7- to 14-d old at the beginning of the test, and we loaded 10 into each of four replicate chambers per treatment. A 1.2- by 2.5-cm conditioned maple leaf strip was added to each test chamber for food and substrate, and 200 µl of a 5 g/L Tetramin® suspension (in deionized water) was added each time test solutions were changed. Test solutions were not aerated. Every three to four days, complete water renewals were conducted, with test organisms being transferred to new beakers containing fresh test solutions. Survival was evaluated with every changeover. After the first appearance of mating pairs (day 25), the number of pairs per test chamber was recorded daily, and discarded tests solutions (after changeovers) were carefully searched for young. Young began to appear on day 35, and the number produced was recorded until the end of the test (day 42). At the end of the test, adult amphipods were sexed and then dried in an oven (60 to 70 °C) for at least 48 h before they were weighed to the nearest 0.001 mg. Endpoints calculated included % survival, mean dry weight (per individual), number of mating pairs, # of young per female.

*Pimephales promelas* -- A 32-d, water only, static-renewal, chronic early life-stage toxicity test bioassay was conducted with *P. promelas* using guidelines detailed in ASTM E 241-05 (2005), but with modifications. The primary modification was that the test was conducted as a static-renewal test rather than a flow-through test. Treatments included

five nominal boron concentrations (2.75, 5.5, 11, 22, and 44 mg B/L) and a control with no boron added. The control and dilution water was our "hard 100a" recipe (Table 1). The test was initiated with embryos ~14 h post fertilization; 60 embryos were placed into each of six 1 L beakers containing a test solution (described above). Beakers were aerated vigorously to prevent accumulation of fungus. On day two, percent survival of embryos was assessed and then the number of organisms was thinned to 40 per treatment, and 10 embryos were placed into each of four replicate 600-ml beakers per treatment. Embryos began to hatch on day two, and by day four, hatching was completed. Only two embryos failed to hatch, both in the 5.5 mg/L treatment. Test solutions were not aerated. Fish were fed brine shrimp (Artemia sp.) following ASTM (2005) guidelines. Approximately every three days, complete water renewals were conducted, with test organisms being transferred to new beakers containing fresh test solutions. Survival was evaluated daily until the end of the test (day 32). At the end of the test, fish were dried in an oven (60 to 70 °C) for at least 48 h before they were weighed to the nearest 0.001 mg. Endpoints calculated included % survival of embryos before thinning, % survival after 32 d, total survival (= [% embryo survival before thinning)]/100 \* % survival at the end of 32 d), and mean dry weight per fish.

Fish test water chemistry – Temperature and dissolved oxygen were measured daily in each test replicate for the fish test. Care was taken to minimally disturb the fish during this process. Other standard water chemistry parameters were measured at the beginning of the test and in the "in" and "out" water from every changeover for both species; these included pH, conductivity, alkalinity and hardness. In addition, total ammonia was measured frequently during the fish test. The pH, dissolved oxygen, conductivity, alkalinity and hardness measured as described above. Ammonia was measured using a Thermo® Orion 4-Star ion selective electrode meter with a Thermo® Orion ammonia probe (model # 9512). Renewal "in" water and discarded "out" water samples from each treatment were collected at each changeover and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of boron concentrations by inductively couple plasma - atomic emission spectrometry according to U.S. EPA method 200.7 (Martin et al. 1994).

# Statistical analysis

All LC50 values were calculated using the trimmed Spearman-Karber method (USEPA 2002). For chronic toxicity tests, we followed guidelines detailed in U.S. EPA (2002). Briefly, data for survival, and sub-lethal endpoints (amphipod dry weight, # females, # young per female, fathead minnow dry weight) were tested for normality using the Shapiro-Wilk's Test, and homogeneity of variance using Bartlett's test. Data that passed both of these tests were analyzed for differences among means using Dunnett's test. For the *Hyalella* chronic test, one replicate beaker was lost resulting in unequal numbers of replicates so Bonferroni's test was used to analyze weight and reproduction data, while Fisher's exact test was used to analyze survival data. Those that did not pass normality or homogeneity of variance tests were analyzed using Steel's Many-One test. The No Observable Adverse Effects Concentration (NOAEC) was the highest concentration whose mean for a given endpoint was not significantly different from that of the control,

and the Least Observable Adverse Effect Concentration (LOAEC) was the lowest concentration whose mean was significantly different from the control. We also calculated Maximum Acceptable Toxicant Concentrations (MATC) as the geometric mean of the LOAEC and the NOAEC, and Acute to Chronic Ratios (ACR) as the LC50 divided by the MATC. For the ACR, we used the LC50 that was generated for a given species in the same dilution water as was used in the chronic test.

#### RESULTS

#### Acute toxicity

For the 96-h boron toxicity tests with fish, clams, and the stonefly, mean water temperatures remained within 1 °C of targets, mean pH values ranged from 7.9 to 8.0 with low variability within tests, and hardnesses ranged from 91 to 102 mg/L as CaCO<sub>3</sub>, again with low variability within tests (Table A.3). For the fingernail clam test, both total and dissolved boron was measured, and with the exception of one spurious value (day zero, 100 mg/L treatment), total and dissolved boron measurements were similar for the day zero samples, with a mean ratio of dissolved to total B of 1.009 (Table A.4). On day four, more variability was observed, with some ratios being greater than one, and some being less. Ratios of dissolved to total boron did not appear to be related to concentration, and the day four geometric mean was 0.981, with the overall geometric mean ratio being 0.994.

The 96-h LC50 values based on measured boron concentrations ranged from 79.7 mg B/L (fathead minnow) to >447 mg B/L for *S. simile* (Table A.3). For the *S. simile* test, no clams died in any test concentration, and therefore an LC50 could not be calculated.

For the 48- or 96-h boron toxicity tests with the crustaceans *C. dubia* and *H. azteca*, mean water temperatures remained within 1 °C of targets. Mean pH values and hardness were variable due to the experimental design, but within given tests, pH values were stable from day zero to day four and had low variability (Table A.5). The one exception to this was the *C. dubia* test using only boric acid as the boron source. In this test, pH values ranged from 6.8 in the highest test concentration on day zero to 7.8 in the control. The geometric mean of all measured pH values in this test was 7.4.

The 48-h boron LC50s for *C. dubia* ranged from 91 for the pH 6.5 test to 165 mg B/L for the first hard 100a test (Table A.5). Investigating the effects of pH on boron toxicity, we included all tests conducted at various pH values with hardness of ~90 mg/L (n = 6) and conducted regression analysis of pH versus log LC50. The resulting line was positively sloped, but the regression was not statistically significant at the  $\alpha = 0.05$  level (R<sup>2</sup> = 0.5708, p = 0.0823). Likewise, we investigated the influence of hardness on boron toxicity to *C. dubia* by including all tests conducted at various hardness levels but with pH of ~8.0 (n = 4). Conducting a log hardness versus log LC50 regression resulted in a negatively sloped (the higher the hardness the lower the LC50), but statistically insignificant line (R<sup>2</sup> = 0.5329, p = 0.2700).

We conducted similar analyses of the LC50s for *H. azteca*. The 96-h boron LC50s for this species ranged from 64 for the pH 8.5 test to 269 mg B/L for the hard 100c test (Table A.5). Comparing pH versus log LC50 for the tests with hardness values of ~100 mg/L (n = 4) resulted in a plot that was best fit by an upside down, U-shaped line. While the R<sup>2</sup> value was high (0.9311), the p-value was insignificant (0.2624), due to low sample size. For the hardness solutions based on Smith et al (1997) water (the "b" series), increasing hardness decreased boron toxicity in a marginally significant manner (R<sup>2</sup> = 0.9933, p = 0.0522). However, the 300 and 500 mg/L hardness test solutions also had higher chloride concentrations than the 100 mg/L hardness solution (Table A.1), thus presenting a potential confounding factor. Using Borgmann (1996) water as a base, thus keeping chloride concentration constant (the "c" series), increasing hardness in the "b" series in lower LC50s (Table A.5), suggesting the reduced toxicity at higher hardness in the "b" series tests was actually due to increased chloride.

#### Chronic toxicity

<u>Fathead minnows</u> - Basic water quality parameters in the 32-d chronic static renewal bioassay with *Pimephales promelas* (Table A.6) met the basic acceptability requirements as outlined in ASTM E241-05 (2005). Temperature variability was within acceptable limits, and dissolved oxygen did not drop below 5 mg/L (Table A.6). Unionized ammonia concentrations never reached 0.05 mg/L. Measured boron concentrations were generally similar to nominal concentrations (Table A.7), with no major differences between "in" water and "out" water samples. The overall geometric mean percent difference between nominal and measured concentrations was 2.7%.

Percent survival of embryos before thinning was high, with no treatment having a percent survival lower than 93% (Table A.8). Most larvae emerged on day three with no substantial differences among treatments in average day of hatch, and hatching rates were high with all eggs hatching in every treatment except for two individuals in the 11.2 mg/L treatment (Table A.8). After thinning, survival was relatively high in all treatments until ~day 17, when survival in the 44.5 mg/L treatment began to drop (Fig. A.1). At the end of the 32-d test, three treatments (control, 2.8, and 11.2 mg/L) had greater than 90% survival and 87.5% of the fish had survived in the 5.7 mg/L treatment. Two treatments had significantly lower survival than the control: 23 mg/L (80%) and 44.5 mg/L (15%). Because embryo % survival before thinning was high for all treatments, total survival values were similar to % survival values of thinned fish at the end of the test (Table 8, Fig. A.1). Dry weights of individual fish in controls met acceptability requirements of 0.25 mg, but after excluding treatments for which survival was significantly lower, no significant differences among treatments were observed in mean dry weight per fish (Fig. A.2).

<u>Amphipods</u> - Basic water quality parameters in the 42-d chronic static renewal bioassay with *Hyalella azteca* were similar to those observed in the fish test, but with slightly higher hardness because of the different dilution water used (Table A.6). Temperature variability was within acceptable limits, and dissolved oxygen did not drop below 6.6 mg/L (Table A.6). As with the fathead minnow test, measured boron concentrations were generally similar to nominal concentrations (Table A.9), with no major differences between "in" water and "out" water samples. The overall geometric mean percent difference between nominal and measured concentrations was 3.5%.

At the end of 42 d, % survival of the controls was 90%, and although survival in the four lowest boron treatments (3.2, 6.6, 13.0, and 25.9) ranged from 72.5 to 87.5%, only the highest concentration (51.1 mg/L, 37.5%) had significantly lower survival than the control (Fig. A.3). After excluding the highest treatment (51.1 mg/L) from further analysis because of its lower survival rate, there were no differences among treatments in the number of females present (Fig. A.4) or dry weight of individual amphipods (Fig. A.5). However, there were significant differences from the control in # offspring produced per female, with both the 13.0 and the 25.9 mg/L having significantly lower means (Fig. A.4).

<u>Chronic values</u> – Because there were no significant differences among treatments in fathead minnow dry weight, the NOAEC (23.0 mg/L) and LOAEC (11.2 mg/L) values for *P. promelas* were derived from survival data. The resulting MATC from these values was 16.0 mg/L, and using the 96-h LC50 of 79.7 mg/L produced an ACR of 5.0. For *H. azteca*, the NOAEC (13.0 mg/L) and LOAEC (6.6 mg/L) values were derived from the number of offspring produced per female. This resulted in an MATC of 9.3 mg/L, and with the 96-h LC50 of 107 mg/L, the ACR was 11.5.

dilution waters	used for	<u>or definitive b</u>	<u>oron toxicity</u>	<u>testing with f</u>	<u>reshwater s</u>	pecies.
Water name	KC1	NaHCO3	MgSO <sub>4</sub> (an)	$CaSO_4(an)$	CaCl <sub>2</sub>	B ratio <sup>*</sup>
hard 100a	4	96	60	60	0	82 / 18
hard 100b	4	96	30	50	50	82 / 18
hard 100c	4	84	30	0	111	82 / 18
hard 300a	4	96	192	192	0	82 / 18
hard 300b	4	96	90	150	150	82 / 18
hard 300c	4	84	30	190	111	82 / 18
hard 500a	4	96	320	320	0	82 / 18
hard 500b	4	96	150	250	250	82 / 18
hard 500c	4	84	30	408	111	82 / 18
рН 6.5а	4	4	60	60	0	99.1 / 0.9
pH 6.5b	4	4	30	50	50	99.1 / 0.9
рН 7.5а	4	40	60	60	0	93.2 / 6.8
pH 7.5b	4	40	30	50	50	93.2 / 6.8
pH 8.5a	4	400	60	60	0	75.7 / 24.3
pH 8.5b	4	400	30	50	50	75.7 / 24.3
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Table A.1. Salt concentrations (mg/L) added to deionized water for generation of dilution waters<sup>a,b,c,d</sup> used for definitive boron toxicity testing with freshwater specie

<sup>\*</sup>B ratio = ratio of % boron added to highest test concentration as boric acid / borax.

<sup>a</sup> hard 100a was used for tests with *P. promelas, S. simile, A. vivipara,* and *C. dubia.* For *C. dubia,* and additional acute test was conducted in this water using boric acid only.

<sup>b</sup> hard 300a, hard 500a, pH 6.5a, pH 7.5a, pH 8.5a were used for tests with *C. dubia*.

<sup>c</sup> hard 100b & c, hard 300b & c, hard 500b & c, pH 6.5b, pH 7.5b, pH 8.5b were used for tests with *H. azteca.* 

<sup>d</sup> hard 100c, 300c, and 500c also had 1 mg/L NaBr.

organisms.					
		Organism			
Parameter	P. promelas	C. dubia	H. azteca	A. vivipara	S. simile
1. Temperature (°C)	$25 \pm 1$	$25 \pm 1$	$22 \pm 1$	$12 \pm 1$	$22 \pm 1$
2. Test chamber size	250 ml	50 ml	50 ml	250 ml	150 ml
3. Test solution vol.	200 ml	40 ml	40 ml	200 ml	120 ml
4. Age of organisms	<7-d	<24-h	7-14 d	nymphs	juveniles
5. # org./chamber	10	5	5	5	5
6. # chambers/trt.	4	4	4	4	4
7. Feeding	none	none	none	none	none
8. Aeration	none	none	none	none	none
9. Test duration	96-h	48-h	96-h	96-h	96-h
10. Endpoints	survival	survival	survival	survival	survival
11. Control % Surv.	≥ <b>9</b> 0	≥ 90	≥ 90	$\geq$ 90	$\geq 90$

Table A.2. Test conditions for acute toxicity bioassays with various freshwater organisms.

Species	temp. (s.d)	pH (s.d)	hardness (s.d.)	LC50 (95% C.I.)
-	°C	S.U.	mg/L as CaCO <sub>3</sub>	mg B/L
Pimephales promelas	24.7 (0.3)	8.0 (0.1)	91 (1)	79.7 (72 - 88)
Sphaerium simile	21.1 (0.1)	7.9 (0.1)	102 (3)	>447 (n.a.)
Âllocapnia vivipara	11.2 (0.1)	7.9 (0.1)	98 (3)	476 (401-566)

Table A.3. 96-h boron LC50s and measured water quality conditions<sup>\*</sup> for toxicity tests with three freshwater species.

\*water quality values are geometric means of measurements taken in all test concentrations throughout the duration of the test.

Table A.4. Nominal and measured boron concentrations (mg B/L) for unfiltered (total B) and filtered<sup>a</sup> (dissolved B) samples from the 96-h acute toxicity test with the fingernail clam (*Sphaerium simile*).

Nominal	total B	dissolved B	ratio <sup>b</sup>	total B	dissolved B	ratio
concentration	day 0	day 0	day 0	day 4	day 4	day 4
Control	< 0.2	< 0.2	na	< 0.2	0.51	na
25	26	26	1.000	32	30	0.938
50	54	56	1.037	54	56	1.037
100	110	170°	na	120	110	0.917
200	220	220	1.000	230	240	1.043
400	440	440	1.000	460	450	0.978
<u> </u>	<u>C 1 0</u>	1 1 000		4 1	0.001 0	

Geometric mean of day 0 values = 1.009, and day 4 values = 0.981. Overall geometric mean = 0.994

<sup>a</sup> samples were filtered with 0.45  $\mu$ m pore sized cellulose nitrate filters.

<sup>b</sup> ratio = dissolved B divided by total B.

<sup>c</sup> measurement for this sample was extreme and because the day 4 sample was similar to the nominal concentration, the ratio for day 0 at this concentration was not calculated.

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	Ceriodaphnia dubia 48-h tests								
Test water	temp. (s.d)	pH (s.d)	hardness (s.d.)	LC50 (95% C.I.)					
	°C	S.U.	mg/L as CaCO <sub>3</sub>	mg B/L					
hard 100a (boric acid)	24.0 (0.1)	7.4 (0.3)	90 (4)	102 (82 – 126)					
hard 100a (first)	24.3 (0.1)	8.0 (0.2)	91 (3)	165 (137 – 198)					
hard 100a (second)	25.0 (0.0)	8.1 (0.1)	89 (2)	109 (93 – 128)					
hard 300a	25.0 (0.0)	8.1 (0.1)	282 (3)	104 (87 – 123)					
hard 500a	25.0 (0.0)	8.1 (0.1)	469 (1)	93 (77 – 114)					
рН 6.5а	25.0 (0.2)	6.7 (0.1)	85 (1)	91 (79 – 106)					
pH 7.5a	24.9 (0.1)	7.6 (0.0)	87(1)	115 (108 – 122)					
<u>pH 8.5a</u>	25.0 (0.0)	8.4 (0.1)	84 (1)	<u>142 (130 – 155)</u>					
	Hyalell	a azteca 96-h tests							
Test water	temp. (s.d)	pH (s.d)	hardness (s.d.)	LC50 (95% C.I.)					
	°C	S.U.	mg/L as CaCO <sub>3</sub>	mg B/L					
hard 100b	22.2 (0.4)	8.1 (0.0)	106 (4)	107 (70 – 163)					
hard 300b	21.5 (0.1)	8.1 (0.1)	302 (4)	151 (110 – 207)					
hard 500b	22.2 (0.4)	8.1 (0.1)	507 (9)	170 (121 – 239)					
hard 100c	22.0 (0.2)	8.1 (0.1)	111(1)	269 (223 - 326)					
hard 300c	22.1 (0.1)	8.1 (0.1)	291 (3)	203 (170 - 232)					
hard 500c	22.1 (0.1)	8.1 (0.1)	475 (4)	188 (154 – 230)					
pH = 6.5	21.0 (0.0)	6.6 (0.1)	102 (1)	104 (78 – 140)					
pH = 7.5	21.0 (0.0)	7.6 (0.0)	102 (1)	127 (90 – 178)					
pH = 8.5	21.0 (0.0)	8.4 (0.1)	103 (1)	<u>64 (41 – 101)</u>					
*		•							

Table A.5. Mean boron LC50s for *Ceriodaphnia dubia* and *Hyalella azteca* at various levels of water hardness and pH<sup>\*</sup>.

water quality values are geometric means of measurements taken in all test concentrations throughout the duration of the test.

Pimephales promelas 32-d chronic test							
nean*	5 <sup>th</sup> %ile	95 <sup>th</sup> %ile)	min	max			
24.7	24.4	25.0	23.6	25.5			
6.50	5.75	7.12	5.13	7.50			
8.0	7.6	8.2	7.5	8.2			
89	87	92	84	94			
67	60	80	58	86			
Hya			st				
nean <sup>*</sup>	5 <sup>th</sup> %ile	95 <sup>th</sup> %ile	min	max			
22.5	22.2	23.3	22.1	23.8			
7.3	6.8	7.6	6.6	8.0			
7.9	7.6	8.1	7.5	8.1			
105	102	108	102	110			
69	62	84	60	86			
	nean <sup>*</sup> 24.7 6.50 8.0 89 67 <u>Hya</u> nean <sup>*</sup> 22.5 7.3 7.9 105	$\begin{array}{r cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$			

Table A.6. Water quality data for chronic bioassays with Pimephales promelas and Hvalella azteca.

Table A.7. Boron measurement data from samples collected on 19 occasions throughout the 32-d chronic bioassays with Pimephales promelas.

$\underline{\mathbf{m}}$	onic bloas	isays with I	internutes p				
Nominal	overall	in water	out water	$5^{\text{th}}$	$95^{\text{th}}$	min	max
Conc.	mean <sup>a</sup>	mean	mean	%ile	%ile		
Control <sup>b</sup>	0.03	0.02	0.03	0.0	0.1	< 0.02	0.14
2.75 mg/L	2.8	2.7	2.9	2.7	3.1	2.6	3.1
5.5 mg/L	5.7	5.6	5.8	5.4	6.2	5.2	6.3
11 mg/L	11.2	11.0	11.5	10.0	12.0	10	12
22 mg/L	23.0	22.4	23.6	21.9	24.3	21	27
44 mg/L	44.5	43.5	45.8	41.8	47.0	40	47

<sup>a</sup> All means are geometric means. <sup>b</sup> Means shown for controls are for samples that had measureable boron. Nine of 19 control samples had boron less than detection limit of 0.02 mg/L.

Treatment	embryo % survival	mean (s.d) day <sup>a</sup>	% hatch	total <sup>b</sup>
	before thinning	of hatch	after thinning	survival
Control	93.3	3.0 (0.5)	100	88.6
2.8 mg/L	98.3	3.1 (0.5)	100	90.9
5.7 mg/L	100	3.3 (0.4)	95	87.5
11.2 mg/L	95	2.8 (0.6)	100	90.3
23.0 mg/L	96.6	3.1 (0.5)	100	77.3
44.5 mg/L	93.3	3.4 (0.5)	100	14.0

Table A.8. Embryo survival, total survival, and hatching data for the 32-d chronic bioassays with Pimenhales promelas

<sup>a</sup> days after initiation of test <sup>b</sup> total survival = (% embryo survival before thinning/100)\*% survival on day 32.

Table A.9. Boron measurement data from samples collected on 18 occasions throughout the 42-d chronic bioassays with *Hyalella azteca* 

uic + 2 - u cin	Unic Ulua	says with 1.	i futettu uztet				
Nominal	overall	in water	out water	$5^{\text{th}}$	$95^{\text{th}}$	min	max
Conc.	mean <sup>a</sup>	mean	mean	%ile	%ile		
Control <sup>b</sup>	0.07	0.05	0.09	0.0	0.2	< 0.02	0.29
3.13 mg/L	3.2	3.3	3.1	3.0	3.4	2.8	3.4
6.25 mg/L	6.6	6.7	6.3	5.9	7.1	5.8	7.2
12.5 mg/L	13.0	13.1	12.8	12.0	14.0	12	14
25 mg/L	25.9	26.2	25.3	24.9	27.2	24	27
<u>50 mg/L</u>	51.1	51.2	50.6	48.0	54.0	48	54

<sup>a</sup> All means are geometric means.

<sup>b</sup> Data shown for controls are means and percentiles of samples that had measureable boron. Five of 18 control samples had boron less than detection limit of 0.02 mg/L.

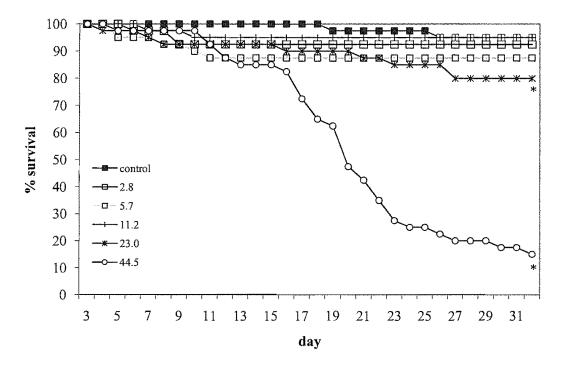


Figure A.1. Mean daily percent survival of fathead minnows (*Pimephales promelas*) in five concentrations of boron plus a control (Hard 100a) in a 32-d chronic, static renewal bioassay. Asterisks indicate mean is significantly different (p < 0.05) from control on day 32.

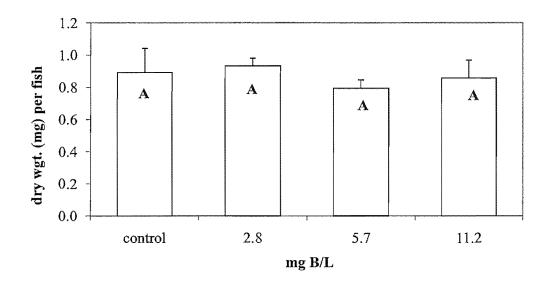


Figure A.2. Mean (error bars = standard deviation) dry weight per 10 fish in three boron concentrations and a control (Hard 100a) at the end of a 32-d chronic, static renewal bioassay with fathead minnows (*Pimephales promelas*). Different capital letters indicate means are significantly different from the control (p < 0.05).

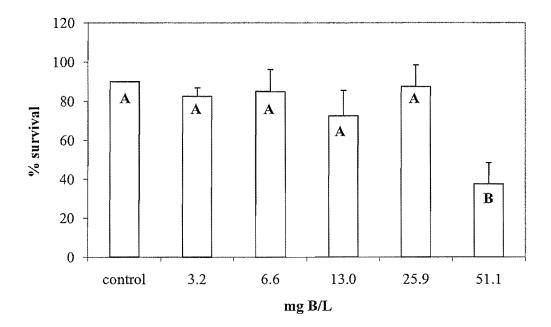


Figure A.3. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control (p < 0.05).

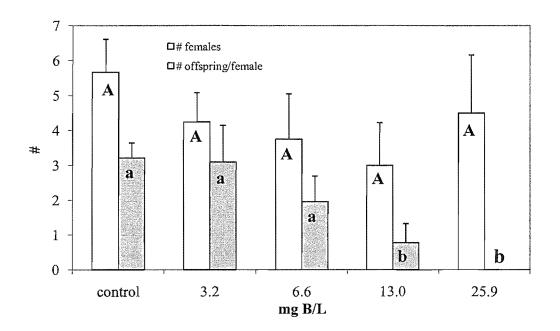


Figure A.4. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

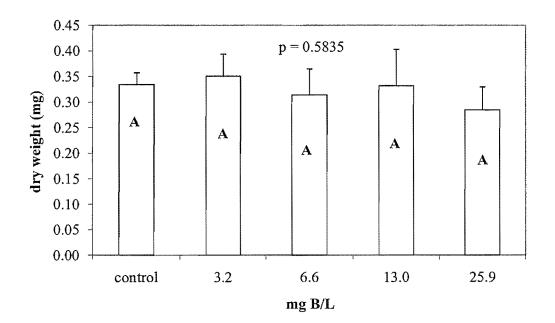


Figure A.5. Mean (error bars = standard deviation) dry weight of individual amphipods in four boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

# **B. FLUORIDE**

#### PURPOSE

The purpose of these experiments was to generate both acute and chronic fluoride toxicity data with *Hyalella azteca* in the same dilution/control water so that an Acute to Chronic Ratio (ACR) can be developed.

### MATERIALS AND METHODS

# Culture of test organisms

The amphipod *Hyalella azteca* was cultured in-house (Soucek laboratory, Illinois Natural History Survey) according to U.S. EPA methods (USEPA 2002) with some modifications. Amphipods were cultured in "Borgmann water" (Borgmann 1996), at 23 °C and a 16:8 (L:D) photoperiod, and were fed ~0.5 mg dry flakes (crushed and sieved to  $<500 \mu$ m) of TetraMin® (TetraWerke, Melle, Germany) daily. Approximately 30 adults were held in a 1-L beaker containing 1 L of Borgmann water. Young were removed at least every week or more frequently when a tighter age range was required.

#### Test chemicals and dilution waters

The fluoride source for both acute and chronic toxicity tests was sodium fluoride (NaF 99+%, CAS # 7681-49-4, Acros Organics, Geel, Belgium). The dilution water for both the acute test and the chronic test was Borgmann water (Table B.1).

#### Acute test procedures

Static, non-renewal, acute toxicity tests were conducted according to guidelines detailed in ASTM E729-96 (2002). Treatments were comprised of a 50% dilution series. Five concentrations were tested using Borgmann water (Table B.1) as both the diluent and control with four replicates tested per concentration. Organisms were 7- to 14-d old at the beginning of the test. The test was conducted for 96 h with a 16:8 (L:D) photoperiod at  $23 \pm 1$  °C. Test chambers were 50 ml glass beakers with 40 ml of test solution and a 2by 2-cm piece of nitex mesh was added to each test chamber to provide substrate for these benthic invertebrates. Tests were not fed or aerated. Percent survival in each replicate was recorded every 24 h and at the end of the exposure period. A dissecting microscope was used to assess survival. Acceptable control survival was set at 90%.

Standard water chemistry parameters were measured at both the beginning and the end of the exposure period, including temperature, pH, conductivity, dissolved oxygen, alkalinity and hardness. The pH measurements were made using an Accumet<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet<sup>®</sup> gelfilled combination electrode (accuracy <  $\pm$  0.05 pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments (RDP, Dayton, OH, USA) model 55 meter. Conductivity measurements were made using a Mettler Toledo<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model MC226 conductivity/TDS meter. Alkalinity and

hardness were measured by titration as described in American Public Health Association (APHA) et al. (2005). At both the beginning and end of the acute test, water samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of fluoride concentrations using an automated electrode according to U.S. EPA method 380-75WE. To address the potential need to account for total versus dissolved fluoride, samples from the acute toxicity test were analyzed for both total and dissolved fluoride, samples were filtered using 0.45  $\mu$ m cellulose nitrate filters (Whatman®, Maidstone, England). Total fluoride was determined with unfiltered samples.

#### Chronic test procedures

Hyalella azteca -- A 42-d, water only, static-renewal, chronic reproduction bioassay was conducted with H. azteca using recommendations detailed in the U.S. EPA sediment toxicity testing guidelines (USEPA 2000), but with modifications. Treatments included five nominal fluoride concentrations (1.75, 3.5, 7, 14, and 28 mg F/L) and a control with no fluoride added. The control and dilution water was Borgmann water (Table 1). Test chambers were 300-ml, high form beakers and 200 ml of test solution was used per test chamber. Organisms were 7- to 14-d old at the beginning of the test, and we loaded 10 in to each of four replicate chambers per treatment. A 2.5- by 5-cm piece of nitex mesh was added to each test chamber as a substrate, and *Pseudokirchneriella subcapitata* (1 mg dry solid) and 200 µl of a 5 g/L Tetramin® suspension (in deionized water) was added each time test solutions were changed. Test solutions were not aerated. Every three to four days, complete water renewals were conducted, with test organisms being transferred to new beakers containing fresh test solutions. After each changeover, "in water" and "out water" samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of fluoride concentrations using an automated electrode according to U.S. EPA method 380-75WE. Survival was evaluated with every changeover. After the first appearance of mating pairs, the number of pairs per test chamber was recorded daily, and discarded tests solutions (after changeovers) were carefully searched for young. Young began to appear on day 28, and the number produced was recorded until the end of the test (day 42). At the end of the test, adult amphipods were sexed and then dried in an oven (60 to 70 °C) for at least 48 h before they were weighed to the nearest 0.001 mg. Endpoints calculated included % survival, mean dry weight (per individual), number of mating pairs, # of young per female.

# Statistical analysis

The LC50 value was calculated using the trimmed Spearman-Karber method (USEPA 2002). For the chronic toxicity test, we followed guidelines detailed in U.S. EPA (2002). Briefly, data for survival, and sub-lethal endpoints (amphipod dry weight, # females, # young per female) were tested for normality using the Shapiro-Wilk's Test, and homogeneity of variance using Bartlett's test. Data that passed both of these tests were analyzed for differences among means using Dunnett's test. Those that did not pass normality or homogeneity of variance tests were analyzed using Steel's Many-One test.

The No Observable Adverse Effects Concentration (NOAEC) was the highest concentration whose mean for a given endpoint was not significantly different from that of the control, and the Least Observable Adverse Effect Concentration (LOAEC) was the lowest concentration whose mean was significantly different from the control. We also calculated Maximum Acceptable Toxicant Concentrations (MATC) as the geometric mean of the LOAEC and the NOAEC, and Acute to Chronic Ratios (ACR) as the LC50 divided by the MATC.

#### RESULTS

#### Acute toxicity

For the 96-h fluoride toxicity test with *Hyalella azteca*, mean water temperatures remained within 1 °C of the target ( $22.7 \pm 0.1$  SD), the mean pH value was  $8.0 \pm 0.1$ , and mean dissolved oxygen was  $8.0 \pm 0.3$  mg/L. Hardness, measured at the beginning of the test only, decreased with increasing fluoride concentration with the control/dilution water having a hardness of 112 mg/L and the 56 mg F/L nominal treatment having a hardness of 50 mg/L. The geometric mean hardness of all the treatments, excluding the highest fluoride concentration was 104 mg/L as CaCO<sub>3</sub>.

Both total and dissolved fluoride were measured for this test (Table B.2). Ratios of dissolved to total fluoride were higher at the beginning of the test as expected with an overall geometric mean ratio of 1.132 on day zero. The geometric mean of the dissolved to total fluoride ratios at the end of the test was 0.941, with ratios tending to be lower at the higher fluoride concentrations (Table B.2).

In the 96-h fluoride toxicity test with *Hyalella azteca*, control survival was 95% at the end of the test, and the measured 96-h LC50 was 25.8 mg F/L (20.1 - 33.1 95% confidence interval).

# Chronic toxicity

Basic water quality parameters in the 42-d chronic static renewal bioassay with *Hyalella azteca* are provided in table B.3. Temperature variability was within acceptable limits, and dissolved oxygen did not drop below 5.6 mg/L. As was the case with the acute fluoride toxicity test, measured fluoride concentrations were generally similar to nominal concentrations up to nominal concentrations of ~14 mg/L, but in the 28 mg/L nominal treatment, measured fluoride concentrations were consistently lower than nominal, likely due to precipitation (Table B.4). However, variability within treatments was relatively low, particularly in the treatments with fluoride concentrations of 14 mg/L or lower.

At the end of 42 d, % survival of the controls was 90%, and although survival in the four lowest fluoride treatments (measured 1.7, 3.3, 6.7, and 11.7 mg/L) ranged from 70 to 95%, only the highest concentration (16.7 mg/L, 22.5%) had significantly lower survival than the control (Fig. B.1). After excluding the highest treatment (16.7 mg/L) from further analysis because of its lower survival rate, there were no differences among treatments in the number of females present (Fig. B.2). However, there were significant

differences from the control in # offspring produced per female, with both the 11.7 mg/L treatment having a significantly lower mean (Fig. B.2). Analyzing dry weight data for individual amphipods, ANOVA indicated that there was no significant difference among treatment means when the 16.7 mg F/L treatment was excluded because of its significantly lower survival; however, when a post-hoc Dunnett's test was performed comparing the individual treatments to the control, the lowest treatment (1.7 mg/L) was significantly different from the control.

<u>Chronic values</u> –The NOAEC (6.7 mg/L) and LOAEC (11.7 mg/L) values were derived from the number of offspring produced per female. This resulted in an MATC of 8.8 mg/L, and with the 96-h LC50 of 25.8 mg/L, the ACR was 2.9. Because the survival and reproductive data indicated significant differences at much higher fluoride concentrations than did the dry weight data, and because the ANOVA for the weight data was not statistically significant, we suggest, that the significant difference at the lowest fluoride concentration be ignored.

Table B.1. Salt concentrations (mg/L) added to deionized water for generation ofdilution waters used for acute and chronic fluoride toxicity testing with Hyalella azteca.Water nameKClNaHCO3MgSO4(an)CaSO4(an)CaCl2NaBrBorgmann4843001111

Table B.2. Nominal and measured fluoride concentrations (mg F/L) for unfiltered (total F) and filtered<sup>a</sup> (dissolved F) samples from the 96-h acute toxicity test with *Hyalella azteca*.

Nominal	total F	dissolved F	ratio <sup>b</sup>	total F	dissolved F	ratio	
concentration	day 0	day 0	day 0	day 4	day 4	day 4	
Control	<0.1	0.2	na	< 0.1	< 0.1	na	
3.5	3.5	3.6	1.029	3.7	3.6	0.973	
7.0	7.1	7.1	1.000	6.8	7.1	1.044	
14	11	14	1.273	13	12	0.923	
28	19	23	1.211	17	15	0.882	
56	35	41	1.171	37	33	0.892	
56 Geometric mean				<i></i>			

Geometric mean of day 0 values = 1.132, and day 4 values = 0.941. Overall geometric mean = 1.032

<sup>a</sup> samples were filtered with 0.45  $\mu$ m pore sized cellulose nitrate filters.

<sup>b</sup> ratio = dissolved F divided by total F.

Parameter	mean <sup>*</sup>	5 <sup>th</sup> %ile	95 <sup>th</sup> %ile	min	max	
Temperature (	°C) 22.7	22.4	22.9	22.0	22.9	
D.O. (mg/L)	7.6	6.6	8.4	5.6	8.8	
pН	7.8	7.4	8.0	7.3	8.2	
Hardness (mg	/L) 114	100	120	86	124	
Alkalinity (mg	g/L) 55	50	60	50	60	

Table B.3. Water quality data for chronic bioassays with Hyalella azteca.

\* Mean of 24 measurements throughout the test.

Table B.4. Fluoride measurement data from samples collected on 22 occasions throughout the 42-d chronic bioassay with *Hyalella azteca* 

unougnout	$u = \frac{1}{2}$		<u>554 y with 11 /</u>	uiciiu uzic	iu.		
Nominal	overall	in water	out water	$5^{th}$	$95^{th}$	min	max
Conc.	mean <sup>a</sup>	mean	mean	%ile	%ile		
Control <sup>b</sup>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
1.75 mg/L	1.7	1.7	1.7	1.6	1.8	1.6	1.8
3.5 mg/L	3.3	3.3	3.2	3.2	3.5	2.8	3.5
7 mg/L	6.7	6.7	6.6	6.6	6.8	6.5	6.8
14 mg/L	11.7	11.4	12.1	10.0	13.0	10	14
<u>28 mg/L</u>	16.7	15.5	18.5	13.1	21.0	14	24
9							

<sup>a</sup> All means are geometric means.
 <sup>b</sup> Fluoride was never found in detectable concentrations in the control.

Table B.5. Nominal and measured fluoride concentrations (mg F/L) for unfiltered (total				
F) and filtered <sup>a</sup> (dissolved F) samples from the 42-d chronic toxicity test with <i>Hyalella</i>				
<i>azteca</i> . Both sample 1 and sample 2 were "out" water samples.				

	mpie i ana	Sample 2 we		ator sample	·0•		
Nominal	total F	dissolved F	ratio <sup>b</sup>	total F	dissolved F	ratio	
<u>concentration</u>	sample 1	sample 1	sample 1	sample 2	sample 2	sample 2	
Control	< 0.05	< 0.05	na	< 0.05	< 0.05	na	
1.75	1.7	1.6	0.941	1.7	1.6	0.941	
3.5	3.3	3.2	0.970	3.2	3.2	1.000	
7	6.6	6.7	1.015	6.5	6.6	1.015	
14	13	13	1.000	12	12	1.000	
28	21	20	0.952	20	20	1.000	
$\overline{C}_{comparison}$ and $\overline{C}_{comparison}$ and $\overline{C}_{comparison}$ and $\overline{C}_{comparison}$ and $\overline{C}_{comparison}$ and $\overline{C}_{comparison}$							

Geometric mean of sample 1 values = 0.975, and sample 2 values = 0.991. Overall geometric mean = 0.983 <sup>a</sup> samples were filtered with 0.45 μm pore sized cellulose nitrate filters. <sup>b</sup> ratio = dissolved F divided by total F.

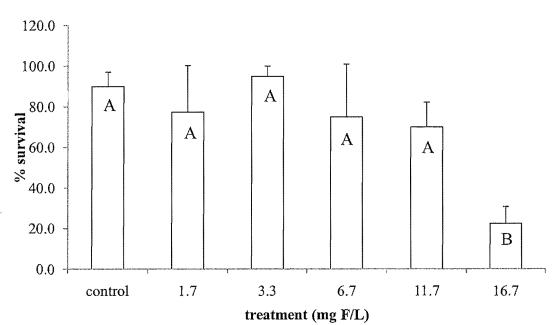


Figure B.1. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five fluoride concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control (p < 0.05).

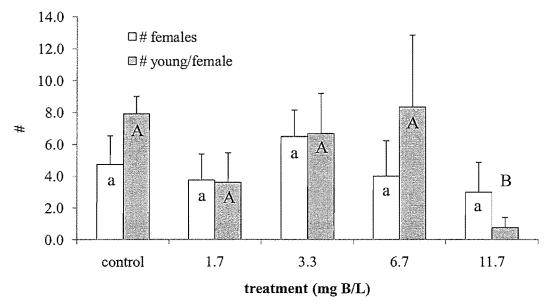


Figure B.2. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four fluoride concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

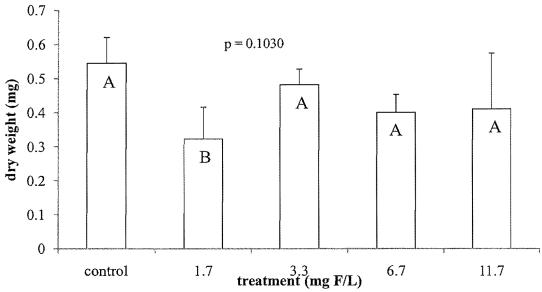


Figure B.3. Mean (error bars = standard deviation) dry weight of individual amphipods in four fluoride concentrations and a control (Borgmann) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

## C. MANGANESE

#### PURPOSE

The purpose of these experiments was to generate both acute and chronic manganese toxicity data with *Hyalella azteca* in the same dilution/control water so that an Acute to Chronic Ratio (ACR) can be developed.

## MATERIALS AND METHODS

#### Culture of test organisms

The amphipod *Hyalella azteca* was cultured in-house (Soucek laboratory, Illinois Natural History Survey) according to U.S. EPA methods (USEPA 2002) with some modifications. Amphipods were cultured in "Borgmann water" (Borgmann 1996), at 23 °C and a 16:8 (L:D) photoperiod, and were fed ~0.5 mg dry flakes (crushed and sieved to  $<500 \mu$ m) of TetraMin® (TetraWerke, Melle, Germany) daily. Approximately 30 adults were held in a 1-L beaker containing 1 L of Borgmann water. Young were removed at least every week or more frequently when a tighter age range was required.

#### Test chemicals and dilution waters

The manganese source for both acute and chronic toxicity tests was a combination of manganese sulfate monohydrate ( $MnSO_4 \cdot H_2O$  Certified ACS, CAS # 10034-96-5, Fisher Scientific, Fairlawn, NJ) and manganese chloride tetrahydrate ( $MnCl_2 \cdot 4H_2O$  Certified ACS, CAS # 13446-34-9, Fisher Scientific, Fairlawn, NJ). For both acute and chronic tests, 44% of the Mn was as manganese sulfate, and 56% was as manganese chloride. This combination was used to keep chloride and sulfate concentrations in solution relatively lower than if either salt was used alone. The dilution water for both the acute test and the chronic test was Borgmann water (Table C.1).

#### Acute test procedures

Static, non-renewal, acute toxicity tests were conducted according to guidelines detailed in ASTM E729-96 (2002). Treatments were comprised of a 50% dilution series. Five concentrations were tested using Borgmann water (Table C.1) as both the diluent and control with four replicates tested per concentration. Organisms were 7- to 14-d old at the beginning of the test. The test was conducted for 96 h with a 16:8 (L:D) photoperiod at  $23 \pm 1$  °C. Test chambers were 50 ml glass beakers with 40 ml of test solution and a 2by 2-cm piece of nitex mesh was added to each test chamber to provide substrate for these benthic invertebrates. Tests were not fed or aerated. Percent survival in each replicate was recorded every 24 h and at the end of the exposure period. A dissecting microscope was used to assess survival. Acceptable control survival was set at 90%.

