ATTACHMENT 1

Facts in Support of Changing Water Quality Standards for Boron, Fluoride, and Manganese

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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 21st Edition of Standard Methods 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 μg/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.
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> The most common form of boron in natural water is H_3BO_3. 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 was significantly lower than that of rainbow trout exposed to 0.11-10.1 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 Water Quality Criteria 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.
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> 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 21st Edition of Standard Methods for the Examination of Water and Wastewater (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.
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> 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 21st Edition of Standard Methods for the Examination of Water and Wastewater (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 µg/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_2) and psilomelane. Manganese is used in steel alloys, batteries, and food additives.
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> 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 µg/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):

> Boron. 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):

> 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.

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):

> Manganese. 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 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 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 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 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. 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 Ageney 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 ≥0.15 mg/L in surface water samples and must have a corresponding finished water sample taken within ±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 ±30 days of surface water samples containing ≥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 µg/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 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. 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 $CaCO_3$) 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 was far less than the typical relationship seen between water hardness and metal 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*(lnX-lnZ))) 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 manganese standards are summarized in Exhibits Q and R, respectively. To aid 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 north-south 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:

Mixing zones: 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.

Cyanide standards: 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.

Toluene standards: 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 µg/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.

Mercury: 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 Ill. 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 µg/L, which is derived by taking the geometric mean of the measured concentrations that resulted in no observable adverse effect (42.3 µg/L) and the lowest observable adverse effect (108 µg/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 *Hyalella 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 µg/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

Second Edition

by

McKEE *and* WOLF

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 $\left(\frac{\text{Na} \times 100}{\text{Na} + \text{Ca} + \text{Mg} + \text{K}}\right)$ 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:

$$\text{SAR} = \frac{\text{Na}}{[\frac{1}{2}(\text{Ca} + \text{Mg})]^{1/2}}$$

where Na, Ca, and Mg are concentrations of the respective ions in milliequivalents per liter of water. If sodium percentage is defined as

$$\text{Na \%} = \frac{100\text{Na}}{\text{Na} + \text{Ca} + \text{Mg}}$$

then SAR can be expressed in terms of the milliequivalents per liter of sodium and the sodium percentage as follows:

$$\text{SAR} = \text{Na}^{1/2}\left[\frac{2\text{Na \%}}{100 - \text{Na \%}}\right]^{1/2}$$

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 cm 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 C1-S1 area of the diagram can be used on almost all soils and for almost all crops without detrimental effects. With increasing salinity, less exchangeable sodium can be tolerated and more leaching will be required to prevent salinity damage. Waters with an SAR value greater than 10 will present an appreciable sodium hazard in fine-textured soil having high cation exchange capacity, especially as the salinity increases. Water in the S2 range may be used on coarse-textured or organic soils with good permeability (1642, 2387). For further analysis of this diagram, the reader should consult U.S.D.A. Handbook No. 60.

Doneen (2385, 2388) uses the term "sodium index" or "permeability index" to combine the effects of the sodium and bicarbonate ions and the total concentration of cations (c) in the irrigation water, all measured in 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 contains a high concentration of bicarbonate ions, for as the soil solution becomes more concentrated there is a tendency for calcium and magnesium to precipitate as carbonates 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:

$$\text{RSC} = (\text{CO}_3^{--} + \text{HCO}_3^{-}) - (\text{Ca}^{++} + \text{Mg}^{++})$$

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 residual 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 borax, 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, boron probably occurs as almost completely undissociated boric 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).

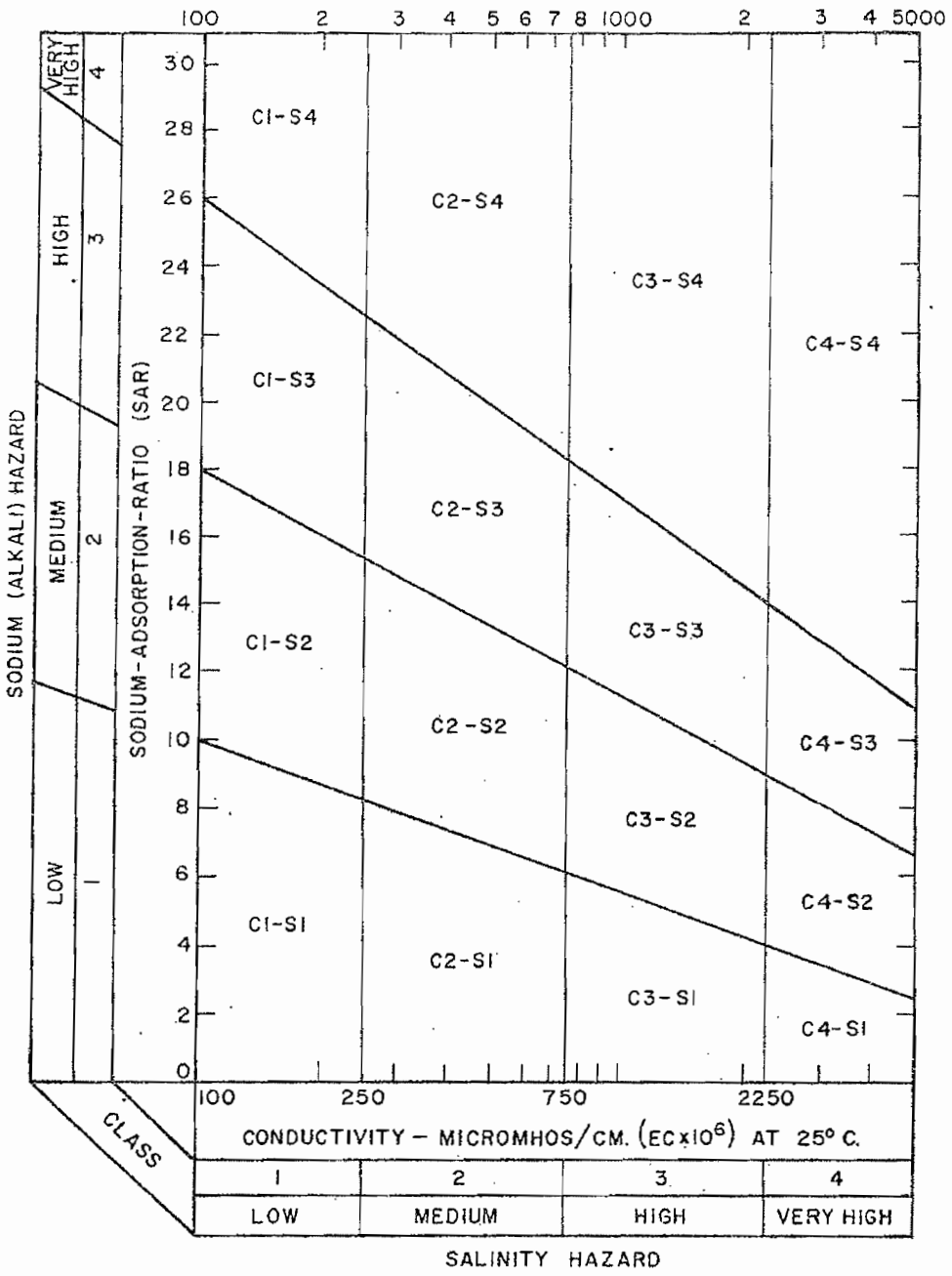

FIGURE 5-1. DIAGRAM FOR THE CLASSIFICATION OF IRRIGATION WATERS (from USDA handbook No. 60)

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, alfalfa, 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 leaf-yellowing 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 more-resistant crops in the area affected. A widely used classification of water according to its boron concentration is shown in Table 5-10.

TABLE 5-10

PERMISSIBLE LIMITS FOR CONCENTRATION OF BORON IN SEVERAL CLASSES OF WATER FOR IRRIGATION

(After Scofield) (263)

	Concentration of Boron in mg/l For Crops That Are		
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

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)

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 salinities 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 disturbances of varying degrees of severity, such as gastrointestinal 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 waters 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:

Animal	*Threshold Salinity Concentrations in grains per Imperial gallon*	*mg/l*
Poultry	200	2860
Pigs	300	4290
Horses	450	6435
Cattle, dairy	500	7150
Cattle, beef	700	10,000
Adult dry sheep	900	12,900

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 slow, 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 reaeration 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

(see Chapter 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).

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_{50} 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:

			Concentration in mg/l		
	Temperature		24-hour	48-hour	96-hour
Chemical	Range	pH Range	TL_m	TL_m	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	3,600

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 toxic 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 Br_2

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 effluents. 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. Hiatt (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 $CH_2=CHCH=CH_2$

A colorless gas, 1, 3-butadiene is insoluble in water. It is used as a polymer component in the synthesis of rubber. According to Garrett (2959), the 24-hour TL_m for the marine pinperch (*Lagodon rhomebodies*) is 71.5 mg/l. No deaths occurred at 50 mg/l.

BUTANONE

(see Methyl Ethyl Ketone)

BUTYL ACETATE $CH_3COOC_4H_9$

Normal butyl acetate is a liquid highly soluble in water. It is used in the manufacture of plastics, lacquer, artificial leather, and photographic films (364). The oral LD_{50} for rats has been reported as 4.13 grams/kg of 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_4H_9OH

Normal butyl alcohol, a colorless liquid, is used extensively in industry, being prepared from cornstarch or 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)

WATER QUALITY CRITERIA

Second Edition

by

McKEE *and* WOLF

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 $FeCl_2$.

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 $FeSO_4$ and $FeSO_4 \cdot 7H_2O$

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 Life. The threshold concentration of ferrous sulfate for immobilization of *Daphnia magna* in Lake Erie water was found to be less than 152 mg/l (358).

The following concentrations of ferrous sulfate have been harmful or lethal to fish in the time specified:

Concentration in mg/l	*Type of 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 hours	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	313
6,950	—	104 minutes	minnows	991
10,000	—	1 week	tench	1459
10,000	—	1 day	other fish	1459
13,900	—	68 minutes	minnows	991

The following concentrations of ferrous sulfate have been reported as not harmful to fish within the time specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Species of Fish*	*Reference*
5	—	24 hours	carp, shiners, suckers	359
17.1	—	1 hour	minnows	362
50	—	7 days	bass, bluegills	1459
50	—	24 hours	trout	359
			bass, sunfish	1035, 1030, 359
100	hard	96 hours	goldfish	313
		7 days	goldfish	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*
1.23	distilled	24 hours	fish	1459
368	—	2-10 hours	goldfish	1466

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 FeS

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 $FeSO_3 \cdot 2\frac{1}{2}H_2O$

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

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 lower	555
0.7	Mild dental fluorosis in 8.5 percent of children	3451
0.8	No effects at this concentration, or lower	36, 1165
0.8 to 0.9	Mild mottling of teeth	1166
0.8 to 1.5	Threshold for mottling of teeth	219
0.9	Mild mottling of teeth	1165, 1167
0.9	Mottling occurred as a result of high water use	353
0.9	Critical concentration for mottling	555, 1168
1.0	Threshold for mottling of teeth	741
1.0	10 percent of children had mottled teeth	1169, 1164
1.0	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 sclerosis found	353
1.5	Limiting concentration for drinking water	1171
1.7 to 1.8	50 percent of children had mottled teeth	1169, 1164
2.0	Gave mottling and weakening of tooth structure	1172
2.0 to 3.0	Retained in system	555
2.0 to 3.0	Moderate to severe mottling	1165
2.5	75 to 80 percent children had mottled teeth	555, 1169
2.5	No evidence of skeletal fluorosis	3452
3 to 4	Not likely to cause endemic cumulative toxic fluorosis in adults	1173, 1174
3 to 6	Gave severe mottling	1165
3.5 to 6.2	No adverse effect on carpol bones of children	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 skeletal system	1175, 1176
5.0	This concentration had no effect on height, weight or bone	1177
6.0	Threshold for appreciable effect on bones	1168
6.0	100 percent of children had mottled teeth	1164
6.0	Gave pitting and chipping of tooth enamel	1165
8.0	No deleterious bone changes except dental mottling	3456
10	Some cases of skeletal fluorosis	3455
11.8	Gave chronic fluorine intoxication to adults	1178
12	Affects deciduous teeth	1179
13.7	100 percent of children had mottled teeth	353
115	Sub-lethal in drinking water	152
180	Toxic to man in drinking water	555
2000	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	1.0
Carbonated beverages	0.2 to 1.0
Food canning and freezing	1.0
Food equipment washing	1.0
Food processing, general	1.0

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 of Fluoride, mg/l	*Effect*	*Reference*
10	No injury to peach, tomato, and buckwheat plants	3458
100	Peach and buckwheat plants severely injured in 3 days	3458
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 time	3458
360	Injurious to peach and buckwheat even at pH 6.5	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 Concentration in mg/l	*Dose*	*Animal*	*Remarks*	*Reference*
1.0	—	cattle	harmless	292
1.0	—	sheep	fluoride poisoning	1183
1.4–4.5	—	mice	mottling of teeth	3460
—	0.4 mg per kg	cattle	no mottling	353
—	1 mg per kg	rats	mottled teeth	353
—	1 mg per kg	cattle	mottled teeth	1190, 3462
—	3 mg per kg	cattle	bone damage and death	353
—	5 mg	dogs	gave hypotension	3461
4.0	—	sheep	mottled and pitted teeth	1184
5.0	—	cows	disliked water	3457
5.0	—	sheep	slight dental mottling	1571
6 to 16	—	hogs, etc.	severe mottling	1005
11.78	—	cows	mottled teeth	1178
15	—	mice	affected thyroid and kidney	3460
18	—	cows	slowly increasing fluorosis	1190
20	—	sheep	5 percent reduction in weight	1571
25–100	—	young cattle	teeth lesions	3464
44–61	—	sheep	chronic fluoride poisoning	1184
50	—	hamsters	dental fluorosis in 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	3452
—	120 mg per day	sheep	threshold for general 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/l	Salt used	Type of fish	Effect	Reference
1.5	--	eggs	slower and poorer hatching	247
2.3–7.3	NaF	trout	TL_m at 18°C. in soft water	3465, 3466
2.5–6.0	NaF	trout	TL_m at 13°C. in soft water	3465, 3466
2.7–4.7	NaF	trout	TL_m	3467
5.9–7.5	NaF	trout	TL_m at 7.5°C. in soft water	3465, 3466
7.7	--	minnows	not harmed in one hour	353
64	KF	--	10-day TL_m	2407
75–91	NaF	carp	TL_m	3467
100	--	goldfish	survived over 4 days	353
120	--	goldfish	killed in 4 days	3468
358	NaF	rainbow trout	toxic in soft water	1756
419	NaF	mosquito-fish	96-hour TL_m in turbid water	2940
678	NaF	*Tinca vulgaris*	lethal dose	3271
1000	--	goldfish	killed in 12 to 29 hours in soft water	353
1000	--	goldfish	killed in 60 to 102 hours in hard water	353

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
b. Industrial water supply	1.0 mg/l
c. Irrigation water	10.0 mg/l
d. Stock watering	1.0 mg/l
e. Aquatic life	1.5 mg/l

FORMALDEHYDE HCHO

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 resins. At ordinary temperatures it is a colorless, flammable gas with a pungent suffocating odor, and it is intensely irritating to mucous 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/l 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_m 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 QUALITY CRITERIA

Second Edition

by

McKEE *and* WOLF

THE RESOURCES AGENCY OF CALIFORNIA
STATE WATER QUALITY CONTROL BOARD
SACRAMENTO, CALIFORNIA

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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	--	goldfish	313

MAGNESIUM OXIDE MgO

(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 $MgSiF_6 \cdot 6H_2O$

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:

Process	Concentration, mg/l Optimum	Maximum	Reference
Brewing, pale ales, I	60-90	---	170
pale ales, II	60-120	---	170
mild ales	60	---	170
stout	60	---	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 in mg/l	Type of Water	Time of Exposure	Type of Fish	Reference
15,500	turbid	96-hour TL_m	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 maleic 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 maleic anhydride in turbid water at 20-23°C. They found the 24- and 48-hour TL_m values to be 240 mg/l and the 96-hour TL_m 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_m to be 150 mg/l and the 48-hour TL_m to be 138 mg/l. They estimated a safe concentration to be 35 mg/l.

MANGANESE Mn

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

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		
Industrial Use	*Manganese in mg/l*	*Iron + Manganese in mg/l*	*Reference*
Air conditioning	0.5	0.5	162
	0.5	--	152
Baking	0.2	0.2	162, 152
Brewing, light and dark	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	162, 152, 234
Milk industry	0.03-0.1	--	2344
Paper and pulp	0	0	36
Groundwood	0.5	1.0	162, 152
	0.1	--	244
Kraft pulp	0.1	0.2	162, 152
Soda and sulfate	0.05	0.1	162, 152
	0.05	--	245
Highgrade paper	0.05	0.1	162, 152
Fine paper	0.05	0.1	350
Kraft paper			
bleached	0.1	--	351
unbleached	0.5	--	351
Photography	0	0	36
Plastics (clear)	0.02	0.02	162, 152
Rayon and viscose			
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
	--	0.1	852
	0.1	--	256
dyeing	0.25	0.25	162, 152
wool scouring	1.0	1.0	162, 152
bandages	0.2	0.2	162, 152

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	*Time of Exposure*	*Type of Fish*	*Reference*
1	--	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 manganese disodium versenate
**from manganese sulfate

Manganese and iron in concentrations above 0.1 mg/l stimulate the growth of certain organisms, such as *Crenothrix, 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 supply ---------- 0.05 mg/l
b. Industrial water supply ---------- 0.05 mg/l
c. Irrigation ---------- 0.50 mg/l
d. Stock watering ---------- 10.0 mg/l
e. Fish and aquatic life ---------- 1.0 mg/l

MANGANESE CHLORIDE $MnCl_2$ and $MnCl_3$

(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 $MnCl_2$. For the small fresh-water fish (*Orizias*), the 24-hour lethal concentration was about 7850 mg/l (1459) and for other fish 5500 mg/l (3545, 3546). The highest concen-

tration 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 $MnCl_2$. In River Havel water at 23°C, the threshold effect of $MnCl_3$ occurred at 50 mg/l of manganese (2158). For the flatworm, *Polycelis nigra*, the threshold concentration of $MnCl_2$ was reported to be 700 mg/l (608).

MANGANESE DIFLUORIDE MnF_2

(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_6H_5SH (thiophenol), a liquid with a repulsive, penetrating, garlic-odor, and tolyl mercaptan $CH_3C_6H_4SH$ (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 $HgCl_2$

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_{50} 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 Mercury, in mg/l	*Time of Exposure*	*Species of Fish*	*Reference*
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

Attachment 1 – Exhibit D

Site-specific relief granted by the IPCB for boron and fluoride to date

Exhibit D: Site-specific relief granted by the IPCB for boron and fluoride to date.

Stream or Lake Name	Discharger	Parameter	Relief (mg/L)
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

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

Exhibit E: Manganese removal estimations at conventional utilities located on impaired Public and Food Processing Water Supply waters with Mn exceeding 150 µg/L.

Facility	Finished Collection Date	Finished Total Mn (µg/L)[1]	Finished Detection Level (µg/L)	Surface Water Intake Site	Intake Total Mn (µg/L)	Intake Collection Date	% Removal	Difference of Sample Dates (±)
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	OI-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	O-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	ROO-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	OI-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	0	15	O-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	ROO-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	O-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	OI-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	OI-08	260	4/8/1993	0.94	16
NASHVILLE	4/14/1999	32	15	ROO-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	0	10	J-36	450	6/27/2001	0.98	21
SLM WATER COMMISSION	10/12/1999	0	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	7/16/2007	18	15	J-36	280	8/15/2007	0.94	30

[1] Where finished Mn results were lower than the detection level, the detection level was used in calculating the removal estimates.

Attachment 1 – Exhibit F

Guidelines for deriving numerical National Water Quality Criteria for the protection of aquatic organisms and their uses

GUIDELINES FOR DERIVING NUMERICAL NATIONAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

by

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CONTENTS

EXECUTIVE SUMMARY

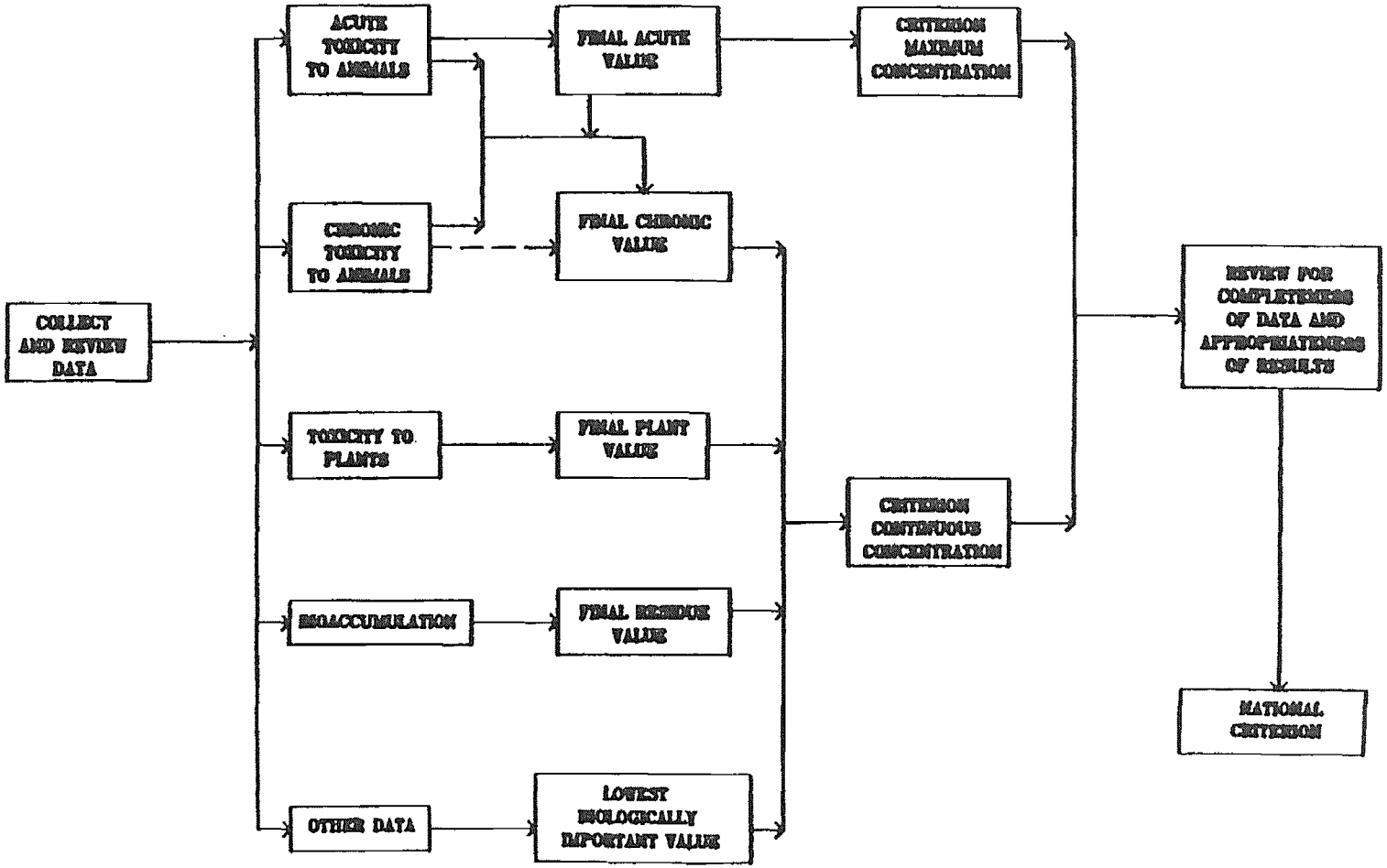
Derivation of numerical national water quality criteria for the protection of aquatic organisms and their uses is a complex process (Figure 1) that uses information from many areas of aquatic toxicology. After a decision is made that a national criterion is needed for a particular material, all available information concerning toxicity to, and bioaccumulation by, aquatic organisms is collected, reviewed for acceptability, and sorted. If enough acceptable data on acute toxicity to aquatic animals are available, they are used to estimate the highest one-hour average concentration that should not result in unacceptable effects on aquatic organisms and their uses. If justified, this concentration is made a function of a water quality characteristic such as pH, salinity, or hardness. Similarly, data on the chronic toxicity of the material to aquatic animals are used to estimate the highest four-day average concentration that should not cause unacceptable toxicity during a long-term exposure. If appropriate, this concentration is also related to a water quality characteristic.

Data on toxicity to aquatic plants are examined to determine whether plants are likely to be unacceptably affected by concentrations that should not cause unacceptable effects on animals. Data on bioaccumulation by aquatic organisms are used to determine if residues might subject edible species to restrictions by the U.S. Food and Drug Administration or if such residues might harm some wildlife consumers of aquatic life. All other available data are examined for adverse effects that might be biologically important.

If a thorough review of the pertinent information indicates that enough acceptable data are available, numerical national water quality criteria are derived for fresh water or salt water or both to protect aquatic organisms

Figure 1

Derivation of Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses



and their uses from unacceptable effects due to exposures to high concentrations for short periods of time, lower concentrations for longer periods of time, and combinations of the two.

Introduction

Of the several possible forms of criteria, the numerical form is the most common, but the narrative (e.g., pollutants must not be present in harmful concentrations) and operational (e.g., concentrations of pollutants must not exceed one-tenth of the 96-hr LC50) forms can be used if numerical criteria are not possible or desirable. If it were feasible, a freshwater (or saltwater) numerical aquatic life national criterion* for a material should be determined by conducting field tests on a wide variety of unpolluted bodies of fresh (or salt) water. It would be necessary to add various amounts of the material to each body of water in order to determine the highest concentration that would not cause any unacceptable long-term or short-term effect on the aquatic organisms or their uses. The lowest of these highest concentrations would become the freshwater (or saltwater) national aquatic life water quality criterion for that material, unless one or more of the lowest concentrations were judged to be outliers. Because it is not feasible to determine national criteria by conducting such field tests, these Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (hereafter referred to as the National Guidelines) describe an objective, internally consistent, appropriate, and feasible way of deriving national criteria, which are intended to provide the same level of protection as the infeasible field testing approach described above.

Because aquatic ecosystems can tolerate some stress and occasional adverse effects, protection of all species at all times and places is not

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*The term "national criteria" is used herein because it is more descriptive than the synonomous term "section 304(a) criteria", which is used in the Water Quality Standards Regulation [1].

deemed necessary. If acceptable data are available for a large number of appropriate taxa from an appropriate variety of taxonomic and functional groups, a reasonable level of protection will probably be provided if all except a small fraction of the taxa are protected, unless a commercially or recreationally important species is very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is too high or too low or just right.