Standard water chemistry parameters were measured at both the beginning and the end of the exposure period, including temperature, pH, conductivity, dissolved oxygen, alkalinity and hardness. The pH measurements were made using an Accumet<sup>®</sup> (Fisher

Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet<sup>®</sup> gelfilled combination electrode (accuracy <  $\pm$  0.05 pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments (RDP, Dayton, OH, USA) model 55 meter. Conductivity measurements were made using a Mettler Toledo<sup>®</sup> (Fisher Scientific, Pittsburgh, PA, USA) model MC226 conductivity/TDS meter. Alkalinity and hardness were measured by titration as described in American Public Health Association (APHA) et al. (2005). At both the beginning and end of acute tests, water samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of manganese concentrations by inductively couple plasma - atomic emission spectrometry according to U.S. EPA method 200.7 (Martin et al. 1994). To address the potential need to account for total versus dissolved manganese, samples from the acute toxicity test were analyzed for both total and dissolved manganese, samples from the acute toxicity test were analyzed for both total and dissolved manganese, samples were filtered using 0.45 µm cellulose nitrate filters (Whatman®, Maidstone, England). Total manganese was determined with unfiltered samples.

#### Chronic test procedures

Hyalella azteca -- A 42-d, water only, static-renewal, chronic reproduction bioassay was conducted with H. azteca using recommendations detailed in the U.S. EPA sediment toxicity testing guidelines (USEPA 2000), but with modifications. Treatments included five nominal manganese concentrations (0.38, 0.75, 1.5, 3, and 6 mg Mn/L) and a control with no manganese added. The control and dilution water was Borgmann water (Table 1). Test chambers were 300-ml, high form beakers and 200 ml of test solution was used per test chamber. Organisms were 7- to 14-d old at the beginning of the test, and we loaded 10 in to each of four replicate chambers per treatment. A 2.5- by 5-cm piece of nitex mesh was added to each test chamber as a substrate, and organisms were fed dry flakes (crushed and sieved to <500 µm) of TetraMin® (TetraWerke, Melle, Germany) three times per week. Feeding rates were as follows: week 1 - 1 mg per test chamber, weeks 2 and 3 - 1.25 mg per test chamber, weeks 4, 5, and 6 - 2.5 mg per test chamber. Test solutions were not aerated. Every three to four days, complete water renewals were conducted, with test organisms being transferred to new beakers containing fresh test solutions. After each changeover, "in water " and "out water" samples from each treatment were collected and submitted to Underwriters Laboratories, South Bend, IN, for confirmation of manganese concentrations by inductively couple plasma - atomic emission spectrometry according to U.S. EPA method 200.7 (Martin et al. 1994). Survival was evaluated with every changeover. After the first appearance of mating pairs, the number of pairs per test chamber was recorded daily, and discarded tests solutions (after changeovers) were carefully searched for young. Young began to appear on day 28, and the number produced was recorded until the end of the test (day 42). At the end of the test, adult amphipods were sexed and then dried in an oven (60 to 70  $^{\circ}$ C) for at least 48 h before they were weighed to the nearest 0.001 mg. Endpoints calculated included % survival, mean dry weight (per individual), number of mating pairs, # of young per female.

# Statistical analysis

The LC50 value was calculated using the trimmed Spearman-Karber method (USEPA 2002). For the chronic toxicity test, we followed guidelines detailed in U.S. EPA (2002). Briefly, data for survival, and sub-lethal endpoints (amphipod dry weight, # females, # young per female) were tested for normality using the Shapiro-Wilk's Test, and homogeneity of variance using Bartlett's test. Data that passed both of these tests were analyzed for differences among means using Dunnett's test. Those that did not pass normality or homogeneity of variance tests were analyzed using Steel's Many-One test. The No Observable Adverse Effects Concentration (NOAEC) was the highest concentration whose mean for a given endpoint was not significantly different from that of the control, and the Least Observable Adverse Effect Concentration (LOAEC) was the lowest concentration whose mean was significantly different from the control. We also calculated Maximum Acceptable Toxicant Concentrations (MATC) as the geometric mean of the LOAEC and the NOAEC, and Acute to Chronic Ratios (ACR) as the LC50 divided by the MATC.

#### RESULTS

#### Acute toxicity

For the 96-h acute manganese toxicity test with *Hyalella azteca*, mean water temperatures remained within 1 °C of the target ( $22.7 \pm 0.3$  SD), the mean pH value was  $7.8 \pm 0.1$ , and mean dissolved oxygen was  $8.2 \pm 0.3$  mg/L. Hardness, measured in the controls only because manganese is a divalent cation that interferes with the hardness measurement was 112 mg/L as CaCO<sub>3</sub>.

In the 96-h fluoride toxicity test with *Hyalella azteca*, control survival was 95% at the end of the test, and the measured 96-h LC50 was 11.0 mg Mn/L (8.6 - 14.195% confidence interval).

# Chronic toxicity

Basic water quality parameters in the 42-d chronic static renewal bioassay with *Hyalella azteca* are provided in table C.2. Temperature variability was within acceptable limits, and dissolved oxygen did not drop below 6.4 mg/L. Measured manganese concentrations were generally similar to nominal concentrations in all treatments, with relatively little variability (Table C.3). Ratios of dissolved to total manganese concentration were determined on six occasions throughout the 42-d test (Table C.4): three times with "In water" samples or new water to be used for changeovers, and three times with "Out water" samples or water removed from test chambers during a changeover. The geometric mean of ratios (dissolved Mn/total Mn) for "in water" sets was 0.989, and for "out water" sets it was 0.973. The overall geometric mean of ratios throughout the test was 0.981.

At the end of 42 d, % survival of the controls was 92.5%, and survival in the three lowest manganese treatments (measured 0.3, 0.7, and 1.4 mg/L) was relatively high, ranging

from 80 to 94.7%. Both of the highest two concentrations (2.9 and 5.7 mg/L) had significantly lower survival than the control (Fig. C.1). After excluding the highest two treatments from further analysis because of their lower survival rates, there were no differences among treatments in the number of females present, the number of young produced per female (Fig. C.2) or mean dry weight per individual (Fig. C.3).

<u>Chronic values</u> –The NOAEC (1.4 mg/L) and LOAEC (2.9 mg/L) values were derived from the survival data as no significant differences were observed in the sub-lethal endpoints. This resulted in an MATC of 2.0 mg/L, and with the 96-h LC50 of 11.04 mg/L, the ACR was 5.5.

Table C.1. Salt concentrations (mg/L) added to deionized water for generation of dilution waters used for acute and chronic manganese toxicity testing with *Hyalella azteca*.

Water name	KCl	NaHCO <sub>3</sub>	MgSO <sub>4</sub> (an)	CaSO <sub>4</sub> (an)	CaCl <sub>2</sub>	NaBr
Borgmann	4	84	30	0	111	1

Parameter	mean*	5 <sup>th</sup> %ile	95 <sup>th</sup> %ile	min	max	
Temperature (°	C) 22.8	22.1	23.3	22.0	23.6	
D.O. (mg/L)	7.8	7.0	8.4	6.4	8.5	
pH	7.8	7.6	8.2	7.5	8.3	
Hardness <sup>a</sup> (mg	/L) 115	112	118	112	125	
Alkalinity (mg	/L) 52	50	54	50	60	

Table C.2. Water quality data for 42-d chronic Mn bioassay with Hyalella azteca.

\* Mean of 24 measurements throughout the test.

<sup>a</sup> Hardness measured in control only. Mn is a divalent cation and interferes with hardness measurement.

Table C.3. Manganese measurement data from unfiltered (total Mn) samples collected on
24 occasions throughout the 42-d chronic bioassay with Hyalella azteca

<u>27 000001011</u>	o unou sinc		emonie oros			1000	
Nominal	overall	in water	out water	$5^{\text{th}}$	95 <sup>th</sup>	min	max
Conc.	mean <sup>a</sup>	mean	mean	%ile	%ile		
Control <sup>b</sup>	< 0.01	< 0.01	< 0.01	< 0.01	0.011	< 0.01	0.011
0.38 mg/L	0.3	0.4	0.3	0.3	0.4	0.2	0.4
0.75 mg/L	0.7	0.7	0.7	0.6	0.8	0.6	0.8
1.5 mg/L	1.4	1.4	1.4	1.3	1.5	1.3	1.6
3 mg/L	2.9	2.9	2.9	2.8	3.1	2.8	3.1
<u>6 mg/L</u>	5.7	5.7	5.7	5.5	6.1	5.5	6.1

<sup>a</sup> All means are geometric means.

<sup>b</sup> Manganese was detected on one occasion in the control.

<u>Hyalella azteca.</u>	Six differe	ent sets of sar	nples were	e measured	for total and	dissolved Mn.
Nominal	set 1 (in) set 2 (out)					
concentration	total	dissolved	ratio <sup>b</sup>	total	dissolved	ratio
Control	< 0.01	< 0.01	na	< 0.01	< 0.01	na
0.38	0.38	0.38	1.000	0.38	0.34	0.895
0.75	0.77	0.76	0.987	0.78	0.71	0.910
1.5	1.6	1.5	0.938	1.5	1.4	0.933
3	3.1	3.0	0.968	3.1	2.8	0.903
6	6.1	6.1	1.000	6.1	5.5	0.902
Nominal		set 3 (in)			set 4 (out)	
<u>concentration</u>	total	dissolved	ratio <sup>b</sup>	total	dissolved	ratio
Control	< 0.01	0.01	na	< 0.01	0.013	na
0.38	0.35	0.35	1.000	0.35	0.35	1.000
0.75	0.70	0.69	0.986	0.68	0.73	1.074
1.5	1.4	1.4	1.000	1.4	1.5	1.071
3	2.8	2.8	1.000	2.9	2.9	1.036
6	5.5	5.5	1.000	5.6	5.9	1.054
Nominal		set 5 (in)			set 6 (out)	
<u>concentration</u>	total	dissolved	ratio <sup>b</sup>	total	dissolved	ratio
Control	< 0.01	0.029	na	< 0.01	0.014	na
0.38	0.36	0.35	0.972	0.23	0.22	0.957
0.75	0.72	0.71	0.986	0.59	0.58	0.983
1.5	1.4	1.4	1.000	1.3	1.3	1.000
3	2.8	2.8	1.000	2.9	2.7	0.931
6	5.7	5.7	1.000	5.7	5.6	0.982

Table C.4. Nominal and measured manganese concentrations (mg Mn/L) for unfiltered (total Mn) and filtered<sup>a</sup> (dissolved Mn) samples from the 42-d chronic toxicity test with *Hvalella azteca*. Six different sets of samples were measured for total and dissolved Mn.

Geometric mean of ratios for "in water" sets = 0.989, and for "out water" sets = 0.973. Overall geometric mean of ratios = 0.981

<sup>a</sup> samples were filtered with 0.45  $\mu$ m pore sized cellulose nitrate filters.

<sup>b</sup> ratio = dissolved Mn divided by total Mn.

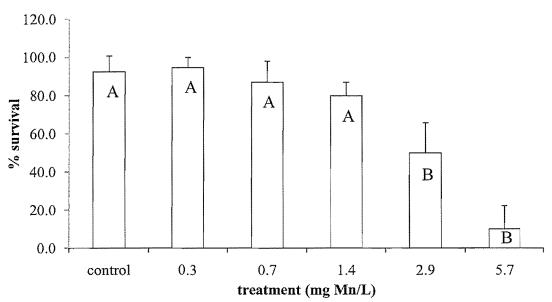


Figure C.1. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five manganese concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control (p < 0.05).

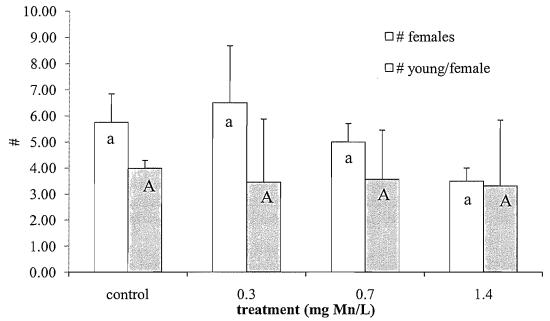


Figure C.2. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four manganese concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

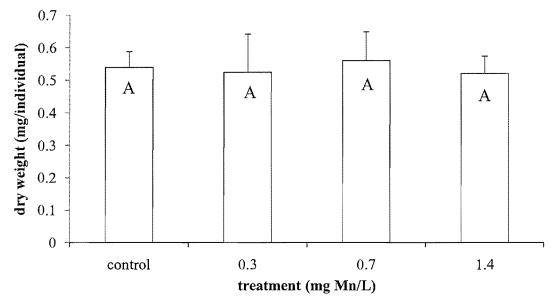


Figure C.3. Mean (error bars = standard deviation) dry weight of individual amphipods in four manganese concentrations and a control (Borgmann) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control (p < 0.05).

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# Attachment 1 – Exhibit V

Excerpts from Exhibit S to Agency Rulemaking Proposal in R02-11

Daphnia magna         LC         Cl         45         <140.3	Species Test Cher	n H Limit	CV/MATC (µg/I)	corrected for H	Reference
LC       CI       45 $<140.3$ $<153.4$ EPA8;         LC       CI       52 $97.190$ 135.8       131.4       EPA8;         LC       CI       104 $43.52$ $47.29$ $47.29$ EPA8;         LC       CI       211 $42.52$ $46.73$ 13.80       EPA8;         21d       - $64.9$ -       122.5 $98.21$ $42$ $40.50d$ S       50       - $25.0$ $25.0$ $52.0$ $22.0$ $31.72$ 16         D. similis       surv S       -       - $59$ $Ceriodaphnia dubia$ $7d$ $40$ $14.25$ $40$ 7d surv CI       169       - $40$ $14.25$ $40$ $50.66$ Moina macrocopa       -       SMCV       gm 26.66 $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.66$ $50.67$ $50.67$ $50.67$ $50.67$ $50.67$	Invertebrates				
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Daphrila magna 👘		٤		
LC       Cl       104       43-52       47.29       47.29       EPA87         LC       Cl       211       42-52       46.73       13.80       EPA87         21d       -       64.9       -       122.5       98.21       42         40-50d S       50       -       25.0       25.0       52       21D7       Cl       225       -       120       31.72       16       .         D. similis       surv S       -       -       <1		45 <140.3	<140.3	<153.4	EPA87
LC       Cl       211 $42.52$ $46.73$ $13.80$ EPA8;         21d       - $64.9$ - $122.5$ $98.21$ $42$ $40.50d$ S       50       - $25.0$ $25.0$ $52$ $21D7$ Cl $225$ - $120$ $31.72$ $16$ D. similis       surv       S       -       <        SMCV       gm < $51.97$ D. similis       surv       S       - $40$ $14.25$ $40$ 7d surv       Cl       169       - $40$ $14.25$ $40$ 7d surv       Cl       169       - $40$ $14.25$ $40$ SMCV       gm 26.66       Moina macrocopa       -       SMCV       gm 674.6         Gammarus pulex       -       Cl       <       -	LC CI	52 97-190	135.8	131.4	EPA87
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	LC Cl	104 43-52	47.29	47.29	EPA87
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	LC CI	211 42-52	46.73	13.80	EPA87
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	21d -	64.9 -	122.5	98.21	42
SMCV       gm <51.97         D. similis       surv S       -       <1	40-50ä S	50 -	25.0	25.0	52
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	21D7 Cl	225 -	120	31.72	16
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	•		SMCV	gm <51.97	
Ceriodaphnia dubia       7d surv Cl       169       40       14.25       40         7d surv Cl       169       140       49.88       40         SMCV       gm 26.66       9       9         Moina macrocopa       5 $\leq$ (?)       353.6       2488       68         M. irrasa       -       Cl $\leq$ -       26.30       182.9       72         Gammarus pulex       -       Cl $\leq$ -       <100	D. similis	•		-	
7d surv Cl       169       -       40       14.25       40         7d surv Cl       169       -       140       49.88       40         SMCV       gm 26.66       9       9       9       9       40         Moina macrocopa       -       S $5(?)$ -       353.6       2488       68         M. irrasa       -       Cl $5$ -       26.30       182.9       72         GMCV       gm 674.6       9       9       72       9       74         Gammarus pulex       -       -       <100	surv S	۲۰۰۰ ۲۰۰۰	<1		59
7d  surv Cl $169$ $40$ $14.25$ $40$ $7d  surv Cl$ $169$ $140$ $49.88$ $40$ $SMCV$ $gm 26.66$ $M$ $macrocopa$ $5$ $5(?)$ $353.6$ $2488$ $68$ $M$ irrasa $ Cl$ $5$ $26.30$ $182.9$ $72$ $GMCV$ $gm 674.6$ $gm 674.6$ $gm 674.6$ $39$ $Hyalella azteca$ $100 - 180$ $42.25$ $18.80$ $10$ $Chironomus riparius$ $pLC$ $ <100$ $ 62$ $Clistornia magnifica$ $LC$ $Cl$ $31$ $>5243$ $>7861$ $EPA8$ $Physa gyrina$ $21d$ $Cl$ $20$ $ 570$ $1219$ $43$ $Erpobdella octulata$ $70d$ LC-50 S $ 100$ $ 67$ $70d$ LC-50 S $ 60$ $ 67$ $67$	Ceriodaphnia dubia				
SMCV       gm 26.66         Moina macrocopa       353.6       2488       68         M. irrasa       -       Cl $5$ 26.30       182.9       72         GMCV       gm 674.6       GMCV       gm 674.6       39         Hyalella azteca       10       -       39         10 wk surv       130       100-180       42.25       18.80       10         Chironomus riparius       -       -       <100		169 -	40	14.25	40
Moina macrocopa       5 $353.6$ $2488$ $68$ M. irrasa       Cl $5$ $26.30$ $182.9$ $72$ GMCV       gm 674.6 $GMCV$ gm 674.6         Gammarus pulex $7d$ GRO S $ <100$ $ 39$ Hyalella azteca       10 wk surv - 130 $100-180$ $42.25$ $18.80$ $10$ Chironomus riparius       pLC $ <100$ $ 62$ Clistornia magnifica $1C$ $Cl$ $31$ $>5243$ $>7861$ $EPA8$ Physa gyrina $21d$ Cl $20$ $ 570$ $1219$ $43$ Erpobdella octulata $70d$ LC-50 S $ 60$ $ 67$	7d surv Cl	169 -	140	49.88	40
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M. irrasa       Cl       <5 $26.30$ 182.9       72         GMCV       gm 674.6         Gammarus pulex $7d$ GRO S       -       <100	Moina macrocopa				
M. irrasa       Cl       <5 $26.30$ 182.9       72         GMCV       gm 674.6 $72$ $GMCV$ gm 674.6         Gammarus pulex $7d$ GRO S $ <100$ $ 39$ Hyalella azteca $100$ $ <100$ $ 39$ Hyalella azteca $100-180$ $42.25$ $18.80$ $10$ Chironomus riparius $pLC$ $ <100$ $ 62$ Clistornia magnifica $LC$ $CI$ $31$ $>5243$ $>7861$ $EPA8$ Physa gyrina $21d$ $CI$ $20$ $ 570$ $1219$ $43$ Erpobdella octulata $70d$ LC-50 S $ 100$ $ 67$ $70d$ LC-50 S $  60$ $ 67$	'- S	-<5(?) -	353.6	2488	68
GMCV       gm 674.6         Gammarus pulex       7d GRO S       -       <100	M. irrasa	.,		,	
Gammarus pulex       7d GRO S       -       <100	- Cl	<5 -	26.30	182.9	72
7d GRO S       -       <100	. 1	4 ,	GMCV	gm 674.6	
7d GRO S       -       <100				×	×
Hyalella azteca       10 wk surv - 130       100-180       42.25       18.80       10         Chironomus riparius       pLC        <100			· ·		
10 wk surv - 130       100-180       42.25       18.80       10         Chironomus riparius       pLC -       -       <100			<100	-	39
Chironomus riparius       9LC       -       <100		•	,		,
pLC       -       <100			42.25	18.80	10
Clistornia magnifica       LC       Cl 31       >5243       >7861       EPA8         Physa gyrina       21d       Cl 20       -       570       1219       43         21d       Cl 20       -       570       1219       43         Erpobdella octulata       -       67       67         70d LC-50       S       -       60       -       67		5	•		
LC       Cl       31       >5243       >7861       EPA8         Physa gyrina       21d       Cl       20       -       570       1219       43         21d       Cl       20       -       570       1219       43         Erpobdella octulata       -       100       -       67         70d LC-50 S       -       -       60       -       67	*		<100		62
Physa gyrina       21d       Cl       20       -       570       1219       43         Erpobdella octulata       70d LC-50       S       -       100       -       67         70d LC-50       S       -       60       -       67			×		
21d       Cl       20       -       570       1219       43         Erpobdella octulata       70d LC-50       S       -       100       -       67         70d LC-50       S       -       60       -       67		31	>5243	>7861	EPA87
Erpobdella octulata 70d LC-50 S 100 - 67 70d LC-50 S 60 - 67	*				
70d LC-50 S       -       100       -       67         70d LC-50 S       -       -       60       -       67		~	570	1219	43.
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SMCV gm 77.46	70d LC-50 S	yang yang yang yang yang yang yang yang	energy and the second		67
		SMCV gn	n 77.46	x	·

19. Chronic Toxicity. List species in descending order of tolerance by MATC.

•

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species Te	st Che	m H	Limit	CV/ MATC (µg/l	) corrected for I	I	Reference
- Labrates					` .	•	
Pimephales	promel	as			+	•	
IC	S	46		78-145	106.3		EPA87
terato	S	100		600	333.5		14
4	S	100		800	444.7		14
gro		159.	т	1039	389.9		24
<i>b</i> ~-	. S	46.5	¢	188	. 199.9		47.
	S	46.5		186	197.8		47
	S	46.5	, ,	186	197.8		47
	S	46.5		183	194.6		47
	S	46.5		183	194.6		47
	S	46.5		187	198.9		47
·	S	46.5		412	438.11		47
<i>.</i> .	S	46.5		405	430.7!		47
¢			ν.	SMCV	gm 254.6		
Poecilia reti	culata				· · ·		w.
LC	S	30		<173	<266.7	۰.	EPA87

# Plants etc: many

.

20. Reject unacceptable data and assign rank as in Item 11 above

Genus	GMCV µg/L
11.) Clistornia	>7861
10.) Physa	1219
9) Moina	674.6
8.) (Poecilia	<266.7)
7.) Pimephales	<254.6
6.) Gammarus	· <100
5.) Chironomus	. <100
4.) Erpobdella	77.46
3.) Daphnia	<51.97
2.) Ceriodaphnia	26.66
1.) Hyalella	18.80

# Attachment 1 – Exhibit W

# Accumulation, regulation and toxicity of copper, zinc, lead and mercury in Hyalella azteca

U. Borgmann, W.P. Norwood & C. Clarke, <u>Hydrobiologia</u>, 259: 79 – 89 (1993)

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### Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*

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#### Abstract

Zinc, lead and mercury accumulation in the amphipod Hyalella azteca increases with increasing exposure to metals. During 10 week chronic toxicity tests, metal accumulated at the highest non-toxic/lowest toxic concentration was  $126/136 \ \mu g \ Tn g^{-1}$ ,  $7.1/16 \ \mu g \ Pb g^{-1}$  and  $56/90 \ \mu g \ Hg g^{-1}$  dry weight. Concentrations of lead and mercury in control animals were substantially lower (1.3  $\mu g \ Pb \ g^{-1}$  and 0.4  $\mu g \ Hg \ g^{-1}$ ), but concentrations of zinc in controls ( $74 \ \mu g \ g^{-1}$ ) were about one half those of the lowest toxic concentration. Copper was completely regulated. Accumulated copper concentrations after 10 weeks exposure to all waterborne copper concentrations resulting in less than 100% mortality were not significantly different from controls ( $79 \ \mu g \ g^{-1}$ ). Lead and mercury concentrations in wild H. azteca should be useful indicators of potential toxicity. Zinc accumulation may also be a useful indicator of zinc toxicity, but careful comparison with control or reference animals is necessary because of the small differences between toxic and control concentrations. Copper is not accumulated by H. azteca under chronic exposure conditions and body burdens of field animals cannot be used as an indicator of exposure or potential toxic effects. Short term exposures to copper, however, result in elevated copper concentrations in H. azteca, even at concentrations below those causing chronic toxicity. Short term bioaccumulation studies might, therefore, provide a useful indication of potential chronic copper toxicity.

#### Introduction

Although most aquatic toxicity studies with metals have related toxicity to waterborne concentrations, the toxicity of non-regulated metals to crustacea may be much easier to predict from concentrations measured in the animals themselves, rather than in the medium. For example, chronic toxicity of cadmium to the amphipod *Hyalella azteca* in the presence of various complexing agents or sediments varied over a 5200-, 36-, or 2.6-fold range depending on whether toxicity was expressed as Cd added, Cd measured in water (*i.e.* not adsorbed to sediments), or Cd accumulated in *H. azteca*, respectively (Borgmann *et al.*, 1991). Similarly, the toxicity of organic and inorganic forms of mercury differs greatly when expressed as concentration in water, but accumulation in equitoxic solutions is often remarkably similar. For example, mercury accumulated at concentrations resulting in approximately 50% mortality in barnacles (*Elminius modestus*) and

Artemia salina after 3 hr ranged from 280 to 920  $\mu$ g g<sup>-1</sup> dry weight, even though A. salina were 100 to 5000 times more resistant to mercury than barnacles, and amylmercuric chloride was 20 to 1000 times more toxic than mercuric chloride (Corner & Rigler, 1958). Organic and inorganic forms of mercury also had approximately equivalent toxicity to Daphnia magna (Biesinger et al., 1982) and the amphipod Bathyporeia pilosa (Khayrallah, 1985) on a body burden basis, but not as a function of concentration in water. These studies suggest that measurements of metal accumulation in field animals should be a much more reliable indicator of potential metal toxicity to natural populations of crustacea than the concentration in water. However, much more data is needed on the relationship between toxicity and accumulation of metals before body burdens can be widely used to estimate the impacts of environmental contamination by metals.

For non-regulated metals such as cadmium, lead and mercury, accumulation within a given medium is usually an allometric function of waterborne concentrations, but copper and zinc are regulated in many fish and higher invertebrates (e.g. Amiard *et al.*, 1987). Lower invertebrates, however, demonstrate varying degrees of copper and zinc regulation (Amiard *et al.*, 1987, Rainbow & White, 1989). If potential copper and zinc toxicity are to be inferred from concentrations in aquatic biota collected from the field, then species displaying poor regulatory capabilities for these metals should be chosen.

Hyalella azteca is an ideal organism for the assessment of metal toxicity because it is very sensitive to metals (Borgmann et al., 1989b), is found throughout most of North America, is easy to identify, and is amenable to laboratory culture and toxicity testing. It is a benthic organism and can be used for testing the toxicity of both waterborne contaminants and sediments (Borgmann & Munawar, 1989). It can also be readily collected in the field for studies on metal levels in wild populations (e.g. Stephenson & Mackie, 1988). It should, therefore, be a useful organism for elucidating the relative contributions of different metals to toxicity, both in the field and in laboratory assays (e.g. sediment toxicity tests), by comparing metal concentrations accumulated by H. azteca with body burdens previously shown to be associated with toxicity. The relationship between cadmium accumulation and toxicity to H. azteca has already been established (Borgmann et al., 1991). This paper describes the relationship between copper, zinc, lead and mercury accumulation and toxicity, and examines the ability of H. azteca to regulate copper and zinc during chronic exposure.

#### Methods

Amphipods were cultured as described in Borgmann et al. (1989b), and bioassay procedures followed Borgmann et al. (1991), except that experiments lasted a full 10 weeks to ensure that effects on reproduction were adequately assessed. Toxicity tests were initiated with twenty 0-1 week old young in 250 ml of dechlorinated Burlington City tap water (originating from Lake Ontario, hardness 130 mg 1<sup>-1</sup>, alkalinity 90 mg 1<sup>-1</sup>, pH 7.9-8.6) in 500 ml Erlenmeyer flasks with loose fitting glass covers and one 5 by 10 cm piece of pre-soaked cotton gauze as substrate. Experiments were conducted in an incubator at 25 °C with a 16 h light: 8 h dark photoperiod. The animals were placed in fresh flasks with renewed water and metals once a week, at which time the number of survivors were counted, young were counted and removed, and 5 mg of Tetra-Min fish food flakes were added as food. Samples of the water were acidified and saved for metal analysis. Additional food was added during the week as required. The animals were weighed on weeks 4, 6, 8 and 10 as described in Borgmann et al. (1989b). Four replicates were run for each control, copper, zinc and lead concentration (2 were set up one week, and 2 another week), and 2 replicates for each mercury concentration.

At the end of the experiments the surviving amphipods were dried at 60 °C, and digested as described in Borgmann *et al.* (1991) using the procedure of Stephenson & Mackie (1988). Twenty-five  $\mu$ l of 70% nitric acid was added to

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1 to 4 amphipods (0.5 to 2 mg dry weight), and allowed to sit for 1 week. Then 17  $\mu$ l of 30% hydrogen peroxide was added, followed after 24 hr by 1 ml of double distilled water.

In addition to the 10 week experiments, copper and zinc accumulation were also determined in 4 week old *H. azteca* exposed to copper or zinc for only 1 week. All other parameters were identical to the 10 week chronic tests.

Water and digested samples were analyzed for copper, zinc and lead using a Varian SpectraAA 400 graphite furnace atomic absorption spectrophotometer with Zeeman background correction. Copper was measured in a partition tube without modifier. Zinc and lead were analyzed using a platform and ammonium phosphate modifier. QC blanks and standards were run every 10th sample.

Mercury samples were analyzed by cold vapour atomic absorption spectrophotometry using a Laboratory Data Control (LDC) UV monitor (Model 1205) with a 30 cm double beam gas flow cell following a procedure modified after Daniels & Wigfield (1989). Four ml of double distilled water and 1 ml of 35% (w/v) sodium hydroxide were delivered to the reduction chamber, a midget impinger, and the top secured. The monitor was then adjusted to zero with the gas flow on. The gas flow was then turned off, 1 ml of reduction solution (2 g stannous chloride, 0.2 g L-cysteine, 1 g sodium chloride and 12.5 ml concentrated sulphuric acid in 100 ml double distilled water) and 1 ml of sample were added, the impinger was sealed and the gas flow turned on. The absorbance peak at 254 nm was recorded on a chart recorder and compared to a standard curve.

#### Results

Measured metal concentrations in water at the end of each week of exposure and prior to the addition of fresh toxicant were always lower than nominal concentrations, except at the lowest copper concentrations (Tables 1 to 4). This decrease was most severe for lead, and least for copper. Detailed studies showed that if the flasks were acidified before the water samples were removed, then most of the metal was recovered. For example, measured concentrations of a  $100 \,\mu g \, l^{-1}$ nominal lead solution were  $96.1 \,\mu g \, l^{-1}$  initially. After one week the measured lead concentrations

Table 1. Percent Survival, wet weight (mg) and total number of young produced per initial animal added ( $\pm$  S.D.) by week 6 and 10, and copper accumulated ( $\mu$ g g<sup>-1</sup> dry weight  $\pm$  S.D.) by week 10. Lowest concentration with significantly reduced survival (chi-square, P < 0.01) indicated with \*\*. Copper accumulated at each concentration was not significantly different from control.

Nominal copper concentration:	Control	5.6 µg 1 <sup>-1</sup>	10 µg l <sup>-1</sup>	18 μg l <sup>-1</sup>	32 μg l <sup>-1</sup>	56 $\mu$ g l <sup>-1</sup>	$100 \mu g  l^{-1}$
Measured concentr	ation						
μgl <sup>-1</sup>	3.5 <u>+</u> 1.4	7.7 <u>+</u> 1.8	10.7 ± 1.3	16.7 <u>+</u> 1.6	25.4 <u>+</u> 2.8	43.8 ± 8.2	81.3 <u>+</u> 9.0
n	20	20	20	20	20	20	16
Week 6 $(n = 4)$							
Survival (%)	71 <u>+</u> 14	68 <u>+</u> 3	69 <u>+</u> 13	63 <u>+</u> 9	41 ± 23**	36 <u>+</u> 22	3 <u>+</u> 3
Weight	$2.1 \pm 0.6$	2.1 ± 0.9	$2.1 \pm 0.9$	$2.0 \pm 0.4$	1.8 <u>+</u> 0.4	$1.4 \pm 0.3$	$0.1 \pm 0$
Young	$0.3 \pm 0.4$	$0.6 \pm 0.4$	$0.6 \pm 1.0$	$0.2 \pm 0.3$	$0.1 \pm 0.3$	$0.04 \pm 0.10$	0
Week 10 $(n = 4)$							
Survival	54 + 18	54 + 13	50 + 4	40 + 14	29 + 25 **	6 + 13	0
Weight	$4.0 \pm 0.7$	$3.5 \pm 1.1$	$4.0 \pm 0.6$	$3.6 \pm 0.7$	$4.3 \pm 0.5$	$3.4 \pm 0$	-
Young	$3.4 \pm 3.0$	$3.9 \pm 1.3$	$3.7 \pm 3.0$	$1.9 \pm 1.3$	$1.3 \pm 1.2$	$0.8 \pm 0$	-
Cu in <i>Hyalella</i>	79 <u>+</u> 20	91 ± 11	92 <u>+</u> 14	95 <u>+</u> 26	88 <u>+</u> 13	80 ± 5	-
n	8	8	8	8	8	4	-

Nominal zinc concentration:	Control	$32 \mu g  l^{-1}$	56 μg l <sup>-1</sup>	$100 \mu g  l^{-1}$	180 µg l <sup>-1</sup>	320 µg 1 <sup>-1</sup>	560 μg l <sup>-1</sup>
Measured concent	tration						
$\mu g l^{-1}$	5.6 ± 3.8	$13.0 \pm 8.9$	21.2 + 8.9	42.3 + 16.6	$108 \pm 32$	185 + 67	316 + 129
n	20	20	20	20	20	20	20
Week 6 $(n = 4)$							
Survival (%)	75 <u>+</u> 5	65 <u>+</u> 9.1	69 <u>+</u> 14	72 ± 10	68 ± 10	32 ± 17**	8 ± 12
Weight	$1.4 \pm 0.3$	$1.8 \pm 0.6$	$1.5 \pm 0.4$	$1.9 \pm 0.2$	$1.7 \pm 0.3$	$1.7 \pm 0.3$	$1.8 \pm 0.6$
Young	$0.1 \pm 0.1$	0.2 <u>+</u> 0.4	$0.3 \pm 0.4$	$0.5 \pm 0.2$	0	0	0
Week $10 (n = 4)$							
Survival	63 <u>+</u> 8	50 ± 8	56 + 23	51 + 11	35 ± 17 **	6±5	3 ± 3
Weight	$3.7 \pm 0.2$	$3.6 \pm 0.9$	$3.7 \pm 1.1$	$3.1 \pm 0.5$	$3.0 \pm 0.9$	$4.2 \pm 1.8$	$3.6 \pm 2.3$
Young	$1.9 \pm 1.0$	$2.2 \pm 1.2$	$2.0 \pm 1.1$	$2.7 \pm 0.9$	$1.0 \pm 0.4$	0	0
Zn in Hyalella	74 <u>+</u> 27	66 <u>+</u> 7	85 ± 14	126 ± 46**	136 ± 39	167 ± 22	167±53
n	15	15	15	28	19	4	2

Table 2. Percent Survival, wet weight (mg) and total number of young produced per initial animal added ( $\pm$  S.D.) by week 6 and 10, and zinc accumulated ( $\mu$ g g<sup>-1</sup> dry weight  $\pm$  S.D.) by week 10. Lowest concentration with significantly reduced survival (chi-square, P < 0.01) or elevated zinc in *Hyalella azteca* (ANOVA, p < 0.01) indicated with \*\*.

Table 3. Percent Survival, wet weight (mg) and total number of young produced per initial animal added ( $\pm$  S.D.) by week 6 and 10, and lead accumulated ( $\mu$ g g<sup>-1</sup> dry weight  $\pm$  S.D.) by week 10. Lowest concentration with significantly reduced survival (P < 0.01) or elevated lead in *Hyalella azteca* (P < 0.01) indicated with \*\* (or with \* at p < 0.05, if different from P < 0.01).

Nominal lead concentration:	Control	$18 \mu g  l^{-1}$	32 µg l <sup>-1</sup>	56 $\mu$ g 1 <sup>-1</sup>	$100 \mu g  l^{-1}$	$180 \mu g  l^{-1}$	320 µg 1 <sup>-1</sup>
Measured concent	tration						
$\mu g l^{-1}$	$0.4 \pm 0.6$	3.3 ± 1.9	$2.6 \pm 1.3$	11.6 ± 8.6	8.8 ± 7.5	12.6 ± 7.9	24.0 ± 19.4
n	25	15	15	15	20	15	11
Week 6 $(n = 4)$							
Survival (%)	73 <u>+</u> 12	69 <u>+</u> 27	74 <u>+</u> 8	68 <u>+</u> 3	35 <u>+</u> 8**	13 <u>+</u> 6	4 <u>+</u> 5
Weight	$1.7 \pm 0.3$	$2.2 \pm 0.6$	$2.0 \pm 0.8$	$2.6 \pm 1.0$	$0.9 \pm 1.0$	$2.0 \pm 0.7$	$1.1 \pm 1.4$
Young	$0.4 \pm 0.4$	$0.5 \pm 0.3$	$0.1 \pm 0.2$	$0.4 \pm 0.6$	$0.1 \pm 0.1$	0	0
Week 10 $(n = 4)$							
Survival	66 ± 10	60 + 25	65 + 6	48 + 13*	31 ± 8**	11 + 5	4+5
Weight	$3.5 \pm 0.7$	$4.0 \pm 0.3$	3.3 + 1.4	4.4 + 0.7	$3.4 \pm 0.9$	$4.5 \pm 1.0$	2.1 + 2.8
Young	$4.2 \pm 3.0$	$5.8 \pm 1.5$	$4.0 \pm 1.9$	3.6 <u>+</u> 3.0	$1.8 \pm 0.7$	$0.4 \pm 0.6$	0
Pb in <i>Hyalella</i>	$1.3 \pm 1.4$	5.8 ± 3.8**	7.1 ± 3.6	15.8 ± 5.7	19.2 ± 16.4	30.0 ± 15.4	20.9 ± 0.9
n	29	17	20	15	11	4	2

in the same flask were 26.8  $\mu$ g l<sup>-1</sup> before acidification, and 96.7  $\mu$ g l<sup>-1</sup> after acidification of the entire flask. The difference between measured and nominal concentrations is, therefore, due to ad-

sorption of metal to the glass, gauze and/or food and detritus in the flasks. This adsorption was relatively fast; measured lead concentrations dropped to approximately one half of nominal

Nominal mercury concentration:	Control	$3.2 \mu g  l^{-1}$	$5.6 \mu g  l^{-1}$	$10 \mu g  l^{-1}$	18 μg 1 <sup>-1</sup>
Measured concentratio	n				
μg1 <sup>-1</sup>	$0.05 \pm 0.48$	$0.62 \pm 0.52$	$1.12 \pm 0.57$	$2.42 \pm 1.46$	$3.96 \pm 1.48$
n	10	10	10	10	5
Week 6 $(n = 2)$					
Survival (%)	88 ± 11	58 ± 4	70 <u>+</u> 14	25±0**	0
Weight	$1.8 \pm 0.1$	$1.3 \pm 0.3$	$2.1 \pm 0.4$	$1.9 \pm 0.7$	-
Young	$0.2 \pm 0.1$	$0.02 \pm 0.04$	0	0	0
Week 10 $(n = 2)$					
Survival	72 ± 4	58 ± 4	65 ± 7	20±0**	0
Weight	$4.0 \pm 0.1$	3.2 + 0.2	$4.3 \pm 0.3$	$5.0 \pm 1.2$	-
Young	$2.4 \pm 1.1$	$2.9 \pm 0.8$	$4.4 \pm 1.9$	$0.9 \pm 1.3$	0
Hg in <i>Hyalella</i>	$0.42 \pm 0.06$	25±6**	56 ± 14	90 ± 32	_
n	4	4	4	4	

Table 4. Percent Survival, wet weight (mg) and total number of young produced per initial animal added ( $\pm$  S.D.) by week 6 and 10, and mercury accumulated ( $\mu g g^{-1}$  dry weight  $\pm$  S.D.) by week 10. Lowest concentration with significantly reduced survival (P < 0.01) or elevated mercury in *Hyalella azteca* (P < 0.01) indicated with \*\*.

within 2 hr. Initially, and during the weekly water changes, therefore, fresh flasks with food and toxicant were set up at least 2–4 hr before the animals were added, ensuring that animals were not exposed to conditions far from equilibrium. The nominal concentrations, therefore, represent the total metal to which the animals were exposed (including metal adsorbed to food, detritus, gauze and the flasks), whereas the measured concentrations are closer to the mean exposure concentrations in water (including free metal, complexed dissolved and fine particulate).

None of the metals resulted in any significant reduction in growth, as judged by wet weight, or reproduction at any concentration which did not also cause significant chronic mortality (Tables 1 to 4). The lowest nominal concentration of copper and mercury resulting in mortality after either 6 or 10 weeks was 32 and 10  $\mu$ g l<sup>-1</sup> respectively (P<0.01). Zinc was significantly toxic at 320  $\mu$ g l<sup>-1</sup> after week 6 and at 180  $\mu$ g l<sup>-1</sup> by week 10. The lowest nominal concentration of lead which was toxic was 100  $\mu$ g l<sup>-1</sup> after 6 weeks, but by week 10 significant mortality occurred at 56  $\mu$ g l<sup>-1</sup> as well (P<0.05, Table 3). The time course of mortality was not the same for all metals. Copper and zinc toxicity continued throughout the 10 week exposure, but mortality due to mercury and  $100 \ \mu g \ l^{-1}$  or higher concentrations of lead was highest in the initial 2 weeks. At 56  $\ \mu g \ l^{-1}$ , lead toxicity was low but continued throughout the 10 week exposure, becoming statistically significant by week 10 (Fig. 1).

In spite of differences in the time course of mortality, the shape of the survival: concentration curve was similar for all metals (Fig. 2), including cadmium (Borgmann *et al.*, 1991). The order of toxicity was Cd>Hg>Pb>Cu>Zn, based on final measured metal concentrations. If expressed as a function of nominal metal concentrations in water, the toxicity of copper is greater than lead; the relative toxicity of the other metals remain the same.

Copper concentrations in *H. azteca* were not significantly different from controls at any exposure concentration (Table 1). Zinc, lead and mercury concentrations in *H. azteca*, however, were always significantly elevated starting at exposure concentrations lower than those resulting in significant mortality (Tables 2 to 4). *Hyalella azteca*, therefore, was capable of regulating copper at all concentrations which are chronically toxic, but it

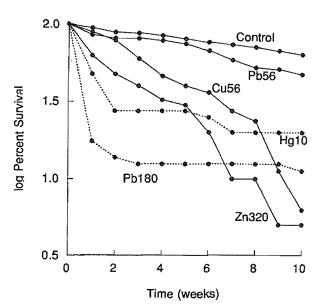


Fig. 1. Time course of mortality at selected metal concentrations. Numbers following the metal symbol represent nominal concentrations in  $\mu g I^{-1}$ .

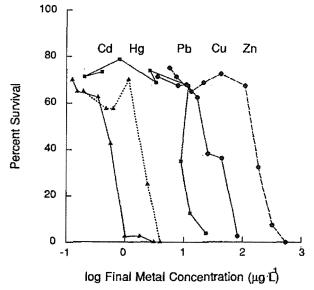


Fig. 2. The relationship between survival after 6 weeks and final measured metal concentrations at the end of each week before water and metal renewal. Data for cadmium are from Borgmann *et al.* (1991).

was unable to regulate zinc as effectively. Although zinc accumulation increased with increasing exposure, the difference between metal

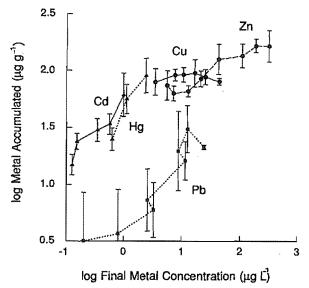


Fig. 3. The relationship between metal accumulation (dry weight basis) and final measured metal concentrations. Data for cadmium are from Borgmann *et al.* (1991). Bars represent  $\pm 1$  standard deviation.

accumulation at the lowest toxic concentration and the control was only about 2-fold, much lower than for lead, mercury or cadmium (Table 5).