Determining the validity of a criterion derived for a particular body of water, possibly by modification of a national criterion to reflect local conditions [1,2,3], should be based on an operational definition of "protection of aquatic organisms and their uses" that takes into account the practicalities of field monitoring programs and the concerns of the public. Monitoring programs should contain sampling points at enough times and places that all unacceptable changes, whether caused directly or indirectly, will be detected. The programs should adequately monitor the kinds of species of concern to the public, i.e., fish in fresh water and fish and macroinvertebrates in salt water. If the kinds of species of concern cannot be adequately monitored at a reasonable cost, appropriate surrogate species should be monitored. The kinds of species most likely to be good surrogates are those that either (a) are a major food of the desired kinds of species or (b) utilize the same food as the desired species or (c) both. Even if a major adverse effect on appropriate surrogate species does not directly result in an unacceptable effect on the kinds of species of concern to the public, it indicates a high probability that such an effect will occur.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and other important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams, and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. Monitoring programs intended to be able to detect unacceptable effects should be tailored to the body of water of concern so that necessary samples are obtained at enough times and places to provide adequate data on the populations of important species, as well as data directly related to the reasons for their being considered important. For example, for substances that are residue limited, species that are consumed should be monitored for contaminants to ensure that wildlife predators are protected, FDA action levels are not exceeded, and flavor is not impaired. Monitoring programs should also provide data on the number of taxa and number of individuals in the above-named assemblages that can be sampled at reasonable cost. The amount of decrease in the number of taxa or number of individuals in an assemblage that should be considered unacceptable should take into account appropriate features of the body of water and its aquatic community. Because most monitoring programs can only detect decreases of more than 20 percent, any statistically significant decrease should usually be considered unacceptable. The insensitivity of most monitoring programs greatly limits their usefulness for studying the validity of criteria because unacceptable changes can occur and not be detected. Therefore, although limited field studies can sometimes demonstrate that criteria are underprotective, only high quality field studies can reliably demonstrate that criteria are not underprotective.

If the purpose of water quality criteria were to protect only commercially and recreationally important species, criteria specifically derived to protect such species and their uses from the direct adverse effects of a material would probably, in most situations, also protect those species from indirect adverse effects due to effects of the material on other species in the ecosystem. For example, in most situations either the food chain would be more resistant than the important species and their uses or the important species and their food chains would be adaptable enough to overcome effects of the material on portions of the food chains.

These National Guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations. All North American bodies of water and resident aquatic species and their uses are meant to be taken into account, except for a few that may be too atypical, such as the Great Salt Lake, brine shrimp, and the siscowet subspecies of lake trout, which occurs in Lake Superior and contains up to 67% fat in the fillets [4]. Derivation of criteria specifically for the Great Salt Lake or Lake Superior might have to take brine shrimp and siscowet, respectively, into account.

Numerical aquatic life criteria derived using these National Guidelines are expressed as two numbers, rather than the traditional one number, so that the criteria more accurately reflect toxicological and practical realities. If properly derived and used, the combination of a maximum concentration and a continuous concentration should provide an appropriate degree of protection of aquatic organisms and their uses from acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms,

without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these Guidelines are intended to be useful for developing water quality standards, mixing zone standards, effluent limitations, etc. The development of such standards and limitations, however, might have to take into account such additional factors as social, legal, economic, and hydrological considerations, the environmental and analytical chemistry of the material, the extrapolation from laboratory data to field situations, and relationships between species for which data are available and species in the body of water of concern. As an intermediate step in the development of standards, it might be desirable to derive site-specific criteria by modification of national criteria to reflect such local conditions as water quality, temperature, or ecologically important species [1,2,3]. In addition, with appropriate modifications these National Guidelines can be used to derive criteria for any specific geographical area, body of water (such as the Great Salt Lake), or group of similar bodies of water, if adequate information is available concerning the effects of the material of concern on appropriate species and their uses.

Criteria should attempt to provide a reasonable and adequate amount of protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a national criterion be the best estimate that can be obtained using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Therefore, these National Guidelines specify certain data that should be available if a numerical criterion is to be derived. If all the required data are not

available, usually a criterion should not be derived. On the other hand, the availability of all required data does not ensure that a criterion can be derived.

A common belief is that national criteria are based on "worst case" assumptions and that local considerations will raise, but not lower, criteria. For example, it will usually be assumed that if the concentration of a material in a body of water is lower than the national criterion, no unacceptable effects will occur and no site-specific criterion needs to be derived. If, however, the concentration of a material in a body of water is higher than the national criterion, it will usually be assumed that a site-specific criterion should be derived. In order to prevent the assumption of the "worst case" nature of national criteria from resulting in the underprotection of too many bodies of water, national criteria must be intended to protect all or almost all bodies of water. Thus, if bodies of water and the aquatic communities in them do differ substantially in their sensitivities to a material, national criteria should be at least somewhat overprotective for a majority of the bodies of water. To do otherwise would either (a) require derivation of site-specific criteria even if the site-specific concentration were substantially below the national criterion or (b) cause the "worst case" assumption to result in the underprotection of numerous bodies of water. On the other hand, national criteria are probably underprotective of some bodies of water.

The two factors that will probably cause the most difference between national and site-specific criteria are the species that will be exposed and the characteristics of the water. In order to ensure that national criteria are appropriately protective, the required data for national criteria include some species that are sensitive to many materials and national criteria are

specifically based on tests conducted in water relatively low in particulate matter and organic matter. Thus, the two factors that will usually be considered in the derivation of site-specific criteria from national criteria are used to help ensure that national criteria are appropriately protective.

On the other hand, some local conditions might require that site-specific criteria be lower than national criteria. Some untested locally important species might be very sensitive to the material of concern, and local water quality might not reduce the toxicity of the material. In addition, aquatic organisms in field situations might be stressed by diseases, parasites, predators, other pollutants, contaminated or insufficient food, and fluctuating and extreme conditions of flow, water quality, and temperature. Further, some materials might degrade to more toxic materials, or some important community functions or species interactions might be adversely affected by concentrations lower than those that affect individual species.

Criteria must be used in a manner that is consistent with the way in which they were derived if the intended level of protection is to be provided in the real world. Although derivation of water quality criteria for aquatic life is constrained by the ways toxicity and bioconcentration tests are usually conducted, there are still many different ways that criteria can be derived, expressed, and used. The means used to derive and state criteria should relate, in the best possible way, the kinds of data that are available concerning toxicity and bioconcentration and the ways criteria can be used to protect aquatic organisms and their uses.

The major problem is to determine the best way that the statement of a criterion can bridge the gap between the nearly constant concentrations used in most toxicity and bioconcentration tests and the fluctuating concentrations that usually exist in the real world. A statement of a criterion as a number

that is not to be exceeded any time or place is not acceptable because few, if any, people who use criteria would take it literally and few, if any, toxicologists would defend a literal interpretation. Rather than try to reinterpret a criterion that is neither useful nor valid, it is better to develop a more appropriate way of stating criteria.

Although some materials might not exhibit thresholds, many materials probably do. For any threshold material, continuous exposure to any combination of concentrations below the threshold will not cause an unacceptable effect (as defined on pages 1-3) on aquatic organisms and their uses, except that the concentration of a required trace nutrient might be too low. However, it is important to note that this is a threshold of unacceptable effect, not a threshold of adverse effect. Some adverse effect, possibly even a small reduction in the survival, growth, or reproduction of a commercially or recreationally important species, will probably occur at, and possibly even below, the threshold. The Criterion Continuous Concentration (CCC) is intended to be a good estimate of this threshold of unacceptable effect. If maintained continuously, any concentration above the CCC is expected to cause an unacceptable effect. On the other hand, the concentration of a pollutant in a body of water can be above the CCC without causing an unacceptable effect if (a) the magnitudes and durations of the excursions above the CCC are appropriately limited and (b) there are compensating periods of time during which the concentration is below the CCC. The higher the concentration is above the CCC, the shorter the period of time it can be tolerated. But it is unimportant whether there is any upper limit on concentrations that can be tolerated instantaneously or even for one minute because concentrations outside mixing zones rarely change substantially in such short periods of time.

An elegant, general approach to the problem of defining conditions (a) and (b) would be to integrate the concentration over time, taking into account uptake and depuration rates, transport within the organism to a critical site, etc. Because such an approach is not currently feasible, an approximate approach is to require that the average concentration not exceed the CCC. The average concentration should probably be calculated as the arithmetic average rather than the geometric mean [5]. If a suitable averaging period is selected, the magnitudes and durations of concentrations above the CCC will be appropriately limited, and suitable compensating periods below the CCC will be required.

In the elegant approach mentioned above, the uptake and depuration rates would determine the effective averaging period, but these rates are likely to vary from species to species for any particular material. Thus the elegant approach might not provide a definitive answer to the problem of selecting an appropriate averaging period. An alternative is to consider that the purpose of the averaging period is to allow the concentration to be above the CCC only if the allowed fluctuating concentrations do not cause more adverse effect than would be caused by a continuous exposure to the CCC. For example, if the CCC caused a 10% reduction in growth of rainbow trout, or a 13% reduction in survival of oysters, or a 7% reduction in reproduction of smallmouth bass, it is the purpose of the averaging period to allow concentrations above the CCC only if the total exposure will not cause any more adverse effect than continuous exposure to the CCC would cause.

Even though only a few tests have compared the effects of a constant concentration with the effects of the same average concentration resulting from a fluctuating concentration, nearly all the available comparisons have shown that substantial fluctuations result in increased adverse effects

[5,6]. Thus if the averaging period is not to allow increased adverse effects, it must not allow substantial fluctuations. Life-cycle tests with species such as mysids and daphnids and early life-stage tests with warmwater fishes usually last for 20 to 30 days. An averaging period that is equal to the length of the test will obviously allow the worst possible fluctuations and would very likely allow increased adverse effects.

An averaging period of four days seems appropriate for use with the CCC for two reasons. First, it is substantially shorter than the 20 to 30 days that is obviously unacceptable. Second, for some species it appears that the results of chronic tests are due to the existence of a sensitive life stage at some time during the test [7], rather than being caused by either long-term stress or long-term accumulation of the test material in the organism. The existence of a sensitive life stage is probably the cause of acute-chronic ratios that are not much greater than 1, and is also possible when the ratio is substantially greater than 1. In addition, some experimentally determined acute-chronic ratios are somewhat less than 1, possibly because prior exposure during the chronic test increased the resistance of the sensitive life stage [8]. A four-day averaging period will probably prevent increased adverse effects on sensitive life stages by limiting the durations and magnitudes of exceedences* of the CCC.

The considerations applied to interpretation of the CCC also apply to the CMC. For the CMC the averaging period should again be substantially less than the lengths of the tests it is based on, i.e., substantially less than

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*Although "exceedence" has not been found in any dictionary, it is used here because it is not appropriate to use "violation" in conjunction with criteria, no other word seems appropriate, and all appropriate phrases are awkward.

48 to 96 hours. One hour is probably an appropriate averaging period because high concentrations of some materials can cause death in one to three hours. Even when organisms do not die within the first hour or so, it is not known how many might have died due to delayed effects of this short of an exposure. Thus it is not appropriate to allow concentrations above the CMC to exist for as long as one hour.

The durations of the averaging periods in national criteria have been made short enough to restrict allowable fluctuations in the concentration of the pollutant in the receiving water and to restrict the length of time that the concentration in the receiving water can be continuously above a criterion concentrations. The statement of a criterion could specify that the four-day average should never exceed the CCC and that the one-hour average should never exceed the CMC. However, one of the most important uses of criteria is for designing waste treatment facilities. Such facilities are designed based on probabilities and it is not possible to design for a zero probability. Thus, one of the important design parameters is the probability that the four-day average or the one-hour average will be exceeded, or, in other words, the frequency with which exceedences will be allowed.

The frequency of allowed exceedences should be based on the ability of aquatic ecosystems to recover from the exceedences, which will depend in part on the magnitudes and durations of the exceedences. It is important to realize that high concentrations caused by spills and similar major events are not what is meant by an "exceedence", because spills and other accidents are not part of the design of the normal operation of waste treatment facilities. Rather, exceedences are extreme values in the distribution of ambient concentrations and this distribution is the result of the usual variations in the flows of both the effluent and the receiving water and the usual

variations in the concentrations of the material of concern in both the effluent and in the upstream receiving water. Because exceedences are the result of usual variation, most of the exceedences will be small and exceedences as large as a factor of two will be rare. In addition, because these exceedences are due to random variation, they will not be evenly spaced. In fact, because many receiving waters have both one-year and multi-year cycles and many treatment facilities have daily, weekly, and yearly cycles, exceedences will often be grouped, rather than being evenly spaced or randomly distributed. If the flow of the receiving water is usually much greater than the flow of the effluent, normal variation and the flow cycles will result in the ambient concentration usually being below the CCC, occasionally being near the CCC, and rarely being above the CCC. In addition, exceedences that do occur will be grouped. On the other hand, if the flow of the effluent is much greater than the flow of the receiving water, the concentration might be close to the CCC much of the time and rarely above the CCC, with exceedences being randomly distributed.

The abilities of ecosystems to recover differ greatly, and depend on the pollutant, the magnitude and duration of the exceedence, and the physical and biological features of the ecosystem. Documented studies of recoveries are few, but some systems recover from small stresses in six weeks whereas other systems take more than ten years to recover from severe stress [3]. Although most exceedences are expected to be very small, larger exceedences will occur occasionally. Most aquatic ecosystems can probably recover from most exceedences in about three years. Therefore, it does not seem reasonable to purposely design for stress above that caused by the CCC to occur more than once every three years on the average, just as it does not seem reasonable

to require that these kinds of stresses only occur once every five or ten years on the average.

If the body of water is not subject to anthropogenic stress other than the exceedences of concern and if exceedences as large as a factor of two are rare, it seems reasonable that most bodies of water could tolerate exceedences once every three years on the average. In situations in which exceedences are grouped, several exceedences might occur in one or two years, but then there will be, for example, 10 to 20 years during which no exceedences will occur and the concentration will be substantially below the CCC most of the time. In situations in which the concentration is often close to the CCC and exceedences are randomly distributed, some adverse effect will occur regularly, and small additional, unacceptable effects will occur about every third year. The relative long-term ecological consequences of evenly spaced and grouped exceedences are unknown, but because most exceedences will probably be small, the long-term consequences should be about equal over long periods of time.

The above considerations lead to a statement of a criterion in the frequency-intensity-duration format that is often used to describe rain and snow fall and stream flow, e.g., how often, on the average, does more than ten inches of rain fall in a week? The numerical values chosen for frequency (or average recurrence interval), intensity (i.e., concentration), and duration (of averaging period) are those appropriate for national criteria. Whenever adequately justified, a national criterion may be replaced by a site-specific criterion [1], which may include not only site-specific criterion concentrations [2], but also site-specific durations of averaging periods and site-specific frequencies of allowed exceedences [3].

The concentrations, durations, and frequencies specified in criteria are based on biological, ecological, and toxicological data, and are designed to protect aquatic organisms and their uses from unacceptable effects. Use of criteria for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of water quality criteria, but a steady-state model might have to be used instead of a dynamic model in some situations. Regardless of the model that is used, the durations of the averaging periods and the frequencies of allowed exceedences must be applied correctly if the intended level of protection is to be provided. For example, in the criterion statement frequency refers to the average frequency, over a long period of time, of rare events (i.e., exceedences). However, in some disciplines, frequency is often thought of in terms of the average frequency, over a long period of time, of the years in which rare events occur, without any consideration of how many rare events occur within each of those eventful years. The distinction between the frequency of events and the frequency of years is important for all those situations in which the rare events, e.g., exceedences, tend to occur in groups within the eventful years. The two ways of calculating frequency produce the same results in situations in which each rare event occurs in a different year because then the frequency of events is the same as the frequency of eventful years.

Because fresh water and salt water have basically different chemical compositions and because freshwater and saltwater (i.e., estuarine and true marine) species rarely inhabit the same water simultaneously, these National Guidelines provide for the derivation of separate criteria for these two kinds of water. For some materials sufficient data might not be available to allow derivation of criteria for one or both kinds of water. Even though absolute toxicities might be different in fresh and salt waters, such

relative data as acute-chronic ratios and bioconcentration factors often appear to be similar in the two waters. When data are available to indicate that these ratios and factors are probably similar, they are used interchangeably.

The material for which a criterion is desired is usually defined in terms of a particular chemical compound or ion, or a group of closely related compounds or ions, but it might possibly be defined in terms of an effluent. These National Guidelines might also be useful for deriving criteria for temperature, dissolved oxygen, suspended solids, pH, etc., if the kinds of data on which the Guidelines are based are available.

Because they are meant to be applied only after a decision has been made that a national water quality criterion for aquatic organisms is needed for a material, these National Guidelines do not address the rationale for making that decision. If the potential for adverse effects on aquatic organisms and their uses is part of the basis for deciding whether an aquatic life criterion is needed for a material, these Guidelines will probably be helpful in the collection and interpretation of relevant data. Such properties as volatility might affect the fate of a material in the aquatic environment and might be important when determining whether a criterion is needed for a material; for example, aquatic life criteria might not be needed for materials that are highly volatile or highly degradable in water. Although such properties can affect how much of the material will get from the point of discharge through any allowed mixing zone to some portion of the ambient water and can also affect the size of the zone of influence in the ambient water, such properties do not affect how much of the material aquatic organisms can tolerate in the zone of influence.

This version of the National Guidelines provides clarifications, additional details, and technical and editorial changes from the previous

version [9]. These modifications are the result of comments on the previous version and subsequent drafts [10], experience gained during the U.S. EPA's use of previous versions and drafts, and advances in aquatic toxicology and related fields. Future versions will incorporate new concepts and data as their usefulness is demonstrated. The major technical changes incorporated into this version of the National Guidelines are:

1. The requirement for acute data for freshwater animals has been changed to include more tests with invertebrate species. The taxonomic, functional, and probably the toxicological, diversities among invertebrate species are greater than those among vertebrate species and this should be reflected in the required data.
2. When available, 96-hr EC50s based on the percentage of fish immobilized plus the percentage of fish killed are used instead of 96-hr LC50s for fish; comparable EC50s are used instead of LC50s for other species. Such appropriately defined EC50s better reflect the total severe acute adverse impact of the test material on the test species than do LC50s or narrowly defined EC50s. Acute EC50s that are based on effects that are not severe, such as reduction in shell deposition and reduction in growth, are not used in calculating the Final Acute Value.
3. The Final Acute Value is now defined in terms of Genus Mean Acute Values rather than Species Mean Acute Values. A Genus Mean Acute Value is the geometric mean of all the Species Mean Acute Values available for species in the genus. On the average, species within a genus are toxicologically much more similar than species in different genera, and so the use of Genus Mean Acute Values will prevent data sets from being biased by an overabundance of species in one or a few genera.

4. The Final Acute Value is now calculated using a method [11] that is not subject to the bias and anomalous behavior that the previous method was. The new method is also less influenced by one very low value because it always gives equal weight to the four values that provide the most information about the cumulative probability of 0.05. Although the four values receive the most weight, the other values do have a substantial effect on the Final Acute Value (see examples in Appendix 2).
5. The requirements for using the results of tests with aquatic plants have been made more stringent.
6. Instead of being equal to the Final Acute Value, the Criterion Maximum Concentration is now equal to one-half the Final Acute Value. The Criterion Maximum Concentration is intended to protect 95 percent of a group of diverse genera, unless a commercially or recreationally important species is very sensitive. However, a concentration that would severely harm 50 percent of the fifth percentile or 50 percent of a sensitive important species cannot be considered to be protective of that percentile or that species. Dividing the Final Acute Value by 2 is intended to result in a concentration that will not severely adversely affect too many of the organisms.
7. The lower of the two numbers in the criterion is now called the Criterion Continuous Concentration, rather than the Criterion Average Concentration, to more accurately reflect the nature of the toxicological data on which it is based.
8. The statement of a criterion has been changed (a) to include durations of averaging periods and frequencies of allowed exceedences that are based on what aquatic organisms and their uses can tolerate, and (b) to

identify a specific situation in which site-specific criteria [1,2,3] are probably desirable.

In addition, Appendix 1 was added to aid in determining whether a species should be considered resident in North America and its taxonomic classification. Appendix 2 explains the calculation of the Final Acute Value.

The amount of guidance in these National Guidelines has been increased, but much of the guidance is necessarily qualitative rather than quantitative; much judgment will usually be required to derive a water quality criterion for aquatic organisms and their uses. In addition, although this version of the National Guidelines attempts to cover all major questions that have arisen during use of previous versions and drafts, it undoubtedly does not cover all situations that might occur in the future. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these Guidelines and should be consistent with the spirit of these Guidelines, i.e., to make best use of the available data to derive the most appropriate criteria. These National Guidelines should be modified whenever sound scientific evidence indicates that a national criterion produced using these Guidelines would probably be substantially overprotective or underprotective of the aquatic organisms and their uses on a national basis. Derivation of numerical national water quality criteria for aquatic organisms and their uses is a complex process and requires knowledge in many areas of aquatic toxicology; any deviation from these Guidelines should be carefully considered to ensure that it is consistent with other parts of these Guidelines.

I. Definition of Material of Concern

A. Each separate chemical that does not ionize substantially in most natural bodies of water should usually be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

B. For chemicals that do ionize substantially in most natural bodies of water (e.g., some phenols and organic acids, some salts of phenols and organic acids, and most inorganic salts and coordination complexes of metals), all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

C. The definition of the material should include an operational analytical component. Identification of a material simply, for example, as "sodium" obviously implies "total sodium", but leaves room for doubt. If "total" is meant, it should be explicitly stated. Even "total" has different operational definitions, some of which do not necessarily measure "all that is there" in all samples. Thus, it is also necessary to reference or describe the analytical method that is intended. The operational analytical component should take into account the analytical and environmental chemistry of the material, the desirability of using the same analytical method on samples from laboratory tests, ambient water,

and aqueous effluents, and various practical considerations, such as labor and equipment requirements and whether the method would require measurement in the field or would allow measurement after samples are transported to a laboratory.

The primary requirements of the operational analytical component are that it be appropriate for use on samples of receiving water, that it be compatible with the available toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection or overprotection of aquatic organisms and their uses. Because an ideal analytical measurement will rarely be available, a compromise measurement will usually have to be used. This compromise measurement must fit with the general approach that if an ambient concentration is lower than the national criterion, unacceptable effects will probably not occur, i.e., the compromise measurement must not err on the side of underprotection when measurements are made on a surface water. Because the chemical and physical properties of an effluent are usually quite different from those of the receiving water, an analytical method that is acceptable for analyzing an effluent might not be appropriate for analyzing a receiving water, and vice versa. If the ambient concentration <u>calculated</u> from a measured concentration in an effluent is higher than the national criterion, an additional option is to <u>measure</u> the concentration after dilution of the effluent with receiving water to determine if the measured concentration is lowered by such phenomena as complexation or sorption. A further option, of course, is to derive a site-specific criterion [1,2,3]. Thus, the criterion should be based on

an appropriate analytical measurement, but the criterion is not rendered useless if an ideal measurement either is not available or is not feasible.

NOTE: The analytical chemistry of the material might have to be taken into account when defining the material or when judging the acceptability of some toxicity tests, but a criterion should not be based on the sensitivity of an analytical method. When aquatic organisms are more sensitive than routine analytical methods, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

II. Collection of Data

A. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants, (b) FDA action levels [12], and (c) chronic feeding studies and long-term field studies with wildlife species that regularly consume aquatic organisms.

B. All data that are used should be available in typed, dated, and signed hard copy (publication, manuscript, letter, memorandum, etc.) with enough supporting information to indicate that acceptable test procedures were used and that the results are probably reliable. In some cases it may be appropriate to obtain additional written information from the investigator, if possible. Information that is confidential or privileged or otherwise not available for distribution should not be used.

C. Questionable data, whether published or unpublished, should not be used. For example, data should usually be rejected if they are

from tests that did not contain a control treatment, tests in which too many organisms in the control treatment died or showed signs of stress or disease, and tests in which distilled or deionized water was used as the dilution water without addition of appropriate salts.

D. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the material of concern should not be used.

E. For some highly volatile, hydrolyzable, or degradable materials it is probably appropriate to use only results of flow-through tests in which the concentrations of test material in the test solutions were measured often enough using acceptable analytical methods.

F. Data should be rejected if they were obtained using:

1. Brine shrimp, because they usually only occur naturally in water with salinity greater than 35 g/kg.
2. Species that do not have reproducing wild populations in North America (see Appendix 1).
3. Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.

G. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with non-resident species in North America or previously exposed organisms may be used to provide auxiliary information but should not be used in the derivation of criteria.

III. Required Data

A. Certain data should be available to help ensure that each of the four major kinds of possible adverse effects receives adequate

consideration. Results of acute and chronic toxicity tests with representative species of aquatic animals are necessary so that data available for tested species can be considered a useful indication of the sensitivities of appropriate untested species. Fewer data concerning toxicity to aquatic plants are required because procedures for conducting tests with plants and interpreting the results of such tests are not as well developed. Data concerning bioaccumulation by aquatic organisms are only required if relevant data are available concerning the significance of residues in aquatic organisms.

B. To derive a criterion for freshwater aquatic organisms and their uses, the following should be available:

1. Results of acceptable acute tests (see Section IV) with at least one species of freshwater animal in at least eight different families such that all of the following are included:

    a. the family Salmonidae in the class Osteichthyes

    b. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)

    c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)

    d. a planktonic crustacean (e.g., cladoceran, copepod, etc.)

    e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)

f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)

g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)

h. a family in any order of insect or any phylum not already represented.

2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that of the three species:

--at least one is a fish

--at least one is an invertebrate

--at least one is an acutely sensitive freshwater species (the other two may be saltwater species).

3. Results of at least one acceptable test with a freshwater alga or vascular plant (see Section VIII). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available (see Section IX).

C. To derive a criterion for saltwater aquatic organisms and their uses, the following should be available:

1. Results of acceptable acute tests (see Section IV) with at least one species of saltwater animal in at least eight different families such that all of the following are included:

a. two families in the phylum Chordata

b. a family in a phylum other than Arthropoda or Chordata

c. either the Mysidae or Penaeidae family

d. three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used above)

e. any other family.

2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that of the three species:

--at least one is a fish

--at least one is an invertebrate

--at least one is an acutely sensitive saltwater species (the other two may be freshwater species).

3. Results of at least one acceptable test with a saltwater alga or vascular plant (see Section VIII). If plants are among the aquatic organisms most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate saltwater species, if a maximum permissible tissue concentration is available (see Section IX).

D. If all the required data are available, a numerical criterion can usually be derived, except in special cases. For example, derivation of a criterion might not be possible if the available acute-chronic ratios vary by more than a factor of ten with no apparent

pattern. Also, if a criterion is to be related to a water quality characteristic (see Sections V and VII), more data will be necessary.

Similarly, if all required data are not available, a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it might be possible to derive a criterion if the available data clearly indicate that the Final Residue Value should be much lower than either the Final Chronic Value or the Final Plant Value.

E. Confidence in a criterion usually increases as the amount of available pertinent data increases. Thus, additional data are usually desirable.

IV. Final Acute Value

A. Appropriate measures of the acute (short-term) toxicity of the material to a variety of species of aquatic animals are used to calculate the Final Acute Value. The Final Acute Value is an estimate of the concentration of the material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acceptable acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value of a commercially or recreationally important species is lower than the calculated Final Acute Value, then that Species Mean Acute Value replaces the calculated Final Acute Value in order to provide protection for that important species.

B. Acute toxicity tests should have been conducted using acceptable procedures [13].

C. Except for tests with saltwater annelids and mysids, results of acute tests during which the test organisms were fed should not be used, unless data indicate that the food did not affect the toxicity of the test material.

D. Results of acute tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between acute toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.