Of the 5 metals studied, lead was accumulated least by H. azteca (Fig. 3). The slope of the accumulation:exposure relationship was highest for mercury (approximately 1) and lowest for copper and zinc (Table 5). Copper is completely regulated and the low slope for zinc may be indicative of partial regulation.

The survival:accumulation curves were similar for each of the non-regulated metals (Fig. 4), but were usually steeper than the survival:exposure curves (Fig. 2). The survival:accumulation curve for zinc was similar to that for the non-regulated metals, except that metal concentrations in the control were much closer to toxic concentrations. The survival:accumulation curve for copper was a vertical line, since this metal was regulated. The order of toxicity was the same as that observed for toxicity as a function of metal concentrations added, except that toxicity was highest for lead (Fig. 4). Concentrations of lead tolerated in the body of *H. azteca* were quite a bit lower than for all other metals, even cadmium and mercury.

et al. (1991)	h.					
Metal	Concentration	$(\mu g g^{-1} dry wt.)$	Regression coefficients			
	Control	Highest non-toxic	Lowest toxic	a	Ь	R <sup>2</sup>
Cu	79	95ª	88ª	(1.94)	0	_
Zn	74	126	136	1.46	0.35	0.82
РЬ	1.3	7.1	16	0.40	0.77	0.44

90

30

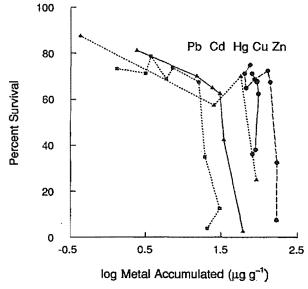
Table 5. Metal concentration in Hyalella azteca for control exposure, the highest exposure concentration showing no significant toxicity, and the lowest concentration with toxicity. Also shown are the intercept (a) and slope (b) coefficients and  $R^2$  for the regression of log metal accumulated against log final measured metal concentration. The data for cadmium are from Borgmann

2.4 <sup>a</sup> Not significantly different from control.

0.4

Hg

Cd



56

23

Fig. 4. The relationship between survival after 6 weeks and metal concentrations accumulated by Hyalella azteca. Data for cadmium are from Borgmann et al. (1991).

#### Discussion

The most sensitive indicator of chronic toxicity was survival. There were no effects on growth or reproduction at any concentration which did not also cause significant chronic mortality (Tables 1 to 4). This is consistent with previous observations on the chronic toxicity of cadmium, pentachlorophenol and PCBs to H. azteca (Borgmann et al., 1989b, 1990), but contrasts with the

chronic toxicity of many metals to Daphnia magna, for which reproductive impairment is often a more sensitive indicator of toxicity than is chronic mortality (Biesinger & Christensen, 1972; Borgmann et al., 1989b). This consistent response of H. azteca to toxicants simplifies comparison of the relative toxicities of different contaminants. It also eliminates the need for measuring growth and reproduction on a routine basis, at least in studies with the toxicants just mentioned.

0.90

0.52

1.62

1.71

Hyalella azteca demonstrated an ability to regulate copper at all concentrations not resulting in complete mortality during chronic exposure, but it was unable to completely regulate zinc (Table 1 and 2, Fig. 3). This was somewhat surprising, since another amphipod, Gammarus zaddachi, regulated zinc reasonably well, but not copper (Amiard et al., 1987). Neither copper nor zinc were completely regulated by the amphipod Echinogammarus pirloti, although zinc accumulation was slow, suggesting some attempt at regulation (Rainbow & White, 1989). Both metals were regulated by the amphipod, Allorchestes compressa, but copper accumulation at all exposure concentrations, although constant, was higher than in the control (Ahsanullah & Williams, 1991). Gammarus duebeni regulated zinc up to external zinc concentrations of 200  $\mu$ g l<sup>-1</sup> (Johnson & Jones, 1989), but Gammarus pulex demonstrated no zinc regulatory ability (Bascombe et al., 1990). The apparent copper and zinc regulatory abilities of

0.76

0.65

amphipods, therefore, appear to vary somewhat from one study to another.

The observed differences in the degree of copper regulation appear to be related, at least in part, to the duration of the experiment. Although H. azteca are excellent regulators of copper during long term exposure (Table 1), this regulation is not instantaneous. Copper was significantly elevated in 4 week old H. azteca exposed to varying copper additions following only 1 week of exposure, even at concentration below those causing chronic toxicity (Table 6, Fig. 5). It is interesting to note that a lack of copper regulation by Gammarus zaddachi was observed after a 4 day exposure (Amiard et al., 1987), whereas regulation by Allorchestes compressa was observed after a 4 week exposure at 19 °C (Ahsanullah & Williams, 1991). Poor regulation by Echinogammarus pirloti was observed after 3 weeks of exposure, but this was done at 10 °C (Rainbow & White, 1989), a lower temperature which may have slowed down the rate of acclimation to copper. Some of the discrepancies regarding copper regulation by amphipods in the literature may, therefore, also be due to the time required for amphi-

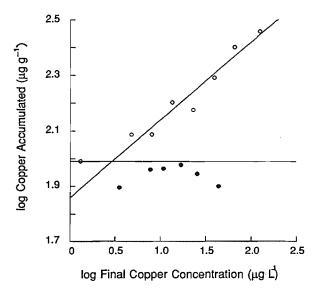


Fig. 5. Comparison of copper accumulation during 10 week chronic (solid symbols) and 1 week (open symbols) exposure to various final copper concentrations. The horizontal line represents the concentration of copper in control amphipods in the 1 week exposure experiment.

pods to adapt to a copper stress, after which regulation is possible.

Our results suggest that copper concentrations in wild *H. azteca*, and in *H. azteca* exposed to copper under chronic conditions in the laboratory, cannot be used to accurately infer the presence or absence of copper toxicity. However, short term exposures in the laboratory will result in elevated copper accumulation at concentrations well below those resulting in chronic toxicity. Short term bioaccumulation could, therefore, potentially be used as an indicator of chronic effects.

Unlike copper, regulation of zinc was not observed during chronic exposure (Table 2). Furthermore, preliminary experiments suggested that 1 week exposures to elevated zinc concentrations in water result in accumulation similar to that obtained following 10 weeks of exposure. Concentrations of zinc in H. azteca can, therefore, indicate exposure to toxic levels of zinc, but only a small (2 fold) elevation in body zinc concentration can be associated with toxicity (Table 5), so careful measurement of zinc concentrations in control animals will be required. This is similar to observations with shrimp. Palaemon elegans, for example, regulates zinc at about 80  $\mu$ g g<sup>-1</sup> dry weight. At external zinc concentrations above 316  $\mu$ g 1<sup>-1</sup> the regulatory mechanism breaks down, resulting in elevated tissue concentrations. The maximum accumulation tolerated is about

Table 6. Copper accumulated ( $\mu g g^{-1}$  dry weight  $\pm$  S.D.) by 4 wk old *Hyalella azteca* after 1 wk exposure to various copper additions ( $\mu g l^{-1} \pm$  S.D.). Amphipod wet weight averaged 0.94  $\pm$  0.40 mg. Accumulation at all concentrations was significantly greater than in the control (P < 0.01).

Nominal concentration	Measured in water	Cu accumulated in <i>Hyalella</i>	n
0	1.3 <u>+</u> 0.4	98 <u>+</u> 21	16
5.6	4.8 ± 0.5	122 <u>+</u> 22	16
10	8.0 <u>+</u> 0.6	123 ± 22	16
18	$13.3 \pm 2.0$	159 <u>+</u> 41	16
32	22.8 ± 1.1	150 <u>+</u> 42	14
56	39.2 <u>+</u> 2.3	196 <u>+</u> 43	16
100	65.1 <u>+</u> 7.7	252 <u>+</u> 38	12
180	124 <u>+</u> 19	288 <u>+</u> 140	9

200  $\mu$ g g<sup>-1</sup> (Rainbow & White, 1989). The total range in body burdens of zinc observed in amphipods and shrimp, from control to toxic concentrations, is, therefore, much less than obtained with non-regulated metals.

The observation of regulation (for copper) or partial regulation (for zinc) does not imply that metal concentrations in amphipods are controlled by active excretion. For example, exposures to elevated zinc concentrations as high as  $1000 \ \mu g$  $1^{-1}$  result in increases in whole body zinc concentrations in talitrid amphipods of only about 2 fold, but all zinc accumulated is retained and there is no evidence of zinc excretion (based on <sup>65</sup>Zn uptake studies). The relatively low degree of metal accumulation is the result of a low uptake rate and dilution of accumulated zinc in the increased body mass as the amphipods grow (Weeks & Rainbow, 1991).

The relationships between toxicity and metal accumulation presented here apply to chronic exposures only. Toxicity could occur at lower body burdens under acute exposure. At higher, acutely toxic, metal concentrations, damage may occur to sensitive tissues (e.g. respiratory epithelia) before extensive metal accumulation occurs. At lower metal concentrations and long term exposures, such as those reported here, gradual metal uptake could result in metal deposition in non-critical tissues (e.g. perhaps the exoskeleton) resulting in a higher overall body metal concentration but a lower metabolically active fraction. In the present study metal accumulation was measured only after 10 weeks of exposure, and the 'safe' concentrations of accumulated metals reported should not be construed as being safe under short term exposure conditions to higher metal concentrations.

There are relatively few published data on the concentrations of accumulated lead and mercury associated with toxicity to crustacea. Mortality was observed at accumulated lead concentrations above 20  $\mu$ g g<sup>-1</sup> in the soft tissues of *Gammarus pulex* (Bascombe *et al.*, 1990), similar to *H. azteca* (Table 5) although our data are for whole animals. Khayrallah (1985) obtained a critical toxic mercury concentration of 3.8  $\mu$ g g<sup>-1</sup> wet weight

for the amphipod *Bathyporeia pilosa*. By comparison, our highest non-toxic body burden (56  $\mu$ g Hg  $g^{-1}$  dry weight) is equivalent to approximately 13  $\mu$ g g<sup>-1</sup> wet weight. Accumulation of mercury by Daphnia magna exposed to the highest nontoxic and lowest toxic mercuric chloride concentrations were 15 and 23  $\mu$ g g<sup>-1</sup> dry weight respectively. Methyl mercuric chloride was toxic at the lowest methyl mercury concentration tested, which resulted in accumulation of 16  $\mu$ g Hg g<sup>-1</sup> (Biesinger et al., 1982). Hvalella azteca, therefore, appears to tolerate slightly higher mercury concentrations in its tissues than Bathyporeia pilosa or Daphnia magna. Mercury accumulated at concentrations resulting in approximately 50% mortality in barnacles (Elminius modestus) and Ar*temia salina* ranged from 280 to 920  $\mu$ g g<sup>-1</sup> dry weight, but the exposure time was only 3 h and mercury accumulated at 50% survival decreased with increasing exposure times (i.e. decreasing concentrations, Corner & Rigler, 1958). These accumulation values are, therefore, probably not directly comparable with the chronic exposure studies.

Data on cadmium accumulation by crustaceans at toxic waterborne concentrations are more numerous than for lead and mercury (Table 7). The reported critical body burdens are all within a factor of approximately 2 of the critical body burden of cadmium to H. azteca.

The tissue concentrations listed in Table 5 can be used for preliminary estimation of the potential toxicity of lead, mercury and cadmium to H. azteca collected from the field. With appropriate control or reference animals, elevated zinc accumulation may also be indicative of exposure to toxic zinc concentrations. However, some caution must be used when interpreting data from the field animals because the relationship between toxicity and accumulation can vary somewhat with variations in water hardness and the presence of sediments (Borgmann et al., 1991). The results in Table 5 are based only on experiments conducted without sediments. Furthermore, the possibility that prolonged, multigeneration exposure to elevated metals might result in metal tolerant populations with different toxicity:accumu-

Species	$\mu g g^{-1}$ (dry wt)	Exposure time	Comments	Reference
Daphnia magna	39 87	20 wk	Highest non-toxic conc. Lowest toxic conc.	Borgmann et al., 1989a
Amphipods:				
Hyalella azteca	23 30	6 wk	Highest non-toxic conc. Lowest toxic conc.	Borgman et al., 1991
Pontoporeia affinis	80-90	265 d	Juvenile mortality	Sundelin 1983
Allorchestes compressa	80	4 wk	Minimum effect concentration	Ahsanullah & Williams, 1
Shrimp:				
Palaemonetes pugio	20-35	21 d	10-25% mortality	Vernberg et al., 1977
Callianassa australiensis	24-29	14 d	14 d LC50	Ahsanullah et al., 1981
Crayfish:				
Orconectes virilis	28	14 d	25% mortality	Mirenda, 1986

5 mo

No significant mortality

Significant mortality

Table 7. Cadmium concentrations accumulated by crustacea at or near toxic waterborne cadmium concentrations.

lation relationships has not been investigated in this species.

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An alternative to measuring metal concentrations in field amphipods is to expose laboratory animals to contamination, either in the lab or in situ. Accumulation during relatively short term exposures should provide an indication of potentially toxic metal concentrations, even for copper, which is regulated during longer exposures.

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1991

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# Attachment 1 – Exhibit X

Revised chronic zinc standard using the corrected Hyalella azteca MATC This spreadsheet calculates the FCV when there are less than 59 MCV's

Number of MCV's in data set 11 Existing Hyalella MCV Normalization Revised MCV Rankings Using Correct MATC of 67.59 µg/L MCV Rankings List of lowest MCV's 1 18.8 (Hyalella) 1 26.66 (Ceriodaphnia) MATC =  $67.59 \, \mu g/L$ at 50 mg/L hardness 2 26.66 (Ceriodaphnia) 2 30.08 (Hyalella) Hardness = 130 mg/L 3 51.97 (Daphnia) 3 51.97 (Daphnia) Slope (B) = 0.8473 MATC = e <sup>[In 67,59 - (0.8473(In 130 - In 50))</sup> 4 (Erpobdella) (Erpobdella) 4 77.46 77.46 MATC =  $e^{3.4039}$ MATC = 30.08 µg/L Number of MAV's entered: 4 Number of MAV's entered: 4 Existing FCV ( $\mu$ g/L) = 12.16 Revised FCV ( $\mu$ g/L) = 17.62 B (slope) = 0.8473 (original rulemaking) B (slope) = 0.8473 (retained from original rulemaking) FCV = e [A + (0.8473(in50))] FCV = e [A + (0.8473(ln50))]  $12.16 = e^{[A + (0.8473(3.912020))]}$  $17.62 = e^{[A + (0.8473(3.912020))]}$ 2.4982 = 3.3147 + A 2.8691 = 3.3147 + A A = -0.8165A = -0.4456Existing Chronic Zinc Equation =  $e^{[A + Bin(H)]}$ Revised Chronic Zinc Equation =  $e^{[A + Bin(H)]}$ where A = -0.4456, B = 0.8473 where A = -0.8165, B = 0.8473

## Attachment 2

Water Quality Standards Stakeholders Meeting Agenda, dated October 19, 2009

Water Quality Standards Stakeholders Meeting Agenda October 19, 2009 Illinois EPA HQ Training Room

- 10:00 Welcome and Introduction to the Rulemaking Proposal Bob Mosher
- 10:15 Proposed Manganese Public Water Supply Intake Standard Brian Koch
- 11:00 Derivation Process for Boron Aquatic Life Use Standards Dave Soucek
- 11:45 Discussion
- 12:00 Lunch on your own
- 1:15 Derivation Process for Manganese and Fluoride Aquatic Life Use Standards Brian Koch
- 2:00 Proposed Housekeeping Changes to the Water Quality Standards Bob Mosher
- 2:30 Open Forum Questions, Comments concerning the proposed standards
- 2:50 What's Next
- 3:00 Dismissal

The length of this meeting is somewhat dependent on the number of questions and comments the stakeholders have. We want to allow plenty of time for this interaction, which is the purpose of this meeting. If questions are few, we will probably get finished before 3:00.

## Attachment 3

Water Quality Standards Stakeholders Meeting, Sign in Sheet, dated October 19, 2009

### Illinois E P A 1021 North Grand Aneme. Esse Springfield, 9.L

Meeting Sign-in

Meeting Date 10-19-09

Meeting Name Water Anality Standards Stakeholders

Participant Name

Representing

Telephone/FAX Number

EEPA Bran Koch 785-4116 314-554-4521 SMALLWOUS AAMA om Jaws 618-206-5931 618 337 1710 538-2012 (mar NU IE PA 558-2012 wait GNA 78 10 ×1108 5 erva CI PA-Sienno 517 77 INHS 217 Z Ø 5 618-451-3013 U.S. STARZ a *k*a a 312-886-14 U.S. FPA Mara 217.691.6696 9 C H Spring. Con North Contran Lare Schoonespr 309-824-1528 IERG 217 522 55 12 CANHA rt.nda 309-557-3153 Vancy Erickson CF 8 217 524-3332 Lilians tepa akil

## Illinois & P A 1021 North Grand Aneme. Esse Springfield, 9.L

Meeting Sign-in

Meeting Date <u>10-19-09</u>

Meeting Name Water Quality Stundards Stakeholders

Participant Name

044 GeFERT Hump hrey MARI ρ NOUSON Ø Å ZAKE INDU ELVENA σħ CHRISTINE ZEMAN Tin King

Representing Telephone/FAX Number ONSOL EVERA 68-625-635-217-520-0491 4517 Praine Rivers Network 217/344-2321 SMSM 217-201-885 MaRyan Minun 926 ,99 シンズ 217 79-198 60 EXXUN MOBIL 815.521.7580 CEULP 217/789-2116 San Dit, Decopy 422-6931

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# Attachment 4

Opinion and Order of the Illinois Pollution Control Board, dated March 6, 1975

#### ILLINOIS POLLUTION CONTROL BOARD March 6, 1975

IN THE MATTER OF: )
PROPOSED AMENDMENTS TO RULES )
R73-15
203 AND 408 OF THE ILLINOIS )
WATER POLLUTION CONTROL )
REGULATIONS

OPINION AND ORDER OF THE BOARD (by Mr. Henss):

Ozark-Mahoning Company and Minerva Oil Company filed a joint proposal seeking changes in Rules 203 and 408 of the Water Pollution Control Regulations as those Rules pertain to fluoride. The proposal was to relax the standard for mining companies by adding the sentence which has been underlined.

Rule 203(f) Water Quality Standards - General Standards

Constituent	Storet Number	Concentration (mg/l)
Fluoride	00950	1.4*

\*Except that fluoride derived from mining and concentrating the mineral fluorspar (CaF<sub>2</sub>) shall not exceed 15 mg/1.

Rule 408(a) Effluent Standards - Additional Contaminants

Constituent	Storet Number	Concentration (mg/l)
Fluoride (total)	00951	2.5*

\*Except that fluoride derived from mining and concentrating the mineral fluorspar (CaF<sub>2</sub>) shall not exceed 15 mg/1.

The proposed amendments and a statement of reasons supporting the proposal were published in Board Newsletter #78, dated December 29, 1973. Public hearings on the proposal were held in Elizabethtown on March 29, 1974 and in Chicago on April 19, 1974. Pursuant to its Petition to Intervene, Olin Corporation was designated a party in interest and granted leave to participate in the hearings. Other participants included the U. S. Environmental Protection Agency, the Illinois Environmental Protection Agency, Allied Chemical Company and private citizens.

The existing effluent limitation of 2.5 mg/l for fluoride was adopted by the Board on January 9, 1972 following extensive public hearings through the State. In setting this limitation the Board stated:

"Fluoride. Our initial proposal for a fluoride effluent standard was 1.0 mg/1. This was somewhat tighter then the water quality standards we later proposed (1.4) for both aquatic life and public water supply, and it posed problems for municipal treatment plants whose influent has been deliberately dosed with as much as 1.0 mg/l of fluoride for dental purposes. Patterson reported that 1.0 mg/l was achievable only through relative exotic and costly methods, such as ion exchange, and that 10.0 mg/lwas a more appropriate standard to achieve by ordinary precipitation. Weston and Dodge both said, however, that 1.0 was readily achievable, Weston specifying the use of alum at cost less than those for achieving most of the metals concentrations here proposed. The most specific information in the record came from Olin, which reports that its fertilizer works at Joliet consistently reduces fluoride concentrations by standard treatment from an influent of 15 mg/l to an effluent of 2.5, but that other ions present prevent reduction as low as 1.0.

We have accepted Olin's figure of 2.5 mg/l, in recognition of the difficulties encountered in going lower and of the likelihood of dilution in many instances to achieve a relatively lenient stream quality standard."

A water quality standard of 1.4 mg/l fluoride was adopted on March 7, 1972, again following extensive public hearings throughout the State. On the fluoride water quality standard the Board stated:

"Fluoride. Fluoride can delay the hatching of fish eggs and has been reported by McKee and Wolf to kill trout at concentrations ranging from 2.3 to 7.2 mg/l. They recommend a standard of 1.5 mg/l. The figure of 1.4, here repeated from the May 12 draft, is in line with that recommendation and also should assure a potable supply."

Both proponents in this matter are actively engaged in the mining and processing of fluorspar (also known as fluorite) for various industrial uses. Operating in Pope and Hardin Counties in Southern Illinois, Proponents extract the fluorspar from bedded and vertical vein deposits 350 to 850 feet below surface. They are the only fluorspar producers in Illinois and their combined production accounts for 80% of the entire amount produced in the United States. Ozark-Mahoning processes about 17,000 tons of crude ore per month at its Rosiclare mill. Minerva processes from 900 to 1300 tons of crude ore per month, from which about 157 tons of fluorspar concentrate, 20 tons of zinc concentrate and 30 tons of barite (BaSO<sub>4</sub>) are extracted. £ < ,

During the concentrating processes, part of the fluorspar in the crude ore is dissolved and discharged in the mill effluent. Some fluoride is also contained in the discharges from the fluorspar mines.

The two counties in which the fluorspar industries operate are described as two of the smallest and most sparsely populated counties in Illinois. The 1970 Census showed that Hardin County had 4914 people on 183 square miles while Pope County had 3,857 people on 381 square miles. Ozark-Mahoning employs 220 persons directly and another 55 to 60 on contract. Minerva employs 210 persons directly and 40 persons indirectly. The majority of the workers reside in either Hardin or Pope County. The only other industries in the two-county area are quarrying, farming and cattle raising. Proponents state that the economy of these two counties is largely dependent upon the fluorspar industry as are the users of the fluorspar product insofar as total domestic production is concerned.

Fluoride-bearing effluent from proponent's mines and mills is discharged to receiving streams which vary from intermittent drainage ditches or creeks to flowing rivers as follows:

#### OZARK-MAHONING COMPANY

Parkinson Mine	- To Big Grand Pierre Creek to Ohio River.
Barnett Mine	- To Big Grand Pierre Creek to Ohio River.
Barnett Air Shaft	- To unnamed creek to Little Grand Pierre Creek to Big Grand
Oxford Mine #7	<ul> <li>Pierre Creek to Ohio River.</li> <li>To unnamed creek to Duck Creek to Rock Creek to Harris Creek to Saline River to Ohio River.</li> </ul>
Knight Mine	- To unnamed creek to Mud Creek to Three Mile Creek to Ohio River.
W. L. Davis Mine #1	- To Davis Branch to Big Sinks to Ohio River (possibly)*
Rosiclare Lead and Fluorspa	r Mine - To Willow Creek to Ohio River
Rosiclare Flotation Plant North Green Mine**	- To settling pond to Ohio River - To Sheridan Branch to Haney Creek to Ohio River
West Green Mine**	- To Sheridan Branch to Haney Creek to Ohio River
MINERVA OIL COMPANY	
Mino #1	- To Bunning Boor Grook to Book

Mine #1

- To Running Bear Creek to Rock Creek to Saline River. MINERVA OIL COMPANY (continued)

- To Rock Creek to Saline River.
- To unnamed creek (sometimes
called Davis Creek) to Big Sinks
to Ohio River* (possibly)
- To Big Grand Pierre Creek to
Ohio River
- To unnamed creek to unnamed creek
to Rock Creek to Harris Creek to
Saline River to Ohio River.
- To Goose Creek to Harris Creek to
Saline River to Ohio River.
- No discharge***

\*The Big Sinks, a natural sinkhole, drains periodically to an unknown receiving stream. It is believed that water drains from Big Sinks through an underground stream to the Ohio River, although dye tests have been unsuccessful in confirming the location of the ultimate receiving stream.

\*\*Initial information showed that both the North and West Green Mines were not consistently discharging water. When operating conditions required the pumping of water from these mines, it was done on an intermittent basis only (1 to 4 hours per day) and the mine water was discharged to the streams shown. New information shows that these mines are now discharging water consistently at a combined rate of 100,800 gpd.

\*\*\*Mine water from this mine flows underground through depleted excavations to Ozark-Mahoning's W. L. Davis Mine. Such flow is minimal.

The other industrial firms participating in this matter have fluoride problems significantly different from those of the mining companies and from each other. At its Blockson Works in Joliet, Olin imports calcium phosphate rock, soda ash and sulfuric acid which are used to manufacture sodium phosphate. Fluoride-based products are also produced at the Blockson Works through the reaction of sulfuric acid and fluorspar to form hydrofluoric acid. The hydrofluoric acid is then reacted with other materials to form the desired fluoride-based final product. Fluoride-bearing effluent from Olin's Blockson Works is discharged to the Des Plaines River.

Allied Chemical operates a facility for the production of uranium hexafluoride  $(UF_6)$ , sulfur hexafluoride  $(SF_6)$ , fluorene, antimony pentafluoride and iodine pentafluoride in Metropolis, Illinois. Allied's liquid discharge, which consists of spent ammonium sulfate solution, sulfide liquors, hydrofluoric acid solution, spent potassium hydroxide solution and uranium recovery leach liquors, flows to the Ohio River through two industrial ditches.

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Corporate positions on these matters vary as widely as do the processes in which the fluoride bearing wastes are generated. As earlier noted, Ozark-Mahoning and Minerva propose to amend the standards only as those standards apply to the fluorspar industry. Olin's position was one of disagreement with Ozark-Mahoning and Minerva over the proposed changes in Rule 408.

Olin proposes to change Rule 408 to allow a fluoride effluent concentration of 10 mg/l for all industries. Olin took no position on the proposed change in Rule 203.

Allied first contended that the effluent standard should be revised to allow 15 mg/l fluoride based on an average of 24 hour composite analysis for 30 consecutive days and 30 mg/l maximum for any one 24 hour composite. Allied took no position on the proposed revision of Rule 203. Neither the U. S. environmental Protection Agency nor the Illinois Environmental Protection Agency took a position on the proposed changes prior to the public hearings. Their post hearing comments will be discussed elsewhere in this Opinion. Of the two Agencies, only the U. S. EPA chose to present any testimony.

Fluorides are widely distributed in the earth's crust, occurring in both igneous and sedimentary rocks. Among the more common fluoride minerals are fluorspar  $(CaF_2)$ , villiaumite (NaF), cryolite  $(Na_3AlF_6)$  and fluorapatite  $[CaF_2.3Ca_3(PO_4)_2]$ . Fluorides in high concentrations are not a common constituent of natural surface waters but they may be prevalent in detrimental concentrations in ground waters.

Small concentrations of fluoride (0.6 mg/l to 1.7 mg/l) in drinking water have been shown to effectively reduce the prevalence of dental carries while excessive amounts cause effects in humans varying from mottled teeth to death. When fluoride is 2.5 mg/l 75% to 80% of children have mottled teeth. In drinking water, fluoride of 180 mg/l is toxic and 2000 mg/l is lethal to man.

Solubility of a fluoride varies according to the nature, pH and temperature of the solvent, cationic partner and prevalence of other chemical constituents in the solvent. The two most discussed fluoride compounds during these proceedings, sodium fluoride and calcium fluoride, vary significantly in their solubility. The solubility of calcium fluoride at 18° C. (64.4° F) is 16 ppm (about 8 ppm fluoride ion) whereas the solubility of sodium fluoride is about 19,000 ppm. This means that sodium fluoride is inherently more soluble in water than is calcium fluoride.

Neither Ozark-Mahoning nor Minerva discharges any effluent that approaches the proposed effluent limit of 15 mg/l fluoride. Discharges from the mines and mills operated by these two companies are less than 5 mg/l, as shown below, with a single exception of the discharge from the Rosiclare flotation plant settling pond.

#### OZARK-MAHONING

### Parkingson Mine - 40 gpm Barnett Mine - 50 gpm Barnett Air Shaft - 40 gpm

Fluoride, mg/l

· · · · ·

Big Grand Pierre above discharge	0.28
Parkinson discharge to Big Grand Pierre	1.40
Barnett Mine discharge to Big Grand Pierre	2.40
Big Grand Pierre below Parkinson and Barnett	0.30
Barnett air shaft discharge to unnamed creek	3.10
Unnamed creek at confluence with Little Grand Pierre	0.50
Little Grand Pierre above confluence with unnamed creek	0.25
Little Grand Pierre below confluence with unnamed creek	0.40
Eig Grand Pierre below all discharges	0.28
Oxford Mine #7 - 10 gpm	
Mine discharge to unnamed creek	2.20
Unnamed creek at confluence with Duck Creek	0.25
Duck Creek above confluence with unnamed creek	1.50
Duck Creek below confluence with unnamed creek	1.00
Duck Creek above confluence with Rock Creek	0.97
Rock Creek above confluence with Duck Creek	0.25
Rock Creek below confluence with Duck Creek	0.63
Knight Mine - 90 gpm	
Knight discharge to unnamed creek	1.40
Unnamed creek above confluence with Mud Creek	0.75
Mud Creek above confluence with unnamed creek	0.25
Mud Creek below confluence with unnamed creek	0.25
W. L. Davis Mine #1 - 1200 gpm	
Mine discharge to unnamed creek	1.4
Unnamed creek above entry to Big Sinks	1.2
Rosiclare Lead and Fluorspar Mine - 20 gpm	
Mine discharge to Willow Creek	1.3
Willow Creek above confluence with Ohio River	1.4
Rosiclare Flotation Plant - 650 gpm	
Plant discharge to settling pond	
Settling pond discharge to Ohio River	10.0

### -7-

#### MINERVA

Fluoride, mg/l

0.40

0.47

4.5 (avg.) 0.6 (avg.)

2.5 (avg.) 0.45

### Mine #1 and Mill - 368 gpm

#3 pond discharge to Rock Creek Rock Creek above #3 pond discharge Rock Creek below #3 pond discharge Harris Creek below confluence with Rock Creek Saline River above confluence with Harris Creek Saline River at confluence with Harris Creek

#### Crystal Mill - 52 gpm

Heavy-media-separation tails	3.62 (avg.)
Unnamed creek above HMS tails	1.34 (avg.)
Big Sinks	1.51 (avg.)

#### Tucker Hill Area - 150 gpm

Churn Drill Hole, underground water	3.02 (avg.)
Unnamed creek upstream	No flow
Unnamed creek downstream	1.14

Gaskins Mine - 875 gpm

Gaskins Shaft	1.58	(avg.)
Big Grand Pierre Creek above discharge	0.39	(avg.)
Big Grand Pierre Creek below discharge	0.50	(avg.)

Spivey Mine - 80 gpm

Spivey Shaft	2.75 (avg.)
Goose Creek above discharge	0.51 (avg.)
Goose Creek below discharge	0.66 (avg.)

C. B. Rash, Ozark-Mahoning's Superintendent of Milling, explained that the proposed 15 mg/l effluent limitation was necessary as a "safeguard" in the event recycling of effluent was imposed upon the industry (R. 41). Ozark-Mahoning's plant in Colorado attempted a waste water recycling effort when the Colorado Department of Public Health requested an effort to achieve "zero flow". Although "zero flow" was not achieved, the effort resulted in the recycling of 80% of the waste water--but at a price. This price was an increase in fluoride concentration to 32 ppm.

When the Board set the effluent standard at 2.5 mg/l it relied heavily upon the testimony of an Olin employee, Emil Stoltz, regarding

the technology available to reduce fluoride in waste water. Stoltz had testified that while Olin had not been able to "obtain it in our specific effluent" they did have the technology to "get down to 2 to 2 1/2 mg/1."

Stoltz testified in the current proceedings that he had meant to inform the Board that this level of fluoride reduction was only a technical feasibility based on laboratory studies made at the corporation's research headquarters in New Haven, Connecticut. This research was primarily based on a lime treatment process which Olin has not used at its Blockson Works. Stoltz testified that, based on the research program, he now believes that Olin could reduce the fluoride in waste water from 15 mg/l to 2.5 mg/l. Blockson Works waste water currently contains about 20 mg/l fluoride before treatment (R. 224).

At this point, it is necessary to review the health related information about fluorides in order to provide a balance to the later discussion on feasibility and economic reasonableness of fluoride treatment.

In setting a 1.4 mg/l fluoride water quality standard, the Board cited a report by McKee and Wolf (McKee, J. E., and Wolf, H. W., <u>Water Quality Criteria</u>, California State Water Resources Control Board, Second Edition, 1963) showing that fluoride can delay the hatching of fish eggs and that concentrations ranging from 2.3 to 7.2 mg/l can kill trout. These references, p. 191 of the McKee-Wolf report, also show that in 15 studies the majority involved the use of sodium fluoride and none of the studies is shown to have involved calcium fluoride.

Under <u>Sodium Fluoride</u>, McKee-Wolf cite research showing the following effects of sodium fluoride on certain aquatic bacteria, algae and small crustaceans:

Species	Results
Daphnua (an order of crustaceans which includes water fleas, found everywhere in fresh waters)	Threshold of NaF at 23° C. was found to be 270 mg/l for a 2 day exposure.
Scenedesmus (a fresh water algae, most common and best known of all algaes, found almost anywhere algae grows)	Threshold of toxic effect was 95 mg/l during 4 days at 24° C.
Microregina (A single cell protozoan often found in fre <b>s</b> h water)	Threshold of toxic effect was 226 mg/l during 4 days at 24° C.

Species	Results
Escherichia coli (a bacteria found abundantly in verte- brate intestine)	Threshold of toxic effect was 180 mg/l during 4 days at 27° C.
Free-living protozoa and fresh water rotifers	Survived and reproduced in water containing 1000 mg/l but were killed at 1700 mg/l.

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This information tends to show that low concentrations of sodium fluoride probably would not present any significant toxicological difficulties for at least some of the more common lower aquatic organisms expected to inhabit Illinois streams. Based on research reported in McKee-Wolf, the same is not true for higher aquatic organisms. This research reported the following effects of sodium fluoride on fish:

Concentration, mg/1	Type Fish	Effect
2.3 to 7.3 2.6 to 6.0 2.7 to 4.7 5.9 to 7.5	Trout Trout Trout Trout	${\rm TL}_m$ at 18° C. in soft water ${\rm TL}_m$ at 13° C. in soft water ${\rm TL}_m$ at 7.5° C. in soft water

Thus, it would appear that some lower aquatic organisms are able to tolerate sodium fluoride concentrations on the order of 100 times that tolerated by trout. Although time of exposure for determining  $TL_m$  is usually specified, this parameter was not provided for the data above, making comparison of results impracticable. Reasons for these phenomenal differences in survivability (for example, osmotic capabilities of membranes of lower aquatic forms vs. higher aquatic forms, significant physiological differences, etc.) were not stated.

In this proceeding, expert testimony indicates that sodium fluoride concentrations in natural waters should be minimal in comparison to concentrations of calcium fluoride. Dr. W. F. Sigler, head of the Wildlife Science Department at Utah State University, testified that all research conducted in the U. S. on fish fluorosis "was done by me and under my direction". Dr. Sigler noted that while small amounts of sodium fluoride might exist, larger amounts do not exist naturally because it dissociates to form calcium fluoride.

A number of opinions on the relative toxicities of sodium and calcium fluoride were aired during the hearings. C. B. Rash testified that his opinion of available research was that sodium

fluoride "would be more toxic than calcium fluoride even at the same concentration, because there is indication that the calcium present with the fluoride ion reduces the toxicity" (R. 45).

Dr. Sigler first testified that sodium fluoride and calcium fluoride have equal toxicities at equal concentrations. (R. 120) Admittedly not a chemist, Dr. Sigler later qualified this statement by testifying that the toxicities would be equal except when other positive ions were present (R. 155). Then later, Dr. Sigler testified that calcium fluoride would be the less toxic of the two fluorides because "calcium and the fluoride have an affinity for each other and reduces the toxicity" (R. 206). Dr. Sigler indicated his preference to let Franklin Davis of the Colorado School of Mines Research Institute answer the questions relating to the chemistry of fluorides. When called upon, Davis testified that he could not "answer that with the proper credentials" because he was not a toxicologist (R. 164).

Significant testimony on fluoride toxicity was produced by Dr. Leonard Krause of Olin Chemical Company. Dr. Krause testified that fluoride entering the system of any living organism will combine with the most prevalent tissue around it, usually tissue containing calcium such as cartilage or bony tissue. Such a combination is known as fluorosis. Fluoride interferes with enzyme systems at the cellular level and interferes with the oxygen uptake in organisms by some mechanism that toxicologists don't yet understand (R. 322).

Fluoride taken into a body in the form of calcium fluoride tends to be excreted almost exclusively as calcium fluoride. This occurs, according to Dr. Krause, because very little, if any, of the fluoride will combine with the body calcium since sufficient calcium is already available for combining with the fluoride.

Dr. Krause testified that his research work involving humans showed that 14 mg/1 of calcium fluoride was not toxic to humans. He did not think a toxic level of calcium fluoride in solution could be reached because it would be precipitating out. Dr. Krause stated that he would not hesitate to drink water containing 14 mg/1 of calcium fluoride but would never put the same amount of sodium fluoride into his body (R. 332). Fluoride in water containing sodium fluoride would not be excreted as would the calcium fluoride. It would be available to bony tissues and kidneys.

Another of the body elements that could be affected by the ingestion of calcium fluoride is potassium, an essential element in nerve tissues. At first Dr. Krause stated unequivocally that potassium in the body would not be replaced by the calcium in calcium fluoride because of the tight chemical bond found in calcium fluoride (R. 335). He later acknowledged that such a replacement possibility did exist (R. 342) although the fluoride itself is more available to cartilaginous and bony tissue than for nerve tissue (R. 351).

Table 6-5 of the McKee-Wolf report shows various levels of fluoride concentrations that caused mottled teeth. In the range from 0.2 to 1.0 mg/l fluoride the mottling is mild with a concentration of 1.0 mg/l listed as the "threshold for mottling". One study reveals a mild to moderate degree of mottling from 1.0 to 2.0 mg/l fluoride. At 6.0 mg/l the references reported pitting and chipping of teeth and that 100% of children had mottled teeth.

E. F. Carter, Jr., Rosiclare postmaster, testified that he knew of no mottling of teeth in the Pope-Hardin County area caused by the discharges of Ozark-Mahoning or Minerva. W. W. Fowler, Ozark-Mahoning Vice President and General Manager, testified that he knew of no adverse effects, including mottling of teeth, that had been suffered by any of his employees. He added that miners have drunk water from the mine seams and walls for a number of years. The highest fluoride concentration in such water was found to be 2.5 mg/l. C. B. Rash also testified that he had observed no ill effects or mottling of teeth in the area.

Rash testified that several farmers in the area depend on the mine discharge water as a source of water for their livestock. The farmers had informed Rash that they had never observed any ill effects in their cattle as a result of drinking the mine discharge water.

Proponents submitted a letter from Truman Louderbach, a Research Biologist at the Colorado School of Mines Research Institute (CSMRI), reporting on results of bioassay testing conducted at CSMRI at the request of Ozark-Mahoning Company (Petitioner's Exhibit 4). For the test, samples were drawn from the tailings dam effluent of Ozark-Mahoning's Cowdrey, Colorado eperation and from Pinkham Creek above the confluence with the tailings dam effluent. These samples had the following properties:

	Tailings Dam Effluent	Pinkham Creek	
Temperature	7° C. (44.6° F.)	6.5° C. (43.7° F.)	
Hq	7.6	7.5	
D.O., ppm	8.3	7.0	
F, ppm	32	2.6	

Six-month-old fingerling rainbow trout were acclimatized for 10 days in Pinkham Creek water at 15° C. ± 2° (59° F.) with a dissolved oxygen concentration above 7 ppm. Following the acclimatization the trout were subjected to testing using various mixtures of Pinkham Creek water and tailings dam effluent up to 100% tailings dam effluent. The tests showed a 100% survival of trout for 96 hours in all mixtures including the undiluted tailings dam effluent. No evidence of distress in the behavior of fish specifimens was observed.

Also submitted by proponents was a report by CSMRI's Senior Research Biologist, Dr. Gary D. Boss, in which Boss summarized his findings on fluoride toxicity based on published reports. According to the Boss report, assignment of specific toxic levels is difficult because of the following major factors:

- 1. Fish species, race, or strain
- 2. Fish size and stage of development
- 3. Physiological state, including age of fish
- 4. Level, type and solubility of fluoride and fluoride containing compounds
- 5. Water temperature
- 6. Individual biological response
- 7. Composition of the water, in particular the content of calcium, magnesium and chloride

Boss cites a Utah State University study (Neuhold and Sigler, 1960) conducted on carp and rainbow trout, using fluoride containing water with a calcium and magnesium content of less than 3 ppm. Results were reported as follows:

Species	Temperature, °F.	TL <sub>50</sub> * at F ion conc. (ppm)
Trout	55	2.7 to 4.7
Carp	65-75	75.0 to 91.0

\*TL<sub>50</sub> - Tolerance limit at which 50% of the fish survived and is nearly equal to  $LD_{50}$  (lethal dose) and  $LC_{50}$  (lethal concentration)

Boss qualifies the above results by stating "Fish populations including rainbow trout flourish in Wyoming and Nevada where fluoride concentrations are 13.0 - 14.0 ppm. Yet reared trout have displayed  $TL_{50}$ 's of about 3.0 ppm of fluoride (Sigler and Neuhold, 1972)".

The Boss report cites another study of response of rainbow trout eggs in water containing less than 3.0 ppm of calcium and magnesium under varying temperatures (Neuhold and Sigler, 1960). Reported results were as follows:

Temperature, °F	TL50, ppm F	Hours
46	222-273	424
55	242-261	214
60	237-281	167

These data show that fluoride toxicity increases for trout eggs with increasing temperature.

Information was also reported on efforts to determine the effect of chloride concentration on rainbow trout (Neuhold and Sigler, 1962). In water containing measured amounts of fluoride and chloride ions, the following results were obtained:

Fion, ppm	Cl <sup>-</sup> ion,	ppm 9
	(Deat	hs)
0	0	0
4	0	0
7	1	0
13	6	1
25	10	1.

Boss states that such evidence indicates that the presence of either calcium, magnesium or chloride ion decreases the toxic level of fish to the fluoride ion. While admitting that the effect of the chloride ion is conditional, Boss asserts that "the weight of the experimental evidence supports the contention that fish acclimated to moderate concentrations of chloride ion have increased resistance to fluoride toxicity."

Summarizing, Boss states: "Fluoride ion has a high affinity for calcium and its presence in the water in significant amounts seems to reduce the effective concentration of calcium in the body of the fish.  $CaF_2$ , however, dissociates to form so few fluoride ions that evidently only light symptoms of fluorosis are produced. Moreover, the calcium ion made available by the dissociation of  $CaF_2$  would seem to provide a replacement for any calcium extracted from the body of the fish."

Boss's overall conclusion based on available information was that "in our opinion, data on fluoride toxicity are too general and vague to establish a valid toxicity level for aquatic life at this time".

As will be noted in the following table, waters used in the tests just described bear little, if any, resemblance to stream conditions applicable to the parties in this proceeding. This table provides definitive stream values in relation to various streams

Average Stream value (1)	Big Grand (2) Pierre Creek, AL01	Saline River South Fork (3)	Saline (4) River, AT04
ph D.O. Fluoride Chloride Hardness	7.5 7.9 0.6 13	0.3 160	6.5 8.0 0.4 49
	Saline (5) <u>River, AT02</u>	Ohio (6) River, A08	Ohio (7) River, A07
ph D.O. Fluoride Chloride Hardness	7.6 8.2 0.2 23 Ohio (8) River	7.9 8.4 0.2 100  Ohio (9) River A01	7.8 8.6 0.1 22  Ohio (10) River, A02
ph D.O. Fluoride Chloride Hardness	0.6 160	7.7 8.6 0.2 24	7.6 8.7 0.1 19
	Ohio (11) River, A06	Ohio (12) River, A04	Ohio (13) <u>River</u>
ph D.O. Fluoride Chloride Hardness	7.5 7.6 0.2 22	7.6 7.8 0.2 20	0.3 178
	Des Plaines (14) River, Gl2	Des Plaines (15 	)
ph D.O. Fluoride Chloride Hardness	7.3 7.0 0.8 120 320	7.4 7.3 0.8 165 290	

receiving proponents effluent, the Des Plaines River near Olin's Blockson Works and the Ohio River near Allied's Metropolis plant:

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- (1) Stream identification followed by an "A" or "G" identification number (i.e., AL01, G12) represents data taken from Illinois EPA Water Quality Network, Summary of Data, 1972. Stream identification without an "A" or "G" identification number represents data taken from Illinois EPA Public Water Supplies Data Book, 1973 (Allied Exhibit #2). Values reported in mg/1.
- (2) Below discharge from Minerva's Gaskins Mine. At or near discharges from Barnett Air Shaft, Barnett Mine and Ozark-Mahoning's Parkinson Mine.
- (3) Above fluorspar mine discharges.
- (4) Far above fluorspar mine discharges.
- (5) Mouth of River below fluorspar mine and mill discharges.
- (6) Near Shawneetown, above fluorspar mine and mill discharges.
- (7) Near Cave-In-Rock, below confluence of Saline and Ohio Rivers.
- (8) Rosiclare water intake below discharge from Ozark-Mahoning's Rosiclare Mill.
- (9) Golconda water intake below fluorspar mine and mill discharges.
- (10) Brookport below all fluorspar mine and mill discharges but above discharge from Allied plant.
- (11) Olmsted below Allied plant discharge.
- (12) Cairo water intake.
- (13) Cairo water intake.
- (14) Above discharge from Olin's Blockson Works.
- (15) Below discharge from Olin's Blockson Works.

From the record it is apparent that the determination of toxicity in this matter depends largely upon the concentration of ions in the receiving waters, particularly calcium and magneisum ions. The reports refer to the concentration of these ions as hardness. (Water hardness in the Des Plaines River near Olin's Blockson Works is about 90% calcium and 10% magnesium [R. 222]). As the above Table shows,Illinois streams are not deficient in calcium and magnesium ion concentrations.

On this basis, toxicity data submitted by Allied Chemical appear to be more pertinent to this proceeding than any other data submitted. Allied contracted Industrial Bio-Test Laboratories Inc. to conduct a 4-day static fish toxicity study using bluegill sunfish (Lepomis macrochirus) and channel catfish (Ictalorus punctatus). A test solution was prepared by using de-ionized water and measured amounts of calcium and magnesium sulfate, sodium bicarbonate and potassium chloride. Water taken from the Ohio River near Metropolis was used as a dilutant.

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Sodium fluoride, calcium fluoride and hydrofluosilicic acid were added at test concentrations of 2.5, 10.0 and 20.0 ppm fluorine to separate vessels, each containing 10 specimens of each species of fish. An untreated sample containing only river water was used as a control. Water temperature was maintained at

about 18° C. (64.4° F.).

In the test using sodium and calcium fluoride no fish fatalities had occurred after 96 hours exposure to the calcium fluoride test solution. One bluegill died after 24 hours exposure to the 10.0 ppm sodium fluoride solution and another died after 72 hours exposure to the 20.0 ppm sodium fluoride solution. No catfish fatalities occurred in the sodium or calcium fluoride solutions. Investigators concluded that the 96-hour  $TL_{50}$  of both sodium and calcium fluoride for unacclimated native fish is in excess of 20 mg/l

These results are particularly important and directly relatable to Illinois streams. They again point to the importance of associating fluoride toxicity levels with calcium and magnesium concentrations in surface streams.

Another document which provides additional insight into the effect of fluoride on stream quality was submitted as Proponent's Exhibit #14. This document reports the results of a biological survey conducted by the Illinois EPA on February 6-7, 1974 to determine the condition of stream environments relative to discharges from Minerva's Gaskins Mine. The survey reveals well balanced benthic invertebrate populations both upstream and downstream from the mine discharge. (An unnamed tributary receiving effluent from the mine was reported to be "semi-polluted" with the cause appearing to be of an "organic origin"). Although fluoride concentrations are not reported in the biological survey, data reported earlier in this Opinion indicate that the fluoride water quality is being met and this receiving stream is adequately protected.

Turning now to the question of economic reasonableness and technical feasibility, we shall first review Proponents' Exhibit #8. Under the direction of Franklin T. Davis, CSMRI, a report titled "Capital and Operating Cost of a Suggested Process for the Removal of Fluoride Ion from Tailings Water" was prepared. The report shows applicability of currently available methods of fluoride removal and also details an as yet unproven method which has a potential of reducing fluoride content from 10 ppm to about 1 ppm at a rate of one million gallons per day.

The Davis report disposes of "state-of-the-art" systems as follows:

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- A. CaF<sub>2</sub> precipitation economically unreasonable because of excessive calcium requirements.
- B. Contacting beds of activated alumina, calcium phosphate, calcium super phosphate or bauxite - prohibitively large bed volume required to treat large amounts of 10 ppm fluoride water, loss of bed material in regeneration and probable addition of phosphate ion to water.
- C. Combined magnesia-lime system restricted to water containing less than 3 ppm fluoride, large amounts of magnesium co-precipitated.
- D. Carbon, zeolites and activated bone best suited for low volume of water with a fluoride concentration of less than 5 ppm and a pH of 7 or less, regeneration losses.
- E. Ion exchange low capacity, slow exchange, low fluoride selectivity and economics.
- F. Reverse osmosis and ion selective membrane economically unattractive and not proven technology.

An alternate method proposed by Davis, but not yet tested, could be labeled as the "Hydroxyapatite Method". In that method water and lime are mixed to produce a 10% slurry which is reacted with 85% phosphoric acid to produce hydroxyapatite by the following reaction:

$$5Ca(OH)_2 + 3H_3PO_4 \rightarrow Ca_5(OH)(PO_4)_3 + 9H_2O$$

Twice the stoichiometric amount of hydrated lime is added to favor complete reaction of the phosphoric acid in the 1 hour reaction time.

Hydroxyapatite slurry is then pumped to an agitated reaction vessel where it contacts the incoming fluoride-bearing waste water. Reaction tank volume allows 1 hour for reaction of the fluoride to fluorapatite. From the reaction vessel the slurry flows to a floculator tank where a flocculating polymer is added. After 15 minutes the treated slurry flows to a clarifier where suspended solids are settled. Overflow from the clarifier is discharged from the plant at a rate of 693 gpm. Sludge from the clarifier is pumped to the tailings dam but can be recirculated in varying amounts to the reaction tank in order to react any remaining unreacted hydroxyapatite. Sludge generation is small for this process and should not present any major disposal problem.

While Davis thinks the method looks good on paper, he quickly adds that additional laboratory studies are required to finalize a numer of **parameters** before final evaluation is possible. Among the parameters to be determined are:

- Ratio of lime to phosphoric acid and required reaction time,
- 2. Rate and absorbtion capacity of the hydroxyapatite, and
- 3. Optimum quantity of flocculant, flocculating time and settling time in the clarifier.

Capital investment for use of the hydroxyapatite method to treat one million gallons per day would be \$287,300, exclusive of roads, power lines and pipe lines. Operating costs for the plant were listed as \$11,278 per month or \$0.376 per 1,000 gallons.

Davis testified that Ozark-Mahoning would require three such plants since 3 million gallons of waste water must be treated (R. 172). Therefore, capital cost for Ozark-Mahoning would be in excess of \$1 million and operating costs would be \$45,000 per month (R. 171). Similar costs on a percentage basis would apply to Minerva's operations (R. 172).

Full-scale laboratory testing remains to be done for the hydroxyapatite method. Davis has performed some laboratory experiments using "artificial hydroxyapatite" with the result being a reduction to less than 1 ppm fluoride (R. 172).

As to other processes for removal of fluorides as described in Waste Water Treatment Technology, Second Edition, IIEQ Document 73-1 (Petitioner's Exhibit #9) Davis testified that none of the processes would be effective on mill tailings water. Davis stated that the processes would not be effective because most of the processes treat water that is relatively free of turbidity. Mill tailings water would have to be clarified or filtered in order to use the process and this "is expensive" (R. 168). Another reason for nonacceptance, according to Davis, was "although they don't say this, ...it is pretty obvious that after they removed it [fluoride] they dumped it back into the river downstream" (R. 167). This option is not open to Proponents.

After reviewing the various methods in the IIEQ document, the Board agrees that they do not directly relate to the fluorspar industry. However, a possible exception might be the use of contact beds of activated alumina. Without committing to the applicability of this process, the Board notes that one such unit in Bartlett, Texas has operated since 1952 on a municipal water plant to reduce fluoride from 8 mg/l to 1 mg/l. Noticeably absent from discussion on the Bartlett plant are flow rates and cost data. According to the report, two investigators experimented with an alumina bed as a polishing unit following lime precipitation. They found that a 30 mg/l residual fluoride concentration could be reduced to 2 mg/l. At a pH of 11.0 to 11.5 they were able to reduce fluoride from 9 mg/l to 1.3 mg/l. Regenerative losses were cited as 4% alumina lost per 100 regenerative cycles. While such information is far too skimpy, it certainly raises the possibility of use on Proponent's mine waters, which are "reasonably clear" (R. 200), or on mill tailings water after clarification. Further, the Board finds nothing in the IIEQ document to indicate that any of the methods discussed involves subsequent dumping of removed contaminants "back into the river downstream".

In his letter dated April 26, 1974 (Petitioner's Exhibit #11), Davis said that new information supplied to Davis showed the mine waters to be free of turbidity. On this basis Davis states that the best process would be the one reported in "Defluoridation of Municipal Water Supplies", by F. J. Maier in the Journal of the American Water Works, August 1953. This is the same alumina contact bed process used in Bartlett, Texas and discussed just above. Davis states the process has a potential for lower capital cost than the hydroxyapatite method but laboratory verification would be required.

A set of figures based on the alumina bed process for mine waters and the hydroxyapatite method for tailings water, adjusted to 1974 prices, was supplied by Davis. These figures show a one million gpd tailings treatment plant with a fixed capital investment of \$298,000 and operating costs of \$12,800 per month. A 650,000 gpd mine water treatment plant to treat water from #7 Oxford Shaft, North Green Mine and West Green Mine and a 650,000 gpd mine water treatment plant to treat water from the Parkinson Mine and Barnett Air Shaft would require a fixed capital investment of \$568,800. Adjusted operating costs are shown as \$0.251 per 1000 gallons for the two mine water treatment plants and \$0.427 per 1000 gallons for the tailings plant for a total of \$0.328 per 1000 gallons. These costs exclude about 10,100 feet of right-of-way for pipeline which Davis warns may be "very substantial".

James N. Pappas, a Sanitarian with the U. S. EPA, attacked Davis' estimates of capital operating cost for the hydroxyapatite method. Pappas testified that these costs most likely would be considerably different if Proponents only treated the blow-down from a recycling process and where fluoride concentration was to be reduced to 2.5 mg/l rather than 1 mg/l. He stated that Proponents had not proved that recycling would be required and had failed to provide data relative to marketing of recovered fluorides as a possible cost reduction.

Davis responded (Petitioner's Exhibit #11) by stating that prior testimony had established "that recycling of tailings water in this type of flotation system is not compatible with the flotation system". He admitted that the water could be purified for recycling purposes but added that such a process would probably

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be more expensive than the hydroxyapatite method because sodium ions and organics would have to be removed. C. B. Rash had testified that recycling adversely affected the efficiency of the flotation process (R. 41). Davis added that recycling efforts at the Colorado plant were not very successful. Solar evaporation ponds were required, which Davis adds, would not be practical in Illinois.

As to the possible sale of recovered fluoride, Davis responded that recovery of acid grade  $CaF_2$  from two million gallons of water would amount to about 240 pounds per day with a market value of about \$10.00. He added that he knows of no process from which  $CaF_2$  is recovered in a marketable form and that the whole idea is "a most impractical consideration".

In a letter dated May 16, 1974 Chris Potos, Chief of Water Quality Standards, U. S. EPA, suggested several possible methods of treatment which, in his opinion, raised doubts regarding the claim of economic hardship. Responding to the concern that during periods of low flow the water quality standard of 1.4 mg/l could be violated by an effluent which would be acceptable during periods of normal flow, Potos suggests that retention basins or lagoons could be utilized to store mine waters until sufficient flows upstream are available to allow release of mine waters without contravention of water quality standards. Potos hastens to add that the U. S. EPA does not necessarily recommend such a solution but merely raises the question "as to consideration of alternatives".

Other alternatives suggested by Potos included relocation of mills to sites near the Saline or Ohio Rivers and transmission of mill waste water from existing sites to the larger receiving streams.

Petitioner's Exhibit #11 was of particular concern to Potos. He questions whether generalized cost figures are applicable for specific projects. He states that treatment costs for reducing fluoride in mill tailings from 5 mg/l at the Minerva Mine #1 Mill to 2.5 mg/l at 580,000 gpd would probably be different than the cost of reducing fluoride in mill tailings from 10 mg/l to 2.5 mg/l at Ozark-Mahoning's Rosiclare Mill at 980,000 gpd. Further reduction of fluoride to 1 mg/l could amount to 90% of the total treatment cost according to Potos.

In his statement of treatment cost, Davis assumed that mine water flows from the Oxford, North Green and West Green Mines were 650,000 gpd. Potos states that Federal NPDES files show the flows to be only about 116,000 gpd. U. S. EPA files containing this information were not made a part of the record. 1 A

The Davis estimate also cited a 650,000 gpd flow to the "Barnett area waste treatment plant" from the Parkinson Mine and the Barnett air shaft. As Potos points out, Petitioner's Exhibit #13 shows flows from the Parkinson Mine, Barnett Mine and Barnett air shaft as 187,200 gpd.

If we were dealing with another type of industry it would be a simple matter at this point to combine the flows each proposed plant was to receive. These figures would show that the two proposed 650,000 gpd plants are substantially larger than required thus showing that the estimates of cost are overstated.

However, this industry must contend with substantial changes in mine discharges. In their Supplemental Submission Petitioners insist that a plant capacity of 650,000 gpd is necessary. Assuming for purposes of argument that it were both possible and practical to combine mine discharges from several mines at one (or more) location, Petitioner states that history would show the inability of the fluorspar industry, or anyone else for that matter, to anticipate increases in mine water as new veins are mined and new faces opened. For examples of the above, Petitioner cites the current discharge from Ozark-Mahoning W. L. Davis Mine which is now three times larger than the original discharge level. Minerva's older Gaskins Mine has a 1,260,000 gpd discharge as opposed to the 115,000 gpd discharge from the new Spivey Mine. Another example is the Crystal Mill facility which has a current discharge of 75,000 gpd during intermittent operations. If both the heavy media separation and flotation mill were placed into operation, this discharge would increase to as much as 480,000 gpd. Thus, Petitioners argue, it would be sheer folly to construct a treatment plant based on current operating requirements when these requirements might increase two, three, or more times in the months and years to come.

The basic premise necessary for such regional treatment plants is that the discharge flows from several points must be combined. Petitioner's concede that a project of this type might be accomplished if reasonableness and ability to finance the project were not to be considered.

Hurdles to be overcome by Petitioners in such a project are numerous and varied. Petitioners would have to commit finances covering the cost of land, easements, pipelines, electrical distribution lines, storage facilities, buildings, labor and maintenance for a theoretical process without any reasonable assurance that compliance would be achieved.

Pipelines and electrical distribution lines would have to cross land in the Shawnee National Forest. Petitioners state that past experiences considered, the U. S. Forest Service would be reluctant and probably unwilling to issue the permits necessary for such a project.

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Petitioners also believe the concept of ponding or lagooning mine discharge is not a feasible alternative. Of the 15 discharge points from Petitioners mines and mills, one flows to the Ohio River and the remaining 14 flow to streams classified as intermittent streams. These discharge points are widely separated in the rock and hill terrain of Hardin and Pope Counties making centralization or combining of discharges impracticable. Numerous small treatment plants would have to be built. Petitioners state that 10 of the 15 discharges are currently in violation of the effluent or water quality standards.

As an example of the problems to be encountered if the ponding concept were implemented, Petitioners cite the following estimated cost for impoundment of discharge water from the Gaskins Mine for a 90 day period:

> Total discharge for period = 113,400,000 gallons Estimated evaporation = 22,000,000 gallons Volume to be retained = 91,400,000 gallons Requires a 60 acre pond with average depth of 4.67 feet. Estimate need to purchase or lease 180 acres for pond site. Levee requires two feet of freeboard - 6.67 feet levee height. Requires moving approximately 31,000 cubic yards of dirt.

Cost:

Building levee at 60¢ per yard = \$18,400180 acres of land at \$300/acre = 54,000Cost of pipeline and pumps = 55,000Major expense total = \$127,000

In addition to the above estimated cost Petitioners would incur fees of \$200 per acre for land leased from the U. S. Forest Service (assuming such leases could be arranged) as well as cost for seed and fertilizer, pipeline right-of-way and maintenance.

However, Petitioner states that the major problem in ponding is that they are simply unaware of any land in the area suitable for ponds or lagoons.

One alternative available to Petitioner is to pump the discharge waters from Gaskin's Mine to the Ohio River, a distance of 7 miles. This project would require a 10" pipe, 40,000 feet long, costing \$320,000 according to Petitioner's estimates. Estimated total cost of this alternative including right-of-way, survey costs, legal fees, leases, piping, pumps and installation is in excess of \$420,000. A second alternative would be to pump the Gaskin's Mine discharge to a central treatment plant serving all Minerva discharges. If this central plant were located at the Minerva Mill, the cost of pipe alone for the 15 mile project would be in excess of \$600,000 at \$8 per foot. Petitioners believe that a project of this magnitude would take longer than the reamining productive life of the Gaskin's Mine.

Responding to the suggestion that Petitioners consider relocation of mills near the Saline or Ohio Rivers, Petitioners state that they have no way of estimating the cost of such a project and that the project would be comparable in difficulty to relocating the Sears Tower.

The Board feels that Petitioners have shown that the many alternatives suggested are not practicable or economically feasible solutions to this complex problem. Hillsides blighted with pipelines and electrical power lines, especially in a national forest, makes these alternatives particularly displeasing from an aesthetic viewpoint in addition to the other drawbacks.

Olin's fluoride problem, as earlier noted, is substantially different from that of Ozark-Mahoning, Minerva or Allied Chemical. Nicholas J. Barone testified that Olin had investigated numerous fluoride removal techniques which were found to be unacceptable from an economic consideration. Olin's corporate engineering department devoted the efforts of some 50 people over a period of years on scaling up laboratory data to a full-scale operation intended for purchase and installation if the effluent standard was not changed.

Waste water from Olin's plant contains phosphate in proportions which enhance utilization of the lime process. Barone testified that the Olin fluoride removal process requires a ratio of phosphate to fluoride of 20 to 1 or greater or the process will fail to achieve the desired reduction (R. 232). An excess of lime of about 200% over stoichiometric is required to reach 2.5 mg/l fluoride.

The Olin process will require a capital investment of \$1.4 million and annual operating costs are estimated to be \$450,000 (R. 238). When operating, the Olin process will require 7 tons of lime and 28 tons of phosphate per day to treat the 1200 gpm waste water flow. About 70,000 lbs. of 35% solid sludge per day will be generated which will either be impounded or hauled to a landfill. Sludge disposal will cost an estimated \$80,000 to \$90,000 a year exclusive of land requirement cost (R. 240). Weighed against these factors will be the removal of an estimated 100 to 200 lbs. per day of fluoride (R. 288). Even with these process disadvantages, Olin believes it has a significant economic advantage over the other parties in this matter because of the phosphate

\* \*

content of its waste water. The other parties would have to add phosphate to their waste water to make them treatable. Barone estimated that phosphate addition would increase operating cost by an additional 10 to 20% (R. 257).

The U. S. EPA's criteria for best practicable treatment of fertilizer industry effluent calls for achieving 15 ppm fluoride or a maximum of 30 mg/l fluoride for any 24 hour period (R. 243). The U. S. EPA's best available technology for the steel industry calls for reduction to levels of 4.2 to 8.3 mg/l fluoride on a 30-day average and 10 to 20 mg/l as maximum allowable for a 24-hour period (R. 246).

However, if the effluent standard were changed to Olin's proposed level of 10 mg/1, Olin could reach this level through "in-process controls" (i.e. pump leakage control, recycling, etc.). Fluoride in Olin's waste water comes in large part from leakage from over 800 pumps at the Blockson Works (R. 241). Barone testified that the reduction to 10 mg/1 is a "very reliable number" (R. 269) based on actual experience at the plant (R. 254). Obviously the cost for in-process control would be far cheaper than installation and operation of a lime treatment process.

Allied Chemical's Metropolis plant effluent currently contains about 410 mg/l fluoride which is equivalent to a discharge of 7,000 lbs. per day fluoride (R. 375). Richard J. Sobel, Director of Environmental and Process Technology for Allied's Special Chemicals Division, testified that it is Allied's belief that technology is available to achieve 15 mg/l fluoride levels in the presence of calcium (R. 370). Allied is committed to a program aimed at an over-all level of 7 mg/l fluoride in the Metropolis plant effluent (R. 370).

Allied presented testimony in 1971 when the Board was considering the fluoride effluent standard. A. J. von Frank, Allied's Director of Air and Water Pollution Control, testified that it was a practical impossibility to achieve a fluoride level of less than 8.3 mg/l. This level represents the theoretical minimum that can be achieved in a water solution of calcium fluoride from the conventional lime method of fluoride removal (R. 371).

Sobel testified that Allied began a search of technical literature and an intensive in-house development program immediately after the Board adopted the 2.5 mg/l standard. This effort was directed toward discovery of a technically feasible and economically reasonable method of achieving the 2.5 mg/l standard. After two years of research and thousands of manhours, Allied concluded that there was no such method available.

Allied sought and was granted a variance from the fluoride effluent standard (and others) on February 28, 1974 upon satisfying

the Board that it was diligently working on fluoride abatement technology. Sobel testified that the abatement program approved in that variance will require about two years for completion at a cost in excess of \$4 million (R. 375). Research on fluoride removal technology will continue during the two year period.

Allied Chemical estimates that it would remove 6,880 lbs. per day of fluoride to achieve 7 mg/l. The capital investment for doing this would be \$2,683,200 and the operating costs would be \$660,000 per year. If the control equipment had a life expectancy of 10 years then capital costs would be approximately \$.107 per lb. of fluoride removed. Operating costs would be approximately \$0.26 per lb. of fluoride removed.

If Allied Chemical then used the most promising and technically feasible method to achieve 4.1 mg/l fluoride (filtration) an additional 33 lbs. of fluoride per day would be removed at a capital cost of \$220,110 (\$1.83 per lb. over a 10 year period) and an operating cost of \$73,000 per year (R. 377). If Allied then used a fixed alumina bed process to reach 2.5 mg/l, an additional 25 lbs. of fluoride per day would be removed at a capital cost of \$330,000 (\$3.62 per lb. over a 10 year period) and operating cost of \$99,000 per year (R. 378).

If the life expectancy of the abatement equipment is 10 years Allied Chemical would have capital costs of \$0.127 per lb. of fluoride removed. If the life expectancy of the equipment is 20 years then the capital costs for fluoride removal would be just \$0.064 per lb. The claim of Allied Chemical that capital costs would amount to \$9,480 per lb. per day is absurd. Allied's mistake was in failing to allocate the cost of the plant over the entire life expectancy. It seems obvious that the entire cost of the capital outlays should not be assigned to the first day of operation. The other companies which were participating in the hearings did not make this same mistake, but Allied Chemical made the mistake for them. (See Appendix A attached to Allied's final position paper). For instance, Allied claimed that capital costs for Ozark-Mahoning would amount to \$11,110 per pound of fluoride removed, apparently assigning a life expectancy of only one day for that proposed facility. Franklin Davis, the designer of the proposed Ozark-Mahoning system indicated that it would have a life expectancy of 20 years. Over a 20-year period the Ozark-Mahoning capital costs per pound of fluoride removed would be around \$1.50.

Allied Chemical did not tell us what the useful life of its control equipment will actually be. We doubt that the equipment installed at the Allied plant would have a life expectancy of 20 years. The U. S. EPA allows a depreciation factor of 10 years, and we have already noted that capital costs over a 10-year period would be less than \$.13 per pound of fluoride removed.

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The Internal Revenue Code allows companies to take depreciation deductions for pollution control facilities over a five year period instead of the "estimated useful life" of the equipment. This practice inflates the cost figures attributable to the equipment during the period of depreciation, a fact Allied Chemical readily concedes. However, such costs could not under any acceptable accounting practice reach \$9,480 per pound.

Proponents mine water discharges do not appear in danger of violating the Mine Related effluent criteria of 8 mg/l. No testimony relating to the Mine Related Pollution Control Regulation was presented by proponents.

A remaining problem unique to Ozark-Mahoning and Minerva comes about as a result of mine discharges. Proponents contend that Rule 302(k) of the Water Pollution Control Regulations "proceeds to designate" as Secondary Contact and Indigenous Aquatic Life Waters "all waters in which, by reason of low flow or other conditions, a diversified aquatic biota cannot be satisfactorily maintained even in the absence of contaminants".

Rule 302(k) (As amended February 14, 1974) states:

"Secondary Contact and Indigenous Aquatic Life Waters"

Secondary contact and indigenous aquatic life waters are those waters which will be appropriate for all secondary contact uses and which will be capable of supporting an indigenous aquatic life limited only by the physical configuration of the body of water, characteristics and origin of the water, and the presence of contaminants in amounts that do not exceed the applicable standards.

The following are designated as secondary contact and indigenous aquatic life waters;

(k) All waters in which by reason of low flow or other conditions, a diversified aquatic biota cannot be satisfactorily maintained even in the absence of contaminants."

In its Opinion on this matter the Board stated:

"Part III contains water use designations. All waters are designated for general use except those in the restricted category, which has here been broadened in response to testimony to include waters whose flow is too low to support aquatic life. This should relieve the burden of treatment beyond the effluent standards for discharges to intermittent streams. Such extra effort is difficult to justify when it will not result in a satisfactory aquatic life because of insufficient flow." (Vol. 3, p. 765).

The request of the mining companies that certain waters be designated "Secondary Contact and Indigenous Aquatic Life Waters" is important, because such designation would substantially increase the allowable fluoride levels in the stream.

Rule 402 of the Water Pollution Regulations provides:

"In addition to the other requirements of this Part, no effluent shall, alone or in combination with other sources, cause a violation of any applicable water quality standard. When the Agency finds that a discharge that would comply with effluent standards contained in this Chapter would cause or is causing a violation of water quality standards, the Agency shall take appropriate action under Section 31 or Section 39 of the Act to require the discharge to meet whatever effluent limits are necessary to ensure compliance with the water quality standards. When such a violation is caused by the cumulative effect of more than one source, several sources may be joined in an enforcement or variance proceeding, and measures for necessary effluent reductions will be determined on the basis of technical feasibility, economic reasonableness, and fairness to all discharges."

Therefore, if we adopt an effluent standard of 15 mg/l, the discharges must meet that effluent standard and also must not cause a violation of the Water Quality Standard beyond the mixing zone. The mining companies could meet a Water Quality Standard of 5 mg/l fluoride.

If on the other hand, the water quality standards were held at the present 1.4 mg/l criteria while the effluent standard is changed to 15 mg/l, the mining companies would still have a problem during periods of low flow when effluent from the mines is proportionately a larger part of the stream. Several alternatives would have to be considered by the mining companies:

1. The mining companies could petition to have the stream declared a "Secondary Contact and Indigenous Aquatic Life Water" under Rule 302(k). Water so designated would have a water quality standard identical to the new 15 mg/1 effluent standard (See Rule 205).

2. Ponding--This concept has already been discussed and found to be impracticable for the mining companies.

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3. Treat the effluent down to the water quality standard of 1.4 mg/l. This alternative would cause undue hardship on the mining companies.

4. Variance--This is available only on a temporary basis while permanent solutions to the problem are brought into play.

The record for reclassification of the streams is woefully inadequate. While numerous streams are known to be receivers of the mine water discharges, proponents sole presentation on the issue is a copy of an Agency report on biological samples taken on Big Grand Pierre Creek. As earlier noted, results of this survey indicate well-balanced benthic invertebrate populations both upstream and downstream from the mine discharge. One stream was found to be "semi-polluted".

If the Board were to act at this time on the information presented, the obvious decision would be to deny the "secondary contact" classification. However, the Board feels that no decision is required at this time on the Rule 302(k) matter simply because Rule 302(k) was not adequately addressed as an issue during these proceedings. Our ruling does not preclude Proponents from raising the Rule 302(k) issue at some later date. Our decision only relates to the inadequacy of the record now before the Board on that matter.

It is the Board's finding that Proponents, with the aid of Olin and Allied, have presented proof sufficient to warrant changing the fluoride effluent limit from 2.5 mg/l to 15 mg/l. Effluent of that quality should be acceptable in Illinois waters. The Water Quality Standard for fluoride remains unchanged at 1.4 mg/l for all dischargers other than the fluorspar mining and concentrating industry. The Water Quality Standard becomes 5 mg/l fluoride in waters which receive effluent from the mines and mills of the fluorspar mining and concentrating industry, and have been designated by the Illinois State Water Survey as streams which once in 10 years have an average minimum seven day low flow of zero.

Throughout these proceedings some degree of importance was attached to information in the Illinois EPA's Public Water Supplies Data Book, July 1973. In that document, fluoride levels in drinking water as high as 7.7 mg/l fluoride for Bureau Junction and 5.8 mg/l for Parkersberg are shown. Proponents state that they are not aware of any Agency initiated proceedings, enforcement or otherwise, because of the fluoride level in these public water supplies. However no evidence was introduced regarding the impact of these fluoride levels in these communities, and we certainly do not infer from the lack of legal action that 5.8 mg/l - 6.6 mg/l is an appropriate standard for the entire state. It is the responsibility of this Board, as charged by the Environmental Protection Act, to protect the quality of the environment. Having reviewed all aspects of these proceedings, the Board feels that an increase in the general water quality standard for streams receiving fluoride containing discharges from the fluorspar mining and concentrating industry, without change for other streams in the State, would not create significant and unwarranted effects on the environment. Unrefuted testimony and evidence in the record shows that no apparent environmental damage has occurred in these streams because of continuous mine discharges over a number of years.

In raising the water quality standard and the effluent limitation for fluoride, the Board has carefully taken into consideration the expected impact upon the receiving streams and the economic impact of the Regulation. Ozark-Mahoning and Minerva will receive relief for operation of their mines and concentrating mills. Ozark-Mahoning's current discharge level of 10 mg/l is below the new effluent limit and should not require any additional treatment barring a major process upset. Minerva, on the other hand, discharges water from its Mill #1 and Crystal Mill that are well within the 15 mg/l limit. Thus, Minerva will not be required to provide any additional fluoride control treatment unless process changes cause the fluoride concentration to increase significantly above the current concentrations.

In those instances where Proponent's mines discharge to flowing streams, current effluent levels appear to be low enough to preclude violation of the 15 mg/l effluent criteria. A different situation confronts Proponents when and if their mine discharges go to dry or intermittent streams. For the most part, mine discharges are well below the new 5 mg/l water quality standard for such streams. Pond #3 of Minerva's Mine #1 and Mill now average 4.5 mg/l and Minerva will have to monitor this discharge closely to insure that this discharge does not violate the new standard. With proper chemical treatment Minerva should be able to maintain this discharge concentration within the new limits.

Increasing the effluent limit to 15 mg/l will provide significant relief for Olin since that level can be reached by implementing "in process controls". In process controls, according to Barone's testimony, will involve some repiping, recycling of certain waste streams, elimination of chronic leaks and possibly some equipment modifications or replacement. Barone testified that Olin considered in process controls to be "a very attractive thing" since the operating costs would be so low as to not even show up as a separate cost (R. 242).

Through the recycling effort Olin would actually receive some benefit since phosphate materials now being discharged would be recovered and end up as product instead of waste. Olin did not

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have any cost figures relating to in process control but Barone testified that the capital investment would be "much lower" than installing a lime treatment plant (R. 242).

This change in effluent criteria for fluoride affects Allied differently since the current fluoride concentration in Allied's effluent is significantly higher than that of any other party in this matter.

At one time in these proceedings Allied sought to change the effluent standard to allow 15 mg/l fluoride based on the average of 24 hour composite analysis for thirty consecutive days and 30 mg/l maximum for any one 24 hour composite. In its last submission Allied states that its recommended standard of 30 mg/l for any one 24 hour composite may prove to be too restrictive for some industries such as hydrofluoric acid manufacturers. Allied now seeks to change the effluent limit to 30 mg/l as the average of 24 hour composites for 30 consecutive days and 60 mg/l for any one 24 hour period.

Allied's original recommendation was based upon criteria published in Volume 39, No. 49 of the Federal Register on March 12, 1974 by the U. S. Environmental Protection Agency. The reasonableness of this U. S. EPA criteria was challenged by the hydrofluoric acid manufacturers in the Fourth Circuit Court of Appeal. One result of this action, according to Allied, is that the U. S. EPA now plans to revise the fluoride effluent limitations to the same limits Allied now seeks in this matter. Although the U. S. EPA has not yet proposed any new limits, Allied states that Region VI of the U. S. EPA granted Allied a permit for its Baton Rouge Works on December 9, 1974 using the new limit.

Allied is now committed to a fluoride reduction program designed to achieve a fluoride concentration in its effluent of 7 mg/l. Undoubtedly, Allied will modify this program to meet the fluoride level now permitted and we would expect this modification to reduce cost.

Having considered all information in this record concerning the technical feasibility and economic reasonableness of alternative methods of fluoride abatement in conjunction with the data from a commercial lime treatment facility now in operation at another Allied facility it is our finding that the 15 mg/l fluoride is both economically reasonable and technically feasible when applied to Allied Chemical.

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#### ORDER

It is the Order of the Pollution Control Board that the Water Quality Standards and the Effluent Standards of the Illinois Water Pollution Control Regulations be amended to specify the following limitations for fluoride:

PART II WATER QUALITY STANDARDS

203.1 Exceptions to Rule 203

- (a) The fluoride standard of Rule 203(f) shall not apply to waters of the State which:
  - receive effluent from the mines and mills of the fluorspar mining and concentrating industry, and
  - (2) have been designated by the Illinois State Water Survey as streams which once in ten years have an average minimum seven day low flow of zero.

Such waters shall meet the following standard with regard to fluoride:

Constituent	Storet Number	Concentration (mg/l)
Fluoride	00950	5

PART IV EFFLUENT STANDARDS

- 408 Additional Contaminants
  - (a) The following levels of contaminants shall not be exceeded by any effluent:

Constituent	Storet Number	Concentration (mg/l)	-
Fluoride (tota	1) 00951	15	

I, Christan L. Moffett, Clerk of the Illinois Pollution Control Board, hereby certify the above Opinion and Order was adopted this \_\_\_\_\_\_ day of \_\_\_\_\_\_, 1975 by a vote of \_\_\_\_\_\_.

Christen In affect

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Attachment 5

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Information from the Illinois State Geological Survey

# Illinois State Geological Survey Geobit 4

Fluorite—Illinois' State Mineral

Deep purple, amethyst, sky blue, sea green, sunny yellow, and crystal clear—the mineral fluorite comes in all colors. Many types of fluorite even glow under ultraviolet light, They're "fluorescent."

Pure fluorite  $(CaF_2)$ , made of the elements calcium (Ca) and fluorine (F), is colorless. The various colors result from tiny amounts of other elements substituting for the calcium in the crystalline structure.

Transparent to translucent, this glass-like mineral may be found as irregular masses filling veins that cut through rocks, or in flat-lying bands or layers parallel with the bedding planes of sedimentary rocks. As the photos show, fluorite also forms as clusters of beautiful cubic crystals.

Light reflects strongly from fluorite's crystal

faces and cleavage surfaces, which can be polished to a high luster. As lovely as a gemstone, fluorite is brittle and relatively soft (4 on Moh's hardness scale), so it's unsuitable for ring settings. Brooches and pendants must be handled carefully to avoid scratching or fracturing the mineral specimens in these settings.

Just for display, miners chipped octahedrons out of coarse crystals of the mineral known to the mining industry as *fluorspar*. They called the eight-sided crystals "diamonds."

#### How did Illinois' fluorite deposits form?

Hot water containing fluorine and other dissolved chemicals rose from deep in the earth during the Jurassic Period, about 150 to 200 million years ago. The water flowed through northeast-trending faults and fractures in limestones laid down earlier in the Mississippian Period, about 330 million years ago.

When the hot brines reached the calcium-rich Mississippian rocks, the temperature and other conditions were just right for crystallizing fluorite along the walls of the faults and in flat-lying layers parallel to the beds of limestone. These host rocks dissolved and were replaced with the fluorite.

#### Country's leading producer of fluorspar

Since the early 1800s, fluorite has been mined in southeastern Illinois. The fluorspar-rich region, which reaches from southeastern Illinois into parts of Kentucky, was called the Illinois–Kentucky Fluorspar Mining District.

In Illinois, fluorite was mined almost exclusively in Hardin and Pope Counties. The main production came from fissure-vein deposits in the Rosiclare district, and stratiform (bedding plane) deposits in the Cave in Rock district (*map*, *p*. 2). Other areas in the two counties yielded smaller amounts of the mineral.

Most mining was underground, as much as 1,300 feet deep. But open-pit mines operated where fluorite deposits intersected land surface.

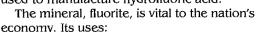
Illinois displaced Kentucky as the country's leading producer of fluorite in 1942. For many years, Illinois accounted for more than 50% of total U.S. fluorspar production. But by 1990, more than 90% of the fluorite used in the U.S. was imported. Illinois was the only remaining domestic producer.

Competition from foreign producers coupled with high costs of underground operations made Illinois' fluorspar mining unprofitable. The last fluorspar mine in Illinois closed in December 1995. Fluorspar is no longer mined anywhere in the United States.

*Illinois' State Mineral* The General Assembly made fluorite the State Mineral in 1965, when fluorspar mining was a multimillion-dollar-per-year industry in Illinois. Over the years, much more fluorite has been mined in Illinois than in any other state.

#### The many uses for fluorite

Native Americans carved fluorspar to make artifacts, but the first recorded use of Illinois' fluorite was in 1823, when fluorspar mined near Shawneetown in Gallatin County was used to manufacture hydrofluoric acid.



Mineral ....

- smelting iron, aluminum, and other metal alloys,
- manufacturing glass, enamel glazes, ceramics, portland cement, and many chemical compounds.

Hydrofluoric acid

- refining aluminum,
- · refining uranium fuel for nuclear reactors,
- · making rocket fuel and metal plating.

Inorganic fluoride chemicals

 toothpastes, special fluxes for welding rods, optical lenses, and concrete hardeners.

Organic fluoride chemicals .

 Plastics, refrigerants, nonstick coatings, lubricants, stain repellents, dyes, herbicides, medicines and anesthetics, cleaning solvents, degreasing agents and foaming agents.

One of the most widely used organic fluoride compounds, the refrigerant Freon 12<sup>®</sup>, is no longer produced in the United States. The chlorine in the compound is thought to damage the protective ozone layer that shields the earth from ultraviolet radiation.

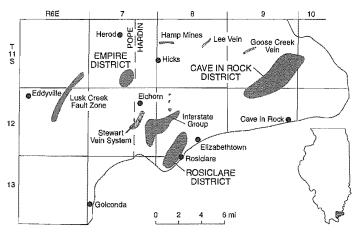
#### Contributed by D.L. Reinertsen and J.M. Masters

ILLINOIS STATE GEOLOGICAL SURVEY 615 East Peabody Drive Champaign, IL 61820-6964 217/333-4747 FAX 217/244-7004





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Principal mining areas in the southeastern Illinois part of the Illinois–Kentucky Fluorspar Mining District.

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Attachment 6

Great Lakes Environmental Commission Final Report (October 22, 2010) (Excerpts pertaining to boron, manganese and fluoride tests only)

#### Final Report on Acute and Chronic Toxicity of Nitrate, Nitrite, Boron, Manganese, Fluoride, Chloride and Sulfate to Several Aquatic Animal Species

Prepared for:



United States Environmental Protection Agency Office of Science and Technology Health and Ecological Criteria Division

EPA Contract: EP-C-09-001 Work Assignments: B-12 and 1-12 Contacts: Tom Poleck and Brian Thompson

Prepared by:



Great Lakes Environmental Center 739 Hastings Street Traverse City, Michigan 49686 Phone: (231) 941-2230 Facsimile: (231) 941-2240 Contacts: Jamie Saxton and Mailee Garton

SUBMISSION DATE: October 22, 2010

#### Boron

Table 60 provides a summary of estimated  $LC_{50}$  values for the nine toxicity tests performed using boron.  $LC_{50}$  values ranged between 28.4 and >544 mg B/L.

Test Species and Duration	LC <sub>50</sub> (mg B/L)
Lampsilis siliquoidea 96 hr	137
Pimephales promelas - 96 hr	101
Pimephales promelas - 32 day	28.4
Ceriodaphnia dubia - 48 hr (pH 7.75)	76.9
Pimephales promelas - 96 hr (pH 6.75)	70.6
Pimephales promelas - 96 hr (pH 7.75)	137
Pimephales promelas - 96 hr (pH 8.75)	133
Ligumia recta - 96 hr	147
Megalonaias nervosa - 96 hr	> 544

Table 60. LC<sub>50</sub> estimates for toxicity tests performed using boron.

For each of the acute toxicity tests completed using boron, two tables were generated: the first summarizes the test results for each toxicity test, including nominal and analytical test concentration and  $LC_{50}$  estimates with confidence intervals; the second table summarizes analytical chemistry data collected throughout the toxicity tests. The results of chronic tests performed with boron were summarized in three tables: the first summarizes nominal and analytical test concentrations,  $LC_{50}$  estimates with confidence intervals, NOEC and LOEC estimates, mean survival and mean biomass; the second table summarizes replicate-specific survival and growth data and the third table summarizes analytical chemistry data collected throughout the toxicity tests. Also discussed, if applicable, are deviations from the guidance provided in the ASTM method used to complete the toxicity testing.

#### 96-hr Toxicity of Boron on Lampsilis siliquoidea

The 96-hr test to determine the toxicity of boron on *L. siliquoidea* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Missouri State University laboratory culture, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 500, 250, 125, 62.5, and 31.3 mg B/L.

Testing was conducted at  $20 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC<sub>50</sub> value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 61; test results are provided in Table 62. Analytical chemistry data are provided in Table 63. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 18.

Summary of Toxicity Test Conditions								
1. Test Species and Age:	Lampsilis siliquoidea, juveniles <5 days old (Missouri State University)							
2. Test Type and Duration:	Static, 96 hours							
3. Test Dates:	June 03 - 07, 2009							
4. Test Temperature (°C):	$20 \pm 1$							
5. Light Quality:	Ambient Laboratory, 10-20 µE/m²/s							
6. Photoperiod:	16 h light, 8 h darkness							
7. Feeding Regime:	None							
8. Size of Test Vessel:	50 mL beaker							
9. Volume of Test Solutions:	40 mL							
10. No. of Test Organisms per Test Vessel:	5							
11. No. of Test Vessels per Treatment:	4							
12. Total No. of Test Organisms per Treatment:	20							
13. Test Concentrations (mg B/L):	500, 250, 125, 62.5, and 31.3							
<ol> <li>Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg B/L):</li> </ol>	524, 260, 140, 72, and 34							
15. Renewal of Test Solutions:	None							
16. Dilution and Primary Control Water:	USEPA MHRW							
17. Test Material:	Boric acid: Acros Organics, 99.6%, ACS Reagent (crystals) Cas. No. 10043-35-3, Lot # B0124654 and Borax (sodium tetraborate decahydrate), 99.5 +% (for analysis ACS), Cas. No. 1303-96-5, Lot # A0256722							
18. Secondary Control Water:	None							
19. Aeration:	None							
20. Endpoints Measured:	Mortality (LC <sub>50</sub> )							

# Table 61. Test conditions for 96-hour toxicity test on *Lampsilis siliquoidea* with boron.

Results of a <i>Lampsilis siliquoidea</i> <u>96-Hour Static Acute Toxicity Test</u> Conducted <u>06/03/09 - 06/07/09</u> <u>Using: Boric acid Cas. No. 10043-35-3 and Borax Cas. No. 1303-96-5</u>											
L	Cumula	tive Perce	nt Affected	] *		LC <sub>50</sub> Values*	(mg/L)				
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr			
Primary Control/ Dilution Water	5	5	5	5	>524	>524	181	137			
31.3 (34) mg/L	0	10	25	35	96-Hour LC	<sub>50</sub> * = 137 mg/I	4				
62.5 (72) mg/L	0	25	30	35	LC <sub>50</sub> 95% Confidence Limits						
125 (140) mg/L	10	15	35	45	24-Hr	48-Hr	72-Hr	96-Hr			
250 (260) mg/L	15	25	60	90	LL NR UL NR	NR NR	110 296	86 220			
500 (524) mg/L	15	20	90	95							
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable Method(s) Used to Determine LC <sub>50</sub> and EC <sub>50</sub> Confidence Limit Values: Spearman-Karber						

Table 62. Test results for 96-hour toxicity test on Lampsilis siliquoidea with boron.