E. Acute values should be based on endpoints which reflect the total severe acute adverse impact of the test material on the organisms used in the test. Therefore, only the following kinds of data on acute toxicity to aquatic animals should be used:

1. Tests with daphnids and other cladocerans should be started with organisms less than 24 hours old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC50 based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC50 is not available from a test, the 48-hr LC50 should be used in place of the desired 48-hr EC50. An EC50 or LC50 of longer than 48 hr can be used as long as the animals were not fed and the control animals were acceptable at the end of the test.

2. The result of a test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea

urchins, lobsters, crabs, shrimp, and abalones should be the 96-hr EC50 based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an EC50 is not available from a test, the lower of the 96-hr EC50 based on the percentage of organisms with incompletely developed shells and the 96-hr LC50 should be used in place of the desired 96-hr EC50. If the duration of the test was between 48 and 96 hr, the EC50 or LC50 at the end of the test should be used.

3. The acute values from tests with all other freshwater and saltwater animal species and older life stages of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimps, and abalones should be the 96-hr EC50 based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed. If such an EC50 is not available from a test, the 96-hr LC50 should be used in place of the desired 96-hr EC50.
4. Tests with single-celled organisms are not considered acute tests, even if the duration was 96 hours or less.
5. If the tests were conducted properly, acute values reported as "greater than" values and those which are above the solubility of the test material should be used, because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species.

F. If the acute toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Acute Equation should be derived based on that water quality characteristic. Go to Section V.

G. If the available data indicate that one or more life stages are at least a factor of two more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the Species Mean Acute Value because a species can only be considered protected from acute toxicity if all life stages are protected.

H. The agreement of the data within and between species should be considered. Acute values that appear to be questionable in comparison with other acute and chronic data for the same species and for other species in the same genus probably should not be used in calculation of a Species Mean Acute Value. For example, if the acute values available for a species or genus differ by more than a factor of 10, some or all of the values probably should not be used in calculations.

I. For each species for which at least one acute value is available, the Species Mean Acute Value (SMAV) should be calculated as the geometric mean of the results of all flow-through tests in which the concentrations of test material were measured. For a species

for which no such result is available, the SMAV should be calculated as the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial concentrations (nominal concentrations are acceptable for most test materials if measured concentrations are not available) of test material.

NOTE: Data reported by original investigators should not be rounded off. Results of all intermediate calculations should be rounded [14] to four significant digits.

NOTE: The geometric mean of N numbers is the N^{th} root of the product of the N numbers. Alternatively, the geometric mean can be calculated by adding the logarithms of the N numbers, dividing the sum by N, and taking the antilog of the quotient. The geometric mean of two numbers is the square root of the product of the two numbers, and the geometric mean of one number is that number. Either natural (base e) or common (base 10) logarithms can be used to calculate geometric means as long as they are used consistently within each set of data, i.e., the antilog used must match the logarithm used.

NOTE: Geometric means, rather than arithmetic means, are used here because the distributions of sensitivities of individual organisms in toxicity tests on most materials and the distributions of sensitivities of species within a genus are more likely to be lognormal than normal. Similarly, geometric means are used for acute-chronic ratios and bioconcentration factors because quotients are likely to be closer to lognormal than normal distributions. In addition,

division of the geometric mean of a set of numerators by the geometric mean of the set of corresponding denominators will result in the geometric mean of the set of corresponding quotients.

J. For each genus for which one or more SMAVs are available, the Genus Mean Acute Value (GMAV) should be calculated as the geometric mean of the SMAVs available for the genus.

K. Order the GMAVs from high to low.

L. Assign ranks, R, to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

M. Calculate the cumulative probability, P, for each GMAV as R/(N+1).

N. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are less than 59 GMAVs, these will always be the four lowest GMAVs).

O. Using the selected GMAVs and Ps, calculate

$$s^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

$A = \ln FAV$

(See [11] for development of the calculation procedure and Appendix 2 for an example calculation and computer program.)

NOTE: Natural logarithms (logarithms to base e, denoted as ln) are used herein merely because they are easier to use on some hand

calculators and computers than common (base 10) logarithms. Consistent use of either will produce the same result.

P. If for a commercially or recreationally important species the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured is lower than the calculated Final Acute Value, then that geometric mean should be used as the Final Acute Value instead of the calculated Final Acute Value.

Q. Go to Section VI.

V. Final Acute Equation

A. When enough data are available to show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be taken into account as described in Sections B-G below or using analysis of covariance [15,16]. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two or more factors affect toxicity, multiple regression analysis should be used.

B. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the acute toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95% confidence limits for each species.

NOTE: Because the best documented relationship is that between hardness and acute toxicity of metals in fresh water and a log-log relationship fits these data, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics, such as pH, temperature, or salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section.

C. Decide whether the data for each species is useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, acute values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic, the acute values available for a species or genus differ by more than a factor of 10, rejection of some or all of the values is probably appropriate. If useful slopes are not available for at least one fish and one invertebrate or if the available

slopes are too dissimilar or if too few data are available to adequately define the relationship between acute toxicity and the water quality characteristic, return to Section IV.G., using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

D. Individually for each species calculate the geometric mean of the available acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

E. Similarly normalize the values of the water quality characteristic for each species individually.

F. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95% confidence limits will be identical to those obtained in Section B above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

G. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope, V, and its 95% confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

H. For each species calculate the geometric mean, W, of the acute toxicity values and the geometric mean, X, of the values of the water quality characteristic. (These were calculated in steps D and E above.)

I. For each species calculate the logarithm, Y, of the SMAV at a selected value, Z, of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

J. For each species calculate the SMAV at Z using the equation: SMAV = e^Y.

NOTE: Alternatively, the SMAVs at Z can be obtained by skipping step H above, using the equations in steps I and J to adjust each acute value individually to Z, and then calculating the geometric mean of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted acute values for each species.

K. Obtain the Final Acute Value at Z by using the procedure described in Section IV.J-O.

L. If the SMAV at Z of a commercially or recreationally important species is lower than the calculated Final Acute Value at Z, then that SMAV should be used as the Final Acute Value at Z instead of the calculated Final Acute Value.

M. The Final Acute Equation is written as: Final Acute Value = $e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = Final Acute Value at Z. Because V, A, and Z are known, the Final Acute Value can be calculated for any selected value of the water quality characteristic.

VI. Final Chronic Value

A. Depending on the data that are available concerning chronic toxicity to aquatic animals, the Final Chronic Value might be calculated in the same manner as the Final Acute Value or by dividing the Final Acute Value by the Final Acute-Chronic Ratio. In some cases it may not be possible to calculate a Final Chronic Value.

NOTE: As the name implies, the acute-chronic ratio (ACR) is a way of relating acute and chronic toxicities. The acute-chronic ratio is basically the inverse of the application factor, but this new name is better because it is more descriptive and should help prevent confusion between "application factors" and "safety factors". Acute-chronic ratios and application factors are ways of relating the acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond the known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it will usually be greater than one; this should avoid the confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

B. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which the concentrations of test material in the test solutions were properly measured at appropriate times during the test.

C. Results of chronic tests in which survival, growth, or reproduction in the control treatment was unacceptably low should not be used. The limits of acceptability will depend on the species.

D. Results of chronic tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between chronic toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.

E. Chronic values should be based on endpoints and lengths of exposure appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

1. Life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species to a different concentration of the test material throughout a life cycle. To ensure that all life stages and life processes are exposed, tests with fish should begin with embryos or newly hatched young less than 48 hours old, continue through maturation and reproduction, and should end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young less than 24 hours old and last for not less than 21 days. Tests with mysids should begin with young less than 24 hours old and continue until 7 days past the median time of first brood release in the controls. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability

(salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female. For mysids, data should be obtained and analyzed on survival, growth, and young per female.

2. Partial life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species of fish to a different concentration of the test material through most portions of a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with immature juveniles at least 2 months prior to active gonad development, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

3. Early life-stage toxicity tests consisting of 28- to 32-day (60 days post hatch for salmonids) exposures of the early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained and analyzed on survival and growth.

   NOTE: Results of an early life-stage test are used as predictions of results of life-cycle and partial life-cycle tests

with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests are possibly not good predictions of the results of comparable life-cycle or partial life-cycle tests.

F. A chronic value may be obtained by calculating the geometric mean of the lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of adverse effect on any of the specified biological measurements, and (c) below which no tested concentration caused an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of adverse effect on one or more of the specified biological measurements, and (c) above which all tested concentrations also caused such an effect.

NOTE: Because various authors have used a variety of terms and definitions to interpret and report results of chronic tests, reported results should be reviewed carefully. The amount of effect that is considered unacceptable is often based on a statistical hypothesis test, but might also be defined in terms of a specified percent reduction from the controls. A small percent

reduction (e.g., 3%) might be considered acceptable even if it is statistically significantly different from the control, whereas a large percent reduction (e.g., 30%) might be considered unacceptable even if it is not statistically significant.

G. If the chronic toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section VII.

H. If chronic values are available for species in eight families as described in Sections III.B.1 or III.C.1, a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values available for the species, and appropriate Genus Mean Chronic Values should be calculated. The Final Chronic Value should then be obtained using the procedure described in Section IV.J-O. Then go to Section VI.M.

I. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of the results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which the concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. The acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted

in the same laboratory and dilution water, but in a different study, may be used. If no such acute tests are available, results of acute tests conducted in the same dilution water in a different laboratory may be used. If no such acute tests are available, an acute-chronic ratio should not be calculated.

J. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios available for that species.

K. For some materials the acute-chronic ratio seems to be the same for all species, but for other materials the ratio seems to increase or decrease as the Species Mean Acute Value (SMAV) increases. Thus the Final Acute-Chronic Ratio can be obtained in four ways, depending on the data available:

1. If the species mean acute-chronic ratio seems to increase or decrease as the SMAV increases, the Final Acute-Chronic Ratio should be calculated as the geometric mean of the acute-chronic ratios for species whose SMAVs are close to the Final Acute Value.
2. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all the species mean acute-chronic ratios available for both freshwater and saltwater species.
3. For acute tests conducted on metals and possibly other substances with embryos and larvae of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones

(see Section IV.E.2), it is probably appropriate to assume that the acute-chronic ratio is 2. Chronic tests are very difficult to conduct with most such species, but it is likely that the sensitivities of embryos and larvae would determine the results of life-cycle tests. Thus, if the lowest available SMAVs were determined with embryos and larvae of such species, the Final Acute-Chronic Ratio should probably be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B).

4. If the most appropriate species mean acute-chronic ratios are less than 2.0, and especially if they are less than 1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B).

If the available species mean acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a Final Chronic Value probably cannot be calculated.

L. Calculate the Final Chronic Value by dividing the Final Acute Value by the Final Acute-Chronic Ratio. If there was a Final Acute Equation rather than a Final Acute Value, see also Section VII.A.

M. If the Species Mean Chronic Value of a commercially or recreationally important species is lower than the calculated Final Chronic Value, then that Species Mean Chronic Value should be used as the Final Chronic Value instead of the calculated Final Chronic Value.

N. Go to Section VIII.

VII. Final Chronic Equation

A. A Final Chronic Equation can be derived in two ways. The procedure described here in Section A will result in the chronic slope being the same as the acute slope. The procedure described in Sections B-N will usually result in the chronic slope being different from the acute slope.

1. If acute-chronic ratios are available for enough species at enough values of the water quality characteristic to indicate that the acute-chronic ratio is probably the same for all species and is probably independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the available species mean acute-chronic ratios.

2. Calculate the Final Chronic Value at the selected value Z of the water quality characteristic by dividing the Final Acute Value at Z (see Section V.M.) by the Final Acute-Chronic Ratio.

3. Use V = pooled acute slope (see section V.M.) as L = pooled chronic slope.

4. Go to Section VII.M.

B. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be taken into account as described in Sections B-G below or using analysis of covariance [15,16]. The two methods are equivalent and produce identical results. The manual method

described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two or more factors affect toxicity, multiple regression analysis should be used.

C. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the chronic toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95% confidence limits for each species.

NOTE: Because the best documented relationship is that between hardness and acute toxicity of metals in fresh water and a log-log relationship fits these data, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics, such as pH, temperature, or salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section. It is probably preferable, but not necessary, to use the same transformation that was used with the acute values in Section V.

D. Decide whether the data for each species is useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A

slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, chronic values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic, the chronic values available for a species or genus differ by more than a factor of 10, rejection of some or all of the values is probably appropriate. If a useful chronic slope is not available for at least one species or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between chronic toxicity and the water quality characteristic, it might be appropriate to assume that the chronic slope is the same as the acute slope, which is equivalent to assuming that the acute-chronic ratio is independent of the water quality characteristic. Alternatively, return to Section VI.H, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

E. Individually for each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

F. Similarly normalize the values of the water quality characteristic for each species individually.

G. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and the 95% confidence limits will be identical to those obtained in Section B above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

H. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope, L, and its 95% confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

I. For each species calculate the geometric mean, M, of the toxicity values and the geometric mean, P, of the values of the water quality characteristic. (These were calculated in steps E and F above.)

J. For each species calculate the logarithm, Q, of the Species Mean Chronic Value at a selected value, Z, of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.
NOTE: Although it is not necessary, it will usually be best to use the same value of the water quality characteristic here as was used in section V.I.

K. For each species calculate a Species Mean Chronic Value at Z using the equation: $SMCV = e^{Q}$.

NOTE: Alternatively, the Species Mean Chronic Values at Z can be obtained by skipping step J above, using the equations in steps J and K to adjust each acute value individually to Z and then calculating the geometric means of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted chronic values for each species.

L. Obtain the Final Chronic Value at Z by using the procedure described in Section IV.J-O.

M. If the Species Mean Chronic Value at Z of a commercially or recreationally important species is lower than the calculated Final Chronic Value at Z, then that Species Mean Chronic Value should be used as the Final Chronic Value at Z instead of the calculated Final Chronic Value.

N. The Final Chronic Equation is written as: Final Chronic Value = $e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = pooled chronic slope and S = Final Chronic Value at Z. Because L, S and Z are known, the Final Chronic Value can be calculated for any selected value of the water quality characteristic.

VIII. Final Plant Value

A. 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.

B. A plant value is the result of a 96-hr test conducted with an alga or a chronic test conducted with an aquatic vascular plant.

NOTE: A test of the toxicity of a metal to a plant usually should not be used if the medium contained an excessive amount of a complexing agent, such as EDTA, that might affect the toxicity of the metal. Concentrations of EDTA above about 200 µg/L should probably be considered excessive.

C. The Final Plant Value should be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured and the endpoint was biologically important.

IX. Final Residue Value

A. The Final Residue Value is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from affecting marketability because of exceedence of applicable FDA action levels and (b) protect wildlife, including fishes and birds, that consume aquatic organisms from demonstrated unacceptable effects. The Final Residue Value is the lowest of the residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level [12] for fish oil or for the edible portion of fish or shellfish, or (b) a maximum acceptable dietary intake based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no

maximum permissible tissue concentration is available, go to Section X because no Final Residue Value can be derived.

B. Bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution in which the organism had been living. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. Some uptake during the bioconcentration test might not be directly from water if the food sorbs some of the test material before it is eaten by the test organisms. A BAF is intended to account for net uptake from both food and water in a real-world situation. A BAF almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey that itself could have accumulated the material from both food and water. The BCF and BAF are probably similar for a material with a low BCF, but the BAF is probably higher than the BCF for materials with high BCFs. Although BCFs are not too difficult to determine, very few BAFs have been measured acceptably because it is necessary to make enough measurements of the concentration of the material in water to show that it was reasonably constant for a long enough period of time over the range of territory inhabited by the organisms. Because so few acceptable BAFs are available, only BCFs will be discussed further. However, if an acceptable BAF is available for a material, it should be used instead of any available BCFs.

C. If a maximum permissible tissue concentration is available for a substance (e.g., parent material, parent material plus metabolites, etc.), the tissue concentration used in the calculation of the BCF should be for the same substance. Otherwise the tissue concentration used in the calculation of the BCF should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

D. 1. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of the test material in tissue and in the test solution, and the exposure continued at least until either apparent steady-state or 28 days was reached. Steady-state is reached when the BCF does not change significantly over a period of time, such as two days or 16 percent of the length of the exposure, whichever is longer. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached, (b) the highest BCF obtained, if apparent steady-state was not reached, and (c) the projected steady-state BCF, if calculated.

2. Whenever a BCF is determined for a lipophilic material, the percent lipids should also be determined in the tissue(s) for which the BCF was calculated.

3. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to a BCF obtained with unaffected organisms of the same species at lower concentrations that did not cause adverse effects.

4. Because maximum permissible tissue concentrations are almost never based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight BCF by 0.1 for plankton and by 0.2 for individual species of fishes and invertebrates [17].

5. If more than one acceptable BCF is available for a species, the geometric mean of the available values should be used, except that if the BCFs are from different lengths of exposure and the BCF increases with length of exposure, the BCF for the longest exposure should be used.

E. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

1. For each available maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of the tested wildlife species.

2. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion (muscle for decapods, muscle with or without skin for fishes, adductor muscle for scallops, and total soft tissue for other bivalve molluscs) of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

F. For lipophilic materials, it might be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percent lipids from one tissue to another and from one species to another [18-20], extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percent lipids.

1. For each BCF for which the percent lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by the percent lipids. This adjustment to a one percent lipid basis is intended to make all the measured BCFs for a material comparable regardless of the species or tissue with which the BCF was measured.

2. Calculate the geometric mean normalized BCF. Data for both saltwater and freshwater species should be used to determine the mean normalized BCF, unless the data show that the normalized BCFs are probably not similar.

3. Calculate all possible residue values by dividing the available maximum permissible tissue concentrations by the mean normalized BCF and by the percent lipids values appropriate to the maximum permissible tissue concentrations, i.e.,

$$\text{Residue value} = \frac{\text{(maximum permissible tissue concentration)}}{\text{(mean normalized BCF)(appropriate percent lipids)}}$$

   a. For an FDA action level for fish oil, the appropriate percent lipids value is 100.

   b. For an FDA action level for fish, the appropriate percent lipids value is 11 for freshwater criteria and 10 for

saltwater criteria because FDA action levels are applied on a species-by-species basis to commonly consumed species. The highest lipid contents in the edible portions of important consumed species are about 11 percent for both the freshwater chinook salmon and lake trout and about 10 percent for the saltwater Atlantic herring [21].

c. For a maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, the appropriate percent lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

G. The Final Residue Value is obtained by selecting the lowest of the available residue values.

NOTE: In some cases the Final Residue Value will not be low enough. For example, a residue value calculated from an FDA action level will probably result in an average concentration in the edible portion of a fatty species that is at the action level. Some individual organisms, and possibly some species, will have residue concentrations higher than the mean value but no mechanism has been devised to provide appropriate additional protection. Also, some chronic feeding studies and long-term field studies with wildlife identify concentrations that cause adverse effects but do not identify concentrations which do not cause adverse effects; again no mechanism has been devised to provide appropriate additional protection. These are some of the species and uses that are not protected at all times in all places.

X. Other Data

Pertinent information that could not be used in earlier sections might be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other adverse effect that has been shown to be biologically important. Especially important are data for species for which no other data are available. Data from behavioral, biochemical, physiological, microcosm, and field studies might also be available. Data might be available from tests conducted in unusual dilution water (see IV.D and VI.D), from chronic tests in which the concentrations were not measured (see VI.B), from tests with previously exposed organisms (see II.F), and from tests on formulated mixtures or emulsifiable concentrates (see II.D). Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important.

XI. Criterion

A. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

B. The Criterion Maximum Concentration (CMC) is equal to one-half the Final Acute Value.

C. The Criterion Continuous Concentration (CCC) is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value, unless other data (see Section X) show that a lower value should be used. If toxicity is related to a water quality

characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the Final Residue Value by selecting the one, or the combination, that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section X) show that a lower value should be used.

D. Round [14] both the CMC and the CCC to two significant digits.

E. The criterion is stated as:

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where a locally important species is very sensitive, (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

where (1) = insert "freshwater" or "saltwater"

(2) = insert name of material

(3) = insert the Criterion Continuous Concentration

(4) = insert the Criterion Maximum Concentration.

XII. Final Review

A. The derivation of the criterion should be carefully reviewed by rechecking each step of the Guidelines. Items that should be especially checked are:

1. If unpublished data are used, are they well documented?
2. Are all required data available?
3. Is the range of acute values for any species greater than a factor of 10?
4. Is the range of Species Mean Acute Values for any genus greater than a factor of 10?
5. Is there more than a factor of ten difference between the four lowest Genus Mean Acute Values?
6. Are any of the four lowest Genus Mean Acute Values questionable?
7. Is the Final Acute Value reasonable in comparison with the Species Mean Acute Values and Genus Mean Acute Values?
8. For any commercially or recreationally important species, is the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured lower than the Final Acute Value?
9. Are any of the chronic values questionable?
10. Are chronic values available for acutely sensitive species?
11. Is the range of acute-chronic ratios greater than a factor of 10?
12. Is the Final Chronic Value reasonable in comparison with the available acute and chronic data?
13. Is the measured or predicted chronic value for any commercially or recreationally important species below the Final Chronic Value?
14. Are any of the other data important?
15. Do any data look like they might be outliers?

16. Are there any deviations from the Guidelines? Are they acceptable?

B. On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific evidence. If it is not, another criterion, either higher or lower, should be derived using appropriate modifications of these Guidelines.

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Appendix 1. Resident North American Species of Aquatic Animals Used in Toxicity and Bioconcentration Tests

Introduction

These lists identify species of aquatic animals which have reproducing wild populations in North America and have been used in toxicity or bioconcentratin tests. "North America" includes only the 48 contiguous states, Canada, and Alaska; Hawaii and Puerto Rico are not included. Saltwater (i.e., estuarine and true marine) species are considered resident in North America if they inhabit or regularly enter shore waters on or above the continental shelf to a depth of 200 meters. Species do not have to be native to be resident. Unlisted species should be considered resident North American species if they can be similarly confirmed or if the test organisms were obtained from a wild population in North America.

The sequence for fishes is taken from A List of Common and Scientific Names of Fishes from the United States and Canada. For other species, the sequence of phyla, classes, and families is taken from the NODC Taxonomic Code, Third Edition, National Oceanographic Data Center, NOAA, Washington, DC 20235, July, 1981, and the numbers given are from that source to facilitate verification. Within a family, genera are in alphabetical order, as are species in a genus.

The references given are those used to confirm that the species is a resident North American species. (The NODC Taxonomic Code contains foreign as well as North American species.) *If no such reference could be found, the species was judged to be nonresident.* No reference is given for organisms not identified to species; these are considered resident only if obtained from wild North American populations. A few nonresident species are listed in brackets and noted as "nonresident" because they were mistakenly identified as resident in the past or to save other investigators from doing literature searches on the same species.

Freshwater Species

Class	Family	Species		Reference
		Common Name	Scientific Name	
PHYLUM: PORIFERA (36)				
Demospongia 3660	Spongillidae 366301	Sponge	Ephydatia fluviatilis	P93
PHYLUM: CNIDARIA (COELENTERATA) (37)				
Hydrozoa 3701	Hydridae 370602	Hydra	Hydra oligactis	E318, P112
		Hydra	Hydra littoralis	E321, P112

Freshwater (Concinued)

Class	Family	Species: Common Name	Species: Scientific Name	Reference
PHYLUM: PLATYHELMINTHES (39)				
Turbellaria 3901	Planariidae	Planarian	Dugesia dorotocephala	D22
		Planarian	Dugesia lugubris (Dugesia polychroa)	D24
		Planarian	Planaria gonocephala	[Footnote 1]
		[Planarian]	[Polycelis felina]	[nonresident]
	Dendrocoelidae 391501	Planarian	Procotyla fluviatilis (Dendrocoelum lacteum)	E334, P132, D63
PHYLUM: GASTROTRICHA (44)				
Chaetonotoida 4402	Chaetonotidae 440201	Gastrotrich	Lepidodermella squamatum	E413
PHYLUM: ROTIFERA (ROTATORIA) (45)				
Bdelloidea 4503	Philodinidae 450402	Rotifer	Philodina acuticornis	Y
		Rotifer	Philodina roseola	E487
Monogononta 4506	Brachionidae 450601	Rotifer	Keratella cochlearis	E442, P188
		Rotifer	Keratella sp.	[Footnote 2]
PHYLUM: ANNELIDA (50)				
Archiannelida 5002	Aeolosomatidae 500301	Worm	Aeolosoma headleyi	E528, P284
Oligochaeta 5004	Lumbriculidae 500501	Worm	Lumbriculus variegatus	E533, P290
	Tubificidae 500902	Tubificid worm	Branchiura sowerbyi	E534, P289, GG
		Tubificid worm	Limnodrilus hoffmeisteri	E536, GG
		Tubificid worm	Quistadrilus multisetosus (Peloscolex multisetosus)	E535, GG

Freshwater (Continued)

Class	Family	Species: Common Name	Species: Scientific Name	Reference
		Tubificid worm	Rhyacodrilus montana	GG
		Tubificid worm	Spirosperma ferox (Peloscolex ferox)	GG
		Tubificid worm	Spirosperma nikolskyi (Peloscolex variegatus)	E534, GG
		Tubificid worm	Stylodrilus heringianus	GG
		Tubificid worm	Tubifex tubifex	E536, P289, GG
		Tubificid worm	Varichaeta pacifica	GG
	Naididae 500903	Worm	Nais sp.	[Footnote 2]
		Worm	Paranais sp.	[Footnote 2]
		Worm	Pristina sp.	[Footnote 2]
Hirudinea 5012	Erpobdellidae 501601	[Leech]	[Erpobdella octoculata]	[nonresident] (BB16)
PHYLUM: MOLLUSCA (5085)				
Gastropoda 51	Viviparidae 510306	Snail	Campeloma decisum	P731, M216
	Bithyniidae (Amnicolidae) (Bulimidae) (Hydrobiidae) 510317	Snail	Amnicola sp.	[Footnote 2]
	Pleuroceridae 510340	Snail	Goniobasis livescens	P732
		Snail	Goniobasis virginica	E1137
		Snail	Leptoxis carinata (Nitocris carinata) (Mudalia carinata)	X, E1137
		Snail	Nitocris sp.	[Footnote 2]

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Lymnaeidae 511410	[Snail]	[Lymnaea acuminata]	[nonresident]
		Snail	Lymnaea catascopium (Lymnaea emarginata) (Stagnicola emarginata)	M328
		Snail	Lymnaea elodes (Lymnaea palustris)	E1127, M351
		[Snail]	[Lymnaea luteola]	[nonresident] (M266)
		Snail	Lymnaea stagnalis	E1127, P726, M296
		Snail	Lymnaea sp.	[Footnote 2]
	Planorbidae 511412	[Snail]	[Biomphalaria glabrata]	[nonresident] (M390)
		Snail	Gyraulus circumstriatus	P729, M397
		Snail	Helisoma campanulatum	M445
		Snail	Helisoma trivolvis	P729, M452
	Physidae 511413	Snail	Aplexa hypnorum	E1126, P727, M373
		[Snail]	[Physa fontinalis]	[nonresident] (M373)
		Snail	Physa gyrina	E1126, P727, M373
		Snail	Physa heterostropha	M378
		Snail	Physa integra	P727
		Snail	Physa sp.	[Footnote 2]
Bivalvia (Pelecypoda) 55	Margaritiferidae 551201	Mussel	Margaritifera margaritifera	E1138, P748, J11
	Amblemidae	Mussel	Amblema plicata	AA122