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Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All  $LC_{50}$  and  $EC_{50}$  values are determined based on measured concentrations.

### Table 63. Analytical chemistry data for 96-hour toxicity test on *Lampsilis siliquoidea* with boron.

Nominal (Measured) Test Concentration		Boron*	Temperature	pH	D.O.	Conductivity	Alkalinity	Hardnes
		(mg/L)	(°C)	(s.u.)	(mg/L)	(µmhos)	(mg/L)	(mg/L)
	Day 0	0.1	20.0	8.0	7.81	305	60	90
	Day 1		19.9					
	Day 2		20.0					
	Day 3		20.1					
	Day 4	1.1	19.9	8.0	7.10	305	62	90
Dilution water/Control		0.3						
	Day 0	35	20.1	8.0	7.80	322	82	90
	Day 1		19.8					
	Day 2		20.0					
	Day 3		20.1					
	Day 4	33	19.9	8.0	7.05	320	82	92
31.3 (34) mg/L		34						
	Day 0	69	20.0	8.0	7.80	344	90	90
	Day 1		19.8					
	Day 2		20.1					
	Day 3		20.1					
	Day 4	76	20.0	8.0	6.99	350	92	92
62.5 (72) mg/L		72						
	Day 0	130	20.2	8.0	7.92	385	116	90
	Day 1		19.7					
	Day 2		20.1					
	Day 3		20.1					
	Day 4	150	19.9	8.0	6.97	390	120	90
125 (140) mg/L		140						
	Day 0	250	20.0	8.0	7.96	464	164	88
	Day 1		19.9					
	Day 2		20.1					
	Day 3		20.1					
	Day 4	270	19.9	8.0	6.92	465	164	90
250 (260) mg/L		260						
	Day 0	500	20.1	8.1	7.99	619	272	86
	Day 1		20.0					
	Day 2		20.1					
	Day 3		20.1					
	Day 4	550	20.0	8.0	6.89	625	270	90
500 (524) mg/L	-	524						

\*Boron Analysis Method 200.7

#### 96-hr Toxicity of Boron on Megalonaias nervosa

The 96-hr test to determine the toxicity of boron on *M. nervosa* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Genoa National Fish Hatchery, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate. In one replicate of the 250 mg/L treatment, a test organism was inadvertently crushed, but this was accounted for in the  $LC_{50}$  calculation.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 500, 250, 125, 62.5, and 31.3 mg B/L.

Testing was conducted at  $20 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC<sub>50</sub> value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 64; test results are provided in Table 65. Analytical chemistry data are provided in Table 66. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 19.

Summary of Toxicity Test Conditions								
1. Test Species and Age:	Megalonaias nervosa, juveniles <5 days old, Genoa National Fish Hatchery							
2. Test Type and Duration:	Static, 96 hours							
3. Test Dates:	October 16 - 20, 2009							
4. Test Temperature (°C):	$20 \pm 1$							
5. Light Quality:	Ambient Laboratory, 10-20 µE/m²/s							
6. Photoperiod:	16 h light, 8 h darkness							
7. Feeding Regime:	None							
8. Size of Test Vessel:	50 mL beaker							
9. Volume of Test Solutions:	40 mL							
10. No. of Test Organisms per Test Vessel:	5							
11. No. of Test Vessels per Treatment:	4							
12. Total No. of Test Organisms per Treatment:	20							
13. Test Concentrations (mg B/L):	500, 250, 125, 62.5, and 31.3							
<ul> <li>14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg B/L):</li> </ul>	544, 275, 140, 74, and 37							
15. Renewal of Test Solutions:	None							
16. Dilution and Primary Control Water:	USEPA MHRW							
17. Test Material:	Boric acid: Acros Organics, 99.6%, ACS Reagent (crystals) Cas. No. 10043-35-3, Lot # B0124654 and Borax (sodium tetraborate decahydrate), 99.5 +% (for analysis ACS), Cas. No. 1303-96-5, Lot # A0256722							
18. Secondary Control Water:	None							
19. Aeration:	None							
20. Endpoints Measured:	Mortality (LC <sub>50</sub> )							

### Table 64. Test conditions for 96-hour toxicity test on *Megalonaias nervosa* with boron.

Conducted <u>10/16/09 - 10/20/09</u> <u>Using: Boric acid Cas. No. 10043-35-3 and Borax Cas. No. 1303-96-5</u>											
	Cumula	tive Perce	nt Affected	1 •		LC50 Values	" (mg/L)				
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr			
Primary Control/ Dilution Water	0	0	0	0	>544	>544 >544 >544 >5					
31.3 (37) mg/L	0	0	0	0	96-Hour LC	<sub>50</sub> * = >544mg/	L				
62.5 (74) mg/L	0	0	5	10	LC <sub>50</sub> 95% Confidence Limits						
125 (140) mg/L	0	0	5	15	24-Hr	48-Hr	72-Hr	96-Hr			
250 (275) mg/L	0	5	10	20	LL NR UL NR	NR NR	NR NR	NR NR			
500 (544) mg/L	0	0	0	5							
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable						
					Method(s) U	R = Confidence Intervals are not reliable Method(s) Used to Determine $LC_{50}$ and $EC_{50}$ Confidence Limit Values: Spearman-Karber					

#### Table 65. Test results for 96-hour toxicity test on Megalonaias nervosa with boron.

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<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All  $LC_{50}$  and  $EC_{50}$  values are determined based on measured concentrations.

### Table 66. Analytical chemistry data for 96-hour toxicity test on *Megalonaias nervosa* with boron.

Nominal (Measured) Test									
Concentration		Boron"	Temperature	pН	<b>D.O.</b>	Conductivity	Alkalinity	Hardness	
		(mg/L)	(°C)	(s.u.)	(mg/L)	(mmos)	(mg/L)	(mg/L)	
	Day 0	<0.02	21.0	7.8	7.95	300	60	88	
	Day 1		20.8	7.7	8.27	303			
	Day 2		20.5	7.8	8.21	305			
	Day 3		20.3	8.0	8.21	290			
	Day 4	<0.02	20.6	8.0	7.62	337	68	90	
Dilution water/Control		na							
	Day 0	36	21.0	8.0	8.21	320	70	88	
	Day 1		20.5	7.9	8.32	320			
	Day 2		20.5	7.9	8.22	320			
	Day 3		20.4	8.0	8.28	330			
	Day 4	38	20.6	8.0	8.15	351	72	88	
31.3 (37) mg/L		37							
	Day 0	72	21.0	8.0	8.20	340	86	88	
	Day 1		20.8	7.9	8.34	343			
	Day 2		20.5	7.9	8.22	345			
	Day 3		20.3	8.0	8.27	347			
	Day 4	76	20.5	8.0	8.25	364	90	88	
62.5 (74) mg/L		74							
	Day 0	140	21.0	7.9	8.25	381	110	88	
	Day 1		20.9	8.0	8.38	389			
	Day 2	-	20.6	8.0	8.23	390			
	Day 3		20.5	8.1	8.29	401			
	Day 4	140	20.7	8.1	8.27	417	115	88	
125 (140) mg/L		140							
	Day 0	270	21.0	7.9	8.23	460	160	88	
	Day 1		20.8	8.0	8.40	461			
	Day 2		20.6	8.0	8.21	461			
	Day 3		20,5	8.1	8.25	488			
	Day 4	280	20.8	8.1	8.28	504	178	88	
250 (275) mg/L	2	275							
<u>`````````````````````````````````</u>	Day 0	520	21.0	7.9	8.24	613	266	86	
	Day 1		20.9	8.0	8.32	616			
	Day 2		20.6	8.0	8.20	618	1		
	Day 2 Day 3		20.4	8.1	8.25	638	1		
	Day 4	570	20.8	8.2	8.23	654	276	88	
500 (544) mg/L	, ,	544		0.2	0.40				

<sup>a</sup> Boron Analysis Method 200.7

na=not applicable

#### 96-hr Toxicity of Boron on Ligumia recta

The 96-hr test to determine the toxicity of boron on *L. recta* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Missouri State University laboratory culture, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate. One replicate was mistakenly loaded with only four individuals, but this was accounted for in the  $LC_{50}$  calculation.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 500, 250, 125, 62.5, and 31.3 mg B/L.

Testing was conducted at  $20 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC<sub>50</sub> value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 67; test results are provided in Table 68. Analytical chemistry data are provided in Table 69. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 20.

Su	Summary of Toxicity Test Conditions								
1,	Test Species and Age:	<i>Ligumia recta</i> , juveniles <5 days old, Missouri State University							
2.	Test Type and Duration:	Static, 96 hours							
3.	Test Dates:	September 10 - 14, 2009							
4.	Test Temperature (°C):	20 <u>+</u> 1							
5.	Light Quality:	Ambient Laboratory, 10-20 µE/m²/s							
6.	Photoperiod:	16 h light, 8 h darkness							
7.	Feeding Regime:	None							
8.	Size of Test Vessel:	50 mL beaker							
9.	Volume of Test Solutions:	40 mL							
10.	No. of Test Organisms per Test Vessel:	5							
11.	No. of Test Vessels per Treatment:	4							
12.	Total No. of Test Organisms per Treatment:	20							
13.	Test Concentrations (mg B/L):	500, 250, 125, 62.5, and 31.3							
14.	Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg B/L):	510, 255, 130, 64, and 33							
15.	Renewal of Test Solutions:	None							
16.	Dilution and Primary Control Water:	USEPA MHRW							
17.	Test Material:	Boric acid: Acros Organics, 99.6%, ACS Reagent (crystals) Cas. No. 10043-35-3, Lot # B0124654 and Borax (sodium tetraborate decahydrate), 99.5 +% (for analysis ACS), Cas. No. 1303-96-5, Lot # A0256722							
18.	Secondary Control Water:	None							
19.	Aeration:	None							
20.	Endpoints Measured:	Mortality (LC <sub>50</sub> )							

#### Table 67. Test conditions for 96-hour toxicity test on Ligumia recta with boron.

Results of a <i>Ligumia recta</i>	<u>96-Hou</u>	r <u>Static A</u>	cute Toxici	ity Test								
Conducted <u>09/10/09 - 09/14/09</u> Using: Boric acid Cas. No. 10043-35-3 and Borax Cas. No. 1303-96-5												
	Cumula	tive Percei	nt Affected	a		LC <sub>50</sub> Values*	' (mg/L)					
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr				
Primary Control/ Dilution Water	0	0	0	0	>510	>510	>510	147				
31.3 (33) mg/L	0	0	0	30	96-Hour LC	<sub>:0</sub> * = 147 mg/I						
62.5 (64) mg/L	0	0	0	30	LC <sub>50</sub> 95% Confidence Limits							
125 (130) mg/L	0	0	0	50	24-Hr	48-Hr	72-Hr	96-Hr				
250 (255) mg/L	0	0	0	58	LL NR UL NR	NR NR	NR NR	88 246				
500 (510) mg/L	0	0	0	100	ob tit			210				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable							
					$NR = Confidence Intervals are not reliable$ $Method(s) Used to Determine LC_{50} and EC_{50}$ $Confidence Limit Values: Spearman-Karber$							

#### Table 68. Test results for 96-hour toxicity test on Ligumia recta with boron.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All  $LC_{50}$  and  $EC_{50}$  values are determined based on measured concentrations.

### Table 69. Analytical chemistry data for 96-hour toxicity test on *Ligumia recta* with boron.

Nominal (Measure Concentration		Boron <sup>a</sup>	Temperature	pH	<b>D.O.</b>	Conductivity	Alkalinity	Hardness
		(mg/L)	(°C)	(s.u.)	(mg/L)	(µmhos)	(mg/L)	(mg/L)
	Day 0	<0.02	20.4	7.9	8.18	301	60	92
	Day 1		19.5					
	Day 2		19.1					
	Day 3	1	19.2					
	Day 4	<0.02	19.2	8,1	8.14	312	60	92
Dilution water/Control		па						
	Day 0	33	20.5	8.0	8.14	320	68	92
	Day 1	1	19.5					
	Day 2		19.0					
	Day 3		19.3	1				
	Day 4	34	19.3	8.1	8.10	334	68	92
31.3 (33) mg/L	<u>_</u>	33				kuunna 1997 juli 2000 juli 200		
	Day 0	62	20.5	8.0	8.13	341	90	90
	Day 1		19.5			0.7		
	Day 2		19.0					
	Day 3		19.3					
	Day 4	66	19.3	8.1	8.11	353	90	90
62.5 (64) mg/L		64						
	Day 0	130	20.4	8.0	8.12	382	112	90
	Day 1		19.5			ULBERRAR		
	Day 2		19.0					
	Day 3		19.3					
	Day 4	130	19.3	8.1	8.05	394	112	90
125 (130) mg/L	\$	130						
	Day 0	250	20.5	8.1	8.12	460	170	90
	Day 1		19.5					
	Day 2		19.2					
	Day 3		19.3					
	Day 4	260	19.3	8.1	8.03	472	170	90
250 (255) mg/L		255						
	Day 0	500	20.5	8.1	8.11	616	270	90
	Day 1		19.5					
	Day 2		19.4					
	Day 3		19.1					
	Day 4	520	19.1	8.1	8.12	634	270	90
500 (510) mg/L		510						

<sup>a</sup> Boron Analysis Method 200.7 na = not applicable

#### 96-hr Toxicity of Boron on Pimephales promelas

The 96-hr test to determine the toxicity of boron on *P. promelas* was completed by GLEC. Test organisms, collected from the GLEC laboratory culture, were acclimated to the dilution water (de-chlorinated Lake Michigan water), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); two replicates were used per treatment with ten organisms per replicate.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 38.9, 64.8, 108, 180, 300 and 500 mg B/L.

Testing was conducted at  $25 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC<sub>50</sub> value was determined using the Probit method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 70; test results are provided in Table 71. Analytical chemistry data are provided in Table 72. Accompanying information, including raw laboratory data, analytical chemistry data, reference toxicant data and statistical analyses, is provided in Appendix 21.

Summary of Toxicity Test Conditions							
1. Test Species and Age:	<i>Pimephales promelas</i> , (weight 0.12g and 19.8 mm length), GLEC Culture						
2. Test Type and Duration:	Static, 96 hours						
3. Test Dates:	September 2-September 6, 2009						
4. Test Temperature (°C):	25 ± 1						
5. Light Quality:	Ambient Laboratory, 10-20 µE/m <sup>2</sup> /s						
6. Photoperiod:	16 h light, 8 h darkness						
7. Feeding Regime:	None						
8. Size of Test Vessel:	4000 mL beaker						
9. Volume of Test Solutions:	3500 mL						
10. No. of Test Organisms per Test Vessel:	10						
11. No. of Test Vessels per Treatment:	2						
12. Total No. of Test Organisms per Treatment:	20						
13. Target or Nominal Test Concentrations (mg B/L):	500, 300, 180, 108, 64.8, and 38.9						
<ol> <li>Analytical Test Concentrations (average of samples collected at test initiation and termination-mg B/L):</li> </ol>	546, 352, 200, 123, 71.5, and 46.1						
<ul><li>15. Renewal of Test Solutions:</li><li>16. Dilution and Primary Control Water:</li></ul>	None						
17. Test Material:	De-chlorinated Lake Michigan Water						
	Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, $\ge$ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172						
18. Secondary Control Water:	None						
19. Aeration:	None						
20. Endpoints Measured:	Mortality (LC <sub>50</sub> )						

# Table 70. Test conditions for 96-hour toxicity test on *Pimephales promelas* with boron.

Results of a <i>Pimephales promelas</i> 96 <u>-Hour Static Acute Toxicity Test</u> Conducted <u>09/02/09 - 09/06/09 Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043 35-3)</u> (Borax: Sigma Aldrich Cas No. 1303-96-4)										
	Cumula	tive Perce	nt Affecte	ed "	LC <sub>50</sub> * Values (mg/L)					
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr		
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	0 (0)	>546	312	173	101		
38.9 (46.1) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	96-Hour LC <sub>50</sub> *= 101 mg/L					
64.8 (71.5) mg/L	0 (0)	0 (0)	0 (5)	15 (15)	LC <sub>50</sub> *95% Confidence Limits					
108 (123) mg/L	0 (0)	0 (0)	20 (20)	70 (70)	24-Hr	48-Hr	72-Hr	96-Hr		
180 (200) mg/L	0 (0)	5 (5)	60 (60)	100 (100)	LL NA UL NA	271 353	150 200	88.3 116		
300 (352) mg/L	0 (0)	65 (65)	100 (100)	100 (100)						
500 (546) mg/L	45 (45)	100 (100)	100 (100)	100 (100)	LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable					
					Method(s) Used to Determine LC <sub>50</sub> Confidence Limit Values: Probit					

#### Table 71. Test results for 96-hour toxicity test on *Pimepehales promelas* with boron.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All LC<sub>50</sub> values are determined based on measured concentrations.

Nominal (and M	easured)									
Test Concentrations		Boron	Temperature	pН	D.O. (mg/L)		Conductivity	Alkalinity	Hardness	
	Day 0	(mg/L) 0.03	(°C) 25.0	(s.u.)	8.0	g/L)	(mmos) 290	(mg/L)	(mg/L)	(mg/L)
	the second s	0.03	24.7	8.01	6.3	6.0	290	104	144	ND
	Day 1 Day 2		24.7	7.54	5.9	6.0				
Dilution							-			
water/Control	Day 3 Day 4	0.04	24.8 24.7	7.65	5.7 6.0	5.5 6.1	278	100	132	
	Day 4	0.04	24.7	7.90	6.0	0.1	2/8	100	132	
		0.035								·
	Day 0	54.7	25.0	8.16	8.0	<b>I</b>	318		1	<b></b>
	Day 1		24.6	8.12	6.7	6.8			1	
	Day 2	1	24.8	8.02	6.4	6.5	-		1	
38.9 ( 46.1) mg/L	Day 3		24.9	8.05	6.0	5.9		1		
	Day 4	37.4	24.7	8.07	6.4	6.5	323			
		46.1		0.07	<u>.</u> ,					
				0.44	7.0	1		T		I
	Day 0	82	25.0	8.14	7.9		335			
	Day 1		24.7	8.17	7.7	6.7			4	
	Day 2		24.8	8.05	4.6	6.4				
64.8 (71.5) mg/L	Day 3		24.8	8.11	6.3	4.5				
	Day 4	61	24.7	8.02	5.4	6.4	346			<u> </u>
		71.5								
		1		A 44		····-				
108 (123) mg/L	Day 0	146	25.0	8.18	8.0		362			
	Day 1		24.8	8.18	7.0	6.4				
	Day 2		25.0	8.15	6.5	6.2		ļ		
. , -	Day 3		24.9	8.13	5.9	6,4				
	Day 4	99.1 123	24.8	8.15	6.7	6.0	376		+	
	L		L				1			I
180 (200) mg/L	Day 0	223	25.0	8.18	8.0		406			
	Day 1		25.1	8.17	6.2	6.3				
	Day 2		24.9	8.14	6.1	5.8				
	Day 3		24.9	8.11	5.9	4.7				
	Day 4	176	24.8	8.13	5.3	5.3	422	146	136	
	I	200				<u> </u>		]	_l	
	Day 0	388	25.0	8,17	8.0	1	476	T	1	
	Day 1		24.7	8.17	7.0	6.3				
00 (252)	Day 2		24.8	8.17	6.8	5.4				
300 (352) mg/L	Day 3	1	24.8	8.15	5.8	5.0		1		
	Day 4	316				<b></b>				
	I	352	I]			1		]	<u> </u>	
	Day 0	585	25.0	8.14	8.0	[	597	316	168	ND
	Day 1		24.7	8.15	7.0	7.1				
500 (546) mg/L	Day 2		24.8	8.13	7.4	7.5				l
	Day 3					1		1	1	
	Day 4	507				1			1	
	· · · · · · · · · · · · · · · · · · ·	546	1			1		1	1	1

# Table 72. Analytical chemistry data for 96-hour toxicity test on *Pimephales promelas* with boron.

<sup>a</sup> Boron Analysis EPA 200.8 ND Not Detect; below detection limit

#### 32-day Toxicity of Boron on Pimephales promelas

The 32-day test to determine the toxicity of boron on *P. promelas* was completed by GLEC. The fish were continuously exposed for 32 days to five concentrations of boron (nominal concentrations of 6.3, 12.5, 25, 50 and 100 mg B/L) and to a dilution water control using a continuous flow-through system (Benoit et al. 1982). The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were four replicate test chambers for each treatment. The flow through test was conducted at  $25 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with < 24 hour old fertilized embryos. The embryos were randomly assigned to incubation cups until each incubation cup contained 30 embryos. The incubation cups were randomly assigned to the 2.5 L glass test chambers (1 cup per chamber) and suspended in the test solutions from a rocker arm assembly. The rocker arm assembly moves the incubation cups in a reciprocal motion within each test chamber. Embryos were inspected on a daily basis and the number of live, hatched and dead embryos was recorded. On Day 8 of the test (four days after first hatch), the surviving fish were randomly thinned to achieve 20 fish in each test chamber. The remainder of the surviving fish was discarded. The number of surviving fish was recorded at test termination (32 days). In addition, the wet weights were recorded for each fish at test termination. Because of the size range of fish in each test chamber, all of the fish from each test chamber were weighed together to determine average dry weight.

Instantaneous water temperature measurements made on September 12 (Day 2: 23.5°C-24.5°C), October 1 (Day 21: 23.7°C-24.3°C), October 9 (Day 29: 25.9°C-26.5°C), October 11 (Day 31: 23.7°C-24.1°C), and October 12 (Day 32: 23.8°C-24.5°C) exceeded the allowable range of  $25 \pm 1$  °C in the toxicity testing method. However, the overall average water temperatures (across the duration of the test) in each replicate were within  $\pm 0.5$  °C of the target test temperature (25 °C) in all treatments. Therefore, the water temperature exceedances noted above likely had no effect on the results of this study.

On September 19 and 20, 2009 (test days 9 and 10) 60-90 percent mortality occurred in replicates one and two of the laboratory control. It is of GLEC's opinion that the equipment used during the thinning procedure on test day 8 contributed toxicity to these two control replicates, resulting in the high fish mortality. This high mortality was communicated to the EPA Work Assignment Manager and GLEC was advised to continue the test with the assumption that further control mortality would result in test failure. No further toxicity was observed in the remaining control fish throughout the test. However, because of the mortality observed in the control treatments, only replicates three and four of the laboratory control were used in the survival and growth statistical comparisons.

Once the test was complete, the LC<sub>50</sub>, NOEC and LOEC values were determined using the average measured concentrations with the Spearman Karber and ANOVA methods. LC<sub>25</sub>, LC<sub>20</sub> and LC<sub>10</sub> values were determined using the Probit method and EC<sub>50</sub>, EC<sub>25</sub>, EC<sub>20</sub> and EC<sub>10</sub> values were estimated using EPA's TRAP.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 73; test results are provided in Table 74. Survival and growth data are provided in Table 75 and analytical chemistry data are provided in Table 76. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 22.

# Table 73. Test conditions for 32-day toxicity test on *Pimephales promelas* with boron.

Summary of Toxicity Test Conditions								
1. Test Species and Age:	Pimephales promelas, (<24 hour fertilized embryos ), GLEC Culture							
2. Test Type and Duration:	Continuous flow-through; 32 days							
3. Test Dates:	September 10-October 12, 2009							
4. Test Temperature (°C):	25 ± 1							
5. Light Quality:	Ambient Laboratory, 10-20 µE/m <sup>2</sup> /s							
6. Photoperiod:	16 h light, 8 h darkness							
7. Feeding Regime:	Live Brine Shrimp (Artemia nauplii) Twice daily							
8. Size of Test Vessel:	2.5 Liter glass Tank							
9. Volume of Test Solutions:	2000 mL							
10. No. of Test Organisms per Test Vessel:	30 eggs, thinned to 20 larvae after hatch							
11. No. of Test Vessels per Treatment:	4							
12. Total No. of Test Organisms per Treatment:	120 eggs, thinned to 80 larvae after hatch							
13. Target or Nominal Test Concentrations (mg/L):	100, 50.0, 25.0, 12.5 and 6.25 mg/L-boron							
<ol> <li>Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):</li> </ol>	112, 56.5, 27.4, 12.9, and 5.90 mg/L-nitrate							
15. Renewal of Test Solutions:	Continuous flow through, 4 turnovers per day							
16. Dilution and Primary Control Water:	De-Chlorinated Lake Michigan Water							
17. Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent $\geq 99.5\%$ Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, $\geq 99.5\%$ , ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172							
18. Secondary Control Water:	None							
19. Aeration:	None							
20. Endpoints Measured:	Survival (LC <sub>50</sub> , LC <sub>25</sub> , LC <sub>20</sub> and LC <sub>10</sub> , NOEC and LOEC) and Growth (EC <sub>50</sub> , EC <sub>25</sub> , EC <sub>20</sub> and EC <sub>10</sub> , NOEC and LOEC)							

Results of a <u>Pimer</u>	ohales promelas	<u>32-Day C</u>	ontinuous Flow	Chronic Toxic	ity Test	
Conducted <u>09/10/</u>	09 - 10/12/09		oron ( Boric Acie gma Aldrich Ca			<u>43-35-3</u>
Test Solution Concentrations Measured	Primary Control/ Dilution Water	5.90 mg/L	12.9 mg/L	27.4 mg/L	56.5 mg/L	112 mg/L
Embryo Percent Hatch (%)	100	100	100	100	100	100
32-Day Mean Survival (%)	97.5	93.8	92.5	58.8ª	1.3ª	0ª
32-Day Average Biomass <sup>1</sup> (mg )	10.1	7.27 *	7.37 °	4.29°	0.06ª	0ª
Embryo Percent Hatch NOEC:	100 mg/L		32-Day LC <sub>20</sub>	o: 18.6	mg/L (15.9 mg/L	– 21.3 mg/L)
32-Day LC <sub>50</sub> *:	28.4 mg/L (25.5 n	ng/L-31.7 mg/L)	32-Day LC <sub>10</sub>	o: 12.8 s	mg/L (9.6 mg/L	– 16.0 mg/L)
32-Day Survival NOEC:	12.9 mg/L		32-Day EC5	o: 28.7 :	mg/L (23.7 mg/I	2 – 34.6 mg/L)
32-Day Survival LOEC:	27.4 mg/L		32-Day EC <sub>2</sub>	5: 25.1	mg/L (17.1 mg/I	. – 36.7 mg/L)
32-Day Growth NOEC:	<5.9 mg/L		32-Day EC2	o: 24.2	mg/L (14.3 mg/L	. – 41.0 mg/L)
32-Day Growth LOEC:	5.9 mg/L		32-Day EC1	o: 21.9 :	mg/L (8.6 mg/L	– 56.0 mg/L)
32-Day LC <sub>25</sub> :	20.8 mg/L (18.2 n	mg/L - 23.4 mg/L	)			

### Table 74. Test results for 32-day toxicity test on Pimepehales promelas with boron.

\*: All LC, EC, NOEC and LOEC values are determined based on the average measured boron concentration.

NOEC: No-Observed-Effect-Concentration LOEC: Lowest-Observed-Effect-Concentration

<sup>1</sup>Biomass: Biomass is the average dry weight of the four replicates calculated by the total dry weight of surviving organisms divided by the initial number of organisms (20).

Nominal (and Me Concentration	asured) Test	Number of Eggs at Test Initiation	Number of Hatched Larvae	Number of Dead Eggs	Percent Hatched Larvae	Number of Larvae at Test Termination	Percent Survival at Test Termination*	Biomass <sup>1</sup> (mg)
	Replicate # 1	30	30	0	100.0	1	5.0	1.27
	Replicate # 2	30	30	0	100.0	4	20.0	4.83
	Replicate # 3	30	30	0	100.0	19	95.0	9.62
Dilution water/Control	Replicate # 4	30	30	0	100.0	20	100.0	10.52
Average	100.0 97.5 <sup>°</sup>		10.07 2					
	Replicate # 1	30	30	0	100.0	17	85.0	6.69
	Replicate # 2	30	30	0	100.0	18	90.0	6.84
	Replicate # 3	30	30	0	100.0	20	100.0	7.34
6.25 (5.9) mg/L	Replicate # 4	30	30	0	100.0	20	100.0	8.21
Average					100.0		93.8	7.27
	Replicate # 1	30	30	0	100.0	17	85.0	6.99
	Replicate # 2	30	30	0	100.0	19	95.0	8.53
	Replicate # 3	30	30	0	100.0	19	95.0	6.77
12.5 (12.9) mg/L	Replicate # 4	30	30	0	100.0	19	95.0	7.17
Average					100.0		92.5	7.37
	Replicate # 1	30	30	0	100.0	10	50.0	3.76
	Replicate # 2	30	30	0	100.0	10	50.0	3.62
	Replicate # 3	30	30	0	100.0	13	65.0	5.05
25.0 (27.4) mg/L	Replicate # 4	30	30	0	100.0	14	70.0	4.73
Average				<u> </u>	100.0		58.8	4.29
	Replicate # 1	30	30	0	100.0	0	0.0	0.00
	Replicate # 2	30	30	0	100.0	0	0.0	0.00
	Replicate # 3	30	30	0	100.0	1	5.0	0.25
50.0 (56.5) mg/L	Replícate # 4	30	30	0	100.0	0	0.0	0.00
Average	Acquience in 4		06	<u>0</u>	100.0		1,3	0.06
	Replicate # 1	30	30	0	100.0	0	0.0	0.00
	Replicate # 2	30	30	0	100.0	0	0.0	0.00
	Replicate # 3	30	30	0	100.0	0	0.0	0.00
100 (112) mg/L	Replicate # 4	30	30	0	100.0			0.00
Average					100.0		0.0	0.00

#### Table 75. Survival and growth data for 32-day toxicity test on Pimephales promelas with boron.

\* On Day 8 of the test, (four days after first hatch) the surviving fish were randomly thinned to 20 fish in each test chamber. Percent surval at test termination is the number of surviving at test termination divided by 20.
<sup>1</sup> Biomass: Biomass is the total dry weight of surviving organisms divided by the initial number of organisms (20)

<sup>2</sup> Due to a technician error on day 8, only replicates 3 and 4 were used in the growth and survival analysis for the laboratory control

Table 76. Analytical chemistry data for 32-day toxicity test on Pimephales promelas with boron.

WILLI DOFUII. Nominal (and Mea	anrad) Tart	Boron	Temperature		DO	60	TT	A 12	A
Conentrati		(mg/L)	(°C)	рН (s.u.)	(mg/L)	SC (mmos)	Hardness	Alkalinity	Ammonia
CONCILCTAD	Replicate #	0.0	25.1	(s.u.)	7.5	(mmos)	(mg/L)	(mg/L)	(mg/L)
	1	(0.0-0.1)	(23.7-26.0)	8.08	(7.0-8.1)	290	112	100	ND
	Replicate #	0.0	24.9	0.00	7.3	2/0	112	100	IND.
Dilution	2	(0.0-0.1)	(23.8-26.3)	8.11	(6.8-7.8)	296	132	99	ND
Water/Control	Replicate #	0.1	24.9		7.2				
	3	(0.0-0.4)	(24.2-26.0)	8.16	(6.9-7.4)	300	NM	NM	ND
	Replicate #	0.0	24.9		7.3			[	
	4	(0.0-0.1)	(24.1-25.5)	8.17	(7.1-7.5)	304	NM	NM	ND
Average Boron									
mg/L		0							
	Replicate #	5,8	24.6		7.5				
	1	(4.9-6.6)	(23.9-25.6)	8.16	(7.0-7.9)	301	128	106	ND
	Replicate #	6.0	24.9		7.3 (6.7				
6.25 mg/L	2	(4.9-7.0)	(24.0-26.1)	8.17	8.0)	305	132	104	ND
(5.9 mg/L)	Replicate #	6.0	25.0		7.1				
	3	(5.0-7.1)	(24.1-26.2)	8.21	(6.6-7.5)	305	NM	NM	ND
	Replicate #	5.9	24.8		7.1				
	4	(5.1-6.6)	(23.9-25.4)	8.18	(6,7-7.6)	309	NM	NM	ND
Average Boron		5 0							
mg/L		5.9							
	Replicate #	13.0	24.8		7.4				
	1	(11.6-13.9)	(23.7-25.9)	8.17	(6.8-8.1)	307	132	110	ND
	Replicate #	13.1	24,9		7.5				
12.5 mg/L	2	(11.5-14.2)	(24.1-26.1)	8.20	(6.9-8.0)	308	130	107	ND
(12.9 mg/L)	Replicate #	13.1	25.1		7.3				
	3	(11.6-14.7)	(24.2-26.5)	8.25	(7.0-7.6)	310	NM	NM	ND
	Replicate #	12.3	25.0	9.91	7.4	217	201	ND/	
Average Boron	4	(11.0-13.8)	(24.2-25.6)	8.21	(7.1-7.8)	313	NM	NM	ND
mg/L		12.9							
	Replicate #	28.6	24.6		7.5				
	1 Des Forder #	(23.8-34.3)	(23.7-25.7)	8.18	(7.1-8.0)	314	128	116	ND
25.0 mg/L	Replicate #	28.0	24.7	0.01	7.5	216	130	112	
(27.4 mg/L)	2 Replicate #	(25.1-32.5) 26.2	(23.8-26.2) 24.8	8.21	(7.2-8.0)	316	128	113	ND
(27.4 mg/L)	3	(22.7-31.1)	(23.8-26.1)	8,12	(7.1-7.8)	317	NM	NM	ND
	Replicate #	26.3	24.5	0,12	7.4	311		14144	
	4	(22.9-31.2)	(23.5-25.2)	8.22	(7.1-7.7)	320	NM	NM	ND
Average Boron				<u></u>	1.(		L	1	1
mg/L		27.4							
	Replicate #	56.1	24.8	0.01	7.6	221	105	100	
	l Deplicate #	(48.2-65.4)	(23.9-25.7)	8.21	(7.1-8.5)	331	132	128	ND
50.0 mg/L	Replicate # 2	56.4 (46.9-63.3)	24.7	0 33	7.5 (7.2-8.1)	333	130	127	ND
(56.5 mg/L)	Z Replicate #	<u>(46.9-63.3)</u> 57.4	(24.1-26.0) 24.8	8.23	7.5	333	1.30	121	
(ooro meru)	3	(45.8-67.9)	(23.9-26.0)	8.27	(7.1-8.3)	351	NM	NM	ND
	Replicate #	56.2	24.8	<i></i>	7.5				1
	4	(45,3-63,4)	(23.9-25.4)	8.24	(7.3-8.0)	337	NM	NM	ND
Average Boron	<b></b>		I						L
mg/L		56.5							
	Replicate #	111	24.8		7.6		1		1
	Replicate #	(98.9-117)	(23.6-26.0)	8.22	(7.0-8.4)	363	128	152	ND
	Replicate #	111	(23.6-26.0)	0.22	7.6	202	120	132	
100 mg/L (112	2	(93.7-117)	(23.9-26.2)	8.23	(7.2-8.4)	367	130	150	ND
mg/L)	Replicate #	111	25.0	0.20	7.4	507	1.50	1.50	t
	3	(97.2-122)	(24.4-26.2)	8.27	(7.0-8.0)	367	NM	NM	ND
	Replicate #	113	24.9		7.6		1		1
	4	(103-123)	(24.0-25.6)	8.24	(7.3-8.0)	370	NM	NM	ND
Average Boron					···· ·				
me/L	and a she in the first of the second of the state of the state	112		ماستور المرابع وماسية الأومار ويوا	of store is in the admittle and stript, but	11-141-111-1-1-1-1-1-1-1-1-1-1-1-1-1-1-	A well of a Grand Science Strategy and A		

DO: Dissolved Oxygen SC: Specific Conductance

ND: Non Detect; below detection limit.

NM: Not Measured

### 48-hr Toxicity of Boron (pH 7.75) on Ceriodaphnia dubia

The 48-hr test to determine the toxicity of boron (pH 7.75) on *C. dubia* was completed by GLEC. The *C. dubia* were continuously exposed for 48-hours to five concentrations of boron and to a dilution water control with a target test pH of 7.75 (range of 7.65 to 7.85) using a continuous flow-through system (modified Benoit mini-dilutor) and an in-line pH adjustment/metering unit. The pH of both the dilution water and stock solution were adjusted by using a 1:1 ratio of sulfuric acid and hydrochloric acid (acid solution). The pH adjusted stock solution was delivered to mixing cells and diluted with pH adjusted, de-chlorinated Lake Michigan water to achieve target nominal concentrations of boron and a target test pH of 7.75.

Due to the buffering capacity of the borax and boric acid solution used to prepare the boron concentrations, EPA agreed that GLEC should target the dilution water control pH at 7.75 (range of 7.65 to 7.85). The five test concentrations were targeted to a pH of  $\pm$  0.1 pH unit from the pH value defined at test initiation in each test concentration (i.e. regardless of whether or not the pH in the test concentrations were  $\pm$  0.1 pH units from that observed in the control water). The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were four replicate test chambers for each treatment. The flow through test was conducted at 25  $\pm$  1 °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with < 24 hour old *C. dubia* collected from the GLEC laboratory culture. Although these organisms were cultured in, and acclimated to, the dilution water's "natural" pH (typically between a pH of 7.9 and 8.2), they were not acclimated to the pH-adjusted dilution water prior to test initiation for two reasons: 1) GLEC does not maintain a laboratory culture of *C. dubia* in de-chlorinated Lake Michigan water maintained at a pH of 7.75 (and this was outside the scope of work for this Work Assignment) and 2) adequate acclimation of the organisms to the pH-adjusted dilution water would have jeopardized the age requirement (< 24 hour old at test initiation) for test organisms required under the toxicity testing method (ASTM 2007).

The *C. dubia* were randomly assigned to test cups until each test cup contained five *C. dubia*. The test cups were randomly assigned to the 2.5 L glass test chambers (1 cup per chamber) and suspended in the test solutions from a rod. *C. dubia* were counted on a daily basis and the number of live *C. dubia* was recorded. pH was recorded twice a day, at a minimum of eight hours apart (i.e. morning and evening). The number of surviving *C. dubia* was recorded at test termination (48-hours).

The test was completed at the following nominal boron concentrations: 25.0, 50.0, 100, 200, and 400 mg/L. The average pH for the dilution water control measured in the *C. dubia* toxicity test for the 48-hour test period was 7.77. The average pH over the 48-hour test duration for the five test concentrations of 27.6, 49.8, 118, 223, and 391 mg/L was 7.92, 8.03, 8.03, 8.07, and 8.06, respectively.

Once the test was complete, the  $LC_{50}$  was determined using the average measured test concentrations with the Probit method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 77; test results are provided in Table 78. Analytical chemistry data are provided in Table 79. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 23.

Table 77. Test conditions for 48-hour toxicity test on 6	Ceriodaphnia dubia with boron
(pH 7.75).	

Su	Summary of Toxicity Test Conditions								
1.	Test Species and Age:	Ceriodaphnia dubia, < 24 hours old, GLEC Culture							
2.	Test Type and Duration:	Continuous flow-through, 48 hours							
3.	Test Dates:	October 23-October 25, 2009							
4.	Test Temperature (°C):	$25 \pm 1$							
5.	Light Quality:	Ambient Laboratory, 10-20 µE/m <sup>2</sup> /s							
6.	Photoperiod:	16 h light, 8 h darkness							
7.	Feeding Regime:	None							
8.	Size of Test Vessel:	2.5 Liter glass Tank							
9.	Volume Test Solutions:	2000 mL							
10.	No. of Test Organisms per Test Vessel:	10							
11.	No. of Test Vessels per Treatment:	2							
12.	Total No. of Test Organisms per Treatment:	20							
13.	Target or Nominal Test Concentrations (mg/L):	400, 200, 100, 50.0, and 25.0							
14.	Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):	391, 223, 118, 49.8, and 27.6							
15.	Renewal of Test Solutions:	Continuous flow through, 4 turnovers per day							
16.	Dilution and Primary Control Water:	De-Chlorinated Lake Michigan Water							
17.	Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent $\geq 99.5\%$ Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, $\geq 99.5\%$ , ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172							
18.	Secondary Control Water:	None							
19.	Aeration:	None							
20.	Endpoints Measured:	Mortality (LC <sub>50</sub> )							

Results of a <u>Cerio</u> Conducted <u>10/2</u>	<u>48-Hour Static Acute Toxicity Test</u> <u>Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043-35-3)</u> (Borax: Sigma Aldrich Cas No. 1303-96-4)									
	Cumula	tive Percei	nt Affected	( ai		LC50 Values	* (mg/L)			
Nominal (Measured)	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr		
Primary Control/ Dilution Water	0 (0)	0 (0)			172	76.9	NA	NA		
25.0 (27.6) mg/L	0 (0)	0 (0)			48-Hour LC	<sub>50</sub> * = 76.9 mg/	L			
50.0 (49.8) mg/L	0 (0)	25 (25)			LCs	<sub>:0</sub> * 95% Confi	dence Limi	ts		
100 (118) mg/L	15 (15)	75 (75)			24-Hr	48-Hr	7 <b>2-Hr</b>	96-Hr		
200 (223) mg/L	75 (75)	100 (100)			LL 144 UL 202	62.3 94.9	NA NA	NA NA		
400 (391) mg/L	100 (100)	100 (100)								
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable					
					Method(s) Us Limit Values	sed to Determi : Probit	ne LC <sub>50</sub> Cor	ifidence		

# Table 78. Test results for 48-hour toxicity test on *Ceriodaphnia dubia* with boron at pH 7.75.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All LC<sub>50</sub> values are determined based on measured concentrations.

Table 79. Analytical chemistry data for 48-hour toxicity test on Ceriodaphnia a	lubia
with boron at pH 7.75.	