Freshwater (Continued)

Class	Family	Species: Common Name	Species: Scientific Name	Reference
	Unionidae 551202	Mussel	Anodonta imbecillus	J72, AA122
		Mussel	Carunculina parva (Toxolasma texasensis)	J19, AA122
		Mussel	Cyrtonaias tampicoenis	P759, AA122
		Mussel	Elliptio complanata	J13
	Corbiculidae 551545	Asiatic clam	Corbicula fluminea	E1159
		Asiatic clam	Corbicula manilensis	P749
	Pisidiidae (Sphaeriidae) 551546	Fingernail clam	Eupera cubensis (Eupera singleyi)	E1158, P763, G9
		Fingernail clam	Musculium transversum (Sphaerium transversum)	M160, G11
		Fingernail clam	Sphaerium corneum	G12
PHYLUM: ARTHROPODA (58-69)				
Crustacea 61	Lynceidae 610701	Conchostracan	Lynceus brachyurus	E580, P344
	Sididae 610901	Cladoceran	Diaphanosoma sp.	[Footnote 2]
	Daphnidae 610902	Cladoceran	Ceriodaphnia acanthina	E618
		Cladoceran	Ceriodaphnia reticulata	E618, P368
		Cladoceran	Daphnia ambigua	E607, P369
		Cladoceran	Daphnia carinata	[Footnote 3]
		[Cladoceran]	[Daphnia cucullata]	[nonresident]
		Cladoceran	Daphnia galeata mendotae	E610, P370
		Cladoceran	Daphnia hyalina	[Footnote 4]
		Cladoceran	Daphnia longispina	[Footnote 5]

Freshwacer (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Cladoceran	Daphnia magna	E605, P367
		Cladoceran	Daphnia parvula	E611
		Cladoceran	Daphnia pulex	E613, P367
		Cladoceran	Daphnia pulicaria	A
		Cladoceran	Daphnia similis	E606, P367
		Cladoceran	Moina macrocopa	E622, P372
		Cladoceran	Moina rectirostris	E623
		Cladoceran	Simocephalus serrulatus	E617, P370
		Cladoceran	Simocephalus vetulus	E617, P370
	Bosminidae 610903	Cladoceran	Bosmina longirostris	E624, P373
	Polyphemidae 610905	Cladoceran	Polyphemus pediculus	E599, P385
	Cyprididae (Cypridae) 611303	[Ostracod]	[Cypretta kawatai]	[nonresident] (U)
		Ostracod	Cypridopsis vidua	E720, P430
	Diaptomidae 611818	[Copepod]	[Eudiaptomus padanus]	[nonresident]
	Temoridae 611820	Copepod	Epischura lacustris	E751, P407
	Cyclopidae 612008	[Copepod]	[Cyclops abyssorum]	[nonresident]
		Copepod	Cyclops bicuspidatus	E807, P405
		Copepod	Cyclops vernalis	E804, P405
		Copepod	Cyclops viridis (Acanthocyclops viridis)	E803, P397
		Copepod	Acanthocyclops sp.	[Footnote 2]

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Copepod	Diacyclops sp.	[Footnote 2]
		Copepod	Eucyclops agilis	P403
		Copepod	Mesocyclops leuckarti	E812, P403
	Asellidae 616302	[Isopod]	[Asellus aquaticus]	[nonresident] (I2)
		Isopod	Asellus bicrenata (Caecidotea bicrenata)	HH (I1,2)
		Isopod	Asellus brevicaudus	E875, P447, I
		Isopod	Asellus communis	E875, P448, I
		Isopod	Asellus intermedius	E875, P448, I
		[Isopod]	[Asellus meridianus]	[nonresident]
		Isopod	Asellus racovitzai	P449, I
		Isopod	Lirceus alabamae	E875, I
	Gammaridae 616921	Amphipod	Crangonyx pseudogracilis	P459, T68, FF28
		Amphipod	Gammarus fasciatus	E877, P458, T53
		Amphipod	Gammarus lacustris	E877, P458, FF23
		Amphipod	Gammarus pseudolimnaeus	E877, P458, T48
		[Amphipod]	[Gammarus pulex]	[nonresident]
		Amphipod	Gammarus tigrinus	L51, FF17
		Amphipod	Gammarus sp.	[Footnote 2]

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Hyalellidae (Talitridae) 616923	Amphipod	Hyalella azteca (Hyalella knickerbockeri)	E876, P457, T154
	Palaemonidae 617911	[Prawn]	[Macrobrachium lamarrei]	[nonresident]
		Malaysian prawn	Macrobrachium rosenbergii	[Footnote 6]
		Prawn	Palaemonetes kadiakensis	E881, P484
	Astacidae 618102	Crayfish	Cambarus latimanus	E897
		Crayfish	Faxonella clypeatus	E890
		Crayfish	Orconectes immunis	E894, P482
		Crayfish	Orconectes limosus	E893, P482
		Crayfish	Orconectes propinquus	E894, P482
		Crayfish	Orconectes nais	E894
		Crayfish	Orconectes rusticus	E893, P482
		Crayfish	Orconectes virilis	E894, P483
		Crayfish	Pacifastacus trowbridgii	E883
		Crayfish	Procambarus acutus	P482
		Crayfish	Procambarus clarki (Procambarus clarkii)	E885, P482
		Crayfish	Procambarus simulans	E888, P482
		Crayfish	Procambarus sp.	[Footnote 2]
Insecta 62-65	Heptageniidae 621601	Mayfly	Stenonema ithaca	S173, 0205
		Mayfly	Stenonema rubrum	S178, 0205
	Baetidae 621602	Mayfly	Callibaetis skokianus	S116, N9

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Mayfly	Callibaetis sp.	[Footnote 2]
		Mayfly	Cloeon dipterum	0173
	Leptophlebiidae 621701	Mayfly	Paraleptophlebia praepedita	S89, 0233
	Ephemerellidae 621702	Mayfly	Ephemerella doddsi	0245
		Mayfly	Ephemerella grandis	0245
		Mayfly	Ephemerella subvaria	N9, 0248, S71
		Mayfly	Ephemerella sp.	[Footnote 2]
	Caenidae 621802	Mayfly	Caenis diminuta	S51, 0268
	Ephemeridae 622003	Mayfly	Ephemera simulans	S36, N9, 0283
		Mayfly	Hexagenia bilineata	N9, S39, 0290
		Mayfly	Hexagenia rigida	0290, S41, N9
		Mayfly	Hexagenia sp.	[Footnote 2]
	Libellulidae 622601	Dragonfly	Pantala hymenea (Pantala hymenaea)	N15, V603
	Coenagrionidae (Agrionidae) (Coenagriidae) 622904	Damselfly	Enallagma aspersum	DD
		[Damselfly]	[Ischnura elegans]	[nonresident]
		Damselfly	Ischnura verticalis	N15, E918
		Damselfly	Ischnura sp.	[Footnote 2]
	Pteronarcidae (Pteronarcyidae) 625201	Stonefly	Pteronarcella badia	L172
		Stonefly	Pteronarcys californica	L173
		Stonefly	Pteronarcys dorsata	E947

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Stonefly	Pteronarcys sp.	[Footnote 2]
	Nemouridae 625204	[Stonefly]	[Nemoura cinerea]	[nonresident
	Perlidae 625401	Stonefly	Acroneuria lycorias	N4, E953
		Stonefly	Acroneuria pacifica	E953, L180
		Stonefly	Claassenia sabulosa	E953
		Stonefly	Neophasganophora capitata (Phasganophora capitata)	E953, CC407
	Perlodidae 625402	Stonefly	Arcynopteryx parallela	E954
	Nepidae 627206	Water scorpion	Ranatra elongata	[nonresident]
	Dytiscidae 630506	Beetle		[Footnote 2]
	Elmidae (Elminthidae) 631604	Beetle	Stenelmis sexlineata	W21
	Hydropsychidae 641804	Caddisfly	Arctopsyche grandis	L251, II98
		Caddisfly	Hydropsyche betteni	N24
		Caddisfly	Hydropsyche californica	L253
		Caddisfly	Hydropsyche sp.	[Footnote 2]
	Limnephilidae 641807	Caddisfly	Clistornia magnifica	II206
		Caddisfly	Philarctus quaeris	II272
	Brachycentridae 641815	Caddisfly	Brachycentrus sp.	[Footnote 2]
	Tipulidae 650301	Crane fly	Tipula sp.	[Footnote 2]

Freshwater (Continued)

Class	Family	Species: Common Name	Species: Scientific Name	Reference
	Ceratopogonidae 650504	Biting midge	-	[Footnote 2]
	Culicidae 650503	Mosquito	Aedes aegypti	EE3
		Mosquito	Culex pipiens	EE3
	Chironomidae (Tendipedidae) 650508	Midge	Chironomus plumosus (Tendipes plumosus)	L423
		Midge	Chironomus tentans	Q
Chironomus decorus ?		[Midge]	[Chironomus thummi]	[nonresident]
		Midge	Chironomus sp.	[Footnote 2]
		Midge	Paracanytarsus parthenogeneticus	[Footnote 7]
		Midge	Tanytarsus dissimilis	R11
	Rhagionidae (Leptidae) 651603	Snipe fly	Atherix sp.	[Footnote 2]
PHYLUM: ECTOPROCTA (BRYOZOA) (78)				
Phylactolaemata 7817	Pectinatellidae	Bryozoan	Pectinatella magnifica	E502, P269
	Lophopodidae	Bryozoan	Lophopodella carteri	E502, P271
	Plumatellidae 781701	Bryozoan	Plumatella emarginata	E505, P272
PHYLUM: CHORDATA (8388)				
Agnatha 86	Petromyzontidae 860301	Sea lamprey	Petromyzon marinus	F11
Osteichthyes 8717	Anguillidae 874101	American eel	Anguilla rostrata	F15
	Salmonidae 875501	Pink salmon	Oncorhynchus gorbuscha	F18
		Coho salmon	Oncorhynchus kisutch	F18

Freshwater (Continued)

Class	Family	Species: Common Name	Species: Scientific Name	Reference
		Sockeye salmon	Oncorhynchus nerka	F19
		Chinook salmon	Oncorhynchus tshawytscha	F19
		Mountain whitefish	Prosopium williamsoni	F19
		Golden trout	Salmo aguabonita	F19
		Cutthroat trout	Salmo clarki	F19
		Rainbow trout (Steelhead trout)	Salmo gairdneri	F19
		Atlantic salmon	Salmo salar	F19
		Brown trout	Salmo trutta	F19
		Brook trout	Salvelinus fontinalis	F19
		Lake trout	Salvelinus namaycush	F19
	Esocidae 875801	Northern pike	Esox lucius	F20
	Cyprinidae 877601	Chiselmouth	Acrocheilus alutaceus	F21
		Longfin dace	Agosia chrysogaster	F21
		Central stoneroller	Campostoma anomalum	F21
		Goldfish	Carassius auratus	F21
		Common carp	Cyprinus carpio	F21
		[Zebra danio] [(Zebrafish)]	[Danio rerio] [(Brachydanio rerio)]	[nonresident] (F96)
		Silverjaw minnow	Ericymba buccata	F21
		Golden shiner	Notemigonus crysoleucas	F23
		Pugnose shiner	Notropis anogenus	F23

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Emerald shiner	Notropis atherinoides	F23
		Striped shiner	Notropis chrysocephalus	F23
		Common shiner	Notropis cornutus	F23
		Pugnose minnow	Notropis emiliae	F24
		Spottail shiner	Notropis hudsonius	F24
		Red shiner	Notropis lutrensis	F24
		Spotfin shiner	Notropis spilopterus	F25
		Sand shiner	Notropis stramineus	F25
		Steelcolor shiner	Notropis whipplei	F25
		Northern redbelly dace	Phoxinus eos	F25
		Bluntnose minnow	Pimephales notatus	F25
		Fathead minnow	Pimephales promelas	F25
		Northern squawfish	Ptychocheilus oregonensis	F25
		Blacknose dace	Rhinichthys atratulus	F25
		Speckled dace	Rhinichthys osculus	F25
		Bitterling	Rhodeus sericeus	F26
		Rudd	Scardinius erythrophthalmus	F26
		Creek chub	Semotilus atromaculatus	F26
		Pearl dace	Semotilus margarita	F26
		Tench	Tinca tinca	F26

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Catostomidae 877604	White sucker	Catostomus commersoni	F26
		Mountain sucker	Catostomus platyrhynchus	F26
	Ictaluridae 877702	Black bullhead	Ictalurus melas	F27
		Yellow bullhead	Ictalurus natalis	F27
		Brown bullhead	Ictalurus nebulosus	F27
		Channel catfish	Ictalurus punctatus	F27
	Clariidae 877712	Walking catfish	Clarias batrachus	F28
	Oryziidae	Medaka	[Oryzias latipes]	[nonresident] (F96)
	Cyprinodontidae 880404	Banded killifish	Fundulus diaphanus	F33
		Flagfish	Jordanella floridae	F33
	Poeciliidae 880408	Mosquitofish	Gambusia affinis	F33
		Amazon molly	Poecilia formosa	F34
		Sailfin molly	Poecilia latipinna	F34
		Molly	Poecilia sp.	
		Guppy	Poecilia reticulata (Lebistes reticulatus, Obs.)	F34
		Southern platyfish	Xiphophorus maculatus	F34
	Gasterosteidae 881801	Brook stickleback	Culaea inconstans	F35
		Threespine stickleback	Gasterosteus aculeatus	F35
		Ninespine stickleback	Pungitius pungitius	F35

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Percichthyidae	White perch	Morone americana (Roccus americanus, Obs.)	F36
		Striped bass	Morone saxatilis (Roccus saxatilis, Obs.)	F36
	Centrarchidae 883516	Rock bass	Ambloplites rupestris	F38
		Green sunfish	Lepomis cyanellus	F38
		Pumpkinseed	Lepomis gibbosus	F38
		Orangespotted sunfish	Lepomis humilis	F38
		Bluegill	Lepomis macrochirus	F38
		Longear sunfish	Lepomis megalotis	F38
		Redear sunfish	Lepomis microlophus	F38
		Smallmouth bass	Micropterus dolomieui	F39
		Largemouth bass	Micropterus salmoides	F39
		White crappie	Pomoxis annularis	F39
		Black crappie	Pomoxis nigromaculatus	F39
	Percidae 883520	Rainbow darter	Etheostoma caeruleum	F39
		Johnny darter	Etheostoma nigrum	F40
		Orangethroat darter	Etheostoma spectabile	F40
		Yellow perch	Perca flavescens	F41
		Walleye	Stizostedion vitreum vitreum	F41
	Sciaenidae 883544	Freshwater drum	Aplodinotus grunniens	F45
	Cichlidae 883561	Oscar	Astronotus ocellatus	F47

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Blue tilapia	Tilapia aurea	F47
		Mozambique tilapia	Tilapia mossambica	F47
	Cottidae 883102	Mottled sculpin	Cottus bairdi	F60
Amphibia 89	Ranidae 890302	Bullfrog	Rana catesbeiana	B206
		Green frog	Rana clamitans	B206
		Pig frog	Rana grylio	B206
		River frog	Rana heckscheri	B206
		Leopard frog	Rana pipiens	B205
		Wood frog	Rana sylvatica	B206
		[Frog]	[Rana temporia]	[nonresident
		Leopard frog	Rana spenocephala	JJ
	Microhylidae 890303	Narrow-mouthed toad	Gastrophryne carolinensis	B192
	Bufonidae 890304	American toad	Bufo americanus	B196
		[Toad]	[Bufo bufo]	[nonresident
		Green toad	Bufo debilis	B197
		Fowler's toad	Bufo fowleri	B196
		Red-spotted toad	Bufo punctatus	B198
		Woodhouse's toad	Bufo woodhousei	B196
	Hylidae 890305	Northern cricket frog	Acris crepitans	B203
		Southern gray treefrog	Hyla chrysoscelis	B201
		Spring peeper	Hyla crucifer	B202

Freshwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Barking treefrog	Hyla gratiosa	B201
		Squirrel treefrog	Hyla squirella	B201
		Gray treefrog	Hyla versicolor	B200
		Northern chorus frog	Pseudacris triseriata	B202
	Pipidae	African clawed frog	Xenopus laevis	Z16
	Ambystomatidae 890502	Spotted salamander	Ambystoma maculatum	B176
		[Mexican axolotl]	[Ambystoma mexicanum]	[nonresident]
		Marbled salamander	Ambystoma opacum	B176
	Salamandridae 890504	Newt	Notophthalmus viridescens (Triturus viridescens)	B179

Footnotes:

1. Apparently this is an outdated name (D19, 20). Organisms identified as such should only be used if they were obtained from North America.

2. Organisms not identified to species are considered resident only if they were obtained from wild populations in North America.

3. If from North America, it is resident and should be called D. similis (C). If not from North America, it should be considered nonresident.

4. If from North America, it is resident and may be any one of a number of species such as D. laevis, D. dubia, or D. galeata mendota (C). If not from North America, it should be considered nonresident.

5. If from North America, it is resident and may be any one of a number of species, such as D. ambigua, D. longiremis, or D. rosea (C). If not from North America, it should be considered nonresident.

6. This species might be established in portions of the southern United States.

7. The taxonomy of this species and this and similar genera has not been clarified, but this species should be considered resident.

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Saltwater Species

Class	Family	Species: Common Name	Species: Scientific Name	Reference
PHYLUM: CNIDARIA (COELENTERATA) (37)				
Hydrozoa 3701	Campanulariidae 370401	Hydroid	Campanularia flexuosa	B122, E81
		Hydroid	Laomedea loveni	[nonresident]
		Hydromedusa	Phialidium sp.	[Footnote 1] (E81)
	Campanulinidae 370404	[Hydroid]	[Eirene viridula]	[nonresident]
PHYLUM: CTENOPHORA (38)				
Tentaculata 3801	Pleurobrachiidae 380201	Ctenophore	Pleurobrachia pileus	B218, E162
	Mnemiidae 380302	Ctenophore	Mnemiopsis mccrdayi	C39, I94
PHYLUM: RHYNCHOCOELA (43)				
Heteronemertea 4303	Lineidae 430302	Nemertine worm	Cerebratulus fuscus	B252
PHYLUM: ROTIFERA (ROTATORIA) (45)				
Monogononta 4505	Brachionidae 450601	Rotifer	Brachionus plicatilis	B272
PHYLUM: ANNELIDA (50)				
Polychaeta 5001	Phyllodocidae 500113	Polychaete worm	Phyllodoce maculata (Anaitides maculata) (Nereiphylla maculata)	E334
	Nereidae 500124	Polychaete worm	Neanthes arenaceodentata (Nereis arenaceodentata)	E377
		[Polychaete worm]	[Neanthes vaali]	[nonresident]
		Polychaete worm	Nereis diversicolor (Neanthes diversicolor)	E337, F527

Saltwater (Continued)

Class	Family	Species: Common Name	Species: Scientific Name	Reference
		Sand worm	Nereis virens (Neanthes virens)	B317, E337, C58
		Polychaete worm	Nereis sp.	
	Dorvilleidae 500136	Polychaete worm	Ophryotrocha diadema	P23
		[Polychaete worm]	[Ophryotrocha labrunica]	[nonresident]
	Spionidae 500143	Polychaete worm	Polydora websteri	E338
	Cirratulidae 500150	Polychaete worm	Cirriformia spirabranchia	G253
	Ctenodrilidae 500153	Polychaete worm	Ctenodrilus serratus	G275
	Capitellidae 500160	Polychaete worm	Capitella capitata	B358, E337
	Arenicolidae 500162	Polychaete worm	Arenicola marina	B369, E337
	Sabellidae 500170	Polychaete worm	Eudistylia vancouveri	DD
Oligochaeta 5004	Tubificidae 500902	Oligochaete worm	Limnodriloides verrucosus	Z
		Oligochaete worm	Monopylephorus cuticulatus	Z
		Oligochaete worm	Tubificoides gabriellae	Z
PHYLUM: MOLLUSCA (5085)				
Gastropoda 51	Haliotidae 510203	Black abalone	Haliotis cracherodii	C88, D17
		Red abalone	Haliotis rufescens	D18
	Calyptraeidae 510364	Common Atlantic slippershell	Crepidula fornicata	C90, D141
	Muricidae 510501	Oyster drill	Urosalpinx cinerea (Urosalpinx cinereus)	B646, D179, E264

Saltwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Melongenidae (Neptuneidae) 510507	Channeled whelk	Busycon canaliculatum	B655, D223, E264
	Nassariidae (Nassidae) 510508	Mud snail	Nassarius obsoletus (Nassa obsoleta) (Ilyanassa obsoleta)	B649, D226, E264
Bivalvia (Pelecypoda) 55	Mytilidae 550701	Northern horse mussel	Modiolus modiolus	D434
		Blue mussel	Mytilus edulis	B566, C101, D428, E299
		[Mediterranean mussel]	[Mytilus galloprovinciallis]	[nonresidenc
	Pectinidae 550905	Bay scallop	Argopecten irradians	D447
	Ostreidae 551002	Pacific oyster	Crassostrea gigas	C102, D456, E300
		Eastern oyster	Crassostrea virginica	D456, E300
		Oyster	Crassostrea sp.	[Footnote 1]
		Oyster	Ostrea edulis	E300
	Cardiidae 551522	[Cockle]	[Cardium edule]	[nonresidenc
	Mactridae 551525	Clam	Mulina lateralis	D491
		Common rangia	Rangia cuneata	D491, E301
		Surf clam	Spisula solidissima	B599, D489, E301
	Tellinidae 551531	Clam	Macoma inquinata	D507
		[Bivalve]	[Tellina tenuis]	[nonresidenc
	Veneridae 551547	Quahog clam	Mercenaria mercenaria	D523, E301

Saltwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Common Pacific littleneck	Protothaca staminea	D526
		Japanese littleneck	Tapes philippinarum	D527
	Myidae (Myacidae) 551701	Soft-shell clam	Mya arenaria	B602, D536, E302
PHYLUM: ARTHROPODA (58-69)				
Merostomata 58	Limulidae 580201	Horseshoe crab	Limulus polyphemus	B533, E403, H30
Crustacea 61	Artemiidae 610401	[Brine shrimp]	[Artemia salina]	[Footnote 2]
	Calanidae 611801	Copepod	Calanus helgolandicus	Q25
		Copepod	Undinula vulgaris	Q29
	Eucalanidae 611803	Copepod	Eucalanus elongatus	AA
		Copepod	Eucalanus pileatus	AA
	Pseudocalanidae 611805	Copepod	Pseudocalanus minutus	E447, I155, Q43
	Euchaetidae 611808	Copepod	Euchaeta marina	Q63
	Metridiidae 611816	Copepod	Metridia pacifica	X179, Y
	Pseudodiaptomidae 611819	Copepod	Pseudodiaptomus coronatus	E447, I154, Q101
	Temoridae 611820	Copepod	Eurytemora affinis	E450, I155, Q111
	Pontellidae 611827	Copepod	Labidocera scotti	R157
	Acartiidae 611829	Copepod	Acartia clausi	E447

Salcwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Copepod	Acartia tonsa	E447, I154
	Harpacticidae 611910	Copepod	Tigriopus californicus	J78
		[Copepod]	[Tigriopus japanicus]	[nonresidenc
	Tisbidae 611913	Copepod	Tisbe holothuriae	BB
	Canthocamptidae 611929	Copepod	Nitocra spinipes	Q240
	Balanidae 613402	Barnacle	Balanus balanoides	B424, E457
		Barnacle	Balanus crenatus	B426, E457
		Barnacle	Balanus eburneus	B424, E457
		Barnacle	Balanus improvisus	B426, E457
	Mysidae 615301	Mysid	Heteromysis formosa	E513, K720
		Mysid	Mysidopsis bahia	U173
		Mysid	Mysidopsis bigelowi	E513, K720
		Mysid	Neomysis sp.	[Footnote 1]
	Idoteidae 616202	Isopod	Idotea baltica	B446, E483
		[Isopod]	[Idotea emarginata]	[nonresidenc]
		[Isopod]	[Idotea neglecta]	[nonresidenc]
	Janiridae 616306	[Isopod]	[Jaera albifrons]	[nonresidenc]
		[Isopod]	[Jaera albifrons sensu]	[nonresidenc]
		[Isopod]	[Jaera nordmanni]	[nonresidenc]
	Ampeliscidae 616902	Amphipod	Ampelisca abdita	E488, L136

Saltwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Eusiridae (Pontogeneiidae) 616920	Amphipod	Pontogeneia sp.	[Footnote 1]
	Gammaridae 616921	Amphipod	Gammarus duebeni	L56
		Amphipod	Gammarus oceanicus	E489, L50
		Amphipod	Gammarus tigrinus	L51
		[Amphipod]	[Gammarus zaddachi]	[nonresident]
		Amphipod	Marinogammarus obtusatus	L58
	Lysianassidae 616934	Amphipod	Anonyx sp.	[Footnote 1]
	Euphausiidae (Thysanopodidae) 617402	Euphausiid	Euphausia pacifica	M15
	Penaeidae 617701	Brown shrimp	Penaeus aztecus	E518, N17
		Pink shrimp	Penaeus duorarum	E518, N17
		White shrimp	Penaeus setiferus	E518, N17
		Blue shrimp	Penaeus stylirostris	[nonresident]
	Palaemonidae 617911	[Shrimp]	[Leander paucidens]	[nonresident]
		[Prawn]	[Leander squilla] [(Palaemon elegans)]	[nonresident]
		Prawn	Macrobrachium rosenbergii	[Footnote 3]
		Korean shrimp	Palaemon macrodactylus	T380
		Grass shrimp	Palaemonetes pugio	E521, N59
		Grass shrimp	Palaemonetes vulgaris	B500, E521, N56

Saltwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Hippolytidae 617916	Sargassum shrimp	Latreutes fucorum	N78
	Pandalidae 617918	Coon stripe shrimp	Pandalus danae	T306, W163
		Shrimp	Pandalus goniurus	W163
		Pink shrimp	Pandalus montagui	B494, E522, W163
	Crangonidae 617922	[Sand shrimp]	[Crangon crangon]	[nonresident
		Bay shrimp	Crangon franciscorum (Crago franciscorum)	V176, W164
		Shrimp	Crangon nigricauda	V176, W164
		Sand shrimp	Crangon septemspinosa	B500, E522,
	Nephropsidae (Nephropidae) (Homaridae) 618101	American lobster	Homarus americanus	B502, E532
		[Lobster]	[Homarus gammarus]	[nonresident]
	Paguridae 618306	Hermit crab	Pagurus longicarpus	B514, E537, N125
	Cancridae 618803	Rock crab	Cancer irroratus	B518, E543, N175
		Dungeness crab	Cancer magister	T166, V185, W177
	Portunidae 618901	Blue crab	Callinectes sapidus	B521, C80, E543, N168
		Green crab	Carcinus maenas	C80, E543
	Xanthidae (Pilumnidae) 618902	Mud crab	Eurypanopeus depressus	B522, E543, N195
		Crab	Leptodius floridanus	S80
		Mud crab	Rhithropanopeus harrisii	E543, N187

Saltwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Grapsidae 618907	Shore crab	Hemigrapsus nudus	CC
		Shore crab	Hemigrapsus oregonensis	CC
		Drift line crab	Sesarma cinereum	B526, E544, N222
		[Crab]	[Sesarma haematocheir]	[nonresident]
	Ocypodidae 618909	Fiddler crab	Uca pugilator	B526, E544, N232
PHYLUM: ECHINODERMATA (81)				
Asteroidea 8104	Asteriidae 811703	Starfish	Asterias forbesi	B728, E578, O392
Ophiuroidea 8120	Ophiochricidae 812904	Brittle star	Ophiochrix spiculata	O672, T526
Echinoidea 8136	Arbaciidae 814701	[Sea urchin]	[Arbacia lixula]	[nonresident]
		Sea urchin	Arbacia punctulata	B762, E572
	Toxopneustidae 814802	Sea urchin	Lytechinus pictus	T253
		[Sea urchin]	[Pseudocentrotus depressus]	[nonresident]
	Echinidae 814901	[Echinoderm]	[Paracentrotus lividus]	[nonresident]
	Echinometridae 814902	[Coral reef echinoid]	[Echinometra mathaei]	[nonresident] [Hawaii only]
	Strongylocentrocidae 814903	Sea urchin	Strongylocentrotus purpuratus	O574, T202
	Dendrasteridae 815501	Sand dollar	Dendraster excentricus	O537, V363
PHYLUM: CHAETOGNATHA (83)		Arrow worm	Sagitta hispida	E218

Saltwater (Continued)

Class	Family	Species: Common Name	Species: Scientific Name	Reference
PHYLUM: CHORDATA (8388)				
Chondrichthyes 8701	Rajidae 871304	[Thornback ray]	[Raja clavata]	[nonresidenc
Osteichthyes 8717	Anguillidae 874101	American eel	Anguilla rostrata	A15
	Clupeidae 874701	Atlantic menhaden	Brevoortia tyrannus	A17
		Gulf menhaden	Brevoortia patronus	A17
		Atlantic herring	Clupea harengus harengus	A17
		Pacific herring	Clupea harengus pallasi	A17
		Herring	Clupea harengus	A17
	Engraulidae 874702	Northern anchovy	Engraulis mordax	A18
		[Nehu]	[Stolephorus purpureus]	[nonresidenc] [Hawaii only]
	Salmonidae 875501	Pink salmon	Oncorhynchus gorbuscha	A18
		Chum salmon	Oncorhynchus keta	A18
		Coho salmon	Oncorhynchus kisutch	A18
		Sockeye salmon	Oncorhynchus nerka	A19
		Chinook salmon	Oncorhynchus tshawytscha	A19
		Rainbow trout (Steelhead trout)	Salmo gairdneri	A19
		Atlantic salmon	Salmo salar	A19
	Gadidae 879103	Atlantic cod	Gadus morhua	A30
		Haddock	Melanogrammus aeglefinus	A30
	Cyprinodontidae 880404	Sheepshead minnow	Cyprinodon variegatus	A33

Saltwater (Continued)

Class	Family	Species: Common Name	Species: Scientific Name	Reference
		Mummichog	Fundulus heteroclitus	A33
		Striped killifish	Fundulus majalis	A33
		Longnose killifish	Fundulus similis	A33
	Poeciliidae 880408	Mosquitofish	Gambusia affinis	A33
		Sailfin molly	Poecilia latipinna	A34
	Atherinidae 880502	Inland silverside	Menidia beryllina	A34
		Atlantic silverside	Menidia menidia	A34
		Tidewater silverside	Menidia peninsulae	A34
	Gasterosteidae 881801	Threespine stickleback	Gasterosteus aculeatus	A35
		Fourspine stickleback	Apeltes quadracus	A35
	Syngnathidae 882002	Northern pipefish	Syngnathus fuscus	A36
	Percichthyidae	Striped bass	Morone saxatilis (Roccus saxatilis, Obs.)	A36
	Kuhliidae 883514	[Mountain bass]	[Kuhlia sandvicensis]	[nonresident] [Hawaii only]
	Carangidae 883528	Florida Pompano	Trachinotus carolinus	A43
	Sparidae 883543	Pinfish	Lagodon rhomboides	A45
	Sciaenidae 883544	Spot	Leiostomus xanthurus	A46
		Atlantic croaker	Micropogonias undulatus	A46

Saltwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Red drum	Sciaenops ocellatus	A46
	Embiotocidae .883560	Shiner perch	Cymatogaster aggregata	A47
		Dwarf perch	Micrometrus minimus	A48
	Pomacentridae 883562	Blacksmith	Chromis punctipinnis	A48
	Labridae 883901	Cunner	Tautogolabrus adspersus	A49
		Bluehead	Thalassoma bifasciatum	A49
	Mugilidae 883601	[Mullet]	[Aldrichetta forsteri]	[nonresident]
		Striped mullet	Mugil cephalus	A49
		White mullet	Mugil curema	A49
	Ammodytidae 884501	Pacific sand lance	Ammodytes hexapterus	A53
	Gobiidae 884701	Longjaw mudsucker	Gillichthys mirabilis	A54
		Naked goby	Gobiosoma bosci	A54
	Cottidae 883102	Tidepool sculpin	Oligocottus maculosus	A61
	Bothidae 885703	Speckled sanddab	Citharichthys stigmaeus	A64
		Summer flounder	Paralichthys dentatus	A64
	Pleuronectidae 885704	[Dab]	[Limanda limanda]	[nonresident]
		[Plaice]	[Pleuronectes platessa]	[nonresident]
		English sole	Parophrys vetulus	A65
		Winter flounder	Pseudopleuronectes americanus	A65
	Balistidae 886002	Planehead filefish	Monacanthus hispidus	A66

Saltwater (Continued)

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Tetraodontidae 886101	Northern puffer	Sphoeroides maculatus	A66

Footnotes:

1. Organisms not identified to species are considered resident only if obtained from wild populations in North America.

2. This species should not be used because it might be too atypical.

3. This species might be established in portions of the southern United States.

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Appendix 2. Example Calculation of Final Acute Value, Computer Program, and Printouts

A. Example calculation

N = total number of MAVs in data set = 8

Rank	MAV	lnMAV	$(\ln MAV)^2$	P=R/(N+1)	$\sqrt{P}$
4	6.4	1.8563	3.4458	0.44444	0.66667
3	6.2	1.8245	3.3290	0.33333	0.57735
2	4.8	1.5686	2.4606	0.22222	0.47140
1	0.4	-0.9163	0.8396	0.11111	0.33333
Sum:		4.3331	10.0750	1.11110	2.04875

$$S^2 = \frac{10.0750 - (4.3331)^2/4}{1.11110 - (2.04875)^2/4} = 87.134$$

$$S = 9.3346$$

$$L = [4.3331 - (9.3346)(2.04875)]/4 = -3.6978$$

$$A = (9.3346)(\sqrt{0.05}) - 3.6978 = -1.6105$$

$$FAV = e^{-1.6105} = 0.1998$$

B. Example computer program in BASIC language for calculating the FAV

```
10 REM THIS PROGRAM CALCULATES THE FAV WHEN THERE ARE LESS THAN
20 REM 59 MAVS IN THE DATA SET.
30 X=0
40 X2=0
50 Y=0
60 Y2=0
70 PRINT "HOW MANY MAVS ARE IN THE DATA SET?"
80 INPUT N
90 PRINT "WHAT ARE THE FOUR LOWEST MAVS?"
100 FOR R=1 TO 4
110 INPUT V
120 X=X+LOG(V)
130 X2=X2+(LOG(V))*(LOG(V))
140 P=R/(N+1)
150 Y2=Y2+P
160 Y=Y+SQR(P)
170 NEXT R
180 S=SQR((X2-X*X/4)/(Y2-Y*Y/4))
190 L=(X-S*Y)/4
200 A=S*SQR(0.05)+L
210 F=EXP(A)
220 PRINT "FAV = "F
230 END
```

C. Example printouts from program

```
HOW MANY MAVS ARE IN THE DATA SET?
? 8
WHAT ARE THE FOUR LOWEST MAVS?
? 6.4
? 6.2
? 4.8
? .4
FAV = 0.1998


HOW MANY MAVS ARE IN THE DATA SET?
? 16
WHAT ARE THE FOUR LOWEST MAVS?
? 6.4
? 6.2
? 4.8
? .4
FAV = 0.4365
```

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.

Species	Chemical	Test Type	Duration (days)	LC50 (mg/L)	SMAV	GMAV	Reference	Notes
Water flea	Sodium tetraborate	SR,M	2	141	154.9	154.9	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
Daphnia magna	Boric acid	S,U	2	226			Lewis and Valentine 1981	pH and hardness not reported, carbon-filtered well water used for dilution water
	Boric acid	S,U	2	133			Gersich 1984	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	~~62.4~~			MELP 1996 (unpublished)	**Study is unattainable,** hardness = 100
	Boric acid	UNK	2	~~139.2~~			MELP 1996 (unpublished)	**Study is unattainable,** hardness = 250
	Boric acid	UNK	2	~~21.3~~			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			Dethloff et al. 2009	Hardness/alkalinity = 290/95 mg/L
	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_2 **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~~			Dethloff et al. 2009	Hardness/alkalinity = 90/188 mg/L, pH = 7.4, CO_2 **introduced to lower pH, elevated control mortality**
	Boric acid	S,M	2	83.8			Dethloff et al. 2009	Low Cl (5.7 mg/L)
	Boric acid	S,M	2	60.9			Dethloff et al. 2009	High Cl (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~~	~~123.4~~	~~123.4~~	Hickey 1989	**24 hour test**
Simocephalus vetulus								
Bluegill	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
Lepomis macrochirus								
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	~~157.3~~			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	~~201.3~~	136.6	136.6	MELP 1996 (unpublished)	**Study is unattainable,** hardness = 100 mg/L
Hyalella azteca	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	S,M	4	170			Soucek and Dickinson 2010	Hardness = 507 mg/L, Geomean pH 8.1, Smith water used, chloride increased to 161 mg/L
	82% boric acid:18% borax	S,M	4	104			Soucek and Dickinson 2010	Hardness = 102 mg/L, pH 6.6, Smith water used (chloride = 34 mg/L)
	82% boric acid:18% borax	S,M	4	127			Soucek and Dickinson 2010	Hardness = 102 mg/L, pH 7.6, Smith water used (chloride = 34 mg/L)
	82% boric acid:18% borax	S,M	4	64			Soucek and Dickinson 2010	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	S,M	4	203			Soucek and Dickinson 2010	Hardness = 291 mg/L, pH 8.1, Borgmann water used (chloride = 72 mg/L)
	82% boric acid:18% borax	S,M	4	188			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 5.6 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	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	UNK	4	~~304.1~~			MELP 1996 (unpublished)	Study is **unattainable,** hardness = 100 mg/L
	Boric acid	UNK	4	~~477.1~~			MELP 1996 (unpublished)	Study is **unattainable,** hardness = 250 mg/L
	Boric acid	UNK	4	~~357.4~~			MELP 1996 (unpublished)	Study is **unattainable,** hardness = 25 mg/L
Chinook salmon	Boric acid	S,U	4	566	640.6		Hamilton and Buhl 1990	Soft water (hardness = 41.7 mg/L, pH = 7.57 in dilution water)
O. tsha wytscha	Boric acid	S,U	4	725			Hamilton and Buhl 1990	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	~~379.6~~			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
	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	F,M	4	137			GLEC 2010	pH 7.75, hardness = 134.2 mg/L
	Boric acid	F,M	4	133			GLEC 2010	pH 8.75, hardness = 113.9 mg/L
Brown planarian	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
Dugesia tigrina								
Colorado Squawfish	Boric acid	S,U	4	279	279.0	279.0	Hamilton 1995	Youngest, most sensitive life stage used (swimup fry, 17-31 days)
Ptychocheilus lucius								Hardness = 196 mg/L, pH= 7.8 in dilution water; results during tests not reported
Razorback Sucker	Boric acid	S,U	4	233	233.0	233.0	Hamilton 1995	Youngest, most sensitive life stage used (swimup fry, 10-17 days)
Xyrauchen texanus								Hardness = 196 mg/L, pH= 7.8 in dilution water; results during tests not reported
Bonytail	Boric acid	S,U	4	280	280.0	280.0	Hamilton 1995	Youngest, most sensitive life stage used (swimup fry, 11-18 days)
Gila elegans								Hardness = 196 mg/L, pH= 7.8 in dilution water; results during tests not reported
Flannelmouth Sucker	Boric acid	S,U	4	125	125.0	125.0	Hamilton and Buhl 1997	13 day old larvae used
Catostomus latipinnis								Geomean pH in treatments 7.72 (range of 6.7-8.9), hardness was 144 mg/L in dilution water
Mosquitofish	Sodium tetraborate	S,U	4	~~408~~	~~632.3~~	~~632.3~~	Wallen et al. 1957	**Turbid water used, potential organics problem,** 3600 Na tetraborate, 11.3% B
Gambusia affinis	Boric acid	S,U	4	~~980~~			Wallen et al. 1957	**Turbid water used, potential organics problem,** 5600 boric acid, 17.5% B
Grooved Fingernail Clam	90% boric acid:10% borax	S,M	4	>447	>447	>447	Soucek and Dickinson 2010	Hardness = 102 mg/L, pH = 7.9
Sphaerium simile								
Winter stonefly	90% boric acid:10% borax	S,M	4	476	476	476	Soucek and Dickinson 2010	Hardness = 98 mg/L, pH = 7.9
Allocapnia vivipara								
Fatmucket	90% boric acid:10% borax	S,M	4	137	137	137	GLEC 2010	Hardness = 90 mg/L, pH = 8.0
Lampsilis siliquoidea								
Black sandshell	82% boric acid:18% borax	S,M	4	147	147	147	GLEC 2010	Hardness = 91 mg/L, pH = 8.1
Ligumia recta								
Washboard	82% boric acid:18% borax	S,M	4	>544	>544	>544	GLEC 2010	Hardness = 88 mg/L, pH = 8.0
Megalonaias nervosa								

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 from 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 rainbow trout growth at concentrations ≥0.123 mg/L, with greatest growth at 10 mg/L B (growth was significantly lower in treatments with <0.123 mg/L boron).

Species	Chemical	Test Type	Duration (days)	Endpoint	NOEC (mg/L)	LOEC (mg/L)	MATC (mg/L)	SMAV	ACR	Reference	Notes
Water flea	Boric acid	SR,M	21	Brood size	6	13	8.83	226	25.6	Lewis and Valentine 1981	pH was 7.1-8.7, hardness averaged 166 mg/L, no pH/hardness reported in acute test
Daphnia magna	Boric acid	SR,M	21	Brood size	6.4	13.6	9.33	133	14.3	Gersich 1984	pH and hardness not reported in chronic test, same dilution water used in acute test
	Boric acid	SR,UNK	UNK	UNK	~~13.1~~	~~25.4~~	~~18.2~~	~~52.4~~	~~2.9~~	MELP 1996 (unpublished)	Study is unattainable, 100 mg/L hardness
	Boric acid	SR,UNK	UNK	UNK	~~12.4~~	~~26.4~~	~~18.1~~	~~139.2~~	~~7.7~~	MELP 1996 (unpublished)	Study is unattainable, 250 mg/L hardness
	Boric acid	SR,M	21	Brood size	~~10~~	~~16~~	~~12.6~~	NA	NA	Hooftman et al 2000	No corresponding acute test available for ACR determination
				GeoMeans (valid data):				9.1	173.4	19.1	
Rainbow trout	Boric acid	F,M	28	Survival	~~23.5~~	~~45.5~~	~~32.7~~	-	-	Birge and Black 1977	50 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable
Oncorhynchus mykiss	Boric acid	F,M	28	Survival	~~9.5~~	~~1~~	~~9.71~~	-	-	Birge and Black 1977	200 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable
	Borax	F,M	28	Survival	~~9.7~~	~~22.6~~	~~14.84~~	-	-	Birge and Black 1977	50 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable
	Borax	F,M	28	Survival	~~9.63~~	~~49.7~~	~~21.88~~	-	-	Birge and Black 1977	200 mg/L hardness, no corresponding acute test, Birge data deemed unacceptable
	Boric acid	F,M	87	Growth/survival	~~18~~	~~>18~~	~~>18.0~~	-	-	Black et al. 1993	Well water used (hardness = 38.5 mg/L), no corresponding acute test
Fathead minnow	Boric acid	SR,M	7	Growth	~~11~~	~~18~~	~~14.1~~	75.9	~~5.4~~	Sanders and Associates 2007	7 day ELS test not sufficient for ACR determination
Pimephales promelas	90% boric acid:10% borax	SR,M	32	Growth/survival	11.2	23	16.0	79.7	5.0	Soucek and Dickinson 2010	
	Boric acid	F,M	32	Survival	12.9	27.4	18.8	101	5.4	GLEC 2010	pH = 8.2, hardness = 129.8 mg/L, GLEC acute test at pH 8.15 used for ACR determination
				GeoMeans (valid data):				17.4	89.7	5.2	
Amphipod	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
Hyalella azteca											
Protozoan	Boric acid	S,U	2	EC50	-	~~63.7~~	-	-	-	Nalecz-Jawecki and Sawicki 1998	Unicellular organism, chronic test (1985 guidelines), no acute test for ACR determination
Spirostomum ambiguum	Boric acid	S,U	2	LC50	-	~~852~~	-	-	-	Nalecz-Jawecki and Sawicki 1998	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 Type	Duration (days)	Endpoint	NOEC (mg/L)	LOEC (mg/L)	MATC (mg/L)	EC/IC50 (mg/L)	ACR	Reference	Notes
Greater duckweed	Boric acid	SR,M	10	Growth rate	~~6.1~~	~~18.9~~	~~10.7~~	~~11.7~~	-	Davis et al. 2002	pH ranged from 5.2-5.8
Spirodella polyrhiza	Boric acid	SR,M	10	Frond number	~~0.9~~	~~3.6~~	~~1.8~~	~~14.3~~	-	Davis et al. 2002	pH ranged from 5.2-5.8, LOEC is misleading, EC20 = ~9 mg/L
	Boric acid	SR,M	10	Abnormalities	~~18.9~~	~~22.4~~	~~20.6~~	~~17.7~~	-	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	S,M	7	Weight	~~5-10~~	~~10-20~~	~~UNK~~	-	-	Frick 1985	Unclear methods, results not quantified, pH was 5
	Boric acid	S,M	7	Weight	~~20~~	~~50~~	~~31.6~~	-	-	Frick 1985	Unclear methods, results not quantified, pH was 5
Lemna gibba	Boric acid	S,M	12	Weight	~~>10~~	~~>10~~	~~>10~~	-	-	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~~	~~61.3~~	-	-	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	~~50~~	~~75~~	~~61.3~~	-	-	Martinez et al. 1986	No pH or hardness measurements, no acute tests for ACR development
Green Algae	Boric acid	S,U	4	Growth	~~10~~	~~>10~~	~~>10~~	~~>10~~	-	Fernandez et al. 1984	B unmeasured, no pH or hardness reported, no effect on cell composition at 10 mg/L
Chlorella pyrenoidosa											
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

Boron Standard Derivation Using 1985 Guidelines Methodology

This spreadsheet calculates the FAV when there are less than 59 GMAV's

Number of MAV's in data set		17
List of lowest MAV's	1	84.8
	2	96.1
	3	125
	4	136.6
Number of MAV's entered		4

FAV= **80.1**

Acute Standard = **40.1 mg/L**

	GMAV (mg/L)
Chironomus	1513
Dugesia	1358
Megalonaias	>544
Allocapnia	476
Sphaerium	>447
Oncoryhnchus	>360.8
Gila	280
Ptychocheilus	279
Lumbriculus	267.4
Xyrauchen	233
Lepomis	>201.1
Daphnia	154.9
Ligumia	147
Lampsilis	137
Hyalella	136.6
Catostomus	125
Pimephales	96.1
Ceriodaphnia	84.8

1985 Guidelines General Use Boron CATC Calculation

Chronic Standard = FAV / Geomean of all ACRs (FACR)

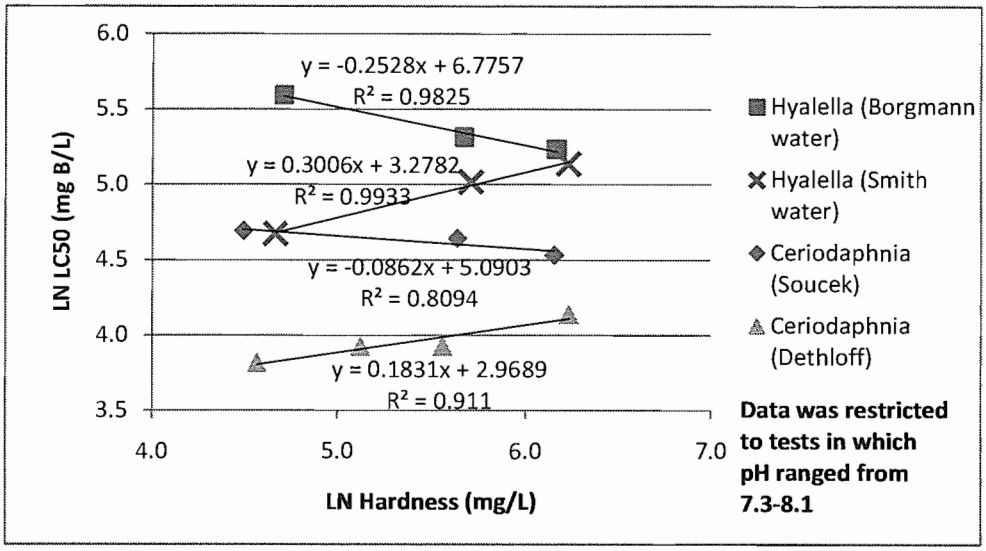
Chronic Standard = 80.1 / Geomean of 19.1, 5.2, and 11.6

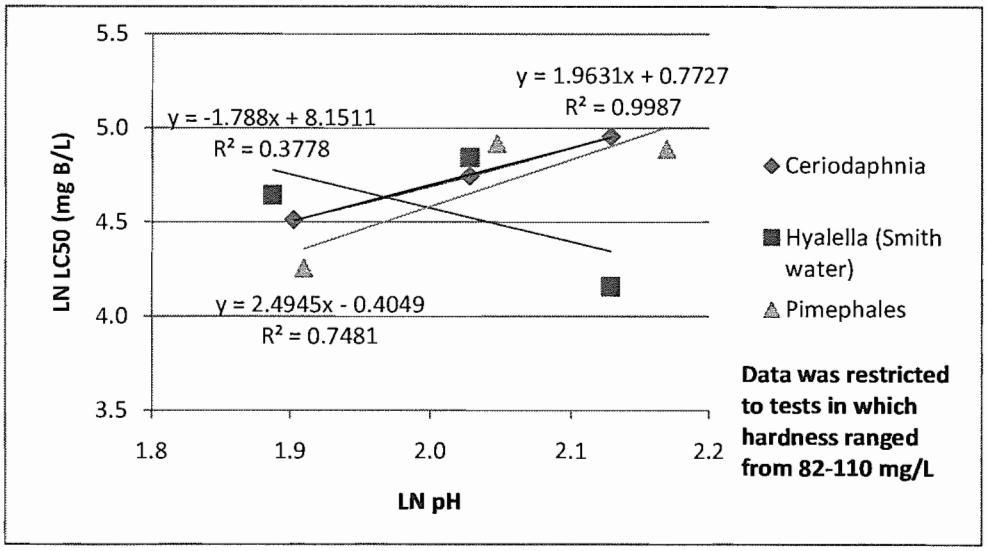
Chronic Standard = 80.1 / 10.5 = **7.6 mg/L**

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	LC50 (mg/L)	Hardness (mg/L)	Geometric Mean LC50 W	GeoMean Hardness X	LN (LC50 / GeoMean LC50)	LN (Hardness / GeoMean Hardness)	Slope	R squared	
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			
	114	70			-0.595694237	-0.667097416			
Pimephales promelas	112.2	67	144.9	118.7	-0.255886289	-0.572208025	0.388007883	0.936861966	
	190	260			0.27085479	0.783780987			
	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	186			0.014353519	0.230343066			
	182	117			0.025403355	-0.233229672			
	122	76			-0.374582287	-0.664670267			
Gasterosteus aculeatus	340	78	390.2	150.6	-0.137835502	-0.657990481	~~0.225405768~~	0.987652284	*Low slope, precipitation may have occurred w/ increased hardness
	380	146			-0.026609867	-0.031092686			
	460	300			0.16444537	0.689083167			
Actinonaias pectorosa	259	28	277.8	48.5	-0.070132712	-0.549306144	~~0.127675092~~	1	*Low slope, poor relationship w/ hardness across all four tests
	298	84			0.070132712	0.549306144			
Hyallela azteca	25.8	112	25.8	112.0	0	0			
Lepomis macrochirus	375.6	40	375.6	40.0	0	0			
Ceratopsyche bronta	17	40.2	17.0	40.2	0	0			
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	17.5	48.2	17.5	0	0			
Hydropsyche pellucidula	38.5	18.2	38.5	18.2	0	0			
Chimarra marginata	44.9	12.6	44.9	12.6	0	0			
Cheumatopsyche pettiti	42.5	40.2	42.5	40.2	0	0			
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	145	124.1	145.0	0	0			
Brachionus calyciflorus	183.33	90	183.3	90.0	0	0			
Physa sp.	163.1	36.1	163.1	36.1	0	0			
Lumbriculus variegatus	93.5	49.5	93.5	49.5	0	0			
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	0			
					Pooled Slope (V) =	**0.539423386**	*Calculated from Ceriodaphnia, Daphnia, and Pimephales data		
					R squared =	**0.86**			

	ln W	V	ln X	Z	LN Z	Y	SMAV	GMAV	GMAV Rank
Hyallela azteca	3.250374492	0.539423	4.718498871	50	3.912023005	2.815342549	16.70	16.70	1
Hexagenia limbata	3.47506723	0.539423	4.976733742	50	3.912023005	2.900737359	18.19	18.19	**2**
Ceratopsyche bronta	2.833213344	0.539423	3.693866996	50	3.912023005	2.950891798	19.12	19.12	**3**
Sphaerium simile	4.130355	0.539423	4.564348191	50	3.912023005	3.778475539	43.75	43.75	4
Cheumatopsyche pettiti	3.749504076	0.539423	3.693866996	50	3.912023005	3.867182529	47.81	47.81	
Hydropsyche occidentalis	3.546739687	0.539423	3.693866996	50	3.912023005	3.66441814	39.03	56.57	
Hydropsyche bulbifera	3.269568939	0.539423	2.827313622	50	3.912023005	3.854686548	47.21	56.57	
Hydropsyche exocellata	3.277144733	0.539423	2.533696814	50	3.912023005	4.020646115	55.74	56.57	
Hydropsyche lobata	3.875359021	0.539423	2.862200881	50	3.912023005	4.441657626	84.92	56.57	
Hydropsyche pellucidula	3.650658241	0.539423	2.901421594	50	3.912023005	4.195800277	66.41	56.57	
Chironomus tentans	4.821087692	0.539423	4.976733742	50	3.912023005	4.246757821	69.88	69.88	
Simocephalus vetulus	5.305789381	0.539423	5.521460918	50	3.912023005	4.437620933	84.57	84.57	
Pimephales promelas	4.976169282	0.539423	4.776900644	50	3.912023005	4.509634057	90.89	90.89	
Lumbriculus variegatus	4.537961436	0.539423	3.90197267	50	3.912023005	4.543382822	94.01	94.01	
Chimarra marginata	3.804437795	0.539423	2.533696814	50	3.912023005	4.547939176	94.44	94.44	
Ceriodaphnia dubia	5.178603332	0.539423	4.995403607	50	3.912023005	4.594202499	98.91	98.91	
Daphnia magna	5.331892685	0.539423	4.915592658	50	3.912023005	4.790543745	120.37	120.37	
Brachionus calyciflorus	5.211287808	0.539423	4.49980967	50	3.912023005	4.894221934	133.52	133.52	
Physa sp.	5.09436351	0.539423	3.586292865	50	3.912023005	5.270069965	194.43	194.43	
Gasterosteus aculeatus	5.96678112	0.539423	5.014699308	50	3.912023005	5.371971735	215.29	215.29	
Lampsilis fasciola	5.147494477	0.539423	3.465735903	50	3.912023005	5.388232177	218.82	218.82	
Philodina acuticornis	5.356586275	0.539423	3.688879454	50	3.912023005	5.476955125	239.12	239.12	
Actinonaias pectorosa	5.626960774	0.539423	3.881510655	50	3.912023005	5.64341985	282.43	282.43	
Utterbackia imbecillis	5.455321115	0.539423	3.526360525	50	3.912023005	5.663356477	288.11	288.11	
Alasmidonta raveneliana	5.713732806	0.539423	3.33220451	50	3.912023005	6.026500462	414.26	414.26	
Lepomis macrochirus	5.928524747	0.539423	3.688879454	50	3.912023005	6.048893597	423.64	423.64	