Nominal (and M	easured) Test	Boron	Temperature	pH	DO	SC	Hardness	Alkalinity	Ammonia
Conentr	ations	(mg/L)	(°C)	(s.u.)	(mg/L)	(mmos)	(mg/L)	(mg/L)	(mg/L)
	Replicate #	0.05	24.6	7.65	7.7				
	1	(0.04-0.05)	(24.5-24.6)	(7.53-7.75)	(6.5-8.7)	320	146	47	ND
	Replicate #	0.06	25.0	7.75	7.9				
Dilution	2	(0.04-0.07)	(24.9-25.1)	(7.70-7.82)	(6.9-8.6)	328			
Water/Control	Replicate #	0.05	25.2	7.84	7.7				
	3	(0.03-0.07)	(25.1-25.2)	(7.77-7.94)		322			
	Replicate #	0.06	24.9	7.71	7.5				
	4	(0.05-0.07)	(24.9-24.9)	(7.56-7.79)	(6.4-8.1)				
Average		0.05	24.8	7.77	7.7	323	146	47	ND
	Replicate #	27.9	24,7	7.89	7.3		r		
	· · · ·			(7.80-7.95)		246	120	60	
	l Replicate #	(27.2-28.5) 27.3	(24.5-24.8)		(5.9-8.1)	346	138	50	ND
A.F	1 -		1	7.96		350			
25 mg/L	2	(25.9-28.0)	(25.0-25.2)	(7.89-8.06)	(7.2-8.0)	350			
(27.6 mg/L)	Replicate #	27.4	24.8	7.92	7.6				
	3	(27.1-27.8)	(24.7-24.9)	(7.87-8.00)					
	Replicate #	28.0	24.8	7.97	7.7				
	4	(27.2-28.9)	(24.8-24.9)	(7.89-8.06)		*			
Average		27.6	24.7	7.92	7.6	348	138	50	ND
	Replicate #	49.8	24.6	8.00	7.4				
	1	(47.4-52.9)	(24.5-24.7)	(7.96-8.07)	(6.4-7.9)	364	144	57	ND
	Replicate #	50,1	24.8	8.04	7.7		144	57	110
50 mg/L	2	(47.8-53.7)	(24.7-24.8)	(7.97-8.11)		365			
(49.8 mg/L)	Replicate #	49.3	24.8	8.00	7.8	505			
(47.0 mg/L)	3	(47.5-52.7)	(24.7-24.9)	(7.96-8.07)	8				
	Replicate #	49.8	24.8	8.04	7.5				
	4	(47.8-53.5)	(24.7-24.9)	(7.97-8.11)					
		49.8	24.6	8.03	7.6	264	144	57	NID
Average		47.0	24.0	0.03	7.0	364	144	57	ND
	Replicate #	120	24.8	8.07	7.5				
	1	(111-127)	(24.6-24.9)	(8.03-8.13)	(6.7-8.0)	411	128	77	ND
	Replicate #	120	24.9	8.08	7.6				
100 mg/L	2	(112-124)	(24.8-25.0)	(8.02-8.14)	(6.8-8.0)	412			
(118 mg/L)	Replicate #	118	24.8	8.07	7.5		1		
	3	(110-130)	(24.7-24.9)	(8.02-8.13)	(6.4-8.1)			1	
	Replicate #	115	24.9	8.07	7.6				
	4	(105-125)	(24.6-25.1)	(8.00-8.14)			1		
Average		118	24.8	8.03	7.5	411	128	77	ND
					L		A	· · · · · · · · · · · · · · · · · · ·	
	Replicate #	227	24.7	8.08	7.7				
	1	(221-232)	(24.6-24.8)	(8.02-8.15)	(7.2-8.0)	475	132	105	ND
	Replicate #	222	24.5	8.08	7.5				
200 mg/L	2	(216-228)	(24.4-24.7)	(8.03-8.15)	(6.6-8.0)	477			
(223 mg/L)	Replicate #	218	24.7	8.09	7.7				
	3	(211-224)	(24.6-24.8)	(8.02-8.15)	(7.1-8.0)				
	Replicate #	225	25.1	8.07	7,6				
	4	(222-228)	(24.8-25.3)	(8.01-8.14)	(6.7-8.1)				
Average		223	24.8	8.07	7.6	476	132	105	ND
		200		0.54		1		1	
	Replicate #	392	24.5	8.06	7.6		1		
	1	(381-399)	(24.4-24.6)	(8.00-8.13)		587	132	167	ND
	Replicate #	391	24.6	8.07	7.7				
400 mg/L	2	(386-396)	(24.4-24.8)	(8.01-8.14)		589			
(391 mg/L)	Replicate #	391	24.5	8.07	7.7				
	3	(388-394)	(24.3-24.8)	(8.01-8.14)		ļ	<u> </u>		
	Replicate #	391	24.6	8.06	7.5				
	4	(384-401)	(24.4-24.7)	(8.01-8.13)	(6.4-8.0)	]	1	1	
Average		391	24.6	8.06					

DO: Dissolved Oxygen SC: Specific Conductance ND: Non Detect; below detection limit. NM: Not Measured

### 96-hour Toxicity of Boron (pH 6.75) on Pimephales promelas

The 96-hr test to determine the toxicity of boron (pH 6.75) on P. promelas was completed by GLEC. The P. promelas (collected from the GLEC laboratory culture) were continuously exposed for 96-hours to five concentrations of boron and to a dilution water control with a target test pH of 6.75 (range of 6.65 to 6.85) using a continuous flow-through system (modified Benoit mini-dilutor) and an in-line pH adjustment/ metering unit. The pH of both the dilution water and stock solution were adjusted by using a 1:1 ratio sulfuric acid and hydrochloric acid (acid solution). The pH adjusted stock solution was delivered to mixing cells and diluted with pH adjusted Lake Michigan water to achieve target nominal concentrations of boron and a target test pH of 6.75. Due to the buffering capacity of the borax and boric acid solution used to prepare the boron concentrations, EPA agreed that GLEC should target to a pH of  $\pm 0.1$  pH unit from the pH value defined at test initiation in each test concentration (i.e. regardless of whether or not the pH in the test concentrations were  $\pm 0.1$  pH unit from that observed in the control water). The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were two replicate test chambers for each treatment. The flow through test was conducted at  $25 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with the *P. promelas*. Although these organisms were cultured in, and acclimated to, the dilution water's "natural" pH (typically between a pH of 7.9 and 8.2), they were not acclimated to the pH-adjusted dilution water prior to test initiation for two reasons: 1) GLEC does not maintain a laboratory culture of *P. promelas* in dechlorinated Lake Michigan water maintained at a pH of 6.75 (and this was outside the scope of work for this Work Assignment) and 2) adequate acclimation of the organisms to the pH-adjusted dilution water may have jeopardized the weight requirement (0.1–5 g at test initiation) for test organisms required under the toxicity testing method (ASTM 2007).

The *P. promelas* were randomly assigned to the 2.5 L glass test chambers until each test chamber contained ten *P. promelas*. *P. promelas* were counted on a daily basis and the number of live *P. promelas* was recorded. pH was recorded twice a day, at a minimum of eight hours apart (i.e., morning and evening). The number of surviving *P. promelas* was recorded at test termination (96-hours).

The test was completed at the following nominal boron concentrations: 25.0, 50.0, 100, 200, and 400 mg/L (dilution factor of 0.5). The average pH for the dilution water control measured in the *P. promelas* toxicity test for the 96-hour test period was 6.67. The average pH over the 96-hour test duration for the five test concentrations of 32.9, 55.2, 122, 224, and 394 mg/L was 6.85, 6.93, 7.18, 7.28, and 7.33, respectively.

The hourly water temperatures as recorded by the continuous temperature logger did show that test temperatures fell outside the temperature allowance in the early morning on October 29, 2009 (readings of 23.8 and 23.9 °C). However, instantaneous water temperatures measured by GLEC technicians on October 28 and later in the morning on October 29 never fell outside the acceptable range as outlined in the method. Therefore, these water temperature exceedances were very brief and likely had no effect on the results of this study.

Once the test was complete, the  $LC_{50}$  was determined using the average measured test concentrations with the Probit and Spearman method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 80; test results are provided in Table 81. Analytical chemistry data are provided in Table 82. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 24.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	Pimephales promelas, (weight 0.11g and 18.4 mm length), GLEC Culture
2. Test Type and Duration:	Continuous flow-through, 96 hours
3. Test Dates:	October 29-November 02, 2009
4. Test Temperature (°C):	25 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 µE/m <sup>2</sup> /s
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	2.5 Liter glass Tank
9. Volume of Test Solutions:	2000 mL
10. No. of Test Organisms per Test Vessel:	10
11. No. of Test Vessels per Treatment:	2
12. Total No. of Test Organisms per Treatment:	20
13. Target or Nominal Test Concentrations (mg/L):	400, 200, 100, 50.0, and 25.0
<ol> <li>Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):</li> </ol>	394, 224, 122, 55.2, and 32.9
15. Renewal of Test Solutions:	Continuous flow through, 4 turnovers per day
16. Dilution and Primary Control Water:	De-chlorinated Lake Michigan Water
17. Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent $\geq 99.5\%$ Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, $\geq 99.5\%$ , ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC <sub>50</sub> )

# Table 80. Test conditions for 96-hour toxicity test on *Pimephales promelas* with boron (pH 6.75).

Results	of aPimeph	ales prome	las_	96 <u>-Hour S</u>	Static Acute To	xicity Test					
Conducted 10/29/09-11/02/09Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043-35-3)(Borax: Sigma Aldrich Cas No. 1303-96-4)											
	Cumula	tive Percei	nt Affected	1 <sup>2</sup>		LC <sub>50</sub> * Values	s (mg/L)				
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr			
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	0 (0)	>394	297	163	70.6			
25.0 (32.9) mg/L	5 (5)	5 (5)	10 (10)	10 (10)	96-Hour LC	<sub>50</sub> *= 70.6 mg/	L				
50.0 (55.2) mg/L	5 (5)	5 (5)	5 (5)	20 (20)	LC5	<sub>0</sub> *95% Confi	dence Limi	ts			
100 (122) mg/L	0 (0)	0 (0)	30 (30)	90 (90)	24-Hr	48-Hr	72-Hr	96-Hr			
200 (224) mg/L	5 (5)	5 (5)	65 (65)	100 (100)	ll na Ul na	285 310	130 205	58.3 86.3			
400 (394) mg/L	10 (10)	95 (95)	100 (100)	100 (100)							
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable						
					Method(s) Used to Determine LC <sub>50</sub> Confidence Limit Values: Probit and Spearman						

## Table 81. Test results for 96-hour toxicity test on *Pimephales promelas* with boron at pH 6.75.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All  $LC_{50}$  values are determined based on measured concentrations.

Nominal (and M	· · · ·	Boron	Temperature	pH	DO	SC	Hardness	Alkalinity	Ammonia
Conentra		(mg/L)	(°C)	(s.u.)	(mg/L)	(mmos)	(mg/L)	(mg/L)	(mg/L)
	Replicate #	0.04	24.4	6.62	7.3				
Dilution		(0.04-0.04)	(24.0-24.8)	(6.48-6.69)		327	130	70	ND
Water/Control	Replicate #	0.06	24.5	6.73	7.2				
	4	(0.05-0.06)	(24.0-24.8)	(6.63-6.88)		343			
Average		0.05	24.5	6.67	7.3	332	130	7	ND
	Replicate #	32.8	24.5	6.82	7.2		1		
25 mg/L	1	(30.3-38.6)	(24.1-24.9)	(6.73-6.93)	(6.6-8.0)	362	130	10	ND
(32.9 mg/L)	Replicate #	33.1	24.6	6.88	7.6 (7.2				
	3	(30.4-39.0)	(24.1-25.0)	(6.80-6.98)	8.2)	365			
Average		32.9	24.5	6.85	7.4	363	130	10	ND
	Replicate #	55.2	24.4	6.94	7.4		1	r	Γ
50 mg/L	1 ICopilcate #	(51.8-62.5)	(24.0-24.6)	(6.86-7.04)		382	126	11	ND
(55.2 mg/L)	Replicate #	55.2	24.6	6.93	7.3	102	120		
(33.2 mg/D)	3	(51.1-61.1)	(24.3-24.8)	(6.83-7.04)		351			
Average		55.2	24.5	6.93	7.4	371	126	11	ND
		100	<u> </u>		·		γ·····		
	Replicate #	120	24.5	7.17	7.4				
100 mg/L	1	(112-126)	(24.0-25.1)	(7.11-7.25)		440	130	18	ND
(122 mg/L)	Replicate #	123	24.5	7.19	7.6				
	3	(119-129)	(24.1-24.9)	(7.13-7.26)		447			
Average		122	24.5	7.18	7.5	442	130	18	ND
	Replicate #	223	24.3	7.28	7.2		T	T	
200 mg/L	2	(219-234)	(24.0-24.9)	(7.22-7.35)	(6.5-8.2)	518	128	25	ND
(224 mg/L)	Replicate #	224	25.3	7.28	7.3			1	
(	4	(217-232)	(25.1-25.5)	(7.20-7.35)	(6.8-8.2)	527			
Average		224	24.8	7.28	7.2	521	128	25	ND
	Replicate #	391	24.3	7.33	7.4		T	1	
400 mg/L	1	(372-407)	(24.0-24.6)	(7.26-7.38)		670	126	41	ND
(394 mg/L)	Replicate #	397	24.6	7.32	7.5	070	120	+	
(224 mg/L)	4	(368-426)	(24.3-25.1)	(7.24-7.37)		696			
Average		394	24.4	7.33	7.4	678	126	41	ND

### Table 82. Analytical chemistry data for 96-hour toxicity test on Pimephales promelas with boron at pH 6.75.

DO: Dissolved Oxygen SC: Specific Conductance ND: Non Detect; below detection limit. NM: Not Measured

### 96-hr Toxicity of Boron (pH 7.75) on Pimephales promelas

The 96-hr test to determine the toxicity of boron (pH 7.75) on P. promelas was completed by GLEC. The P. promelas (collected from the GLEC laboratory culture) were continuously exposed for 96-hours to five concentrations of boron and to a dilution water control with a target test pH of 7.75 (range of 7.65 to 7.85) using a continuous flow-through system (modified Benoit mini-dilutor) and an in-line pH adjustment/metering unit. The pH of both the dilution water and stock solution were adjusted by using a 1:1 ratio of sulfuric acid and hydrochloric acid (acid solution). The pH adjusted stock solution was delivered to mixing cells and diluted with pH adjusted Lake Michigan water to achieve target nominal concentrations of boron and a target test pH of 7.75. Due to the buffering capacity of the borax and boric acid solution used to prepare the boron concentrations, EPA agreed that GLEC should target the dilution water control pH at 7.75 (range of 7.65 to 7.85). The five test concentrations were targeted to a pH of  $\pm 0.1$  pH unit from the pH value defined at test initiation in each test concentration (i.e. regardless of whether or not the pH in the test concentrations were  $\pm 0.1$  pH unit from that observed in the control water). The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were two replicate test chambers for each treatment. The flow through test was conducted at  $25 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with the *P. promelas*. Although these organisms were cultured in, and acclimated to, the dilution water's "natural" pH (typically between a pH of 7.9 and 8.2), they were not acclimated to the pH-adjusted dilution water prior to test initiation for two reasons: 1) GLEC does not maintain a laboratory culture of *P. promelas* in dechlorinated Lake Michigan water maintained at a pH of 7.75 (and this was outside the scope of work for this Work Assignment) and 2) adequate acclimation of the organisms to the pH-adjusted dilution water may have jeopardized the weight requirement (0.1–5 g at test initiation) for test organisms required under the toxicity testing method (ASTM 2007).

The *P. promelas* were randomly assigned to the 2.5 L glass test chambers until each test chamber contained ten *P. promelas*. *P. promelas* were counted on a daily basis and the number of live *P. promelas* was recorded. pH was recorded twice a day, at a minimum of eight hours apart (i.e. morning and evening). The number of surviving *P. promelas* was recorded at test termination (96-hours).

The test was completed at the following nominal boron concentrations: 25.0, 50.0, 100, 200, and 400 mg/L (dilution factor of 0.5). The average pH for the dilution water control measured in the *P. promelas* toxicity test for the 96-hour test period was 7.68. The average pH over the 96-hour test duration for the five test concentrations of 28.6, 50.9, 121, 223, and 392 mg/L was 7.88, 7.98, 8.05, 8.07, and 8.06, respectively.

Once the test was complete, the  $LC_{50}$  was determined using the average measured test concentrations with the Spearman method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 83; test results are provided in Table 84. Analytical chemistry data are provided in Table 85. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 25.

Summar	Summary of Toxicity Test Conditions							
1. Test S	pecies and Age:	Pimephales promelas, (weight 0.12g and 22.0 mm length), GLEC Culture						
2. Test T	ype and Duration:	Continuous flow-through, 96 hours						
3. Test D	ates:	October 23-October 27, 2009						
4. Test Te	emperature (°C):	25 ± 1						
5. Light (	Quality:	Ambient Laboratory, 10-20 µE/m <sup>2</sup> /s						
6. Photop	period:	16 h light, 8 h darkness						
7. Feedin	g Regime:	None						
8. Size of	Test Vessel:	2.5 Liter glass Tank						
9. Volum	e of Test Solutions:	2000 mL						
10. No. of	Test Organisms per Test Vessel:	10						
11. No. of	Test Vessels per Treatment:	2						
12. Total M	No. of Test Organisms per Treatment:	20						
13. Target	or Nominal Test Concentrations (mg/L):	400, 200, 100, 50.0, and 25.0						
	ical Test Concentrations (average of samples ted at test initiation and termination-mg/L):	392, 223, 121, 50.9, and 28.6						
	ral of Test Solutions: on and Primary Control Water: faterial:	Continuous flow through, 4 turnovers per day De-chlorinated Lake Michigan Water Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, ≥ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172						
18. Second	lary Control Water:	None						
19. Aeratio	on:	None						
20. Endpo	ints Measured:	Mortality (LC <sub>50</sub> )						

# Table 83. Test conditions for 96-hour toxicity test on Pimephales promelas withboron (pH 7.75).

<b>Results of a</b> Pimephales promelas96-Hour Static Acute Toxicity Test										
Conducted <u>10</u>	/23/09 -	10/27/09	_ <u>Usi</u> (Bo	ng: Boron rax: <u>Sigm</u>	n (Boric Acid: Sigma Aldrich Cas No. 10043-35-3) ma Aldrich Cas No. 1303-96-4)					
	Cumula	tive Percei	nt Affected	a a		LC <sub>50</sub> * Values	s (mg/L)			
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr		
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	0 (0)	>392	289	202	137		
25.0 (28.6) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	96-Hour LC	<sub>50</sub> *= 137 mg/l	L			
50.0 (50.9) mg/L	0 (0)	0 (0)	0 (0)	0 (5)	LC <sub>50</sub> *95% Confidence Limits					
100 (121) mg/L	0 (0)	0 (0)	0 (0)	21 (21)	24-Hr	48-Hr	72-Hr	96-Hr		
200 (223) mg/L	5 (5)	10 (10)	65 (65)	100 (100)	LL NA UL NA	260 320	178 229	118 158		
400 (392) mg/L	10 (10)	95 (95)	100 (100)	100 (100)						
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable					
					Method(s) Used to Determine LC <sub>50</sub> Confidence Limit Values: Spearman					

### Table 84. Test results for 96-hour toxicity test on *Pimephales promelas* with boron at pH 7.75.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All  $LC_{50}$  values are determined based on measured concentrations.

Nominal (and Measured) Test Conentrations		Boron	Temperature	pН	DO	SC	Hardness	Alkalinity	Ammonia
		(mg/L)	(°C)	(s.u.)	(mg/L)	(mmos)	(mg/L)	(mg/L)	(mg/L)
	Replicate #	0.05	24.8	7.63	7.3				
Dilution	1	(0.04-0.05)	(24.5-25.3)	(7.49-7.75)	(6.5-8.0)	351	146	41	ND
Water/Control	Replicate #	0.06	25.1	7.72	7.3				
	4	(0.05-0.07)	(24.9-25.4)	(7.56-7.85)	(6.4-8.1)	321			
Average		0.05	24.9	7.68	7.3	336	146	41	ND
	Replicate #	28.7	24.9	7.86	7.2		1	[	
25 mg/L	1	(27.2-31.2)	(24.5-25.4)	(7.70-7.95)		375	138	49	ND
(28.4 mg/L)	Replicate #	28.6	25.0	7.91	7.4	0.70			110
(2011 mg 2)	3	(27.1-32.1)	(24.7-25.4)	(7.80-8.00)		345			
Average		28.6	24.9	7.88	7.3	360	138	49	ND
	Replicate #	51.0	24.7	7.97	7.2			1	
50 mg/L	1	(47.4-54.7)	(24.5-25.1)	(7.80-8.07)		393	146	57	ND
(50.9 mg/L)	Replicate #	50.9	24.9	7.98	7.4		1	<u>-</u>	
(con	3	(47.5-55.5)	(24.7-25.4)	(7.90-8.07)		361			
Average		50.9	24.8	7.98	7.3	377	146	57	ND
	Replicate #	121	24.9	8.06	7.4			<b>1</b>	
100 mg/L	1	(111-127)	(24.6-25.3)	(7.97-8.13)		436	128	77	ND
(121 mg/L)	Replicate #	121	25.0	8.05	7.1				112
(121 mg 1)	3	(110-130)	(24.7-25.4)	(7.96-8.13)		410			
Average		121	24.9	8.05	7.3	423	128	77	ND
							L		
	Replicate #	222	24.6	8.07	7.3			ţ.	
200 mg/L	2	(216-228)	(24.4-25.1)	(7.99-8.15)		500	132	107	ND
(223 mg/L)	Replicate #	225	25.2	8.07	7.3				
	4	(222-228)	(24.8-25.6)	(8.00-8.14)	the second s	476			
Average		223	24.9	8.07	7.3	488	132	107	ND
	Replicate #	392	24.7	8.07	7.3		T	r	l
400 mg/L	1	(381-399)	(24.4-25.1)	(8.00-8.13)		616	128	168	ND
(392 mg/L)	Replicate #	392	24.7	8.06	7.3		1	1	
	4	(384-401)	(24.4-25.2)	(8.01-8.13)	(6.4-8.0)	590	]		
Average		392	24.7	8.06	7.3	603	128	168	ND

# Table 85. Analytical chemistry data for 96-hour toxicity test on *Pimephales promelas* with boron at pH 7.75.

DO: Dissolved Oxygen SC: Specific Conductance

ND: Non Detect; below detection limit. NM: Not Measured

### 96-hr Toxicity of Boron (pH 8.75) on Pimephales promelas

The 96-hr test to determine the toxicity of boron (pH 8.75) on *P. promelas* was completed by GLEC. The *P. promelas* (collected from the GLEC laboratory culture) were continuously exposed for 96-hours to five concentrations of boron and to a dilution water control with a target test pH of 8.75 (range of 8.65 to 8.85) using a continuous flow-through system (modified Benoit mini-dilutor) and an in-line pH adjustment/metering unit. The stock solutions used for the test were prepared by diluting a known weight (grams) of borax and boric acid to a known volume of dilution water (dechlorinated Lake Michigan water). The pH of both the dilution water and stock solution were adjusted by using sodium hydroxide. The pH adjusted stock solution was delivered to mixing cells and diluted with pH adjusted Lake Michigan water to achieve target nominal concentrations of boron and a target test pH of 8.75. The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were two replicate test chambers for each treatment. The flow through test was conducted at  $25 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with the *P. promelas*. Although these organisms were cultured in, and acclimated to, the dilution water's "natural" pH (typically between a pH of 7.9 and 8.2), they were not acclimated to the pH-adjusted dilution water prior to test initiation for two reasons: 1) GLEC does not maintain a laboratory culture of *P. promelas* in dechlorinated Lake Michigan water maintained at a pH of 8.75 (and this was outside the scope of work for this Work Assignment) and 2) adequate acclimation of the organisms to the pH-adjusted dilution water may have jeopardized the weight requirement (0.1–5 g at test initiation) for test organisms required under the toxicity testing method (ASTM 2007).

The *P. promelas* were randomly assigned to the 2.5 L glass test chambers until each test chamber contained ten *P. promelas*. *P. promelas* were counted on a daily basis and the number of live *P. promelas* was recorded. pH was recorded twice a day, at a minimum of eight hours apart (i.e., morning and evening). The number of surviving *P. promelas* was recorded at test termination (96-hours).

The test was completed at the following nominal boron concentrations: 25.0, 50.0, 100, 200, and 400 mg/L (dilution factor of 0.5). The average pH for the dilution water control measured in the *P. promelas* toxicity test for the 96-hour test period was 8.75. The average pH over the 96-hour test duration for the five test concentrations of 21.1, 42.4, 112, 219, and 376 mg/L was 8.72, 8.70, 8.70, 8.70, and 8.67, respectively.

Instantaneous water temperature measurements made on February 13, 14, 15 and 16 (Days 1-4: 23.6°C-24.7°C) exceeded the allowable range of  $25 \pm 1$  °C outlined in the toxicity testing method. However, the average water temperatures across the duration of the test in each replicate were always  $\pm 0.9$  °C of the target test temperature (25 °C) in all treatments. In addition, the water temperature as recorded by the continuous temperature

logger did show that the test temperature fell outside the temperature allowance during the times of 0430 to 0830 on those days. However, due to the relatively small number of temperature readings measured outside the range, these water temperature exceedances likely had little effect on the results of this study.

Once the test was complete, the  $LC_{50}$  was determined using the average measured test concentrations with the Probit method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 86; test results are provided in Table 87. Analytical chemistry data are provided in Table 88. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 26.

Su	Summary of Toxicity Test Conditions							
1.	Test Species and Age:	Pimephales promelas, (weight 0.13g and 22.8 mm length), GLEC Culture						
2.	Test Type and Duration:	Continuous flow-through, 96 hours						
3.	Test Dates:	February 12-February 16, 2010						
4.	Test Temperature (°C):	25 ± 1						
5.	Light Quality:	Ambient Laboratory, 10-20 µE/m <sup>2</sup> /s						
6.	Photoperiod:	16 h light, 8 h darkness						
7.	Feeding Regime:	None						
8.	Size of Test Vessel:	2.5 Liter glass Tank						
9.	Volume of Test Solutions:	2000 mL						
10.	No. of Test Organisms per Test Vessel:	10						
11.	No. of Test Vessels per Treatment:	2						
12.	Total No. of Test Organisms per Treatment:	20						
13.	Target or Nominal Test Concentrations (mg/L):	400, 200, 100, 50.0, and 25.0						
14.	Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):	376, 219, 112, 42.4, and 21.1						
15.	Renewal of Test Solutions:	Continuous Reputterante A terra ana des						
16.	Dilution and Primary Control Water:	Continuous flow through, 4 turnovers per day						
17.	Test Material:	De-chlorinated Lake Michigan Water Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, ≥ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172						
18.	Secondary Control Water:	None						
19.	Aeration:	None						
20.	Endpoints Measured:	Mortality (LC <sub>50</sub> )						

# Table 86. Test conditions for 96-hour toxicity test on *Pimephales promelas* with boron (pH 8.75).

Results of aPimephales promelas_ 96-Hour Static Acute Toxicity Test								
Conducted02/12/1002/16/10Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043-35- (Borax: Sigma Aldrich Cas No. 1303-96-4)								
	Cumula	tive Percei	nt Affected	a		LC <sub>50</sub> * Values	s (mg/L)	
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	5 (5)	>373	325	195	133
25.0 (21.1) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	96-Hour LC	<sub>50</sub> *= 133 mg/l	["	
50.0 (42.4) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	LC <sub>50</sub> *95% Confidence Limits			
100 (112) mg/L	0 (0)	0 (0)	5 (5)	30 (30)	24-Hr	48-Hr	72-Hr	96-Hr
200 (219) mg/L	5 (5)	15 (15)	60 (60)	95 (95)	LL NA UL NA	276 412	165 227	110 157
400 (376) mg/L	10 (10)	65 (65)	100 (100)	100 (100)				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC <sub>50</sub> Confidence Limit Values: Probit			

### Table 87. Test results for 96-hour toxicity test on *Pimephales promelas* with boron at pH 8.75.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All LC50 values are determined based on measured concentrations.

Nominal (and Measured) Test Conentrations		Boron	Temperature	pН	DO	SC	Hardness	Alkalinity	Ammonia
		(mg/L)	(°C)	(s.u.)	(mg/L)	(mmos)	(mg/L)	(mg/L)	(mg/L)
	Replicate #	0.04	24.2	8.74	7.6				
Dilution	1	(0.03-0.04)	(23.8-24.6)	(8.68-8.83)		290	126	111	ND
Water/Control	Replicate #	0.03	24.4	8.75	7.4				
	2	(0.03-0.03)	(23.8-25.2)	(8.71-8.81)	(7.1-7.6)	299			
Average		0.03	24.3	8.75	7.5	295	126	111	ND
	Replicate #	21.9	24.3	8.72	7.5		1	1	Г
25 mg/L	1 Kephcate #	(21.7-22.2)	(23.8-24.8)	(8.67-8.77)		331	110	133	ND
(21.1  mg/L)	Replicate #	20.2	24.2	8.71	7.3	551	110	155	IND .
(21.1 mg/L)	3	(18.0-21.7)	(23.8-25.2)	(8.63-8.77)		336			
A 110 10 10		21.1	24.2	8.72	7.4	333	110	133	ND
Average		41.1	24.2	0.14	/.4	333	110	135	
	Replicate #	42.6	24.3	8.69	7.1				
50 mg/L	2	(37.0-46.0)	(23.8-25.2)	(8.62-8.72)	(6.8-7.6)	368	116	156	ND
(42.4 mg/L)	Replicate #	42.7	24.3	8.72	7.2				······
· • ·	4	(36.3-45.4)	(23.8-24.8)	(8.67-8.77)	(7.1-7.4)	370			
Average		42.4	24.3	8.70	7.2	369	116	156	ND
	Replicate #	111	24.3	8.71	7.3				r
100	Replicate #					100		225	200
100 mg/L	Replicate #	(93.5-125)	(23.9-25.2) 24.3	(8.65-8.76) 8.70	(7.1-7.5) 7.4	472	116	235	ND
(112 mg/L)						14			
	4	(96.3-128)	(23.6-25.0)	(8.63-8.76) 8.70		464		225	NID
Average		112		8.70	7.3	471	116	235	ND
	Replicate #	207	24.3	8,70	7.3			1	[
200 mg/L	1	(185-254)	(23.6-24.8)	(8.64-8.73)	(7.0-7.6)	628	120	340	ND
(219 mg/L)	Replicate #	231	24.5	8.70	7.2		*		
(	4	(202-264)	(23.9-25.2)	(8.64-8.74)	(7.1-7.4)	638			
Average		219	24.4	8.70	7.3	633	120	340	ND
	Replicate #	382	24.1	8.67	7.2				
400 mg/L	1	(359-412)	(24.0-24.5)	(8.62-8.73)		844	108	510	ND
(376 mg/L)	Replicate #	370	24.1	8.67	7.2				
	4	(353-399)	(23.8-24.8)	(8.62-8.71)	(7.1-7.3)	845			
Average		376	24.1	8.67	7.2	845	108	510	ND

### Table 88. Analytical chemistry data for 96-hour toxicity test on *Pimephales promelas* with boron at pH 8.75.

DO: Dissolved Oxygen SC: Specific Conductance

ND: Non Detect; below detection limit. NM: Not Measured

#### Manganese

Table 89 provides a summary of estimated  $LC_{50}$  values for the two toxicity tests performed using manganese.  $LC_{50}$  values ranged between 31.5 and 43.3 mg Mn/L.

Test Species and Duration	LC <sub>50</sub> (mg Mn/L)
Lampsilis siliquoidea 96 hr	43.3
Megalonaias nervosa - 96 hr	31.5

For each of the acute toxicity tests completed using manganese, two tables were generated: the first summarizes the test results for each toxicity test, including nominal and analytical test concentration and  $LC_{50}$  estimates with confidence intervals; the second table summarizes analytical chemistry data collected throughout the toxicity tests. Also discussed, if applicable, are deviations from the guidance provided in the ASTM method used to complete the toxicity testing.

### 96-hr Toxicity of Manganese on Lampsilis siliquoidea

The 96-hr test to determine the toxicity of manganese on *L. siliquoidea* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Missouri State University laboratory culture, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate. One replicate was mistakenly loaded with only 4 individuals, but this was accounted for in the  $LC_{50}$  calculation.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 150, 75, 37, 18.8, 9.4, and 4.7 mg Mn/L.

Testing was conducted at  $20 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC<sub>50</sub> value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 90; test results are provided in Table 91. Analytical chemistry data are provided in Table 92. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 27.

Summary of Toxicity Test Conditions							
1. Test Species and Age:	Lampsilis siliquoidea, juveniles <5 days old, Missouri State Univbersity						
2. Test Type and Duration:	Static, 96 hours						
3. Test Dates:	September 8 - 12, 2009						
4. Test Temperature (°C):	$20 \pm 1$						
5. Light Quality:	Ambient Laboratory, 10-20 µE/m²/s						
6. Photoperiod:	16 h light, 8 h darkness						
7. Feeding Regime:	None						
8. Size of Test Vessel:	50 mL beaker						
9. Volume of Test Solutions:	40 mL						
10. No. of Test Organisms per Test Vessel:	5						
11. No. of Test Vessels per Treatment:	4						
12. Total No. of Test Organisms per Treatment:	20						
13. Test Concentrations (mg Mn/L):	150, 75, 37, 18.8, 9.4, and 4.7						
<ul><li>14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination- Mn mg/L):</li></ul>	154.9, 72.5, 34.5, 18.5, 10.1 and 4.5						
15. Renewal of Test Solutions:	None						
16. Dilution and Primary Control Water:	USEPA MHRW						
17. Test Material:	Manganese sulfate monohydrate: Fisher Scientific, ACS grade assay, 98.7%, Cas. No. 7785-87-7, Lot # 086316 and manganese chloride tetrahydrate, Fisher Scientific, certified ACS Assay 99.8% Cas. No. 7773-01-5, Lot # 081484						
<ul><li>18. Secondary Control Water:</li><li>19. Aeration:</li></ul>	None						
20. Endpoints Measured:	Mortality (LC <sub>50</sub> )						

# Table 90. Test conditions for 96-hour toxicity test on *Lampsilis siliquoidea* with manganese.

Results of a <u>Lampsilis siliquoidea)</u> Conducted <u>09/08/09 - 09/12/09</u>	<u>96-Hour Static Acute Toxicity Test</u> <u>Using: Manganese sulfate Cas. No. 7785-87-7 &amp; manganese</u> chloride Cas. No. 7773-01-5							
	Cumula	tive Percei	nt Affected	1 <sup>8</sup>		LC <sub>50</sub> Values'	* (mg/L)	
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr
Primary Control/ Dilution Water	0	0	0	0	62.8	50.0	48.2	43.3
4.7 (4.5) mg/L	0	0	0	0	96-Hour LC	<sub>50</sub> * = 43.3 mg/	L	
9.4 (10.1) mg/L	0	0	0	0	LC <sub>50</sub> 95% Confidence Limits			
18.8 (18.5) mg/L	0	0	0	0	24-Hr	48-Hr	72-Hr	96-Hr
37 (34.5) mg/L	0	0	5.2	21.0	LL 52.7 UL 74.9	NR NR	45.0 51.7	38.1 49.2
75 (72.5) mg/L	70	100	100	100				
150 (154.9) mg/L	95	100	100	100	LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
						sed to Determi imit Values: S		

# Table 91. Test results for 96-hour toxicity test on *Lampsilis siliquoidea* with manganese.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All  $LC_{50}$  and  $EC_{50}$  values are determined based on measured concentrations.

Concentrati	on	_ Manganese <sup>a</sup>	Temperature	pН	D.O.	Conductivity	Alkalinity	Hardness
		(mg/L)	(°C)	(s.u.)	(mg/L)	(mmos)	(mg/L)	(mg/L)
	Day 0	<0.01	20,4	7.9	7.08	316	62	92
	Day 1		20.3					
	Day 2		20.4					
	Day 3		19.6					
	Day 4	<0.01	19,1	7.9	7.50	322	62	90
Dilution water/Control		na						
	Day 0	4.5	20.5	7.9	7.83	324	62	98
	Day 1		20.2					
	Day 2		20.3					
	Day 3		19.5					
	Day 4	4.6	19.1	7.9	7.69	328	62	100
4.7 (4.5) mg/L		4.5						
	Day 0	9.2	20.4	7.9	7.87	341	62	110
	Day 1		20.3	ſ				
	Day 2		20.4					
	Day 3		19.6					
	Day 4	11.0	19.0	7.9	7.65	345	62	110
9.4 (10.1) mg/L		10.1						
	Day 0	19.0	20.4	7.7	7.83	376	62	120
	Day 1		20.2					
	Day 2		20.3					
	Day 3		19.6	1				
	Day 4	18.0	19.0	7.9	7.72	380	62	124
18.8 (18.5) mg/L		18.5						
······	Day 0	34.0	20.5	7.7	7.94	447	62	152
	Day 1		20.2					
	Day 2	<u>-</u>	20.3					
	Day 3	-	19.6					
	Day 4	35.0	19.0	7.9	7.76	450	62	152
37 (34.5) mg/L		34.5						
	Day 0	73.0	20.5	7.7	7.88	582	62	220
	Day 1		20.2		1.00			
	Day 2	-	20.3	1				
	Day 3		19.7				1	
	Day 4	72.0	19.1	7.9	7.70	590	62	224
75 (72.5) mg/L		72.5		1	1.70			
	Day 0	150.0	20.5	7.7	7.88	840	62	*
	Day 0 Day 1	1.50.0	20.3	† <i></i>	/.00			
	Day 2		20.4					
	Day 2 Day 3		19.7					
	Day 5 Day 4	160.0	19.2	7.9	7.80	850	62	*
150 (154.9) mg/L	Day 4	154.9		+ <u>'.</u> ,	1.00	0.00		· · · · · · · · · · · · · · · · · · ·

# Table 92. Analytical chemistry data for 96-hour toxicity test on *Lampsilis siliquoidea* with manganese.

\* Manganese Analysis Method 200.7

\* interference in hardness measurement

na = not applicable

#### 96-hr Toxicity of Manganese on Megalonaias nervosa

The 96-hr test to determine the toxicity of manganese on *M. nervosa* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Genoa National Fish Hatchery, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate. In one replicate of the control a test organism was inadvertently crushed, but this was accounted for in the  $LC_{50}$  calculation.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 300, 150, 75, 37.5, and 18.8 mg Mn/L.

Testing was conducted at  $20 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC<sub>50</sub> value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 93; test results are provided in Table 94. Analytical chemistry data are provided in Table 95. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 28.

Summary of Toxicity Test Conditions							
1. Test Species and Age:	<i>Megalonaias nervosa,</i> juveniles <5 days old (Genoa National Fish Hatchery)						
2. Test Type and Duration:	Static, 96 hours						
3. Test Dates:	October 23 - 27, 2009						
4. Test Temperature (°C):	$20 \pm 1$						
5. Light Quality:	Ambient Laboratory, 10-20 μE/m²/s						
6. Photoperiod:	16 h light, 8 h darkness						
7. Feeding Regime:	None						
8. Size of Test Vessel:	50 mL beaker						
9. Volume of Test Solutions:	40 mL						
10. No. of Test Organisms per Test Vessel:	5						
11. No. of Test Vessels per Treatment:	4						
12. Total No. of Test Organisms per Treatment:	20						
13. Test Concentrations (mg Mn/L):	300, 150, 75, 37.5, and 18.8						
<ol> <li>Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg Mn/L):</li> </ol>	290, 140, 72, 34, and 18						
15. Renewal of Test Solutions:	None						
16. Dilution and Primary Control Water:	USEPA MHRW						
17. Test Material:	Manganese sulfate monohydrate: Fisher Scientific, ACS grade assay, 98.7%, Cas. No. 7785-87-7, Lot # 086316 and manganese chloride tetrahydrate, Fisher Scientific, certified ACS Assay 99.8% Cas. No. 7773-01-5, Lot # 081484						
18. Secondary Control Water:	None						
19. Aeration:	None						
20. Endpoints Measured:	Mortality (LC <sub>50</sub> )						

# Table 93. Test conditions for 96-hour toxicity test on *Megalonaias nervosa* with manganese.

### Table 94. Test results for 96-hour toxicity test on *Megalonaias nervosa* with manganese.

Results of a <u>Megalonaias nervosa</u>		96-Hour	Static Acu	te Toxicity	/ Test			
Conducted 10/23/09 - 10/27/09Using: Manganese sulfate Cas. No. 7785-87-7 & manganese chloride Cas. No. 7773-01-5								
	Cumulative Percent Affected <sup>a</sup>			LC <sub>50</sub> Values* (mg/L)				
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr
Primary Control/ Dilution Water	0	0	0	0	41.6	37.5	31.5	31.5
18.8 (18) mg/L	0	0	0	0	96-Hour LC	<sub>50</sub> * = 31.5 mg/	L	
37.5 (34) mg/L	30	40	65	65		nfidence Limi		
75 (72) mg/L	95	100	100	100	24-Hr _	48-Hr	72-Hr	96-Hr
150 (140) mg/L	100	100	100	100	LL 35.6 UL 48.8	32.2 43.6	27.2 36.6	27.2 36.6
300 (290) mg/L	100	100	100	100				
					LL = Lower I UL = Upper I NR = Confide		are not relia	ble
					Method(s) Us	sed to Determi Limit Values: S	ne LC <sub>50</sub> and	EC <sub>50</sub>

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All  $LC_{50}$  and  $EC_{50}$  values are determined based on measured concentrations.

### Table 95. Analytical chemistry data for 96-hour toxicity test on Megalonaias nervosa with manganese.

Nominal (Measure	d) Test Concentration	Manganese <sup>#</sup>	Temp.	pН	D.O.	Cond.	Alkalinity	Hardness	Ammonia
		(mg/L)	<u>(°C)</u>	(s.u.)	(mg/L)	(mmos)	(mg/L)	(mg/L)	(mg/L)
	Day 0	< 0.01	20.8	8.0	8.34	303	60	90	<0.05
	Day 1		20.9	8.1	8.04	297			
	Day 2		20.9	8,1	8.13	303			
	Day 3		20.9	8.0	8.31	305			
	Day 4 total	0.1	20.8	8.0	8.00	315	60	92	<0.05
Dilution	Day 4 dissolved	0.7							
water/Control		0.1							
	Day 0	18.0	20.8	7.9	8.33	375	60	112	<0.05
	Day 1		20.9	7.9	8.10	362			
	Day 2		20.9	7.7	8.19	371			
	Day 3		20.9	7.6	8.31	379			
	Day 4 total	18.0	20.8	7.8	7.92	383	60	112	< 0.05
	Day 4 dissolved	16.0							
18.8 (18.0) mg/L		18.0							
	Day 0	33.0	20.8	7.9	8.26	445	60	*	< 0.05
	Day 1		20.9	7.7	8.11	442			
	Day 2		20.9	7.6	8.25	458			
	Day 3	·····	20.9	7.6	8.42	478			
	Day 4 total	35.0	20.8	7.7	7.94	495	60	*	< 0.05
	Day 4 dissolved	34.0	1						
37.5 (34.0) mg/L		34.0							
<u>, , , , , , , , , , , , , , , , , , , </u>	Day 0	70.0	20.8	7.9	8.29	582	60	*	< 0.05
	Day 1		20.9	7.7	8.07	569			
	Day 2		20.8	7.6	8.26	567			
	Day 3		20.9	7.5	8.32	581		-	
	Day 4 total	74.0	20.9	7.7	7.91	589	62	*	< 0.05
	Day 4 dissolved	68.0	-						
75 (72.0) mg/L		72.0	1	1					
<u> </u>	Day 0	140.0	20.8	7.8	8.33	841	60	*	<0.05
:	Day 1		20.9	7.7	8.03	826		[	
	Day 2		20.8	7.6	8.22	817			
	Day 3		20.9	7.5	8.40	851		1	
	Day 4 total	140.0	20.9	7.6	7.88	848	62	*	<0.05
	Day 4 dissolved	150.0		1				1	
150 (140.0) mg/L		140.0		1					
100 (1100) mg L	Day 0	290.0	20.8	7.7	8.27	1333	60	*	<0.05
	Day 1		20.9	7.7	7.98	1301	<u> </u>	<u> </u>	
	Day 1 Day 2		20.8	7.6	8.20	1300			
	Day 3		20.9	7.5	8.22	1325		1	
	Day 4 total	290.0	20.9	7.6	7.94	1340	62	*	<0.05
	Day 4 dissolved	290.0		1.0		1340			-0.05
300 (290) mg/L	Day 7 uissourcu	290.0	1	+		<u> </u>		1	

Nominal (Magaured) Test Concentration Manganeses Temp nH Alkalinity Hardness Ammonia DO. Cond

> \*Manganese Analysis Method 200.7 \*interference in hardness measurement Temp. = temperature; Cond. = conductivity

#### Fluoride

Table 96 provides a summary of estimated  $LC_{50}$  values for the two toxicity tests performed using fluoride.  $LC_{50}$  values ranged between 13.4 and 62.0 mg F/L.

Test Species and Duration	LC <sub>50</sub> (mg F/L)
Sphaerium simile - 96 hr	62.0
<i>Hyalella azteca -</i> 96 hr	13.4

For each of the acute toxicity tests completed using fluoride, two tables were generated: the first summarizes the test results for each toxicity test, including nominal and analytical test concentration and  $LC_{50}$  estimates with confidence intervals; the second table summarizes analytical chemistry data collected throughout the toxicity tests.

#### 96-hr Toxicity of Fluoride on Sphaerium simile

The 96-hr test to determine the toxicity of fluoride on *S. simile* was completed by INHS. Test organisms, juveniles released from field collected adults, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 800, 400, 200, 100, and 50 mg F/L.

Testing was conducted at  $22 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC<sub>50</sub> value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 97; test results are provided in Table 98. Analytical chemistry data are provided in Table 99. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 29.

Summary of Toxicity Test Conditions						
1. Test Species and Age:	Sphaerium simile, juveniles (released from field-collected adults)					
2. Test Type and Duration:	Static, 96 hours					
3. Test Dates:	July 13 - 17, 2009					
4. Test Temperature (°C):	$22 \pm 1$					
5. Light Quality:	Ambient Laboratory, 10-20 µE/m <sup>2</sup> /s					
6. Photoperiod:	16 h light, 8 h darkness					
7. Feeding Regime:	None					
8. Size of Test Vessel:	150 mL beaker					
9. Volume of Test Solutions:	120 mL					
10. No. of Test Organisms per Test Vessel:	5					
11. No. of Test Vessels per Treatment:	4					
12. Total No. of Test Organisms per Treatment:	20					
13. Test Concentrations (mg F/L):	800, 400, 200, 100, and 50					
<ul> <li>14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg F/L);</li> </ul>	800, 390, 185, 88 and 44					
15. Renewal of Test Solutions:	None					
16. Dilution and Primary Control Water:	USEPA MHRW					
17. Test Material:	Sodium fluoride: Acros Organics, 99+% for analysis ACS, Cas. No. 7681-49-5, Lot # A0243428					
18. Secondary Control Water:	None					
19. Aeration:	None					
20. Endpoints Measured:	Mortality (LC <sub>50</sub> )					

## Table 97. Test conditions for 96-hour toxicity test on *Sphaerium simile* with fluoride.

Results of a Sphaerium simile96-Hour Static Acute Toxicity TestConducted 07/13/09 - 07/17/09Using: Sodium fluoride: Sigma Aldrich Cas. No. 7681-49-5									
	Cumula	tive Percei	nt Affected	l <sub>8</sub>		LC <sub>50</sub> Values <sup>4</sup>	Values* (mg/L)		
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr	
Primary Control/ Dilution Water	0	0	0	5	>800	>800	>800	62	
50 (44) mg/L	0	0	0	25	96-Hour LC <sub>50</sub> * = 62 mg/L				
100 (88) mg/L	0	0	0	75	LC <sub>50</sub> 95% Confidence Limits				
200 (185) mg/L	0	0	35	100	24-Hr	48-Hr	72-Hr	96-Hr	
400 (390) mg/L	0	5	50	100	LL NR UL NR	NR NR	NR NR	51 75	
800 (800) mg/L	0	5	25	100					
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable				
						ed to Determi imit Values: S			

### Table 98. Test results for 96-hour toxicity test on Sphaerium simile with fluoride.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

• All LC<sub>50</sub> and EC<sub>50</sub> values are determined based on measured concentrations.

# Table 99. Analytical chemistry data for 96-hour toxicity test *Sphaerium simile* with fluoride.

Nominal (Measure Concentratio		Fluoride"	Temperature	pН	D.O.	Conductivity	Alkalinity	Hardness
		- (mg/L)	(°C)	(s.u.)	(mg/L)	(µmhos)	(mg/L)	(mg/L)
	Day 0	<0.5	22,9	8.0	7.76	307	62	96
	Day 1		22.9		7.97			
	Day 2		22.8		8.00			
	Day 3		22.7		7.63			
	Day 4	<0.5	22.9	8.0	8.05	310	62	96
Dilution water/Control		na						
····	Day 0	45	22.8	8.1	7,82	555	64	94
	Day 1		22.8		7.92			
	Day 2		22.8		7.83			
	Day 3		22.7		7.70			
	Day 4	44	22.9	8.0	8.04	558	68	80
50 (44) mg/L		44						
· · · · · · · · · · · · · · · · · · ·	Day 0	86	22.8	8.1	7.80	785	66	80
	Day 1		22.9		8.11			
	Day 2		22.8		7.95			
	Day 3		22.7		7.67			
	Day 4	91	22.9	8.1	7.53	784	68	62
100 (88) mg/L		88						
	Day 0	190	22.9	8.0	7.82	1264	78	76
	Day 1		22.8		8.05			
	Day 2		22.9		7.82			
	Day 3		22.8		6.63			
	Day 4	180	22.9	8.1	6.03	1268	82	50
200 (185) mg/L		185						
	Day 0	400	22.9	8.2	7.78	2210	120	64
	Day 1		22.9		7.99			
	Day 2		22.8		7.79			
	Day 3		22.8		4.88			
	Day 4	380	23.0	7.9	5.49	2210	120	30
400 (390) mg/L		390						1
	Day 0	800	23.0	8.2	7.82	4050	160	14
	Day 1		22.9		8.05			
	Day 2		22.9	1	8.02			
	Day 3		22.8	1	6.51			
	Day 4	800	22.9	8.0	6,89	4090	160	2
800 (800) mg/L		800						

\* Fluoride Analysis Method 300.0

na = not applicable

### 96-hr Toxicity of Fluoride on Hyalella azteca

The 96-hr test to determine the toxicity of fluoride on *H. azteca* was completed by GLEC. *H. azteca* were collected from GLEC's laboratory culture. These organisms are maintained in 10 gallon glass aquaria; plastic artificial turf and screen mesh serve as a substrate for the culture. The tanks are filled with de-chlorinated Lake Michigan water (City of Traverse City, Michigan water passed through an activated carbon filter). Cultures are fed 50 mL of 4 g/L Tetrafin slurry daily. When visible algae are not observed within the glass aquaria, algae (*Selenastrum* sp.) are used as a supplement to the Tetrafin slurry. Additionally, on occasion, dried Aspen (*Populus* sp.) leaves are prepared as a food supplement. The culture is maintained in a 16-hour light: 8-hour dark photoperiod at a temperature between 23 and 26 °C.

Test organisms were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 7.8, 12.9, 21.6, 36.0, 60.0, and 100 mg F/L.

Testing was conducted at  $22 \pm 1$  °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC<sub>50</sub> value was determined using the Probit and Spearman Karber methods.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 100; test results are provided in Table 101. Analytical chemistry data are provided in Table 102. Accompanying information, including raw laboratory data, analytical chemistry data, reference toxicant data and statistical analyses, is provided in Appendix 30.

Su	Summary of Toxicity Test Conditions						
1.	Test Species and Age:	Hyalella azteca, 14 days old, GLEC culture					
2.	Test Type and Duration:	Static, 96 hours					
3.	Test Dates:	September 17- September 21, 2009					
4.	Test Temperature (°C):	$22 \pm 1$					
5.	Light Quality:	Ambient Laboratory, 10-20 µE/m²/s					
6.	Photoperiod:	16 h light, 8 h darkness					
7.	Feeding Regime:	None					
8.	Size of Test Vessel:	150 mL beaker					
9.	Volume of Test Solutions:	100 mL					
10.	No. of Test Organisms per Test Vessel:	5					
11.	No. of Test Vessels per Treatment:	4					
12.	Total No. of Test Organisms per Treatment:	20					
13.	Target or Nominal Test Concentrations (mg F/L):	100, 60, 36, 21.6, 12.9, and 7.8					
14.	Analytical Test Concentrations (average of samples collected at test initiation and termination-mg F/L):	89.4, 58.9, 32.7, 22.8, 14.3, and 8.5					
15.	Renewal of Test Solutions:						
16.	Dilution and Primary Control Water:	None					
17.	Test Material:	USEPA MHRW					
10		Sodium Fluoride: Sigma Aldrich, 99+% ACS Reagent Cas. No. 7681-49-4, Batch # 06810JJ					
18.		None					
	Aeration:	None					
20.	Endpoints Measured:	Mortality (LC <sub>50</sub> )					

## Table 100. Test conditions for 96-hour toxicity test on *Hyalella azteca* with fluoride.

Results of a <u>Hyalella azteca</u> 96-Hour Static Acute Toxicity Test         Conducted 09/17/09 - 09/21/09       Using: Fluoride (Sodium Fluoride Sigma Aldrich Cas. No. 7681-49-4)								
	Cumulative Percent Affected <sup>a</sup> LC <sub>50</sub> Values* (mg/L)					* (mg/L)		
Nominal (Measured) Concentrations	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	0 (0)	25.1	16.0	13.8	13.4
7.8 (8.5) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	96-Hour LC	<sub>i0</sub> * = 13.4 mg/	L	
12.9 (14.3) mg/L	0 (0)	45 (45)	70 (70)	70 (70)	LC	50 95% Confid	lence Limit	S
21.6 (22.8) mg/L	20 (20)	80 (80)	90 (90)	95 (95)	24-Hr	48-Hr	72-Hr	96-Hr
36 (32.7) mg/L	100 (100)	100 (100)	100 (100)	100 (100)	LL 23.3 UL 27.1	13.9 18.3	12.0 15.6	11.8 15.2
60 (58.9) mg/L	100 (100)	100 (100)	100 (100)	100 (100)				
100 (89.4) mg/L	100 (100)	100 (100)	100 (100)	100 (100)	LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC <sub>50</sub> Confidence Limit Values: Probit and Spearman-Karber			

### Table 101. Test results for 96-hour toxicity test on Hyalella azteca with fluoride.

<sup>a</sup> Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

\* All LC<sub>50</sub> values are determined based on measured concentrations.

### Table 102. Analytical chemistry data for 96-hour toxicity test on Hyalella azteca with fluoride.

Nominal (and M Test Concent		Fluoride*	Temperature	рH	D.O.	Conductivity	Alkalinity	Hardness	Ammonia
	l	(mg/L)	(°C)	(s.u.)	(mg/L)	(mmhos)	(mg/L)	(mg/L)	(mg/L)
	Day 0	ND	22.0	7.84	8.0	300	54	80	ND
	Day 1		21.9	7.79	7.5				
	Day 2		22.1	7.79	7.1				
Dilution	Day 2 Day 3		22.0	7.84	7.9				
water/Control	Day 3 Day 4	ND	22.2	7.81	8.0	325	68	88	
	Day 4	ND	<u> </u>	7.01	0.0	323	00		
		ND							
w					L	L	1		
	Day 0	8.4	22.0	7.84	8.0	368	1	1	
	Day 1		22.0	7.78	7.5				
	Day 2		22.1	7.83	7.3				
7.8 (8.5) mg/L	Day 3		22.0	7.91	7.7	4			
(0.07 mg/ 2	Day 4	8.6	22.2	7.87	8.0	363			
	Duj 4		L.L	1.01	<u> </u>				
		8.5							
	David	444		7.05			r	·····	
	Day 0	14.1	22.0	7.85	8.0	393			
	Day 1		22.0	7.85	7.5				
12.9 (14.3) mg/L	Day 2		22.1	7.88	7.4	L			
	Day 3		21.9	7.97	8.1				
	Day 4	14.5	22.2	7.89	8.0	377			
		14.3					I		
	Day 0	22.0	22.0	7.85	8.0	432	1		
	Day 0 Day 1	22.0	21.9	7.90	7.5	402			
	Day 1 Day 2		22.0	7.89	7.3				
21.6 (22.8) mg/L	Day 2 Day 3		22.0	8.02	8.0				
	Day 3 Day 4	23.5	21.0	7.97	8.0	422	66	100	
	Day 4	23.5	21.9	1,91	0.0	426	00	100	
		22.0			I	1			
	Day 0	33.4	22.0	7.88	8.0	502	1	1	
	Day 1	32.0	21.9	7.91	7.5				
	Day 2	1	21.0	7.01	<u>/.9</u>				
36 (32.7) mg/L	Day 3								
	Day 4								
		32.7							
	L	1			I		J	J	
	Day 0	58.1	22.0	7.88	8.0	622			
	Day 1	59.6	21.9	7.92	7.5	1		1	
	Day 2							1	
50 (58.9) mg/L	Day 3							1	
	Day 4				i	1	1	1	
		58.9				1			
	•				•				
	Day 0	91.0	22.0	7.89	8.0	781	72	68	ND
	Day 1	87.7	21,9	7.99	7.5		70	68	
	Day 2						1	1	
100 (89.4) mg/L	Day 3	[						1	-
	Day 4					1		1	
	,	89.4			1	1	1	+	

\* Fluoride Analysis EPA 300.0 ND Not Detect; below detection limit

## Attachment 7

Facilities with NPDES Permit Limits Based on the Incorrect Chronic Standard for Zinc

		Parameter	Limit	
NPDES ID	Permit Name	Description	The ter is a work in the work	Limit Unit
IL0034631	ALCOA EXTRUSIONS INC	Zinc, total (as Zn)	.061	Milligrams per Liter
IL0021130	BLOOMINGDALE, VILLAGE OF	Zinc, total (as Zn)	.042	Milligrams per Liter
IL0032735	BOLINGBROOK, VILLAGE OF	Zinc, total (as Zn)	.084	Milligrams per Liter
IL0021083	CASEYVILLE TOWNSHIP EAST STP	Zinc, total (as Zn)	.038	Milligrams per Liter
IL0027979	CENTRALIA, CITY OF	Zinc, total (as Zn)	.028	Milligrams per Liter
IL0028321	DECATUR SD MAIN STP	Zinc, total (as Zn)	.075	Milligrams per Liter
IL0028517	DUQUOIN, CITY OF	Zinc, total (as Zn)	.054	Milligrams per Liter
IL0028622	EFFINGHAM, CITY OF	Zinc, total (as Zn)	.028	Milligrams per Liter
IL0034479	HANOVER PARK, VILLAGE OF	Zinc, total (as Zn)	.044	Milligrams per Liter
IL0029173	HIGHLAND, CITY OF	Zinc, total (as Zn)	.025	Milligrams per Liter
IL0026280	ITASCA, VILLAGE OF	Zinc, total (as Zn)	.045	Milligrams per Liter
IL0022519	JOLIET, CITY OF	Zinc, total (as Zn)	.073	Milligrams per Liter
IL0022055	LAKE COUNTY PUBLIC WORKS DEPAI	Zinc, total (as Zn)	.053	Milligrams per Liter
IL0004073	MARATHON PETROLEUM COMPANY, I	Zinc, total (as Zn)	.055	Milligrams per Liter
IL0029874	METROPOLIS, CITY OF	Zinc, total (as Zn)	.026	Milligrams per Liter
IL0036218	MONMOUTH, CITY OF	Zinc, total (as Zn)	.043	Milligrams per Liter
IL0078786	NL Properties, LLC	Zinc, total (as Zn)	.135	Milligrams per Liter
IL0035297	NUCOR STEEL INC-BOURBONNAIS	Zinc, total (as Zn)	.052	Milligrams per Liter
IL0021636	OFALLON, CITY OF	Zinc, total (as Zn)	.0379	Milligrams per Liter
IL0036382	ROCK ISLAND SW STP	Zinc, total (as Zn)	.048	Milligrams per Liter
IL0048721	ROSELLE, VILLAGE OF	Zinc, total (as Zn)	.04	Milligrams per Liter
IL0026859	SCOTT AIR FORCE BASE	Zinc, total (as Zn)	.044	Milligrams per Liter
IL0000329	US STEEL CORP GRANITE CITY WKS	Zinc, total (as Zn)	.17	Milligrams per Liter
IL0079073	VILLAGAE OF ITASCA	Zinc, total (as Zn)	0.45	Milligrams per Liter

Attachment 7 Facilities with NPDES Permit Limits Based on the Incorrect Chronic Standard for Zinc

Attachment 8

Agency Errata Sheet Numbers 1, 2 and 3 from R02-11

#### BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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IN THE MATTER OF:

WATER QUALITY AMENDMENTS TO 35 III. Adm. Code 302.208(e)-(g), 302.504(a), 302.575(d), 303.444, 309.141(h); and PROPOSED 35 III. Adm. Code 301.267, 301.313, 301.413, 304.120, and 309.157

R02-11 (Rulemaking - Water)

#### AGENCY'S ERRATA SHEET

THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY submits this ERRATA

SHEET for the above-entitled matter to the Illinois Pollution Control Board and the participants on

the Service List. The revisions suggested below are based on the Agency's ongoing review of the

proposal.

Section 304.120 Deoxygenating Wastes

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g) Compliance with the BOD<sub>5</sub> numerical standard in Part 304 Section 304.120 for Publicly Owned Treatment Works, Publicly Regulated Treatment Works or other domestic sewage treatment works will be determined by the analysis of 5 day carbonaceous biochemical oxygen demand (CBOD<sub>5</sub>) (STORET number 80082). Effluent from the treatment works subject to the requirements of Section 304.120(a) shall not exceed 25 mg/L CBOD<sub>5</sub>.

(Source: Amended at 13 Ill. Reg. 7754, effective May 4, 1989, amended in \_\_\_\_\_\_ at Ill. Reg. \_\_\_\_\_\_, effective \_\_\_\_\_\_, 2002).

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#### BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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#### IN THE MATTER OF:

WATER QUALITY AMENDMENTS TO 35 Ill. Adm. Code 302.208(e)-(g), 302.504(a), 302.575(d), 303.444, 309.141(h); and PROPOSED 35 Ill. Adm. Code 301.267, 301.313, 301.413, 304.120, and 309.157

R02-11 (Rulemaking - Water)

#### AGENCY'S ERRATA SHEET NUMBER 2

THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY submits this ERRATA

SHEET NUMBER 2 for the above-entitled matter to the Illinois Pollution Control Board and the

participants on the Service List. The revisions suggested below (double underlined) are based on

the information gathered at the January 29, 2002 hearing and are in addition to the revisions

suggested in the Agency's ERRATA SHEET.

#### Section 302.504 Chemical Constituents

· · ·

The following concentrations of chemical constituents must not be exceeded, except as provided in Sections 302.102 and 302.530:

 a) The following standards must be met in all waters of the Lake Michigan Basin. Acute aquatic life standards (AS) must not be exceeded at any time except for those waters for which the Agency has approved a zone of initial dilution (ZID) pursuant to Sections 302.102 and 302.530. Chronic aquatic life standards (CS) and human health standards (HHS) must not be exceeded outside of waters in which mixing is allowed pursuant to Section 302.102 and 302.530 by the arithmetic average of at least four consecutive samples collected over a period of at least four days. The samples used to demonstrate compliance with the CS or HHS must be collected in a manner which assures an average representation of the sampling period.

Constituent	STORET	<u>Unit</u>	AS	CS	HHS
	Number				
******					

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Constituent	<u>STORET</u> <u>Number</u>	<u>Unit</u>	<u>AS</u>	<u>CS</u>	<u>HHS</u>
***					
Cadmium (dissolved)	01025	μg/L	exp[A +Bln(H)] <u>X</u> <u>{1.138672-</u> [(lnH)(0.0418 <u>38)]}*</u> A=-3.6867, <u>and</u> B=1.128	exp[A +Bln(H)] X / (1.101672-10.00000000000000000000000000000000000	NA
		*	**		

(Source: Amended at 21 Ill. Reg. 1356, effective December 24, 1997, amended in \_\_\_\_\_\_ at \_\_\_\_\_\_, effective \_\_\_\_\_\_\_, 2002)

\*\*\*

#### Section 302.575 Procedures for Deriving Tier I Water Quality Criteria and Values in the Lake Michigan Basin to Protect Wildlife

\*\*\*

d) Calculation of TSV. The TSV, measured in milligrams per liter (mg/L), is calculated according to the equation:

 $TSV = \{ [TD x Wt] / [UF_a x UF_s x UF_1] \} / \{ W + \sum [F_{TLi} x BAF_{WLTLi}] \}$ Where:

TSV = target species value in milligrams of substance per liter (mg/L).

TD = test dose that is toxic to the test species, either NOAEL or LOAEL.

 $UF_a$  = the uncertainty factor for extrapolating toxicity data across species (unitless). A species-specific UF<sub>a</sub> shall be selected and applied to each target species, consistent with the equation

 $UF_s$  = the uncertainty factor for extrapolating from subchronic to chronic exposures (unitless)

UF<sub>1</sub> = the uncertainty factor for extrapolation from LOAEL to NOAEL (unitless) Wt = average weight in kilograms (kg) of the target species

W = average daily volume of water in liters consumed per day (L/d) by the target species

 $F_{TLi}$  = average daily amount of food consumed by the target species in kilograms (kg/d) for trophic level i

 $BAF_{WLTLi}$  = aquatic life bioaccumulation factor with units of liter per kilogram

#### (L/kg), as derived in Section 302.570 for trophic leveli

Respectfully Submitted

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

By:\_\_\_\_\_

Sanjay K Sofat Assistant Counsel Division of Legal Counsel

DATED: February 20, 2002

Illinois Environmental Protection Agency 1021 North Grand Avenue East P.O. Box 19276 Springfield, Illinois 62794-9276 (217) 782-5544

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#### BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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#### IN THE MATTER OF:

WATER QUALITY AMENDMENTS TO 35 III. Adm. Code 302.208(e)-(g), 302.504(a), 302.575(d), 303.444, 309.141(h); and PROPOSED 35 III. Adm. Code 301.267, 301.313, 301.413, 304.120, and 309.157

R02-11 (Rulemaking - Water)

#### AGENCY'S ERRATA SHEET NUMBER 3

THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY submits this

ERRATA SHEET NUMBER 3 for the above-entitled matter to the Illinois Pollution

Control Board and the participants on the Service List. The revisions suggested below

are based on the Agency's ongoing review of the proposal and the information gathered at

the January 29, 2002 hearing. These revisions are in addition to the revisions suggested

in the Agency's ERRATA SHEET and ERRATA SHEET NUMBER 2.

#### Section 302.208 Numeric Standards for Chemical Constituents

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b) The chronic standard (CS) for the chemical constituents listed in subsection (e) shall not be exceeded by the arithmetic average of at least four consecutive samples collected over any period of at least four days, except as provided in subsection (d). The samples used to demonstrate attainment compliance or lack of attainment compliance with a CS must be collected in a manner that which assures an average representative of the sampling period. For the metals that have water quality based standards dependent upon hardness, the chronic water quality standard will be calculated according to subsection (e) using the hardness of the water

body at the time the metals sample was collected. To calculate attainment status of chronic metals standards, the concentration of the metal in each sample is divided by the calculated water quality standard for the sample to determine a quotient. The water quality standard is attained if the mean of the sample quotients is less than or equal to one for the duration of the averaging period.

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e) Numeric Water Quality Standards for the Protection of Aquatic Organisms

Constituent	Storet Number	AS (μ <u>g/L</u> ) ( <del>ug/L</del> )	СS ( <u>µg/L</u> ) ( <del>ug/L</del> )			
Cyanide <u>(weak acid</u> <u>dissociable)</u>	00718	<u>49</u> <del>22</del>	<u>11</u> 9-9 5-2			
Zinc (dissolved)	01090	exp[A+Bln(H)] X 0.978*	exp[A+Bln(H)] X 0.986*			
		<u>where A= 0.9035</u> 0.8875, and	where A=- 0.8165 0.8227, and			
		<u>B=0.8473</u>	<u>B=0.8473</u>			
where: <u>µg/L</u> <del>ug/</del>	L = microgram	per liter,				
$\exp[x] = base \underline{n}$	<u>atural</u> <del>neutral</del> log	garithms raised to the x- pov	ver, and			
ln(H) = natural	logarithm of Har	dness (STORET 00900).				
* = conversion factor multiplier for dissolved metals						
(Source: Amended at 20 III. Reg. 7682, effective May 24, 1996; amended in at at						
Section 304.120 Deoxygenating Wastes						

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g) <u>Compliance with the BOD<sub>5</sub> numerical standard in Part 304 Section-304.120 for Publicly</u> <u>Owned Treatment Works, Publicly Regulated Treatment Works or other domestic</u>

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sewage treatment works will be determined by the analysis of 5 day carbonaceous biochemical oxygen demand  $(CBOD_5)$  (STORET number 80082), unless federal regulations require treatment works treating industrial wastes to comply with more stringent requirements determined by the analysis of 5 day biochemical oxygen demand (BOD<sub>5</sub>). Effluent from the treatment works subject to the requirements of Section 304.120(a) shall not exceed 25 mg/L CBOD<sub>5</sub>.

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**Respectfully Submitted** 

#### ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

By:\_\_\_\_\_

Sanjay K Sofat Assistant Counsel Division of Legal Counsel

DATED: March 6, 2002

Illinois Environmental Protection Agency 1021 North Grand Avenue East P.O. Box 19276 Springfield, Illinois 62794-9276 (217) 782-5544

## Appendix D

ENVIRON Curriculum Vitae (Not Confidential)



## Phyllis Fuchsman | Manager

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Phyllis Fuchsman has 20 years' experience in environmental consulting, conducting ecotoxicological studies, biological surveys and ecological risk assessments. She is a Senior Manager in ENVIRON's Ecology and Sediment Management practice, working on projects in the United States and abroad. Her primary expertise is in the ecology and toxicology of aquatic and terrestrial systems, including sediment toxicology, aquatic toxicology, and the effects of bioaccumulative chemicals on wildlife. With more than 90 publications and presentations, Phyllis is a recognized leader in the development of ecological risk assessment methods for contaminated sediments and has served as a peer reviewer for USEPA. She has developed and applied innovative approaches to assessing the environmental effects of PCBs, VOCs and other organic chemicals, mercury, and other metals. She also co-authored a guidance document for the US Department of Defense on monitored natural recovery at contaminated sediment sites.

#### **EDUCATION**

1990 BA, Environmental Studies, Swarthmore College

1993 MM, The Juilliard School

#### EXPERIENCE

#### Ecological Risk Assessment

- Prepared environmental assessments for pharmaceutical substances, for submittal to the U.S. Food and Drug Administration and the European Medicines Agency. Projects have included new drugs and a new combination of existing drugs. Also preparing an environmental assessment of a veterinary medicinal substance for submittal to the Food and Drug Administration.
- Evaluated ecological risks associated with an anti-microbial used in various personal care products. Prepared manuscripts detailing triclosan's fate during the wastewater treatment process, ecological risks associated with its occurrence in land-applied biosolids, and aquatic ecological risks. The assessment used probabilistic techniques to assess risk to soil-dwelling and aquatic organisms and terrestrial and aquatic-feeding wildlife. Fugacity modeling was used to supplement measured exposure concentrations in biota tissue and abiotic media, and a chronic species sensitivity distribution was constructed as part of the aquatic effects assessment.
- On behalf of the Sediment Management Work Group, currently preparing a set of manuscripts detailing a critical review of mercury toxicity reference values for protection of benthic invertebrates, fish, and wildlife. The review is informed by a comprehensive literature review of mercury effects on these organisms.
- Managing a mammalian toxicology study with Michigan State University, investigating effects of Aroclor 1268 on mink health and reproductive success. Study is designed to inform evaluation of Aroclor 1268 concentrations in dolphin blubber near a Superfund site. Aroclor 1268 is an unusual PCB mixture with a higher chlorine content and lower concentrations of the most toxic PCB congeners, compared to typical PCB mixtures.
- Led ecological risk analyses for the Deloro Mine Site, a former mining and smelting site in Ontario. Key
  contaminants include arsenic, cobalt, copper, and nickel. Designed sampling and analysis program for stream
  and floodplain areas, including a sediment triad study—toxicity testing, invertebrate community
  characterization, and chemical analyses—and biota tissue analyses, as well as abiotic sampling. Metal
  bioavailability to benthic invertebrates was assessed through analyses of amphipods exposed to site sediments

as part of toxicity tests, following Environment Canada methods. Managed ecological risk analyses and developed risk-based remediation goals for sediments. Soil remediation for ecological protection was not warranted, because any ecological benefits would be outweighed by habitat destruction.

- Developed risk-based sediment remediation goals for DDT, arsenic, and lead, for a site on the Delaware River. The proposed remediation goals were based on an in-depth literature review and were designed to protect benthic invertebrates, fish, birds, and humans consuming fish from the river. By focusing on cause-effect, concentration-response relationships in the risk evaluation for benthic invertebrates, remediation goals were identified that are considerably higher than frequently used sediment screening values. The resulting remediation goals were accepted by EPA, without the need for costly site-specific toxicity testing or bioaccumulation analyses.
- Assessed ecological risks due to mercury and DDT in a southern European alpine freshwater system, composed of a high energy river and two alpine lakes. A sediment triad approach—integrating site-specific invertebrate community, sediment toxicity, and chemistry data—was used to evaluate risks to benthic invertebrates and was supplemented by invertebrate tissue analyses and an in-depth literature review. Fish health was examined through measurements of fish condition and sex ratio, as well as evaluation of chemical concentrations in fish tissue. The risk assessment for birds and mammals (specifically grebes and bats) used a food web model and also drew upon a site-specific risks were found to be minimal. Additional support for these findings was gained through a peer review by risk assessment experts at the U.S. Army Corps of Engineers.
- Evaluated aquatic ecological risks due to mercury in sediments downstream of a former chlor-alkali plant along the St. Clair River, Ontario. Incorporated site-specific benthic invertebrate survey and toxicity data and identified risks to fish and fish consumers associated with measured and estimated tissue residues. Analyses included an assessment of mercury effects on fish sex ratios.
- Assessed aquatic ecological risks due to PCBs in sediments of Wheatley Harbour, Ontario, adjacent to a fish
  processing facility, on behalf of the Essex Region Conservation Authority. Estimated risks to mink using
  techniques from a meta-analysis of extensive published data relating PCB exposures to mink reproductive
  success. Sediment management actions were not needed, based on risk assessment results.
- Analyzed aquatic ecological risks due to PCBs and mercury in sediments adjacent to a former chlor-alkali plant on the shore of Lake Superior (Peninsula Harbour, Ontario), on behalf of Environment Canada. Incorporated site-specific benthic invertebrate survey and toxicity data and identified risks to fish and fish consumers associated with measured and estimated tissue residues.
- Conducted an ERA for a former ceramic manufacturing site in Ontario. Barium and zinc were present at high concentrations in soil due to their use in glazes. Risk estimates were refined based on site-specific bioaccumulation data and analyses of exchangeable (bioavailable) metals in soil. Risk assessment was prepared in accordance with Ontario regulations to obtain a Record of Site Condition.
- Provided technical support for a utility consortium advocating development of PCB criteria based on fish tissue
  rather than water concentrations, for protection of fish-eating wildlife. Reviewed the basis of existing fish tissue
  criteria for PCBs and developed alternatives based on in-depth literature review of PCB effects on birds and
  mink.
- Prepared an ERA for Dicks Creek in Middletown, Ohio, adjacent to a steel manufacturing facility. Chemicals
  of interest included PCBs, PAHs, and metals. Data collection included analyses of chemical concentrations in
  whole-body fish, macroinvertebrates, and aquatic plants. Sediment analyses were also selected using riskbased data use objectives. Integrated survey data measuring fish and invertebrate community quality.

Developed alternative sediment quality benchmarks for PCBs, based on cause-effect, concentration-response data from the scientific literature. Using an extensive data set of published PCB toxicity data for mink, compared the utility of various exposure metrics in explaining the observed dose-response data.

- Critically evaluated, on behalf of a consortium of trade associations, ecological components of the multimedia, multireceptor, multipathway risk assessment (3MRA) model developed by USEPA under the Hazardous Waste Identification Rule (HWIR). Prepared comments for submission to USEPA.
- Managed the design and completion of screening and baseline ecological risk assessments and associated work plans for the Hertel Landfill Superfund Site (New York). Directed sampling, analyses, and toxicity testing in support of the baseline assessment. Potential impacts of discharging groundwater on adjacent wetlands and streams were of concern, due to the presence of waste below the water table. Chemicals of interest included metals, PAHs, pesticides, and cyanide. Successfully limited the extent of additional sampling by applying indepth knowledge of the ecotoxicology literature in the screening assessment. The baseline assessment demonstrated that ecological risks are limited to iron toxicity in small seep areas immediately adjacent to the landfill. These results were used to change the Record of Decision for the site, eliminating the need for an extensive pump-and-treat system.
- Managed the revision of a major ecological risk assessment of Calcasieu estuary waterways adjacent to a chemical production facility in Louisiana, in response to Agency comments. Identified appropriate technical revisions and clarifications to a detailed, multi-chemical, multi-area risk assessment. Major components of the assessment included interpretation of a site-specific sediment toxicity evaluation program, assessment of chemical tissue burdens in fish and invertebrates, food web modeling for assessment of risks to predatory fish and wildlife, identification of regional background concentrations, and interpretation of estimated risks in the context of physical habitat limitations. Used probabilistic techniques to develop site-specific biota-sediment accumulation factors.
- Conducted an ecological risk assessment for a lake in the former floodplain of the Columbia River. Completed a screening assessment of available sediment data and developed a work plan for further sampling to support a site-specific risk characterization. Tissue sampling and wildlife modeling were then implemented to address potential bioaccumulation of PCBs, pesticides, and selenium. Toxicity testing was conducted to address potential effects on benthic invertebrates. Ecological risks were assessed in accordance with Oregon DEQ rules, which specify the calculation of population-level risk estimates. Although marginal sediment toxicity (attributed to DDT and petroleum hydrocarbons) was noted, the magnitude and spatial extent of effects did not qualify as a significant population-level effect under the Oregon rules.
- Conducted an ecological risk assessment for PCBs and metals in river sediment adjacent to a New Jersey
  manufacturing facility, as well as in on-site soil and ditches. Applied detailed, homologue-based models to
  demonstrate a lack of PCB-related risk to aquatic-feeding mink and sediment-dwelling invertebrates in the river.
  Developed clean-up goals to address risks related to PCB-contaminated soils and metal-contaminated ditch
  sediments. Also provided an assessment of relative risks associated with various remedial options, to focus
  remediation planning on options providing the greatest net environmental benefit.
- Completed a baseline ecological risk assessment addressing terrestrial and aquatic areas potentially affected by the Cam-Or Superfund Site (Westville, Indiana). Reviewed EPA's screening-level risk assessment and identified refinements needed to finalize selection of chemicals of interest for further evaluation. Supplemental sampling addressed issues of site-specific bioavailability, toxicity, and bioaccumulation. No site-related aquatic risks were identified, and terrestrial risks were found to be limited to certain metals potentially ingested by invertebrate-eating wildlife on-site.

#### Sediment Toxicity Evaluation

- Developed a conceptual site model to support sediment management decision-making for the St. Marys River Area of Concern, located in Ontario and Michigan. Based on existing data, identified and described primary sources and source control status, migration pathways, key risk drivers, sediment stability, and evidence of natural recovery. Key contaminants of concern include petroleum hydrocarbons and wood-derived wastes, and toxicity to benthic invertebrates appears to be the key risk driver. Identified key issues, knowledge gaps, and recommendations, toward the goal of defining the areas and toxicants requiring sediment management. Also conducted an in-depth literature review of the chemical and physical effects of petroleum hydrocarbons on benthic invertebrates. Provided strategic advice to maximize the utility of information gathered in Environment Canada's sediment sampling efforts, particularly with regard to identifying causes of sediment toxicity.
- Identified and corrected a mathematical error in the USEPA's equilibrium partitioning (EqP) method for assessing organic chemicals in sediment. Although the standard EqP method is accurate for hydrophobic organic chemicals, for less-hydrophobic chemicals (such as volatile organic compounds [VOCs], phenolic compounds) it produces erroneous results that are lower than screening values developed by assuming 100% bioavailablity. The corrected method, now published in the peer-reviewed literature and incorporated in USEPA and state guidance, has been approved for use in several risk assessments under the Superfund program and in other settings.
- Served as an invited peer reviewer for the USEPA's Procedures for the Derivation of Equilibrium Partitioning Benchmarks (ESBs) for the Protection of Benthic Organisms: Compendium of Tier 2 Values for Nonionic Organics.
- Managed a site-specific sediment toxicity evaluation program applicable to the waterways of the Calcasieu Estuary adjacent to a chemical production facility in Louisiana. Prepared reports detailing the derivation of sediment quality benchmarks for mercury, hexachlorobenzene, other chlorinated benzenes, and hexachlorobutadiene. Approaches included spiked sediment toxicity testing, sediment dilution toxicity testing, interpretation of site-specific no-effect concentrations, and the probabilistic application of equilibrium partitioning theory to published aquatic toxicity data. Demonstrated ability to predict sediment toxicity using site-specific benchmarks in a chemical mixture model.
- Applied cause-effect evidence to assess likely contributors to observed sediment toxicity for a southern California shipyard. Exposure data included concentrations of mercury, tributyltin, PCBs, metals, and PAHs in whole sediment, porewater, and biota tissue. None of the chemicals examined clearly explained the observed toxicity test results, and unmeasured compounds such as pyrethroid insecticides may have played a role.
- Currently managing a sediment evaluation for Duck and Otter Creeks in Ohio, under a cooperative agreement between a local industry group and USEPA's Great Lakes National Program Office. The creeks are located on the Lake Erie shoreline near Toledo, Ohio, downstream from multiple oil refineries. Prior risk assessments indicated widespread sediment toxicity to invertebrates and suggested risks to fish, wildlife, and humans. Designed a data gap investigation to verify the previously observed sediment toxicity and identify its cause. Toxicity was found to be limited to a discrete area and was closely related to porewater PAH concentrations. ENVIRON completed a feasibility study for the affected area and is currently engaged in predesign work, with the potential to secure USEPA matching funds for sediment removal.
- Analyzed results of a sediment triad investigation, including sediment chemistry, toxicity tests, bioaccumulation
  tests, and benthic invertebrate surveys, for a site on the Hackensack River, New Jersey. All detected chemicals
  were evaluated using cause-effect evidence (EqP benchmarks, spiked sediment studies, and field studies from
  sites where the chemical was the predominant contaminant). Toxicity to amphipods exhibited a dose-response

relationship with PAH concentrations, consistent with predicted PAH-related risks. Despite elevated concentrations of total chromium, chromium did not contribute to the observed biological effects, based on sediment geochemistry, porewater analyses, and a lack of effects on the most chromium-sensitive test species. Developed a critique of the Effects-Range Median screening value for chromium and identified an alternative remediation goal.

Developed an innovative application of the EqP approach to assess PCBs in sediment of Dicks Creek in Ohio. The approach was based on cause-effect data, which provides more realistic information than published "consensus" guidelines. Applied this method, together with USEPA's sediment guidelines for PAHs and metals, as part of a comprehensive assessment of risks to benthic invertebrates in a small Ohio stream. Other lines of evidence included invertebrate community surveys, chemical concentrations in invertebrate tissue, and *in situ* toxicity data collected by other researchers. Taken together, these lines of evidence indicated that physical habitat limitations due to historical channelization are the most important factors affecting the benthic invertebrate community, with chemical toxicity playing a role only on a very limited spatial scale.

#### Aquatic Toxicity Evaluation

- Reviewed technical issues, on behalf of a national trade association, associated with the translation of USEPA's mercury fish tissue criterion to a water quality value for purposes of National Pollutant Discharge Elimination System (NPDES) compliance and total maximum daily load (TMDL) development. Reviewed draft USEPA guidance and supporting documentation, identifying relevant errors and oversimplifications. Evaluated the utility of existing mercury bioaccumulation models and default translators and identified sampling requirements for establishing site-specific mercury translators.
- Successfully petitioned Michigan Department of Environmental Quality (MDEQ) to increase state water quality standards for barium, manganese, and 2,4-dimethylphenol. Determined the basis for existing criteria, identified additional, published toxicity data, and developed proposed criteria revisions for submission to the MDEQ. Also identified flaws in the criteria for acetic acid; as a result, the criteria were revised following additional toxicity testing by MDEQ.
- Designed and implemented an assessment of fish and invertebrate community quality in the Menominee River adjacent to an area of contaminated groundwater. Benthic invertebrates were collected using direct sampling and artificial substrate samplers. Fish were surveyed using electrofishing. Habitat characteristics and chemical concentrations in sediment and porewater were evaluated. Used sophisticated statistical analyses, including curve-fitting techniques, to demonstrate that variations in biological community characteristics were due to physical habitat conditions, not chemical concentrations. In addition to limiting natural resource damages liability, the lack of impact on the river served as the basis for proposed site-specific groundwater quality criteria.
- Developed detailed toxicity profiles for cyanide and ammonia as part of a predictive risk assessment for hypothetical fire retardant spill scenarios. Developed species sensitivity distributions and identified the relative sensitivity of various categories of freshwater species. Identified relationships between exposure duration and acute toxicity, to increase the accuracy of risk predictions for pulse exposures in streams.

#### Remediation and Restoration

 Coordinated a multi-disciplinary team of experts from industry, the U.S. Navy, the U.S. Army Corps of Engineers, and the U.S. Environmental Protection Agency in preparing a technical guide to facilitate the understanding and application of monitored natural recovery (MNR) at contaminated sediment sites. MNR involves leaving contaminated sediments in place while monitoring the performance of the natural physical, chemical, and biological processes that physically isolate, transform, and/or reduce the bioavailability and

mobility of the contaminants. The technical guide was prepared under a grant from the U.S. Department of Defense's Environmental Security Technology Certification Program.

- Managed an evaluation of key issues affecting the Saginaw Bay (Michigan) watershed, to help promote scientifically sound prioritization of conservation and restoration projects, identify opportunities to integrate work to improve sediment and surface water quality, and identify watershed processes that could affect the success of conservation and restoration efforts. Key issues included habitat loss, invasive species, dams, altered hydrology, sediment and nutrient loading, and bacterial and chemical contamination.
- Designed the phytoremediation component of a groundwater remedy targeting chlorinated solvents at the Crab Orchard Superfund Site, Marion, Illinois. Design considerations included existing vegetation, existing physical structures, depth to groundwater, and tree species characteristics. A mix of four tree species will be established to decrease solvent migration to an adjacent lake, by increasing groundwater transpiration and promoting rhizosphere degradation, phytoextraction, phytodegradation, and phytovolatilization.
- Participated in design of riparian tree plantings adjacent to a creek in southwestern Ohio. Objectives are to decrease the quantity of groundwater seepage into the creek and to reduce pH levels in any residual groundwater seepage from their current highly alkaline levels, thus creating conditions that are not conducive to the transport of PCBs via groundwater. Additional benefits include improving riparian habitat conditions, improving aquatic habitat conditions in the adjacent stream (e.g., by providing shade and woody debris input), and stabilizing floodplain soils along the bank.

### CREDENTIALS

#### Certifications

Certified Senior Ecologist – Ecological Society of America

#### Professional Affiliations and Activities

Society of Environmental Toxicology and Chemistry

Peer reviewer for Environmental Toxicology and Chemistry, Integrated Environmental Assessment and Management, Archives of Environmental Contamination and Toxicology, and Science of the Total Environment

### PUBLICATIONS

- Fuchsman P., K.S. Bell, K. Merritt, V. Magar. 2014. Monitored natural recovery. In: D. Reible (ed). Processes, Assessment and Remediation of Contaminated Sediment. Springer, New York, NY. pp. 227-262.
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- Lyndall J, Fuchsman P, Bock M, Barber T, Lauren D, Leigh K, Perruchon E, Capdevielle M. 2010. Probabilistic risk evaluation for triclosan in surface water, sediments, and aquatic biota tissues. Integr. Environ. Assess. Manage. 6:419-440.
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- Magar VS, Chadwick DB, Bridges TS, Fuchsman PC, Conder JM, Dekker TJ, Steevens JA, Gustavson KE, Mills MA. 2009. Technical Guide: Monitored Natural Recovery at Contaminated Sediment Sites. ESTCP-ER-0622. Environmental Security Technology Certification Program (ESTCP), Arlington, VA, USA. 276 p. http://www.epa.gov/superfund/health/conmedia/sediment/documents.htm.