Attachment 1 – Exhibit L

Manganese Standard Derivation Using 1985 Guidelines methodology

Manganese Standard Derivation Using 1985 Guidelines Methodology

Species	LC50 (mg/L)	Hardness (mg/L)	Geometric Mean LC50 W	GeoMean Hardness (mg/L) X	LN (LC50 / GeoMean LC50)	LN (Hardness / GeoMean Hardness)	Slope	R squared
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	92			0.508045284	0.275627928		
	27.44	176			0.970555196	0.924323346		
	8.56	28			-0.194696946	-0.913956138		
Hyalella 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	184			0.086931347	0.925049007		
	9.44	25			-0.342261322	-1.071010926		
	11.20	50			-0.171303524	-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			0.588722716	0.967972066		
Chironomus tentans	42.20	100	63.0829612	164.924225	-0.402030484	-0.50031594	0.803553219	1
	94.30	272			0.402030484	0.50031594		
Pytchocheilus oregonensis	189.48	347	189.482	347	0	0		
Anodonta imbecillus	36.20	80	36.2	80	0	0		
Agosia chrysogaster	130.00	224	130	224	0	0		
Bufo 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**		

	ln W	V	ln X	Z	LN Z	Y	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
Megalonaias nervosa	3.449987546	0.746724	4.51085951	50	3.912023005	3.002822083	20.14230004	20.14230004	
Lampsilis siliquoidea	3.768152635	0.746724	4.51085951	50	3.912023005	3.320987173	27.68766958	27.68766958	
Anodonta imbecillus	3.589059119	0.746724	4.38202663	50	3.912023005	3.238096227	25.48515758	25.48515758	
Chironomus lentans	4.144450705	0.746724	5.10548613	50	3.912023005	3.253263399	25.87464157	25.87464157	
Bufo boreus	3.744787086	0.746724	3.96271612	50	3.912023005	3.706933332	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	0.746724	5.08759634	50	3.912023005	4.113420007	61.15551258	61.15551258	

Calculation of Chronic Intercept Based on MATC of *Hyalella azteca*

W (chronic MATC) = 2.01 mg/L, X (test hardness) = 115 mg/L

	ln W	V	ln X	Z	LN Z	Y	MATC
Hyalella azteca (chronic)	0.698134722	0.746724	4.74493213	50	3.912023005	0.076181664	1.079158601

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	16.7
	2	18.19
	3	19.12
	4	43.75
Number of MAV's entered		4

FAV at 50 mg/L hardness = **13.85 mg/L**

FAV / 2 = **6.92 mg/L**

Acute standards at variable hardness = $e^{(V(LN(hardness)) + LN(acute\ standard\ at\ 50\ hardness) - (V(LNZ))}$

Acute standard at 100 mg/L hardness =	**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 = FAV (at 50 mg/L hardness) / FACR (geometric mean of 7.36, 9.88, 1.19, and 2.9 from available ACRs)

= 13.85 / 3.98

= **3.48 mg/L**

Chronic standards at variable hardness = $e^{(V(LN(hardness)) + LN(chronic\ standard\ at\ 50\ hardness) - (V(LNZ))}$

Chronic standard at 100 mg/L hardness =	**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 = **5.07 mg/L**

FAV / 2 = **2.54 mg/L**

Acute standards at variable hardness = $e^{(V(LN(hardness)) + LN(acute\ standard\ at\ 50\ hardness) - (V(LNZ))}$

Acute standard at 100 mg/L hardness =	**4.26**	Equation = EXP((0.746723791250639*(LN(100)))+(LN(2.54)-(0.746723791250639*(3.912023005))))
Acute standard at 200 mg/L hardness =	**7.15**	Equation = EXP((0.746723791250639*(LN(200)))+(LN(2.54)-(0.746723791250639*(3.912023005))))
Acute standard at 300 mg/L hardness =	**9.68**	Equation = EXP((0.746723791250639*(LN(300)))+(LN(2.54)-(0.746723791250639*(3.912023005))))

35 Ill. Adm. Code 302.627(d): 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)

= 5.07 / 3.34

= **1.52 mg/L**

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 = $e^{(V(LN(hardness)) + LN(Hyalella\ MATC\ at\ 50\ hardness) - (V(LNZ))}$

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.

Species	Chemical	Test Type	Duration (hours)	LC50 (mg/L)	Hardness (mg/L)	Reference	Notes
Water flea	Na F	S	48	~~120~~	UNK	OPP 2000	**Can't access the data, no hardness**
Daphnia magna	Na F	S,U	48	~~98~~	250	Dave 1984	**Precipitate and pH problems**
	Na F	S,U	48	~~454~~	173	LeBlanc 1980	**Unmeasured, poor relationship with other data**
	Na F	S,U	48	~~282.8~~	145	Metcalfe-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	S,U	48	~~284~~	169	Feiser et al. 1986	Publication reports the total (nominal) fluoride in the modhard water (table 3), **data listed for comparison only**
	Na F	S,U	24	~~352~~	UNK	Kuhn et al. 1989	**24 hour test, hardness and fluoride not measured, not used because 48 hour tests are available**
	Na F	S,U	24	~~319.8~~	250	Hickey 1989	**24 hour test (D. carinata also available), not used because 48 hour tests are available**
	Na F	S,U	24	~~292.3~~	90	Calleja et al. 1994	**24 hour test, not used because 48 hour tests are available**
	Na F	S,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	~~220.8~~	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	Fieser 1985	Results are in ionic fluoride, total (nominal) fluoride was added but only ionic was measured, temp=20
	Na F	S,U	24	~~157.9~~	250	Hickey 1989	**24 hour test, not used because 48 hour tests are available**
Water flea	Na F	S,U	24	201.5	250	Hickey 1989	Native species, similar tolerance to other Daphnidae
Simocephalus vetulus							
Mosquitofish	Na F	S,U	96	~~418~~	UNK	Wallen et al. 1957	Turbid water, no hardness, **turbidity decreased through test, unmeasured, possible precipitate**
Gambusia affinis							
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**
Gasterosteus 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/precipitate problems, **not used for slope derivation**
Bluegill	Na F	SR,M	96	375.6	40	OPP 2000 (43648201)	
Lepomis macrochirus							
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 Bulkley 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	temp=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	~~317~~	UNK	OPP 2000	**Can't access the data, no hardness**
Brown trout	Na F	S,M	96	164.5	21.2	Camargo and Tarazona 1991	
Salmo trutta							
Fathead minnow	Na F	S,M	96	~~315~~	20-48	Smith et al. 1985	**Major calcium fluoride precipitation occurred, see hardness data**
Pimephales promelas	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	~~225.1~~	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
Midge	Na F	S,U	96	124.1	145	Metcalffe-Smith et al. 2003	Organisms were fed on the day of the test
Chironomus 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
	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
Amphipod	Na F	S,U	48	~~14.6~~	145	Metcalffe-Smith et al. 2003	**Test was only 48 hours**, supplemental tests are needed
Hyalella azteca	Na F	S,M	96	~~13.4~~	82.4	GLEC 2010	**Improper lab water used, chloride too low**
	Na F	S,M	96	25.8	112	Soucek and Dickinson 2010	Correct lab water used for Hyalella testing, higher chloride, bromide present
Wavyrayed lampmussel *Lampsilis fasciola*	Na F	S,M	96	172	32	Keller and Augspurger 2005	Only juveniles were tested, data reported as ionic fluoride
Paper pondshell *Ulterbackia imbecillis*	Na F	S,M	96	234	34	Keller and Augspurger 2005	Results are from juveniles, glochidia were more tolerant, data reported as ionic fluoride
Appalachian elktoe *Alasmidonta raveneliana*	Na F	S,M	96	303	28	Keller and Augspurger 2005	Juvenile test, glochidia were equally tolerant, data reported as ionic fluoride
Pheasantshell	Na F	S,M	96	259	28	Keller and Augspurger 2005	Only juveniles were tested, data reported as ionic fluoride
Actinonaias pectorosa	Na F	S,M	96	~~178~~	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 limits uncalculable**
	Na F	S,M	96	298	84	Keller and Augspurger 2005	Only juveniles were tested, data reported as ionic fluoride
Net-spinning caddisfly *Ceratopsyche bronta*	Na F	S,M	96	17	40.2	Camargo et al. 1992 (#3882)	Author states no preciptate formed, species native to Illinois
Net-spinning caddisfly							
Hydropsyche occidentalis	Na F	S,M	96	34.7	40.2	Camargo et al. 1992 (#3882)	Native genera
H. bulbifera	Na F	S,M	96	26.3	16.9	Camargo and Tarazona 1990	Chloride = 7.4 mg/L, native genera
H. exocellata	Na F	S,M	96	26.5	12.6	Camargo and Tarazona 1990	Chloride = 6.3 mg/L, native genera
H. lobata	Na F	S,M	96	48.2	17.5	Camargo and Tarazona 1990	Chloride = 10.7 mg/L, native genera
H. pellucidula	Na F	S,M	96	38.5	18.2	Camargo and Tarazona 1990	Chloride = 5.1 mg/L, native genera
Net-spinning caddisfly *Chimarra marginata*	Na F	S,M	96	44.9	12.6	Camargo and Tarazona 1990	Chloride = 6.3 mg/L, native genera
Net-spinning caddisfly *Cheumatopsyche pettiti*	Na F	S,M	96	42.5	40.2	Camargo et al. 1992 (#3882)	Native genera
Rotifier *Brachionus calyciflorus*	Na F	S,U	24	183.3	90	Calleja et al. 1994	Native genera, shows tolerance in differnet phylum
Snail	Na F	R,M	96	~~231.7~~	45.5	The Advent Group, Inc., 2000	**Dose response curve was irregular, hardness increased for unknown reason**
Physa sp.	Na F	R,M	96	163.1	36.1	The Advent Group, Inc., 2000	good dose-response, ion measured
Annelid *Lumbriculus variegatus*	Na F	R,M	96	93.5	49.5	Advent 2000	Other tests had problems with nominal vs. measured concentrations, ion measured
Mayfly *Hexagenia limbata*	Na F	S,U	96	32.3	145	Metcalffe-Smith et al. 2003	Native, organims were fed but showed sensitivity
Rotifer *Philodina acuticornis*	Na F	S,M	96	212	40	Buikema et al. 1977	Native genera, hardness not measured, nominal was 40 mg/L
Grooved fingernailclam *Sphaerium simile*	Na F	S,M	96	62.2	96	GLEC 2010	

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.

Species	Chemical	Test Type	Duration (days)	NOEC (ug/L)	LOEC (ug/L)	MATC/IC25 (ug/L)	Endpoint	Hardness (mg/L)	LC50 @ H	ACR	Reference (ECOTOX#)	Notes
Water flea	Na Fl	SR,U	21	~~3.7~~	~~7.4~~	~~5.23~~	MATC Rep	250	98	~~18.73~~	Dave 1984	**Precipitate formed at ≥7.4 mg/L fluoride, acute test had precipate at ≥30 mg/L fluoride**
Daphnia magna	Na Fl	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 Fl	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 Fl	SR,M	21			~~28.5~~	ENP	114	187	~~6.56~~	Fieser 1985	**ENP = Equivalent neonate production (to controls), use MATC instead**
	Na Fl	SR,M	21			~~31.6~~	ENP	170	251	~~7.07~~	Fieser 1985	**ENP = Equivalent neonate production (to controls), use MATC instead**
	Na Fl	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 Daphnia ACRs (valid data):			7.36		
Water flea	Na Fl	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 Fl	SR,M	7	16.2	26.2	20.60		189	174	8.45	Fieser 1985	Summary at table 43
	Na Fl	SR,M	7	~~9~~	~~6~~	~~<6~~		124	146	~~NA~~	Fieser 1985	**All treatments were sig different from control, unusual given the hardness treatment, see table 34**
	Na Fl	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
							Geomean Ceriodaphnia ACRs (valid data):			9.88		
Fathead minnow	Na Fl	R,U	7	~~125~~	~~250~~	~~176.78~~	Survival	280	145		Metcalffe-Smith et al. 2003	**Duration too short**
Pimephales promelas	Na Fl	R,U	7	~~63~~	~~125~~	~~88.74~~	Survival	160	145		Metcalffe-Smith et al. 2003	**Duration too short**
	Na Fl	F,M	28	66.6	134.3	94.57	Surv/Growth	67	112.2	1.19	The Advent Group, Inc., 2000	IC25 estimate used for chronic value
	Na Fl	F,M	28			84.1	IC25 Biomass	67	112.2	~~1.33~~	The Advent Group, Inc., 2000	EPA prefers MATC over IC25
									Geomean Pimephales ACRs:	1.19		
Rainbow trout *Oncorhynchus mykiss*	Na Fl	S,M	8			~~27.6~~	MATC Surviva	22.4			Camargo and Tarazona 1991	**Duration too short**
Wavyrayed lampmussel *Lampsilis fasciola*	Na Fl	S,M	9			~~177~~	Survival	32			Keller and Augspurger 2005	**Duration too short**
Long fingernailclam *Musculium transversum*	Na Fl	S,M	56			~~2.8~~	LC50	-			Sparks 1983	**High control mortality (25%), no hardness, no acute test**
Amphipod *Hyalella azteca*	Na Fl	SR,M		6.7	11.7	8.9	Reproduction	114	25.8	2.9	Soucek and Dickinson 2010	

Attachment 1 – Exhibit Q

Acute toxicity used in manganese Standards Derivation

Acute 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.

** GMAVs are not listed because values must be hardness-normalized. See associated derivation worksheet.

*** 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). (acute rainbow trout study). Soft-water data is being excluded from analysis.

Species	Chemical	Test Type	Duration (hours)	LC50 (mg/L)	Hardness (mg/L)	Reference	Notes
Rainbow trout	$MnSO_4$	F,M	96	3.17	27.6	Davies et al. 1998b	Fry
Oncorhynchus mykiss	$MnSO_4$	F,M	96	16.20	147.8	Davies et al. 1998b	Fry
	$MnCl_2$	S,U	96	~~2.10~~	47.6	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**
	$MnCl_2$	S,U	96	~~20.70~~	100	Reimer 1999	**Mn and hardness were unmeasured, use Davies data**
	$MnCl_2$	S,M	96	~~12.70~~	259	Reimer 1999	**Hardness and Mn measured on Day 0, nominal Mn was 19.1 and measured was 12.7 mg/L, not measured on day 4**
	$MnSO_4$	F,M	96	4.83	34	Davies and Brinkman 1994	42 mm fish, ELS/fingerling stage
Brown trout	$MnSO_4$	F,M	96	3.77	38	Davies and Brinkman 1994	Juveniles were tested
Salmo trutta	$MnSO_4$	F,M	96	49.90	454	Davies and Brinkman 1995	Includes corrections to 1994 study, Table 22 hardnesses were wrong
Brook trout	$MnSO_4$	F,M	96	5.12	31.3	Davies et al. 1998b	ELS, 37 mm organisms
Salvelinus fontinalis	$MnSO_4$	F,M	96	27.50	148.1	Davies et al. 1998b	ELS, 37 mm organisms
	$MnSO_4$	F,M	96	~~269.00~~	5	Gonzalez et al. 1990	**Yearlings were used, size unkown, results a factor higher than others**
	$MnSO_4$	F,M	96	~~291.00~~	25	Gonzalez et al. 1990	**Yearlings were used, size unkown, results a factor higher than others**
	$MnSO_4$	F,M	96	~~319.00~~	100	Gonzalez et al. 1990	**Yearlings were used, size unkown, results a factor higher than others**
	$MnSO_4$	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	$MnCl_2$	S,M	96	~~2.40~~	25.2	Reimer 1999	**Mn measured only on day 0, soft-water results not trustworthy**
O. kisutch	$MnCl_2$	S,M	96	13.10	100	Reimer 1999	Mn measured on Day 0 and 4, hardness was not measured
	$MnCl_2$	S,M	96	~~17.40~~	250	Reimer 1999	**Mn measured only on day 0, data not needed for slope derivation (other valid Salmonid data exists). Hardness measured**
Amphipod	$MnCl_2$	S,M	96	3.00	26	Lasier et al. 2000	Chloride = 9 mg/L
Hyalella azteca	$MnCl_2$	S,M	96	8.56	80	Lasier et al. 2000	Chloride = 2 mg/L, geomean of three replicates
	$MnCl_2$	S,M	96	13.70	164	Lasier et al. 2000	Chloride = 2 mg/L
	$MnCl_2$	S,M	96	~~3.60~~	25	Reimer 1999	**Hardness not measured, soft-water data not trustworthy due to control issues in other tests**
	$MnCl_2$	S,M	96	~~22.20~~	100	Reimer 1999	**Hardness not measured, not used for slope derivation because other valid data exists**
	$MnCl_2$	S,M	96	31.00	269	Reimer 1999	Mn and hardness measured
	UNK	UNK	UNK	~~6.63~~	96	ENSR 1996b	Unpublished in-house data used in Colorado standard, can't get the data for review
	UNK	UNK	UNK	~~10.17~~	94	ENSR 1996b	Used in Colorado standard, unpublished, can't get the data for review
	44% $MnSO_4$/56%$MnCl_2$	S,M	96	11.00	112	Soucek and Dickinson 2010	Dilution water hardness = 112 mg/L, hardness increases with higher Mn concentrations
Crangonyx pseudogracilis	$MnCl_2$	S,U	96	~~694.00~~	50	Martin and Holdich 1986	**Results seem artificially high, factor higher than other genera**
Water flea	$MnCl_2$	S,M	48	5.70	26	Lasier et al. 2000	Chloride = 9 mg/L
Ceriodaphnia dubia	$MnCl_2$	S,M	48	14.50	92	Lasier et al. 2000	Chloride = 2 mg/L
	$MnCl_2$	S,M	48	14.50	184	Lasier et al. 2000	Chloride = 2 mg/L
	$MnCl_2$	R,M	48	9.44	25	ENSR 1992b	total manganese
	$MnCl_2$	R,M	48	11.20	50	ENSR 1992b	total manganese
	$MnCl_2$	R,M	48	21.20	100	ENSR 1992b	total manganese
	$MnCl_2$	R,M	48	27.30	200	ENSR 1992b	total manganese
	$MnCl_2$	R,M	48	~~8.76~~	26	ENSR 1992b	**acid-soluble result (nitric acid added), use total**
	$MnCl_2$	R,M	48	~~12.51~~	50	ENSR 1992b	**acid-soluble result (nitric acid added), use total**
	$MnCl_2$	R,M	48	~~20.50~~	100	ENSR 1992b	**acid-soluble result (nitric acid added), use total**
	$MnCl_2$	R,M	48	~~25.48~~	200	ENSR 1992b	**acid-soluble result (nitric acid added), use total**
	UNK	UNK	UNK	~~15.64~~	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	~~>45.0~~	396	ENSR 1990	Unpublished in-house data used in Colorado standard, can't get the data for review
Water flea	$MnCl_2$	S,M	48	~~0.80~~	26.3	Reimer 1999	**Well water diluted with deionized water, chronic study had excess control mortality in soft water**
Daphnia magna	$MnCl_2$	S,M	48	28.70	100	Reimer 1999	Mn measured, hardness was nominal

	$MnCl_2$	S,M	48	76.30	267	Reimer 1999	Mn and hardness measured
	$MnCl_2$	S,M	48	9.80	45.3	Biesinger and Christensen 1972	Lake Superior water used
	$MnCl_2$	S,M	48	~~22.60~~	UNK	Baird et al. 1991	Reported as ionic, hard water used but concentration unknown
	$MnSO_4$	S,M	48	~~10.40~~	UNK	Kimball 1978	Hardness unknown
Midge	$MnCl_2$	S,M	96	~~6.80~~	27.2	Reimer 1999	Soft-water results deemed unacceptable due to control problems in other tests
Chironomus tentans	$MnCl_2$	S,M	96	42.20	100	Reimer 1999	Mn measured on day 0, hardness not measured
	$MnCl_2$	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	$MnCl_2$	SR,M	96	3.54	26	ENSR 1992a	Used in slope derivation by Colorado
Pimephales promelas	$MnCl_2$	SR,M	96	6.23	50	ENSR 1992a	Used in slope derivation by Colorado
	$MnCl_2$	SR,M	96	9.35	100	ENSR 1992a	Used in slope derivation by Colorado
	$MnCl_2$	SR,M	96	15.83	200	ENSR 1992a	Used in slope derivation by Colorado
	$MnCl_2$	SR,M	96	10.30	48	ENSR 1990	Used in slope derivation by Colorado
	$MnCl_2$	SR,M	96	17.28	92	ENSR 1990	Used in slope derivation by Colorado
	$MnCl_2$	SR,M	96	27.44	176	ENSR 1990	Used in slope derivation by Colorado
	$MnCl_2$	SR,M	96	~~>45.0~~	396	ENSR 1990	> data not suitable for slope
	$MnCl_2$	F,M	96	8.56	28	ENSR 1996e	Used in slope derivation by Colorado
	$MnSO_4$	F,M	96	~~33.60~~	UNK	Kimball 1978	Animals were fed, hardness not measured
Paper pond shell *Anodonta imbecillus*	$MnSO_4$	S,R	216	36.20	80	Wade et al. 1989	USEPA tentatively approved this study, tolerant species, fed daily
Rotifer *Brachionus calyciflorus*	$MnCl_2$	S,U	24	38.70	36.2	Couillard et al. 1989	Native, shows tolerance from different phylum
Tubificid worm	$MnSO_4$	S,U	96	26.80	12	Rathore and Khangarot 2003	
Tubifex tubifex	$MnSO_4$	S,U	96	42.70	45	Rathore and Khangarot 2003	
	$MnSO_4$	S,U	96	85.90	173	Rathore and Khangarot 2003	
	$MnSO_4$	S,U	96	464.75	305	Rathore and Khangarot 2003	
	$MnSO_4$	S,U	96	171.61	245	Khangarot 1991	
Western toad	$MnSO_4$	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 *Asellus aquaticus*	$MnCl_2$	S,U	96	~~333.00~~	50	Martin and Holdich 1986	Results seem artificially high, factor higher than other genera, Genus is now Caecidotea
Nematode *Caenorhabditis elegans*	$MnCl_2$	S,M	24	~~5379.90~~	0.1	Tatara et al. 1997	Hardness not reported, test performed in K-agar
Longfin dace *Agosia chrysogaster*	$MnSO_4$	SR,M	96	130.00	224	Lewis 1978	Native genus, juveniles tested
Northern squawfish	$MnSO_4$	S,U	96	~~130.47~~	316	Beleau and Bartosz 1982	Poor confidence limits, older organisms (juveniles)
Ptychocheilus oregonensis	$MnSO_4$	S,U	96	189.48	347	Beleau and Bartosz 1982	post-larval
Snail *Physa integra*	$MnCl_2$	S,U	96	147.12	162	Harding ESE, Inc. 2001	Unpublished data from Michigan
Washboard mussel *Megalonaias nervosa*	44% $MnSO_4$/56%$MnCl_2$	S,M	96	31.50	91	GLEC 2010	
Fatmucket *Lampsilis siliquoidea*	44% $MnSO_4$/56%$MnCl_2$	S,M	96	43.30	91	GLEC 2010	

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.