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- Fuchsman, P.C. and T.R. Barber. 2005. Letter to editor: Comment on "An Ecological Risk Assessment for Hexachlorobutadiene" by Taylor et al. Human Ecol. Risk Assess. 11:1-2.
- Fuchsman, P., M. Bock, L. Yeager, and T. Barber. 2005. How not to measure total organic carbon in soil and sediment. SETAC Globe 6(3):33-34.
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- Henning M, P Fuchsman, E Perruchon, C Dunn, V Magar. 2013. Critical Review of Mercury Toxicity Reference Values: Avian and Mammalian Wildlife. 11<sup>th</sup> International Conference on Mercury as a Global Contaminant, Edinburgh, Scotland.
- Fuchsman P, J Conder, M Grover, V Magar, M Henning. 2013. Critical Review of Mercury Sediment Quality Values for Benthic Invertebrates. 11<sup>th</sup> International Conference on Mercury as a Global Contaminant, Edinburgh, Scotland.
- Brown, L., P. Fuchsman, M. Henning. 2012. Metal Effects on Benthic Invertebrates in Off-Site Ponds near the Deloro Mine Site (Ontario, Canada). Society of Environmental Toxicology and Chemistry North America 33<sup>rd</sup> Annual Meeting, Long Beach, California, November 11-15.
- Leigh, K., M. Henning, A. Fogg, P. Fuchsman, N. Dyck. 2011. To Depurate or Not to Depurate? Using Earthworm Tissue to Estimate Doses in Ecological Risk Assessment. 32<sup>nd</sup> Annual Meeting of the Society of Environmental Toxicology and Chemistry, Boston, Massachusetts.
- Barrett, C.H., K.M. Taillon, K. Kim, D.J. Milani, M.J. Chambers, M. McChristie, M.H. Henning, P.C. Fuchsman, and P.M.C. Antunes. 2011. Use of multiple lines of evidence to support sediment remediation and management decisions for the St. Marys River Area of Concern. 54<sup>th</sup> Annual Meeting of the International Association for Great Lakes Research, Duluth, Minnesota, May 30 – June 3.
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- Fuchsman, P.C., T.R. Barber, and R.L. Wiesner. 2001. Biological community quality in a groundwater discharge zone: Relationship to habitat and chemistry. 22nd Annual Meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD.



## Katrina Leigh | Manager

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Katrina Leigh is a Manager and Certified Wildlife Biologist® in the Ecology and Sediment Management practice at ENVIRON. She has over 12 years of experience, specializing in ecological risk assessment, wildlife ecotoxicology, environmental review under NEPA, sediment and water quality assessment, aquatic and terrestrial toxicology, biological community quality evaluation, toxicity testing, bioaccumulation of environmental contaminants, wildlife and habitat surveys, and ecotoxicology. In addition to her experience in environmental consulting, Katrina has accumulated more than 17 years experience in the fields of wildlife biology, zoology, ecology, and toxicology. Prior to joining ENVIRON, she was a member of a toxicology research team for six years, focusing on the metabolism of perfluorinated compounds for the US Air Force. She has authored numerous peer-reviewed manuscripts, book chapters, and guidance documents. She has presented investigation findings at national and international scientific meetings, as well as chaired scientific sessions on wildlife ecotoxicology and contaminated sediment.

#### EDUCATION

1993 MS, Physiological Ecology/Zoology, Miami University, Oxford, OH

1990 BS, Zoology, Miami University, Oxford, OH

#### **EXPERIENCE**

#### Ecological Risk Assessment

- Completed an environmental risk assessment for the off-site area near a 202-hectare former mining site in Deloro, Ontario, Canada. Chemical characterization, toxicity testing, and benthic community analysis supported a weight-of-evidence evaluation of potential risks to human health and the environment from site-related chemicals in surface water, sediment, and floodplain soil. Results will support environmental decision-making and potential remediation in the affected off-site area.
- Assisted in developing a web-based information management system to compile, evaluate, and facilitate access to publicly available data, reports, articles, and geospatial information related to baseline ecological and human use services provided within a large U.S. water body. Managed two separate teams of staff from multiple offices.
- Participated in the completion of multiple Baseline Ecological Evaluations (BEEs) for various sites in New Jersey. Conducted site-specific threatened and endangered species evaluations. Evaluated potential risk of site-related chemicals to ecological receptors, including direct and cumulative (food chain) effects.
- Completed a site-specific bioavailability assessment for silver in sediment within several Areas of Concern (AOCs) in Little Ferry, New Jersey identified under the Industrial Site Recovery Act (ISRA) and Environmental Cleanup and Recovery Act (ECRA). Potential bioavailability of silver in sediment evaluated for (1) benthic invertebrates, using acid volatile sulfide (AVS) and simultaneously extracted metals (SEM); and (2) aquaticfeeding wildlife, using a bioaccumulation food web model. Based on the results of this evaluation, successfully petitioned New Jersey Department of Environmental Protection (NJDEP) to discontinue planned sediment remediation within the AOCs.
- Conducted ecological sampling (sediment and fish tissue) and subsequent bioaccumulation modeling to inform a focused ecological risk assessment for a beaver pond associated with a former gas compressor station in Thunder Bay, Ontario, Canada. Responsible for obtaining he scientific collection permit and for operating the

backpack electrofishing unit. Evaluated potential effects of PCBs, petroleum hydrocarbons, and toluene to benthic invertebrates, via sediment toxicity testing, and to aquatic-feeding wildlife, via a bioaccumulation model.

- For a five-year review of the Lawrence Livermore National Laboratory experimental test facility, developed a bioaccumulation model to evaluate potential risks to terrestrial and aquatic wildlife inhabiting an operable unit within the facility in Livermore, California. Chemicals of interest included metals, PCBs, and several radioactive isotopes. Evaluated exposure to wildlife, including burrowing animals (i.e., burrowing owl, California ground squirrel, and kit fox), using soil bioaccumulation factors.
- Under the Connecticut corrective action program, prepared a screening level ecological risk assessment for aquatic and terrestrial resources within and adjacent to an electrical equipment manufacturing facility in Bridgeport, Connecticut. Selected conservative, ecotoxicity-based screening values from appropriate sources. Using bioaccumulation factors derived from multiple sources, estimated concentrations of bioaccumulative metals, pesticides, PAHs, and PCBs in prey items for incorporation into a food web model. Successfully limited the scope of subsequent ecological risk activities.
- Completed an ecological risk assessment for identified solid waste management units (SWMUs), areas of concern (AOCs), and adjacent portion of Otter Creek as part of a Resource Conservation and RCRA Facility Investigation (RFI) for a treatment, storage, and disposal facility (TSDF) within an urbanized watershed in Oregon, Ohio. Used equilibrium partitioning and food web modeling to refine exposure and effects assumptions for evaluating the potential risks of metals, pesticides, PCBs, and PAHs to benthic invertebrates, fish, and aquatic-feeding wildlife. Calculated appropriate sediment quality benchmarks for selected VOCs. Successfully demonstrated a lack of evidence connecting the facility to the failure of surface water and sediment standards in Otter Creek.
- Under the Indiana Voluntary Remediation Program (VRP), completed a comprehensive baseline ecological risk assessment and a screening-level ecological risk assessment for Pleasant Run Creek, adjacent to a former gas manufacturing facility in Indianapolis, Indiana. Developed the strategy and managed the collection of colocated surface water, sediment, and sediment porewater samples. Assessed bioaccumulative chemicals via a site-specific wildlife model. Successfully limited the area of impact within the creek using techniques to evaluate bioavailability (e.g., weak acid dissociable cyanide and alkylated polycyclic aromatic hydrocarbons [PAHs]).
- Directed ecological risk assessment activities in areas adjacent to a can manufacturing facility in support of a RCRA corrective action in Cincinnati, Ohio. Developed specialized, site-specific standard operating protocols (SOPs) to address the collection of surface water (Van Dorn sampler), sediment (petite ponar sampler), sediment porewater (direct push point, piezometer), and surface soil (hand auger) samples to evaluate potential groundwater-surface water interaction in a quarry pond. Evaluated the potential for site-related impacts using equilibrium partitioning-based methodology (e.g., alkylated PAHs and AVS/SEM.
- Collected co-located soil and biota tissue (i.e., soil invertebrate and small mammal) samples from the undeveloped floodplain of Stony Creek in Noblesville, Indiana, downgradient of an air spring manufacturing facility as part of corrective measures activities,. License holder for the required scientific collection permit. Evaluated risks to ecological and human receptors due to PCBs within the AOC.
- Conducted a targeted ecological risk assessment for a wetland located on northern Lake Erie in Wheatley
  Harbour, Ontario, Canada on behalf of Essex Regional Conservation Authority, as an agent of Environment
  Canada and Ontario Ministry of the Environment. Risks posed to piscivorous (fish-eating) fish, birds, and
  mammals by PCBs were evaluated using dose-based methods. Additionally, the body burden-based approach
  was used to evaluate risks to mink, the most sensitive of the receptors evaluated. Risk-based fish tissue and
  sediment thresholds protective of mink were calculated. Determined that management of sediment was not

warranted to protect piscivorous species. Findings contributed significantly to delisting Wheatley Harbour as a Great Lakes AOC in 2010.

- Completed an ecological risk assessment in Peninsula Harbour, Lake Superior, Canada, on behalf of Environment Canada, which addressed potential risks due to PCBs and mercury in sediment and aquatic biota within a Great Lakes AOC. Calculated target fish tissue concentrations to develop numerical sediment management goals. Estimated the area and volume of sediment warranting management to achieve these goals, as well as any residual risks following source control measures, to aid Environment Canada, Ontario Ministry of the Environment, and other stakeholders in understanding whether sediment management is warranted.
- Directed ecological activities associated with a pipe plant facility in Pennsylvania under Pennsylvania's Land Recycling and Environmental Remediation Standards Act (Act 2). Developed specialized, site-specific SOPs for the collection of surface water and sediment samples from various wetlands potentially impacted by past releases from the facility. Chemicals of interest included VOCs, SVOCs, PCBs, and various metals.
- Completed a comprehensive baseline ecological assessment under the Indiana VRP, for Fall Creek flowing
  adjacent to a former coke facility in Indianapolis, Indiana. Evaluated existing data relating to the current
  ecological quality of Fall Creek, as well as developed and executed a sampling strategy to examine the
  sediment quality due to ubiquitous urban contaminants, such as PAHs and metals, immediately upgradient of
  the facility. The effect of PAHs to benthic invertebrates was evaluated cumulatively, based on equilibrium
  partitioning.
- Reviewed USEPA's screening ecological risk assessment and identified refinements needed to finalize selection
  of chemicals for further evaluation as part of the site RI/FS of Cam-Or Superfund Site, Indiana. Supplemental
  sediment, surface soil, and biota tissue sampling addressed issues of site-specific bioavailability, toxicity, and
  bioaccumulation, using analyses such as PCB homologues, alkylated PAHs, AVS and SEM, and dissolved
  metals in sediment porewater. Completed a baseline ecological risk assessment addressing on-site terrestrial
  and off-site aquatic areas potentially affected by the site.
- Completed a screening level ecological risk assessment for a former electric manufacturing site in Cape Girardeau, Missouri, based on VOCs and SVOCs in surface water, sediment, and surface soil. Using siteand chemical-specific information, eliminated seven out of eight chemicals (identified following the conservative screening) from further evaluation. Evaluated the risk of PCBs to upper trophic level birds and mammals in the refined assessment.
- Developed work plans for risk assessment activities in 6-acre Johnson Lake within the former floodplain of the Columbia River, Portland, Oregon following completion of a screening assessment of available sediment and surface soil data. Tissue sampling (i.e., plants, benthic macroinvertebrates, and fish) and wildlife modeling were then implemented to address potential bioaccumulation of PCBs, pesticides, and selenium. Toxicity testing was conducted to address potential effects on benthic macroinvertebrates. Ecological effects were assessed in accordance with Oregon Department of Environmental Quality rules, which specify the calculation of population-level risk estimates.
- Revised a previous screening ecological risk assessment for an active tank-trailer terminal at the Bridgeport Superfund Site, New Jersey. Compared maximum detected concentrations of VOCs, SVOCs, PCBs, pesticides, and metals in surface soil to appropriate ecological screening values for soil, including USEPA Ecological Soil Screening Levels (Eco-SSLs) and Region 5 Ecological Screening Levels (ESLs).
- Prepared an ecological risk assessment for a creek system adjacent to a steel manufacturing facility in Middletown, Ohio. Chemicals of interest included PCBs, PAHs, and metals in surface water, sediment, surface soil, and biota (plants, macroinvertebrates, and fish). Sediment analyses were selected using risk-

based data use objectives. Evaluation focused on the potential risk to aquatic organisms and aquatic-feeding wildlife and integrated survey data measuring fish and invertebrate community quality.

- Under RCRA, prepared a screening level ecological risk assessment for a 700-acre steel manufacturing and processing facility in Mansfield, Ohio. Identified significant on-site ecological habitat to focus the evaluation on the appropriate terrestrial and aquatic portions of the site, including Rocky Fork Creek and its associated floodplain. Evaluated potential risks to aquatic and terrestrial plants, invertebrates, fish, and aquatic- and terrestrial-feeding wildlife to PCBs, metals, SVOCs, and VOCs in on-site surface water, sediment, and surface soil.
- Completed a screening level ecological risk assessment for Clear Creek in Bloomington, Indiana to assist with closure activities at the site under Indiana Department of Environmental Management's VRP. The creek is adjacent to a former wood-treating facility. Assessment focused on potential risks of aquatic organisms and aquatic-feeding wildlife to chemicals in sediment and surface water. Chemicals of interest included SVOCs, benzene, toluene, ethylbenzene, and xylene (BTEX), and metals. Based in part on the equilibrium partitioning approach, the bioavailability and bioaccumulation potential of chemicals to aquatic organisms were determined using specialized analyses (i.e., alkylated PAHs, AVS and SEM, and analysis of dissolved metals in sediment porewater).
- Developed a screening level ecological risk assessment for Little Beaver Creek in Lammers Barrel Superfund Site in Ohio, which was potentially affected by groundwater discharge. As part of the site RI/FS, applied a model using the equilibrium partitioning approach (modified for VOCs) to assess cumulative risks of BTEX and PAHs in sediment.
- Prepared screening and baseline ecological risk assessments for an RFI at the research and development facility of a major manufacturer in Granville, Ohio. Chemicals of interest for the screening evaluation included metals, SVOCs, and VOCs in wetland, pond, stream, wooded, and open upland habitat. Focused sampling to support the baseline evaluation, documenting the site-specific bioavailability of lead to benthic organisms and the bioaccumulation of PCBs to aquatic-feeding wildlife.
- Conducted screening and baseline ecological risk assessments at the Hertel Landfill Superfund Site, New York for the potential impacts of discharging groundwater on adjacent wetlands and streams. Chemicals of interest included PAHs, pesticides, metals, and cyanide. Successfully limited the extent of additional sampling by applying in-depth knowledge of the ecotoxicology literature in the screening assessment.
- Developed detailed toxicity profiles for cyanide and ammonia as part of a probabilistic risk assessment for hypothetical fire retardant spill scenarios. Developed species sensitivity distributions and identified the relative sensitivity of various categories of freshwater species. Identified relationships between exposure duration and acute toxicity, to increase the accuracy of risk predictions for pulse exposures in streams.
- Prepared an ecological risk assessment and associated field sampling plan for PCBs and lead in a small stream adjacent to a former scrap yard in Crawfordsville, Indiana. Directed the field effort, which included collection of sediment and surface soil and specialized sampling of biota tissue (plants, benthic invertebrates, terrestrial invertebrates, fish, and small mammals), to support a wildlife food web model and analyses of chemical bioavailability. Chemical fingerprinting techniques were used to distinguish sources of chemical contamination.
- Assisted with closure activities for a portion of an automobile manufacturing facility in Dayton Ohio. Conducted a site visit, following Ohio EPA ecological risk assessment guidance for ecological site assessment, to determine the potential migration pathways for chemicals of interest to ecological receptors and also the presence of ecological habitat on-site and within the locality of the site.

#### Environmental Assessments (NEPA)

- Developed an Environmental Assessment to support USACE environmental review under NEPA for a proposed offshore wind energy project. The pilot project would potentially be North America's first wind energy project in a true offshore freshwater environment and would consist of a six-turbine array, located in Lake Erie near Cleveland, Ohio. Completed activities necessary to secure the required environmental permits, such as Section 404/Section 10 Permit (USACE), Section 401 Water Quality Certification (Ohio EPA), Certificate of Environmental Compatibility and Public Need (Ohio Power Siting Board), and Coastal Zone Management Act Certification/submerged land lease (Ohio DNR). To facilitate NEPA compliance, presented initial findings of the Environmental Assessment to USACE.
- Prepared an Environmental Assessment to support a new drug application submittal to both the Center for Drug Evaluation and Research of the Food and Drug Administration and the European Union. Used probabilistic techniques to assess ecological risk to aquatic and terrestrial organisms and aquatic- and terrestrial-feeding wildlife, incorporating direct contact and dietary exposures to receptors under multiple environmental scenarios. Modeled exposures were based on fugacity and bioaccumulation characteristics and accounted for environmental variability in a wide variety of aquatic and terrestrial systems. Community-level aquatic effects were evaluated through the use of a species sensitivity distribution for multiple, diverse taxa.

#### Biological/Habitat Surveys

- Evaluated the overall suitability of habitat along the Lake Superior Peninsula Harbour AOC shoreline for piscivorous wildlife in order to realistically refine exposure estimates and facilitate remediation decisions for the AOC. Employed the U.S. Fish and Wildlife Services' habitat suitability index (HIS) model for mink (*Mustela vison*), while habitat suitability for river otter (*Lontra canadensis*) was evaluated qualitatively. Data collection focused on identification of the potential presence of mink or river otter and the suitability of the shoreline and riparian habitat to support these species. Prepared field survey methodology, compiled field data, and developed report.
- Evaluated habitat along the Stony Creek floodplain in Noblesville, Indiana for the potential presence of and/or suitability for mink. Assisted with data collection, following the U.S. Fish and Wildlife Services' HSI model for mink. Data facilitated ecological risk assessment activities for the area.
- Conducted an on-site ecological assessment to determine the location and extent of aquatic and terrestrial
  ecological habitat on, adjacent to, and near the site prior to development of an ecological risk assessment for
  an automotive and aerospace manufacturing facility in northwestern Pennsylvania. Also completed a cursory
  biological survey, identifying any potential ecological receptors.
- Performed Qualitative Habitat Evaluation Index (QHEI) assessment of several segments of the lower Buffalo River (Buffalo, New York) to provide ecological baseline information to evaluate alternative remediation measures and establish expected endpoints for improving beneficial uses of selected portions of the river.

#### Sediment and Water Quality Evaluations

• Assigned as field team leader and responsible for oversight, direction, and organization of all off-site field activities conducted near a 202-hectare former Deloro mining site in Ontario, Canada. Collected 17 colocated sediment and benthic invertebrate samples for chemical characterization, toxicity testing, and benthic community analysis (i.e., sediment triad), as well as co-located aquatic plant and surface water samples, from five ponds. Also collected co-located surface soil and terrestrial invertebrate samples for chemical analysis from the associated floodplain areas.

- Developed surface water, sediment, and biological characterisation guidance—including text chapters, SOPs, and checklists— on behalf of Canadian Council of Ministers of the Environment (CCME) and Environment Canada for use by environmental professionals working for federal, provincial, territorial, and/or private land managers and owners throughout Canada. Guidance to aid in the collection of representative, high quality surface water, sediment, and biological tissue (e.g., invertebrates, fish, small mammals) data for environmental and human health risk assessments. Sediment guidance tools included in CCME's Guidance Manual for Environmental Site Characterization in Support of Environmental and Human Health Risk Assessment.
- Collected fish from several locations in the Buffalo River, Buffalo, New York and a reference stream using a
  variety of methods (i.e., electrofishing, seining, trap lines, hoop nets) as part of a site remedial investigation.
  Calculated metrics for the fish community to evaluate key community attributes and individual fish health. Also
  used Index of Biotic Integrity (IBI) to merge data into a single index value. Additional collection efforts targeted
  brown bullhead catfish for histopathology. Tagged selected catfish using Visual Identification alpha tags and
  released to determine home range areas.
- Developed SQBs for several SVOCs in support of a water quality evaluation for a chemical company. Initially
  derived Tier II water quality benchmarks (WQBs) in freshwater, primarily based on the Great Lakes Water
  Quality Initiative approach. Subsequently calculated SQBs using the WQB, fraction of organic carbon, and
  the octanol-water partition coefficient (K<sub>ow</sub>). For chemicals with a low K<sub>ow</sub>, used a modification to the
  equilibrium partitioning equation which incorporates a site-specific estimate of percent solids.
- Evaluated sediment quality in a small stream adjacent to a former electronic manufacturing site in Hanover Township, New Jersey. Conducted a sediment triad (evaluation of co-located benthic community surveys, toxicity tests, and specialized sediment chemistry data) for both on-site and upstream reference areas to complete site delineation of the impacts of metals. Using a weight-of-evidence approach, determined that although metals were present in sediment at concentrations above the NJDEP screening values, the sediment was not toxic, and the resident benthic invertebrate community was not impaired. Therefore, demonstrated that the metals were not sufficiently bioavailable to cause ecological harm. As a result of this investigation, the NJDEP issued a No Further Action determination for this area of the site.
- Conducted a critical review of published exposure and effects information for fish exposed to PAHs in sediment. Review included as a chapter of a compendium developed by the Electric Power Research Institute and designed to aid in the management of contaminated sediments, particularly at manufactured gas plant sites.
- Investigated the effects of trematode infection, leading to limb deformities, in wood frogs. Examined the connection between water quality, eutrophication, and trematode infection in frogs.
- Evaluated surface water and groundwater chemistry following a release of 42,000 gallons of a 50 percent sodium hydroxide (NaOH) solution into a tributary and wetlands associated with Sinnemahoning Portage Creek in Gardeau, Pennsylvania. Effects of high pH on natural buffering capacity and re-equilibration, mobilization of naturally occurring metals, and toxicity, as well as neutralization by citric acid and dilution, were investigated.
- Completed a sediment, surface water, and fish tissue investigation in creeks potentially impacted by groundwater discharge from a pesticide manufacturing disposal landfill at Hardeman County Landfill Superfund Site, Tennessee as part of the USEPA's Second Five Year Review for the site. Prepared the project quality assurance project plan (QAPP) and served as quality assurance director for the project, including subsequent soil gas, groundwater, and subsurface soil investigations, both on and near the site.
- Successfully petitioned the Michigan Department of Environmental Quality (MDEQ) to increase state water quality standards for barium, manganese, and 2,4-dimethylphenol. Determined the basis for existing criteria,

identified additional, published toxicity data, and developed proposed criteria revisions for submission to he MDEQ.

- Provided emergency response and subsequent ecological and human health support for a train derailment that released approximately 28,500 gallons of sodium hydroxide (NaOH) solution into Cowan Creek in Wilmington, Ohio. Implemented and managed initial containment and remedial activities. Completed the following assessments following the initial response activities: (1) physical and ecological description of the study area; (2) pre-release ecological status of the creek; (3) post-release ecological status; (4) human health exposure evaluation; and (5) five-year human use comparison between state parks. Initial emergency response efforts were successful in mitigating the impacts to the creek from the release, and no impact was observed in a nearby state park.
- Participated, on behalf of a client, in a local stakeholders group (Duck & Otter Creek Partnership) focused on watershed planning efforts for two creeks located within the Maumee river basin in Toledo, Ohio. The Partnership included industry and local citizens, with the Ohio EPA's cooperation.
- Developed water quality values for isopropylbenzene, using USEPA-endorsed methods, as alternatives to the highly conservative values used by the New York State Department of Environmental Conservation (NYSDEC). In addition to the USEPA procedures, calculated values based on the quantitative structure activity relationship (QSAR) and equilibrium partitioning.

#### Site Investigation, Remediation, and Restoration

- Supported remedial and restoration activities within a creek system adjacent to a steel manufacturing facility in Middletown, Ohio. Activities included removal of PCB-impacted sediment, via dredging, and adjacent floodplain soil. Subsequent restoration activites of the excavated areas focused on limiting movement of contaminants from adjacent areas, minimizing channel incision, and restoring biological productivity to the maximum extent practical. Restoration of the stream's biological habitat included placement of clean substrate (sand, gravel, and cobble), minimization of down-cutting, under-cutting, and/or incision of the stream, and establishment of a floodplain/floodway and other riparian restoration measures (e.g., native re-vegetation).
- Provided sampling support for a RCRA/Corrective Measure Study (CMS) to evaluate the nature and extent of
  contamination and assess risk to human health and the environment posed by contamination within solid waste
  management units (SWMUs) associated with a steel manufacturing facility in Middletown, Ohio. Intrusive
  investigation included sampling groundwater, surface water, soil, soil gas, and landfill gas.
- Completed quarterly groundwater monitoring for two years at a former peroxide facility in Elyria, Ohio. Supported a site closure request with the Ohio EPA.
- Prepared investigation work plans for the vertical (surface and subsurface) and horizontal delineation of floodplain soil and sediment in a creek system adjacent to a steel manufacturing facility in Middletown, Ohio. Served as field manager during the investigations. PCBs were the chemicals of interest. Analytical results were used to support remedial decision-making for the creek.
- Completed a baseline characterization of the Piles Creek watershed and the nearby reach of the Arthur Kill in New Jersey. Investigated, among others, historical land use; urban/industrial development; location of wastewater treatment plants, combined sewer overflows, wetlands, National Pollutant Discharge Elimination System (NPDES) permits, and state-and federal-listed sites; boat traffic; dredging activity; nutrient load; invasive species; regional sediment chemistry data; and biological communities.

#### **REGISTRATIONS AND CERTIFICATIONS**

Certified Wildlife Biologist (CWB)®, The Wildlife Society

Hazardous Waste Operations and Emergency Response, 40-hour OSHA HAZWOPER

Adult CPR and First Aid, American Red Cross, Cleveland, Ohio

Level 2 Qualified Data Collector for Stream Habitat Assessment (QHEI), Division of Surface Water Volunteer Monitoring Program, Ohio Environmental Protection Agency, 2004

Cartography, Photogrammetry, Remote Sensing, and GIS Certification, Wright State University, Dayton, Ohio, 1998

#### PROFESSIONAL AFFILIATIONS

Member, Society of Environmental Toxicology and Chemistry (SETAC), since 2004

Board Member, Ohio Valley Regional Chapter, Society of Environmental Toxicology and Chemistry (SETAC), since 2006

Member, The Wildlife Society, since 2007

Executive Board Member (Vice Chair), Wildlife Toxicology Working Group, The Wildlife Society, 2013-2014

Member, Wildlife Toxicology Working Group, The Wildlife Society, since 2009

#### **PROFESSIONAL ACTIVITIES**

- Session Co-Moderator, Wildlife Ecotoxicology Supporting Management Decision Making, 33<sup>rd</sup> North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Long Beach, California, 2012
- Symposium Attendee, Ohio's Wildlife in a Changing Climate: Sustaining Habitats and Diversity, workshop cosponsored by Ohio Department of Natural Resources, Ohio Division of Wildlife, Ohio Chapter of The Wildlife Society, The Wilds, The Nature Conservancy, The Ohio State University, Old Woman Creek National Estuarine Research Reserve, Ohio Coastal Training Program, National Oceanic and Atmospheric Administration; Columbus, Ohio, 2011
- Workshop Developer and Co-Presenter, Ecotoxicology for Biologists and Land Managers, 18<sup>th</sup> Annual Conference of The Wildlife Society, Waikoloa, Hawaii, 2011
- President, Ohio Valley Regional Chapter of the Society of Environmental Toxicology and Chemistry (SETAC), 2010
- Session Moderator, Assessing and Managing Contaminated Sediment, 37th Aquatic Toxicity Workshop, Toronto, Ontario, Canada, 2010
- Short Course Participant, Non-destructive Collection of Biological Samples from Wild Species for Contaminant Analysis, 30<sup>th</sup> Nor h American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), New Orleans, Louisiana, 2009
- Short Course Participant, Approaches to Utilizing Amphibians and Reptiles in Environmental Assessments, 28th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Milwaukee, Wisconsin, 2007
- Short Course Participant, Exposure Modeling Food Chain Modeling using Probabilistic Assessment Techniques, 26th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Baltimore, Maryland, 2005

- Workshop Participant, Biological Assessments and Criteria and Habitat Evaluation Training, instructors: Chris Yoder, Ed Rankin, and Jeff DeShon, Midwest Biodiversity Institute & Center for Applied Bioassessment & Biocriteria, Columbus, Ohio, 2004
- Symposium Attendee, Multiple Stressor Effects in Relation to Declining Amphibian Populations, sponsored by the American Society for Testing and Materials (ASTM) Committee E47 on Biological Effects and Environmental Fate, Pittsburgh, Pennsylvania, 2002

#### PUBLICATIONS & PRESENTATIONS

- Leigh, K.B. and M. Striker. 2014. Whence comes the FONSI? Overview of NEPA and major Federal actions. Invited presentation at 29<sup>th</sup> Ohio Environmental Energy and Resources Law Seminar, Ohio State Bar Association, Newark, Ohio.
- Mahaney, W.M., J.L. Lyndall, K.B. Leigh, and T.R. Barber. 2013. Common ground in a heated debate: A role for restoration in facilitating plant species' responses to climate change. 5<sup>th</sup> World Conference on Ecological Restoration, Society for Ecological Restoration, Madison, Wisconsin.
- Wenning, R.J., L. Martello, and K. Leigh. 2012. Important considerations governing the behavior of chemicals in sediments and water in tropical and temperate environments. 33rd North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Long Beach, California.
- Wenning, R.J., L. Martello, J. Conder, K. Leigh, and S.S. Brown. 2011. Important considerations governing the behaviour of chemicals in sediments and water in tropical and temperate environments. 1st International Conference on Deriving Environmental Quality Standards for the Protection of Aquatic Ecosystems (EQSPAE), Hong Kong, China.
- Leigh, K.B., M.H. Henning, A.L. Fogg, P.C. Fuchsman, N.E. Dyck, and J. Conder. 2011. To depurate or not to depurate? Using earthworm tissue to estimate doses to small mammals in ecological risk assessment. 32nd North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Boston, Massachusetts.
- Wenning, R.J., J. Shaw, L. Martello, J. Conder, and K. Leigh. 2011. Behavior of chemicals in sediment in tropical vs. temperate environments. Regional Meeting of the Mexico Chapter of the Society of Environmental Toxicology and Chemistry (SETAC), Meridia, Mexico.
- Leigh, K., M. Henning, and P. Fuchsman. 2010. The role of ecological risk assessment in delisting of the Wheatley Harbour Area of Concern. 37th Annual Aquatic Toxicity Workshop, Toronto, Ontario, Canada.
- Fuchsman, P., J. Lyndall, M. Bock, D. Lauren, T. Barber, K. Leigh, E. Perruchon, and M. Capdevielle. 2010. Terrestrial risk evaluation for triclosan in land-applied biosolids. Integrated Environmental Assessment and Management 6(3): 405-418.
- Lyndall, J., P. Fuchsman, M. Bock, T. Barber, D. Lauren, K. Leigh, E. Perruchon, and M. Capdevielle. 2010. Probabilistic evaluation for triclosan in surface water, sediments, and aquatic biota tissues. Integrated Environmental Assessment and Management 6(3): 419-440.
- Leigh, K., K. Wells, A. Fogg, M. Henning, S. Hall, and C. Allaway. 2009. Development of contaminated site characterization guidance for sampling in support of human health and environmental risk assessments within Canada. 30th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), New Orleans, Louisiana.
- Leigh, K., S. Hall, T. Bradley, L. Minella, and T. Kuykindall. 2009. Toxicity of TiO<sub>2</sub> to freshwater fish, invertebrates, and algae – effects of organic carbon on TiO<sub>2</sub> toxicity. 26th Regional Meeting of the Ohio Valley Chapter of the Society of Environmental Toxicology and Chemistry (SETAC), Cincinnati, Ohio.

- Fuchsman, P., K. Leigh, M. Henning, and P. Welsh. 2009. Risks to mink from PCBs in Muddy Creek, Wheatley Harbour Area of Concern, Ontario. 5th International Conference on Remediation of Contaminated Sediments by Battelle, Jacksonville, Florida.
- Leigh, K., K. McKay, M. Henning, and K. Merritt. 2008. Evaluation of mink and river otter habitat suitability wi hin the Peninsula Harbour shoreline in Lake Superior, Canada. 15th Annual Conference of The Wildlife Society, Miami, Florida.
- Leigh, K., P. Fuchsman, M. Henning, and P. Welsh. 2008. Innovative evaluation of risks to mink from PCBs in Muddy Creek, Wheatley Harbour Area of Concern, Lake Erie, Ontario. 51st Annual Conference on Great Lakes Research by the International Association for Great Lakes Research (IAGLR), Peterborough, Ontario, Canada.
- Henning, M., K. Leigh, K. Merritt, V.M. Magar, and R. Santiago. 2008. Assessment and mitigation of ecological risks posed by mercury and PCBs in Peninsula Harbour sediment, Lake Superior. 2008 Federal Contaminated Sites National Workshop, Vancouver, British Columbia, Canada.
- Henning, M., K. Leigh, P. Fuchsman, T. Barber, and R. Santiago. 2007. An integrated approach to the management of contaminated harbour sediment: Peninsula Harbour ecological risk assessment. 28th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Milwaukee, Wisconsin.
- Barber, T.R., P.C. Fuchsman, M.J. Ferguson, and K.B. Leigh. 2006. Inter-laboratory comparisons of PCB analyses in sediment: Implications for site characterization and risk assessment. 27th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Montreal, Canada.
- Fuchsman, P.C., T.R. Barber, J.C. Lawton, and K.B. Leigh. 2006. An evaluation of cause-effect relationships between polychlorinated biphenyl concentrations and sediment toxicity to benthic invertebrates. Environmental Toxicology and Chemistry 25(10): 2601-2612.
- Lawton, J.C., T.R. Barber, K.B. Leigh, M.J. Bock, H. Verhaar, and M.C. Capdevielle. 2006. Aquatic risk assessment for triclosan. 27th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Montreal, Canada.
- Leigh, K.B., M.H. Henning, M.J. Bock, and M.C. Capdevielle. 2006. Probabilistic exposure distributions developed for the uptake of triclosan by representative avian species in freshwater aquatic environments. 27th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Montreal, Canada.
- Leigh, K., T. Barber, P. Fuchsman, A. Zahradnik, and E. Demarest. 2005. Achieving closure of a sediment area at a New Jersey site: A sediment triad study. 26th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Baltimore, Maryland.
- MacGregor, A., D. Heidlauf, W. Bagley, S. Hayter, and K. Leigh. 2005. Managing environmental data electronically. 42nd Meeting of the American Institute of Professional Geologists, Lexington, Kentucky.
- Leigh, K.B., P.C. Fuchsman, and T.R. Barber. 2004. Comparison of site-specific biological community quality with ecological risk predictions for fish exposed to PCBs. 25th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Portland, Oregon.
- Barber, T.R., K.B. Leigh, D. Glaser, and M. Rury. 2003. The importance of ecosystem-specific factors in predicting the aquatic toxicity of iron-cyanide complexes. Electric Power Research Institute 2003 MGP Forum: Innovative Solutions for MGP Site Closure, Denver, Colorado. Invited Presentation.
- Fuchsman, P.C., T.R. Barber, and K.B. Leigh. 2003. Does PCB congener analysis improve the accuracy of ecological risk assessments? 24th North American Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Austin, Texas.

- Fuchsman, P.C., T.R. Barber, R.L. Wiesner, and K.B. Leigh. 2002. Lines of evidence in a site-specific risk assessment for benthic invertebrates exposed to PCBs. 5th International Symposium on Sediment Quality, Aquatic Ecosystem Health and Management Society, Chicago, Illinois.
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## Michael J. Ferguson | Senior Associate

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Michael Ferguson has more than nine years of experience in environmental consulting. Since joining ENVIRON, Michael has gained experience in biota, soil, sediment, and water quality investigations; standard operating protocol (SOP) writing; environmental monitoring; ecological risk assessments; transport modeling; data analysis; quality assurance/quality control; and the development and execution of field investigations.

#### **EDUCATION**

2005 Graduate Studies, Biology, John Carroll University, University Heights, Ohio

2002 BS, Biology, John Carroll University, University Heights, Ohio

#### EXPERIENCE

- Conducted biota, soil, sediment, and surface water sampling for chemical characterization at a 700-acre steel
  manufacturing and processing facility in Mansfield, Ohio. Data will support an evaluation of potential risks to
  aquatic and terrestrial plants, invertebrates, fish, and aquatic- and terrestrial-feeding wildlife to polychlorinated
  biphenyls (PCBs), metals, semi-volatile organic compounds (SVOCs), and volatile organic compounds (VOCs) in
  on-site surface water, sediment, and surface soil of Rocky Fork Creek and its associated floodplain.
- Participated in the field activities to delineate the horizontal and vertical extent of polychlorinated biphenyls (PCBs) in sediments, floodplain soils, and upland source areas associated with a 5,650-foot creek system adjacent to a steel manufacturing facility in southwestern Ohio. Field activities involved the collection of hundreds of sediment and soil samples. Activities were conducted under terms in a Consent Decree with USEPA, Ohio EPA, Sierra Club, and Natural Resources Defense Council (NRDC). Assisted in the preparation of work plans, data summary reports, and remediation design documents. Provided oversight during implementation of remediation and restorations activities which included dewatering and mechanical excavation of the creek system.
- Participated in field activities to evaluate the nature and extent of contamination originating from two solid waste management units (SWMUs) at a steel manufacturing facility in southwestern Ohio.by collecting and analyzing groundwater, surface water, soil, surface soil, and soil and landfill gas samples. Field activities were conducted under a work plan for RCRA facility investigation (RFI) and Corrective Measure Study (CMS). The analytical results will be used to assess current and potential future risks to human health and the environment both on and off the facility. After completion of the RFI, potential corrective measures technologies will be evaluated using data gathered during the investigation and the results of groundwater modeling to address detected contamination, as appropriate.
- Served as an integral team member in several ecological risk assessments. He has assisted in development and implementation of work plans, sampling programs, riparian zone evaluations, sedimentation assessments, and remediation design.
- Conducted sediment and upland soil investigations for polychlorinated biphenyls (PCBs) at an 18-acre lake in Portland, Oregon. The investigations provided details necessary for of the design of a final remedy, as selected by Oregon Department of Environmental Quality (DEQ) in a Record of Decision. Assisted with supporting tasks throughout project including preparation or revisions to various workplans, data reports, feasibility plan, Health and Safety Plan (HASP), Stormwater Pollution Prevention Plan (SVP3), and Site Security Plan (SSP).

## Michael J. Ferguson

- Conducted the sediment delineation of a central Ohio creek and its tributary. The final report supported of the preparation of the remediation design to excavate major sediment deposits previously determined by others to contain chlordane.
- Assisted with quarterly groundwater monitoring at a former peroxide facility to support a site closure request with the Ohio Environmental Protection Agency.
- Provided litigation support for a large oil spill Natural Resource Damage Assessment (NRDA) in the southeastern United States.
- Participated in the evaluation of ecological risk associated with triclosan, an anti-microbial used in various personal care products. Assisted in the development of a model to determine its fate during the wastewater treatment process and ecological risks associated with its occurrence in treated effluent and land-applied biosolids. The assessment used probabilistic techniques to assess risk to soil-dwelling and aquatic organisms and terrestrial and aquatic-feeding wildlife. A chronic species sensitivity distribution was constructed as part of the aquatic effects assessment.
- Assisted with a probabilistic evaluation of the environmental impacts of a consumer chemical. Provide support for
  the design and implementation of a coupled fugacity-food web model to predict risks to ecological receptors due
  to the release of a consumer chemical. The model followed the fate and transport of a consumer chemical from
  release to wastewater treatment plants then to wildlife receptors. The model predicted the range of exposures and
  effects expect in watersheds throughout the continental United States.

#### CREDENTIALS

#### **Registrations and Certifications**

Hazardous Waste Operations and Emergency Response, OSHA Regulation 29 CFR 1910.120

10 Hour Construction Industry Outreach Training, OSHA Regulation 29 CFR 1926

Adult CPR and First Aid, American Red Cross

#### PUBLICATIONS

- Sgro, G.V., E.D. Reavie, J.C. Kingston, A.R. Kireta, M.J. Ferguson, N.P. Danz, and J.R. Johansen. A diatom quality index from a diatom-based total phosphorus inference model. Environmental Bioindicators, 2: 15-34, 2007.
- Reavie, E.D., R.P. Axler, G.V. Sgro, N.P. Danz, J.C. Kingston, A.R. Kireta, T.N. Brown, T.P. Hollenhorst, M.J Ferguson, Diatom-based weighted-averaging transfer functions for Great Lakes coastal water quality: relationships to watershed characteristics. Journal of Great Lakes Research 32:321-347, 2006.

#### PRESENTATIONS

- Fuschsman, P., E. Perruchon, M. Ferguson, and M. Nielsen. Sediment remediation goals based on cause-effectoriented literature review: Case study. Battelle: Seventh International Conference on Remediation of Contaminated Sediments, Dallas, Texas, 2013.
- Barber, T., R. Webb, D. Pickering, and M. Ferguson. Measures to assess the short- and long-term effectiveness of sediment remedial and restoration projects: An evaluation of two case studies. Sediment Management Work Group Sponsor Forum, Newark, New Jersey, 2012.
- Fuschsman, P., E. Perruchon, M. Ferguson, and M. Nielsen. Development of watershed-specific risk-based DDT sediment benchmarks for the protection of fish. 33rd Annual Meeting of the Society of Environmental Toxicology and Chemistry, Long Beach, California, 2012.

### Michael J. Ferguson

- Barber, T.R., M.J. Ferguson, T.S. Leigh, and B. Patterson. Sediment, floodplain soil, and groundwater remediation and restoration: Review of Year 1 activities in an urban stream system in southwest Ohio. Sediment Management Work Group Sponsor Forum, Philadelphia, Pennsylvania, 2011.
- Bock, M. J, E. H Martin, T. R. Barber, and M. J. Ferguson. The estimation of baseline metals concentrations in sediments from New York/New Jersey Harbor. Battelle: Fifth International Conference on Remediation of Contaminated Sediments, Jacksonville, Florida, 2009.
- Barber, T.R. and M.J. Ferguson. Evaluation of Immunoassay and GC/MS Data for Delineation and Confirmation Sampling Purposes: Total PCBs in Floodplain Soil. 29th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Tampa, Florida, 2008.
- Martin. E., T. Barber, M. Ferguson, E. Perruchon. Anisotropic interpolation in flow-oriented coordinate systems to estimate stream bathymetry and sediment thickness. New England Arc Users Group 2008.
- Barber, T.R., M.J. Ferguson, and E.H. Martin. Integration of ArcView and EVS for 3D modeling of sediment depth data for a creek in southwest Ohio. Sediment Management Work Group Sponsor Forum, Ashtabula, Ohio, 2007.
- Barber, T.R., P.C. Fuchsman, K.B. Leigh, M.J. Ferguson, and M.J. Bock. Inter-laboratory comparisons of PCB analyses in sediment: Implications for site characterization and risk assessment. 27th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Montréal, Québec, 2006.
- Bock, M.J., T.R. Barber, and M. Ferguson. FUGAWEB, A probabilistic fugacity and food web model for assessing ecological risks associated with consumer products. Meeting of the North Atlantic Chapter of the Society of Environmental Toxicology and Chemistry, Portland, Maine, 2006.
- Bock, M.J., T.R. Barber, M. Ferguson, and M. Capdevielle. Assessing the fate of chemicals in consumer products using fugacity modeling within a probabilistic framework. 27th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Montréal, Québec, 2006.
- Martin, E., T. Barber, M. Ferguson, and A. DeDolph. Integration of ArcView and EVS for three-dimensional modeling of sediment depth data for a creek in southwestern Ohio. Meeting of the North Atlantic Chapter of the Society of Environmental Toxicology and Chemistry, Portland, Maine, 2006.
- Ferguson, M.J., K.C. Scotese, E.A. Morales, and J.R. Johansen. Species diversity in *Fragilaria* sensu lato from selected sites in the Great Lakes. Phycological Society of America, Williamsburg, Virginia, 2004.
- Ferguson, M.J., K.S. Yanko, G.V. Sgro, S.W. Haladay and J.R. Johansen. Near shore sediment diatoms of the Great Lakes and their use as biological indicators. Phycological Society of America, Salishan Beach, Oregon, 2003.

Appendix E 385 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page