Species	Chemical	Test Type	Duration (days)	Endpoint	Hardness (mg/L)	NOEC (mg/L)	LOEC (mg/L)	MATC or other endpoint (mg/L)	LC50 at Hardness	ACR	Reference	Notes
Rainbow trout	$MnSO_4$	F,M	65	L,W	29.2	0.76	1.47	1.06	3.17	3.00	Davies et al. 1998b	ELS, fry had very similar results
Oncorhynchus mykiss	$MnSO_4$	F,M	65	L,W	t51.2	3.39	~~6.48~~	4.69	16.2	3.46	Davies et al. 1998b	ELS, LC50s are from fry, no ELS acute tests conducted
	$MnSO_4$	F,M	31	L,W	27.6	~~0.74~~	~~1.42~~	~~1.03~~	3.17	~~3.09~~	Davies et al. t998b	Fry, use longer ELS tests. LC50 is from fry test, no acute ELS tests
	$MnSO_4$	F,M	44	L,W	147.8	~~3.34~~	~~6.46~~	~~4.65~~	16.2	~~3.40~~	Davies et al. 1998b	Fry, use longer ELS tests. LC50 is from fry test, no acute ELS tests
	$MnSO_4$	F,M	65	IC25 L,W	29.2	-	-	~~1.01~~	3.17	~~3.14~~	Davies et al. t998b	ELS, MATC or EC20 preferred over IC25
	$MnSO_4$	F,M	65	IC25 L,W	151.2	-	-	~~4.80~~	16.2	~~3.38~~	Davies et al. 1998b	ELS, MATC or EC20 preferred over IC25
	$MnSO_4$	F,M	65	EC20 L,W	29.2	-	-	~~1.40~~	3.17	~~2.27~~	Davies et al. 1998b	ELS, MATC is preferred method
	$MnSO_4$	F,M	65	EC10 L,W	29.2	-	-	~~1.39~~	3.17	~~2.64~~	Davies et al. 1998b	ELS, MATC is preferred method
	$MnSO_4$	F,M	65	EC20 L,W	151.2	-	-	~~4.26~~	16.2	~~3.80~~	Davies et al. 1998b	ELS, MATC is preferred method
	$MnSO_4$	F,M	65	EC10 L,W	151.2	-	-	~~3.48~~	16.2	~~4.66~~	Davies et al. 1998b	ELS, MATC is preferred method
	$MnCl_2$	SR,M	28	LC50	104	-	-	~~2.91~~	-	-	Birge et al. t978	ELS, NOEC/LOEC not reported, no corresponding acute, longer tests available for ACR development
	$MnSO_4$	F,M	120	Survival	36.8	~~1.04~~	~~1.06~~	~~1.43~~	4.83	~~3.38~~	Davies and Brinkman t994	Used growth instead (Davies 98), used diff N/LOEC because mort was 10% + acclimated fish were more tolerant (table 24)
	$MnCl_2$	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
	$MnCl_2$	S,M	7	Rep EC50	100	-	-	~~20.00~~	-	-	Reimer 1999	Duration too short
	$MnCl_2$	S,M	7	Rep EC50	252	-	-	~~22.70~~	-	-	Reimer 1999	Duration too short
								Geomean Oncorhynchus ACRs (valid data):		3.22		
Brook trout	$MnSO_4$	F,M	65	Length	3 t.2	0.55	0.85	0.68	5.t2	7.49	Davies et al. t998b	ELS, control length=37.5mm, 0.85 mg/L treatment length=35.2mm, significant? Fry test used for ACR, no acute ELS test
Salvelinus fontinalis	$MnSO_4$	F,M	65	Weight	31.2	0.85	2.18	1.36	5.12	3.76	Davies et al. 1998b	ELS, acute fry test used for ACR, no acute test on ELS
	$MnSO_4$	F,M	65	L,W	155.5	3.53	7.53	5. t6	27.5	5.33	Davies et al. 1998b	ELS
	$MnSO_4$	F,M	31	L,W	31.3	~~2.53~~	~~6.35~~	~~3.68~~	5.12	~~1.39~~	Davies et al. 1998b	Fry (60 days old at start of test), use ELS tests
	$MnSO_4$	F,M	44	W	148.1	~~1.74~~	~~3.69~~	~~2.48~~	27.5	~~11.10~~	Davies et al. 1998b	Fry (60 days old at start of test), use ELS tests, length was affected at higher concentrations
	$MnSO_4$	F,M	44	L	t48.1	~~3.69~~	~~7.39~~	~~5.15~~	27.5	~~6.34~~	Davies et al. 1998b	Fry (60 days old at start of test), use ELS tests
	$MnSO_4$	F,M	65	IC25 weight	31.2	-	-	~~1.53~~	5.12	~~3.35~~	Davies et al. 1998b	ELS, MATC or EC20 preferred over IC25
	$MnSO_4$	F,M	31	IC25 weight	31.3	-	-	~~3.01~~	5.12	~~1.70~~	Davies et al. 1998b	Fry (60 days old at start of test), ELS test preferred, MATC or EC20 preferred
	$MnSO_4$	F,M	65	IC25 weight	155.5	-	-	~~4.23~~	27.5	~~6.50~~	Davies et al. 1998b	ELS, MATC or EC20 preferred over IC25
	$MnSO_4$	F,M	44	IC25 weight	155.5	-	-	~~3.09~~	27.5	~~6.80~~	Davies et al. 1998b	Fry (60 days old at start of test), ELS test preferred, MATC or EC20 preferred
	$MnSO_4$	F,M	65	EC20 weight	31.2	-	-	~~2.19~~	5.12	~~2.43~~	Davies et al. 1998b	ELS, MATC is preferred method
	$MnSO_4$	F,M	65	EC t0 weight	3 t.2	-	-	~~1.70~~	5.12	~~3.01~~	Davies et al. t998b	ELS, MATC is preferred method
	$MnSO_4$	F,M	65	EC20 weight	155.5	-	-	~~3.70~~	27.5	~~7.44~~	Davies et al. 1998b	ELS, MATC is preferred method
	$MnSO_4$	F,M	65	EC t0 weight	155.5	-	-	~~2.83~~	27.5	~~0.73~~	Davies et al. 1998b	ELS, MATC is preferred method
								Geomean Salvelinus ACRs (valid data):		5.32		
Brown trout	$MnCl_2$	F,M	62	ELS survival	30.9	~~3.84~~	~~7.38~~	~~5.39~~	-	-	Stubblefield et al. 1997	No corresponding acute test, based on survival, no effect was found on growth
Salmo trutta	$MnCl_2$	F,M	62	ELS growth	151.9	~~2.78~~	~~4.41~~	~~3.50~~	-	-	Stubblefield et al. 1997	No corresponding acute test, LOEC for survival was 8.8, NOEC was 4.4 mg/L
	$MnCl_2$	F,M	62	ELS growth	449.6	~~4.65~~	~~8.68~~	~~6.28~~	-	-	Stubblefield et al. 1997	No corresponding acute test, LOEC for survival was t6.2, NOEC was 8.7 mg/L
	$MnCl_2$	F,M	62	IC25 survival	30.9	-	-	~~4.67~~	-	-	Stubblefield et al. 1997	No corresponding acute test
	$MnCl_2$	F,M	62	IC25 growth	151.8	-	-	~~5.59~~	-	-	Stubblefield et al. 1997	No corresponding acute test
	$MnCl_2$	F,M	62	IC25 growth	449.6	-	-	~~8.68~~	-	-	Stubblefield et al. 1997	No corresponding acute test
	$MnSO_4$	F,M	t20	Survival	37.5	2.03	3.59	2.70	3.77	1.40	Davies and Brinkman 1994	Survival was only endpoint measured
								GeoMean Salmo ACRs (valid data):		1.40		
Fathead minnow	$MnSO_4$	F,M	36	Length/weight	?	~~1.27~~	~~2.48~~	~~1.78~~	-	-	Kimball 1978	Hardness not measured, acute test results was 33.6 mg/L but organisms were fed
Pimephales promelas												
Amphipod	$MnCl_2$	SR,M	28	LC50	alk = 85	-	~~1.62~~	-	-	-	Norwood et al. 2007	No hardness, chloride was 23.9 mg/L, temp = 25 C, renewed weekly, extremely high variability, duration too short
Hyalella azteca	$MnCl_2$	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
	$MnCl_2$	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 short
	$MnSO_4$	S,U	7	LC50	124	-	-	~~6.95~~	-	-	Borgmann et al. 2005	Duration too short, control survival issues, not aerated
	$MnSO_4$	S,M	7	LC50	124	-	-	~~2.73~~	-	-	Borgmann et al. 2005	Duration too short, control survival issues, not aerated
	$MnSO_4$	S,U	7	LC50	18	-	-	~~>1.00~~	-	-	Borgmann et al. 2005	Duration too short, control survival issues, not aerated
	$MnSO_4Cl_2$	SR,M	42	Survival	110	~~0.71~~	~~1.40~~	~~1.00~~	11.01	~~11.04~~	Soucek and Dickinson 2010	44% MnSO4/56%MnCl2, dissolved oxygen problems
	$MnSO_4Cl_2$	SR,M	42	Survival	115	1.4	2.9	2.01	11.04	5.48	Soucek and Dickinson 2010	44% MnSO4/56%MnCl2
Water flea	$MnCl_2$	S,M	21	Reproduction	25	-	-	-	-	-	Reimer 1999	Excess control deaths due to soft water, no results reported by author
Daphnia magna	$MnCl_2$	S,M	21	Reproduction	100	3.60	6.90	4.98	28.7	5.76	Reimer 1999	Hardness was unmeasured
	$MnCl_2$	S,M	2t	IC25% rep	100	-	-	~~6.40~~	28.7	~~6.31~~	Reimer t999	Mn measured, Hardness was nominal, MATC is preferred method
	$MnCl_2$	S,M	21	Reproduction	269	7.30	13.40	9.89	76.3	7.71	Reimer 1999	Mn and hardness meaured
	$MnCl_2$	S,M	2t	IC25% rep	269	-	-	~~9.49~~	76.3	~~8.12~~	Reimer 1999	Mn and hardness measured, MATC is preferred method
	$MnCl_2$	S,M	2t	IC16% 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 detected from control variability

	$MnSO_4$	S,M	28	Reproduction	UNK	< t. t0	<1,100	~~<1,100~~	-	-	Kimball 1978	**Hardness not measured**, hard well water used, treated for iron
							GeoMean Daphnia ACRs (valid data):			4.74		
Water flea	$MnCl_2$	S,M	7	IC50% rep	26	-	-	~~3.90~~	5.7	~~1.46~~	Lasier et al. 2000	**MATC method is preferred over IC50**
Ceriodaphnia dubia	$MnCl_2$	S,M	7	IC50% rep	92	-	-	~~9.00~~	14.5	~~1.61~~	Lasier et al. 2000	**MATC method is preferred over IC50**
	$MnCl_2$	S,M	7	IC50% rep	184	-	-	~~11.40~~	14.5	~~1.27~~	Lasier et al. 2000	**MATC method is preferred over IC50**
	$MnCl_2$	S,M	7	Reproduction	26	2.40	4.90	3.43	5.7	1.66	Lasier et al. 2000	NOEC/LOEC from Michigan database
	$MnCl_2$	S,M	7	Reproduction	92	4.90	9.80	6.93	14.5	2.09	Lasier et al. 2000	NOEC/LOEC from Michigan database
	$MnCl_2$	S,M	7	Reproduction	184	10.00	19.80	14.07	14.5	1.03	Lasier et al. 2000	NOEC/LOEC from Michigan database
	$MnCl_2$	S,M	7	IC25% rep	26	-	-	~~2.10~~	5.7	~~1.84~~	Lasier et al. 2000	**MATC method is preferred over IC25**
	$MnCl_2$	S,M	7	IC25% rep	92	-	-	~~5.20~~	14.5	~~2.70~~	Lasier et al. 2000	**MATC method is preferred over IC25**
	$MnCl_2$	SR,M	7	Reproduction	46	~~2.20~~	~~3.60~~	~~2.81~~	11.2	~~3.98~~	ENSR 1989	**No acute test done with chronic test, acute test from 1992 entered for comparison, use Lasier data instead**
	$MnCl_2$	SR,M	6	Reproduction	156	~~2.60~~	~~4.80~~	~~3.77~~	-	-	ENSR 1989	**Dilution water taken from pond in Alaska, no acute tests performed with this water, ACR not practical**
	$MnCl_2$	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
	$MnCl_2$	SR,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
	$MnCl_2$	SR,M	6-7	Reproduction	100	4.80	9.30	6.68	21.2	3.17	ENSR 1992c	Acute data used from ENSR June 1992, same lab, same researcher, same week
	$MnCl_2$	SR,M	6-7	Reproduction	200	7.82	20.40	12.63	27.3	2.16	ENSR 1992c	Acute data used from ENSR June 1992, same lab, same researcher, same week
	$MnCl_2$	SR,M	6-7	IC25% rep	25	-	-	~~3.37~~	9.44	~~2.80~~	ENSR 1992c	**MATC method is preferred over IC25**
	$MnCl_2$	SR,M	6-7	IC25% rep	50	-	-	~~5.15~~	11.2	~~2.17~~	ENSR 1992c	**MATC method is preferred over IC25**
	$MnCl_2$	SR,M	6-7	IC25% rep	100	-	-	~~6.89~~	21.2	~~3.08~~	ENSR 1992c	**MATC method is preferred over IC25**
	$MnCl_2$	SR,M	6-7	IC25% rep	200	-	-	~~8.01~~	27.3	~~3.06~~	ENSR 1992c	**MATC method is preferred over IC25**
							GeoMean Ceriodaphnia ACRs (valid data):			2.24		
Protozoan	$MnCl_2$	S,U	48	EC50	150	-	-	~~109.00~~	-	-	Nalecz-Jawecki and Sawicki 1998	**Unicellular organism, test is considered chronic (1985 guidelines: IV.E.4), no corresponding acute test for ACR**
Spirostomum ambiguum												
Goldfish	$MnCl_2$	SR,M	7	LC50	195	-	-	~~8.22~~	-	-	Birge 1978	**7 day ELS test (unfed?), not a suitable chronic test**
Carassius auratus												
Narrow-mouthed toad	$MnCl_2$	S,M	7	LC50	195	-	-	~~1.42~~	-	-	Birge 1878	**7 day ELS test (unfed?), not a suitable chronic test, non-native species to IL**
Gastrophryne carolinensis												
Paper pond shell	$MnSO_4$	F,M	90	Shell Growth	80	-	-	~~20-30~~	-	-	Wade et al. 1989	**Insufficient methods and results reported**
Anodonta imbecillus												

Attachment 1 – Exhibit S

Ambient Water Quality Monitoring Network (AWQMN)

Ambient Water Quality Monitoring Network (AWQMN)

Station Code	Stream Name	(4/30/97) Critical Hardness	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. Br., 1.0 miles NE of Belknap
AK 02	Lusk Creek	54	Pope	37 28 20 88 32 50	Co. Rd. Br., 2.8 miles SE of Edgyville
AT 06	Saline River *	233	Gallatin	37 38 53 88 14 30	Peabody Br., 1.3 miles E of Gibsonia
ATF 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 Harrisburg
ATGC 01	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	37 37 22.5 88 48 43 5	Co. Rd. Br., 3.4 miles Crab Orchard
ATH 05	S. Fork Saline River	317	Saline	37 38 16 88 40 40	Rt. 45 Br., 3.8 miles SW of CARrier Mills
ATHG 01	Sugar Creek	1400	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	Wabash River	190	White	38 07 55 87 56 25	Rt. 460 Br.; near New Harmony, IN
BC 02	Bonpas Creek	150	Edwards-Wabash	38 23 11 87 58 32	Rt. 15 Br., 0.6 miles NE of Browns
BE 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 40 88 10 15	Ryan Bridge, County Rd. 9 miles S of Charleston

 * indicates no flow data collected note: critical hardness expressed as CaCO3 (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

Station Code	Stream Name	(4/30/97) Critical Hardness	County	Latitude Longitude	Description
BE 14	Embarras River	280	Douglas	39 47 59 88 10 13	Co. Rd. Br., west edge of Camargo
BEF 05	N Fork Embarras Riv	193	Crawford	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
BN 01	Brouilletts Creek	250	Vermilion	39 40 53 87 31 16	Indiana Rt. 71 Br., 0 5 miles N of Blanford
BO 07	Little Vermilion Riv	244	Vermilion	38 07 55 87 56 25	Co. Rd. Br., 4 miles SE of Georgetown
BP 01	Vermilion River	278	Vermilion	40 05 53 87 35 37	Grape Creek Rd., 3.5 miles SE of Danville
BPG 09	N. Fork Vermilion Riv	231	Vermilion	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	Col. Rd. Br., 3 miles S of Oakwood
BPJ 07	Salt Fork Vermilion Riv	277	Champaign	40 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
BPK 07	Middle Fork Vermilion Riv	334	Vermilion	40 08 12 87 44 45	Kickapoo St. Park Br., upstream of I-74 Br.
C 09	Little Wabash River	135	Edwards	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 Louisville
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. Br., 5 miles SE of Clay City
C 23	Little Wabash River	136	White	38 05 31 88 09 20	Main St. Br. in Carmi

 * indicates no flow data collected note: critical hardness expressed as CaCO3 (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

(4/30/97)

Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description
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 Iuka
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 River	245	Calhoun / Greene	39 09 37 90 36 55	Rt. 100 Br. at Hardin
D 05	Illinois River	221	Peoria / Tazwell	40 34 23 89 39 17	Rt. 9 Br. at Pekin
D 09	Illinois River *	251	Marshall	41 01 30 89 25 02	Rt. 17 Br. at Lacon
D 16	Illinois River	214	Putman	41 15 26 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 Br., 0.5 miles E of Valley City
DA 04	Macoupin Creek	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	Mauvaise Creek	194	Scott	39 43 53 90 24 26	Co. Rd. Br., 1.5 miles NE of Merritt

 * indicates no flow data collected note: critical hardness expressed as $CaCO_3$ (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

(4/30/97)

Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description
DE 01	McKee Creek *	249	Pike	39 49 04 90 39 09	Rt. 104 Br., at Chambersburg
DF 04	Indian Creek	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	McDonough	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	Knox	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
DJ 08	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 north 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
DJBZ 01	Slug Run	1065	Fulton	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	Tazewell	40 26 51 89 41 28	Co. Rd. Br., 4 miles SSW of S. Pekin
DK 13	Mackinaw River	282	Tazewell	40 35 12 89 16 42	Co. Rd. Br., 4 miles SE of Deer Creek
DL 01	Kickapoo Creek *	398	Peoria	40 39 18 89 39 19	US 24 Br., No of Bartonville
DQ 03	Big Bureau Creek	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

* indicates no flow data collected

note: critical hardness expressed as $CaCO_3$ (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

(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 06	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	Grundy	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	Tazewell	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	Rt. 173 Br. near Wisconsin line
DT 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 mile N of Sheridan
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	Winn Rd. Br., 0.6 miles W of Spring Grove

 * indicates no flow data collected note: critical hardness expressed as $CaCO_3$ (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

(4/30/97)

Station Code	Stream Name	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 Cornland
EL 01	Spring Creek	197	Sangamon	39 49 16 89 41 16	Bruns Lane Br., NW edge of Springfield

 * indicates no flow data collected note: critical hardness expressed as $CaCO_3$ (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

Station Code	Stream Name	(4/30/97) Critical Hardness	County	Latitude 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 33 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 River	312	Iroquois	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 Milford
	DES PLAINES RIVER / LAKE MICHIGAN BASIN				
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	Central Ave. Br. at Des Plaines

 * indicates no flow data collected note: critical hardness expressed as $CaCO_3$ (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

(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 Riverside
GB 10	DuPage River	270	Will	41 41 24 88 09 58	Plainfield/Naperville Rd. Br.
GB 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	135th St. Br. at Romeoville
GI 02	Sanitary & Ship Canal	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 Channel *	218	Cook	41 41 45 87 56 11	Rt. 83 Br., 3 mile NE of Lemont
HB 42	Little Calumet R S.	343	~~Lake~~ Cook (IN)	41 34 07 87 31 18	Hohman Ave. Br., N of Munster
HDB 04	Thorn 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 Branch	234	Lake / Cook	42 09 10 87 49 07	Lake/Cook Co. Line Rd. Br. Chicago River

 * indicates no flow data collected note: critical hardness expressed as $CaCO_3$ (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

(4/30/97)

Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description
	MISSISSIPPI RIVER SOUTH BASIN				
I 84	Mississippi River	226	Alexander	37 13 00 89 27 50	at Thebes, IL
II 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 CENTRAL BASIN				
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 Prarie Ln. Br. SE of Horseshoe Lake
JQ 05	Cahokia Creek	130	Madison	38 49 28 89 58 29	Rt. 143 Br. NW of Edwardsville
JR 02	Wood River *	288	Madison	38 53 03 90 07 20	Rt. 3 Br. at Milton Rd. Junction in Alton
	MISSISSIPPI RIVER NORTH CENTRAL BASIN				
K 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	~~Bay~~ 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

 * indicates no flow data collected note: critical hardness expressed as CaCO3 (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

Station Code	Stream Name	(4/30/97) Critical Hardness	County	Latitude Longitude	Description
	MISSISSIPPI RIVER NORTH BASIN				
M O4	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	Big 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

 * indicates no flow data collected note: critical hardness expressed as CaCO3 (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

(4/30/97)

Station Code	Stream Name	Critical Hardness	County	Latitude Longitude	Description
NG 02	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				
O 02	Kaskaskia River	218	Coles	39 34 59 88 24 50	Local Rd. Br. in Cook Mills
O 07	Kaskaskia River	150	Clinton	38 34 28 89 22 09	Rt. 127 Br., 2.3 miles S of Carlyle
O 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
O 11	Kaskaskia River	205	Shelby	39 24 25 88 46 50	Rt. 16 Br. at Shelbyville near dam
O 15	Kaskaskia River *	298	Moultrie	39 34 22 88 31 53	Rt 121 Br., 1 mile N of Allenville
O 20	Kaskaskia River	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	Douglas	39 51 53 88 21 52	Co. Rd. Br., 4 miles W of Hayes
OC 04	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	Rt. 40 Br., 2.7 miles SE of Troy
OD 07	Silver Creek	191	St. Clair	38 24 22 89 52 26	Rt. 460 Br., 2.2 miles SE of Freeburg

 * indicates no flow data collected note: critical hardness expressed as $CaCO_3$ (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

(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	Crooked Creek	118	Marion	38 33 50 89 03 01	Co. Rd Br., 3.1 miles S of Odin
OJ 08	Crooked Creek	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	Rt. 51 Br., 5.2 miles N of Sandoval
OKA 01	North Fork Kaskaskia *	106	Marion	38 46 25 89 09 15	Old Patoka Rd Bridge
OL 02	Hurricane 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 *	329	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	Co. Rd. Br., 0.8 miles N of Sullivan
	ROCK RIVER BASIN				
P 04	Rock River	250	Henry / Rock Island	41 33 35 90 10 55	Rt. 92 Br., 2 miles E of Joslin
P 06	Rock River	235	Whiteside	41 47 00 89 44 58	US Rt. 30 Br., 2 miles W of Rock Falls

 * indicates no flow data collected note: critical hardness expressed as $CaCO_3$ (mg/L)

Ambient Water Quality Monitoring Network (AWQMN)

Station Code	Stream Name	(4/30/97) 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	Winnebago	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 Geneseo
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	Kishwaukee River	279	Winnebago	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
PQC 06	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

 * indicates no flow data collected note: critical hardness expressed as CaCC3 (mg/L)

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

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.

Nominal Mn Concentration (mg/L)	In Set #1	In Set #2	In Set #3	Out Set #1	Out Set #2	Out Set #3	Geometric Mean In	Geometric Mean Out	Geometric 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

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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* (3.0×10^7 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* (3.0×10^7 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 ± 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 ± 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 ($Na_2B_4O_7 \cdot 10H_2O$, 99.5+%, CAS # 1303-96-4) and boric acid (H_3BO_3, 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 $CaCO_3$) 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 $MgSO_4$, $CaSO_4$, and in the case of *H. azteca,* $CaCl_2$ 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 $NaHCO_3$ 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® (Fisher Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 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® (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®, 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 measurements were made 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 $CaCO_3$, 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 ($R^2 = 0.5708$, $p = 0.0823$). 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 ($R^2 = 0.5329$, $p = 0.2700$).

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^2 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^2 = 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 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

Fathead minnows - 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).

Amphipods - 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).

Chronic values – 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.

Table A.1. Salt concentrations (mg/L) added to deionized water for generation of dilution waters[a,b,c,d] used for definitive boron toxicity testing with freshwater species.

Water name	KCl	$NaHCO_3$	$MgSO_4$(an)	$CaSO_4$(an)	$CaCl_2$	B ratio*
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
pH 6.5a	4	4	60	60	0	99.1 / 0.9
pH 6.5b	4	4	30	50	50	99.1 / 0.9
pH 7.5a	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

*B ratio = ratio of % boron added to highest test concentration as boric acid / borax.

[a] 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.

[b] hard 300a, hard 500a, pH 6.5a, pH 7.5a, pH 8.5a were used for tests with *C. dubia.*

[c] 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.*

[d] hard 100c, 300c, and 500c also had 1 mg/L NaBr.

Table A.2. Test conditions for acute toxicity bioassays with various freshwater organisms.

	Organism				
Parameter	*P. promelas*	*C. dubia*	*H. azteca*	*A. vivipara*	*S. simile*
1. Temperature (°C)	25 ± 1	25 ± 1	22 ± 1	12 ± 1	22 ± 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.	≥ 90	≥ 90	≥ 90	≥ 90	≥ 90

Table A.3. 96-h boron LC50s and measured water quality conditions* for toxicity tests with three freshwater species.

Species	temp. (s.d) °C	pH (s.d) S.U.	hardness (s.d.) mg/L as $CaCO_3$	LC50 (95% C.I.) 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)

*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[a] (dissolved B) samples from the 96-h acute toxicity test with the fingernail clam (*Sphaerium simile*).

Nominal concentration	total B day 0	dissolved B day 0	ratio[b] day 0	total B day 4	dissolved B day 4	ratio 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[c]	na	120	110	0.917
200	220	220	1.000	230	240	1.043
400	440	440	1.000	460	450	0.978

Geometric mean of day 0 values = 1.009, and day 4 values = 0.981. Overall geometric mean = 0.994

[a] samples were filtered with 0.45 µm pore sized cellulose nitrate filters.

[b] ratio = dissolved B divided by total B.

[c] 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.

Table A.5. Mean boron LC50s for *Ceriodaphnia dubia* and *Hyalella azteca* at various levels of water hardness and pH*.

Ceriodaphnia dubia 48-h tests				
Test water	temp. (s.d) °C	pH (s.d) S.U.	hardness (s.d.) mg/L as $CaCO_3$	LC50 (95% C.I.) 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)
pH 6.5a	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)
pH 8.5a	25.0 (0.0)	8.4 (0.1)	84 (1)	142 (130 – 155)
Hyalella azteca 96-h tests				
Test water	temp. (s.d) °C	pH (s.d) S.U.	hardness (s.d.) mg/L as $CaCO_3$	LC50 (95% C.I.) 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)	64 (41 – 101)

*water quality values are geometric means of measurements taken in all test concentrations throughout the duration of the test.

Table A.6. Water quality data for chronic bioassays with *Pimephales promelas* and *Hyalella azteca*.

Pimephales promelas 32-d chronic test					
Parameter	mean*	5th %ile	95th%ile)	min	max
Temperature (°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
Hyalella azteca 42-d chronic test					
Parameter	mean*	5th %ile	95th%ile	min	max
Temperature (°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.7. Boron measurement data from samples collected on 19 occasions throughout the 32-d chronic bioassays with *Pimephales promelas*.

Nominal Conc.	overall mean[a]	in water mean	out water mean	5th %ile	95th %ile	min	max
Control[b]	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

[a] All means are geometric means.

[b] 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.

Table A.8. Embryo survival, total survival, and hatching data for the 32-d chronic bioassays with *Pimephales promelas*.

Treatment	embryo % survival before thinning	mean (s.d) day[a] of hatch	% hatch after thinning	total[b] 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

[a] days after initiation of test

[b] 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*

Nominal Conc.	overall mean[a]	in water mean	out water mean	5th %ile	95th %ile	min	max
Control[b]	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

[a] All means are geometric means.

[b] 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.

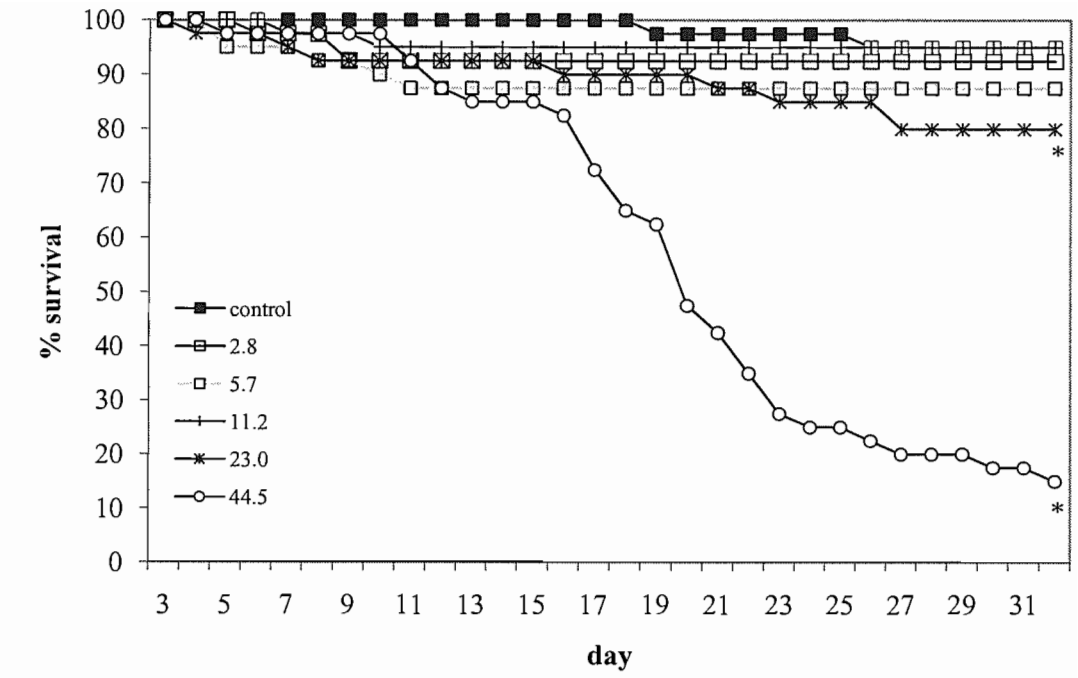

Figure A.1. Mean daily percent survival of fathead minnows (*Pimephales promelas*) in five concentrations of boron plus a control (Hard 100a) in a 32-d chronic, static renewal bioassay. Asterisks indicate mean is significantly different ($p < 0.05$) from control on day 32.

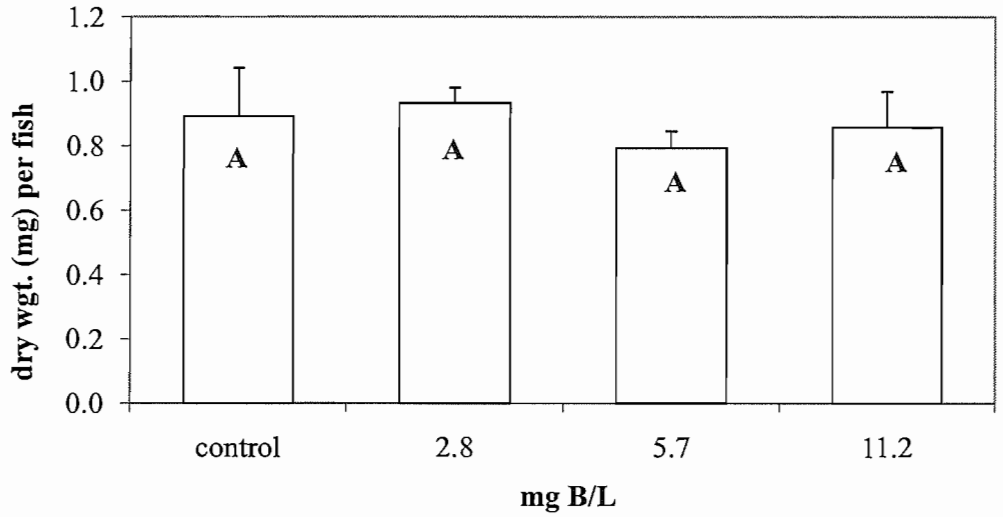

Figure A.2. Mean (error bars = standard deviation) dry weight per 10 fish in three boron concentrations and a control (Hard 100a) at the end of a 32-d chronic, static renewal bioassay with fathead minnows (*Pimephales promelas*). Different capital letters indicate means are significantly different from the control ($p < 0.05$).

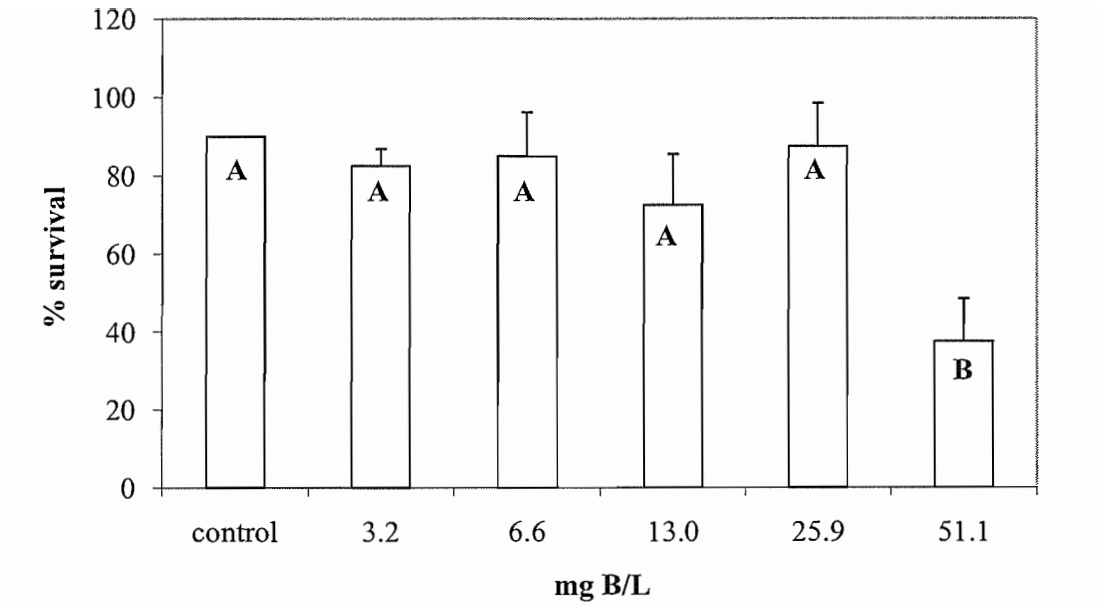

Figure A.3. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

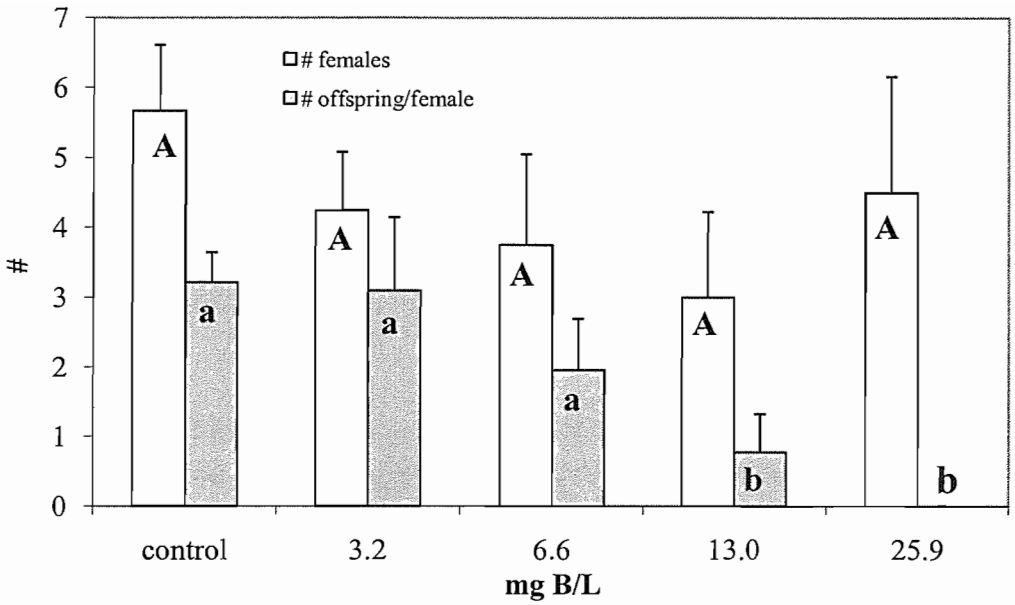

Figure A.4. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

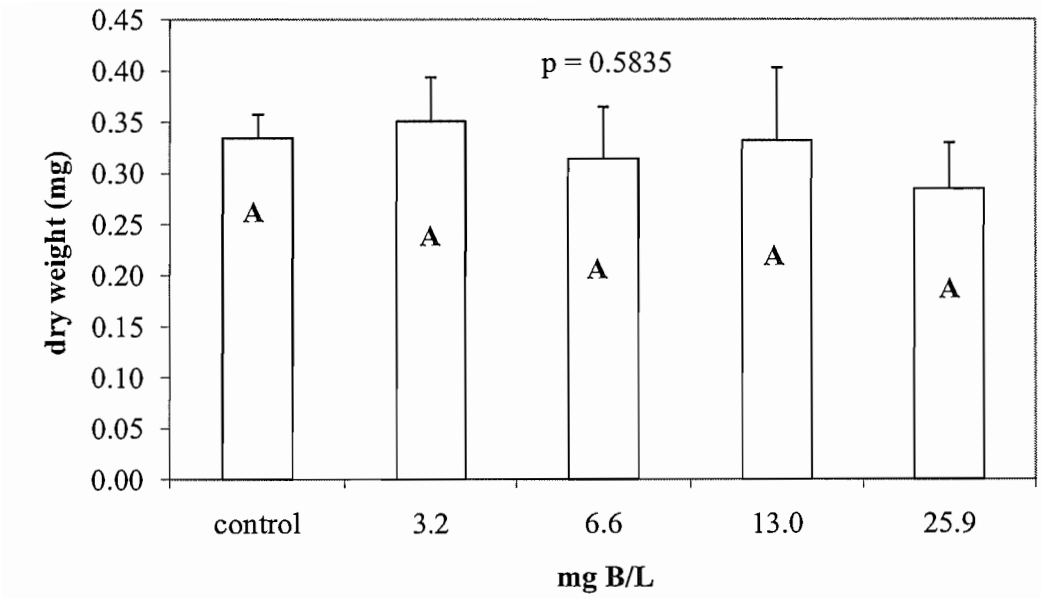

Figure A.5. Mean (error bars = standard deviation) dry weight of individual amphipods in four boron concentrations and a control (Hard 100b) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

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 μm) 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 ± 1 °C. Test chambers were 50 ml glass beakers with 40 ml of test solution and a 2-by 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® (Fisher Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 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® (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 at the beginning and at the end of the test. For measurement of dissolved fluoride, samples were filtered using 0.45 µm 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 ± 0.1 SD), the mean pH value was 8.0 ± 0.1, and mean dissolved oxygen was 8.0 ± 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 $CaCO_3$.

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.

Chronic values –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 of dilution waters used for acute and chronic fluoride toxicity testing with *Hyalella azteca.*

Water name	KCl	$NaHCO_3$	$MgSO_4$(an)	$CaSO_4$(an)	$CaCl_2$	NaBr
Borgmann	4	84	30	0	111	1

Table B.2. Nominal and measured fluoride concentrations (mg F/L) for unfiltered (total F) and filtered[a] (dissolved F) samples from the 96-h acute toxicity test with *Hyalella azteca.*

Nominal concentration	total F day 0	dissolved F day 0	ratio[b] day 0	total F day 4	dissolved F day 4	ratio 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 values = 1.132, and day 4 values = 0.941. Overall geometric mean = 1.032

[a] samples were filtered with 0.45 µm pore sized cellulose nitrate filters.
[b] ratio = dissolved F divided by total F.

Table B.3. Water quality data for chronic bioassays with *Hyalella azteca*.

Parameter	mean*	5th %ile	95th%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
pH	7.8	7.4	8.0	7.3	8.2
Hardness (mg/L)	114	100	120	86	124
Alkalinity (mg/L)	55	50	60	50	60

* 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*.

Nominal Conc.	overall mean[a]	in water mean	out water mean	5th %ile	95th %ile	min	max
Control[b]	<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
28 mg/L	16.7	15.5	18.5	13.1	21.0	14	24

[a] All means are geometric means.

[b] 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[a] (dissolved F) samples from the 42-d chronic toxicity test with *Hyalella azteca*. Both sample 1 and sample 2 were "out" water samples.

Nominal concentration	total F sample 1	dissolved F sample 1	ratio[b] sample 1	total F sample 2	dissolved F sample 2	ratio 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 mean of sample 1 values = 0.975, and sample 2 values = 0.991. Overall geometric mean = 0.983

[a] samples were filtered with 0.45 µm pore sized cellulose nitrate filters.

[b] ratio = dissolved F divided by total F.

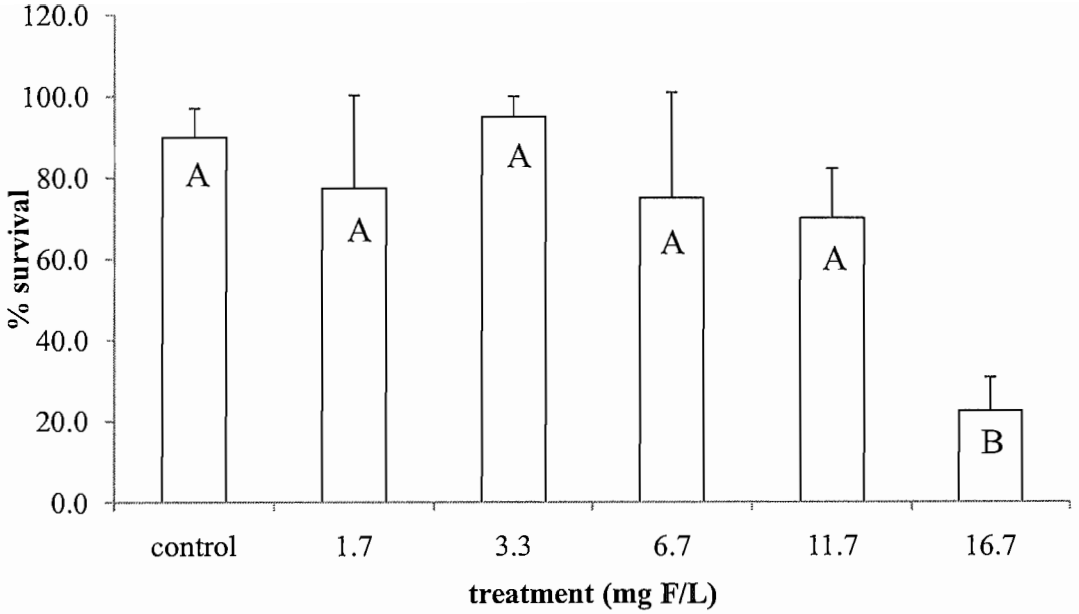

Figure B.1. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five fluoride concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

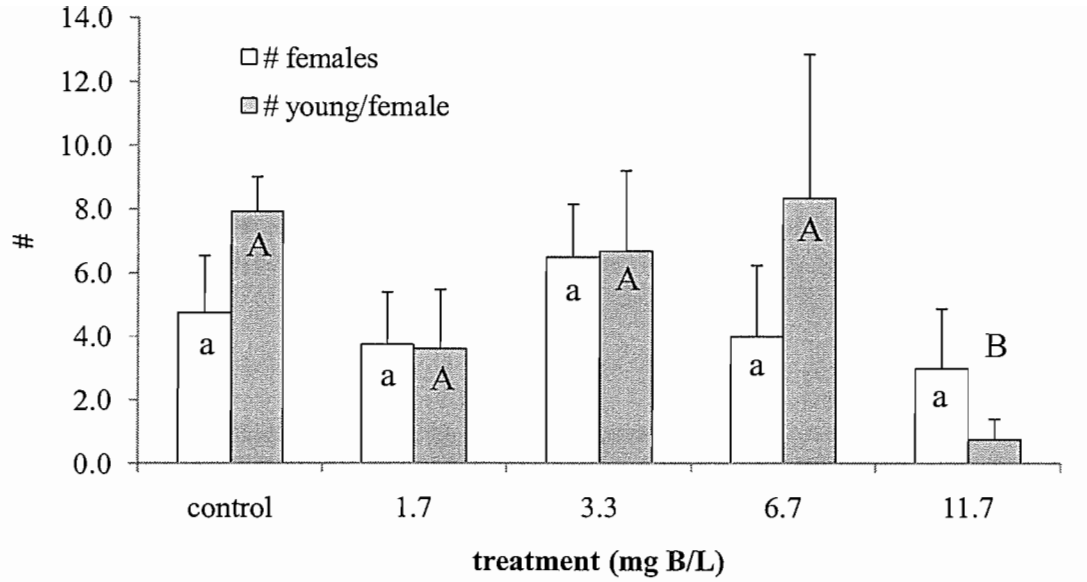

Figure B.2. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four fluoride concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

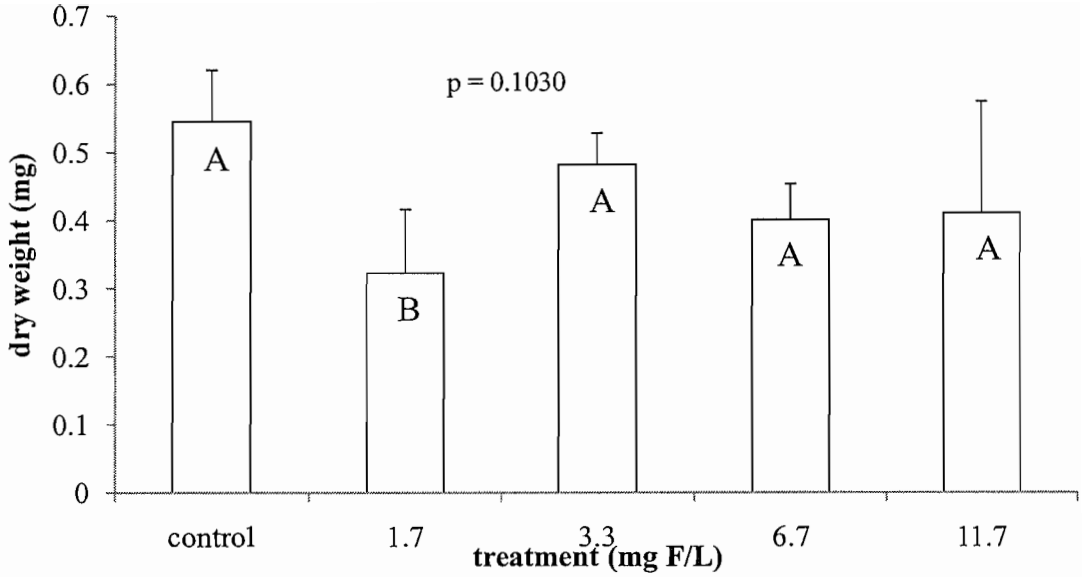

Figure B.3. Mean (error bars = standard deviation) dry weight of individual amphipods in four fluoride concentrations and a control (Borgmann) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

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 μm) 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 ± 1 °C. Test chambers were 50 ml glass beakers with 40 ml of test solution and a 2- by 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® (Fisher

Scientific, Pittsburgh, PA, USA) model AB15 pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 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® (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 at the beginning and at the end of the test. For measurement of 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 °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 ± 0.3 SD), the mean pH value was 7.8 ± 0.1, and mean dissolved oxygen was 8.2 ± 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 $CaCO_3$.

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).

Chronic values –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	$NaHCO_3$	$MgSO_4$(an)	$CaSO_4$(an)	$CaCl_2$	NaBr
Borgmann	4	84	30	0	111	1

Table C.2. Water quality data for 42-d chronic Mn bioassay with *Hyalella azteca*.

Parameter	mean*	5th %ile	95th%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[a] (mg/L)	115	112	118	112	125
Alkalinity (mg/L)	52	50	54	50	60

* Mean of 24 measurements throughout the test.

[a] 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*

Nominal Conc.	overall mean[a]	in water mean	out water mean	5th %ile	95th %ile	min	max
Control[b]	<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
6 mg/L	5.7	5.7	5.7	5.5	6.1	5.5	6.1

[a] All means are geometric means.

[b] Manganese was detected on one occasion in the control.

Table C.4. Nominal and measured manganese concentrations (mg Mn/L) for unfiltered (total Mn) and filtered[a] (dissolved Mn) samples from the 42-d chronic toxicity test with *Hyalella azteca*. Six different sets of samples were measured for total and dissolved Mn.

Nominal		set 1 (in)			set 2 (out)	
concentration	total	dissolved	ratio[b]	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[b]	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)	
concentration	total	dissolved	ratio[b]	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

Geometric mean of ratios for "in water" sets = 0.989, and for "out water" sets = 0.973.
Overall geometric mean of ratios = 0.981

[a] samples were filtered with 0.45 µm pore sized cellulose nitrate filters.
[b] ratio = dissolved Mn divided by total Mn.

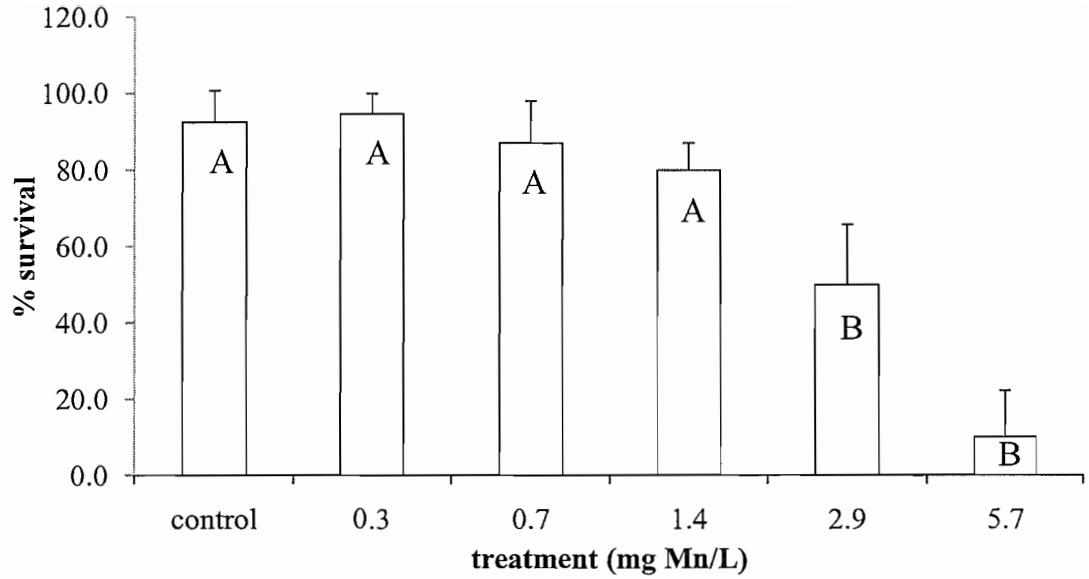

Figure C.1. Mean (error bars = standard deviation) percent survival of *Hyalella azteca* in five manganese concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

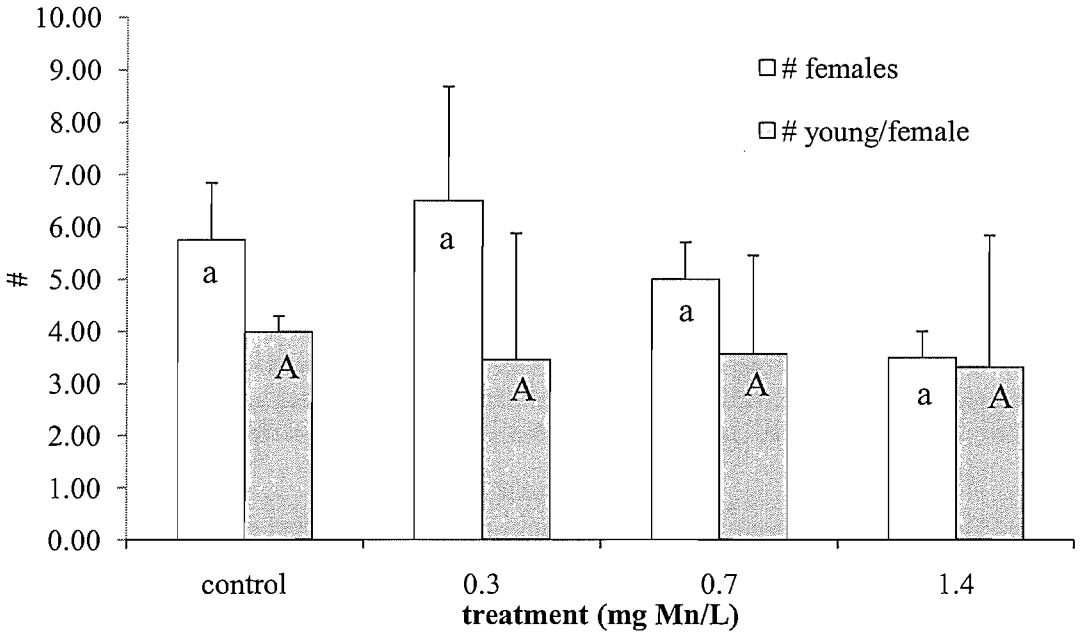

Figure C.2. Mean (error bars = standard deviation) number of females per replicate and number of offspring produced per female in four manganese concentrations and a control (Borgmann water) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

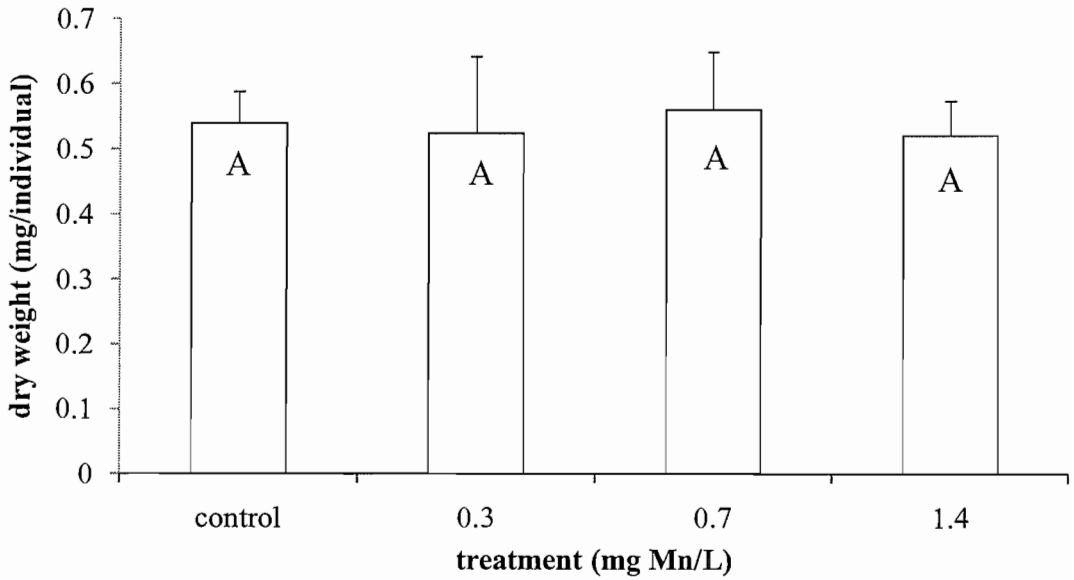

Figure C.3. Mean (error bars = standard deviation) dry weight of individual amphipods in four manganese concentrations and a control (Borgmann) at the end of a 42-d chronic, static renewal bioassay with *Hyalella azteca*. Different capital letters indicate means are significantly different from the control ($p < 0.05$).

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Attachment 1 – Exhibit V

Excerpts from Exhibit S to Agency Rulemaking Proposal in R02-11

19. Chronic Toxicity. List species in descending order of tolerance by MATC.

Species	Test	Chem	H	Limit	CV/ MATC (µg/l)	corrected for H	Reference
Invertebrates							
Daphnia magna							
	LC	Cl	45	<140.3	<140.3	<153.4	EPA87
	LC	Cl	52	97-190	135.8	131.4	EPA87
	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
	21D?	Cl	225	-	120	31.72	16
					SMCV	gm <51.97	
D. similis							
	surv	S	-	-	<1	-	59
Ceriodaphnia dubia							
	7d surv	Cl	169	-	40	14.25	40
	7d surv	Cl	169	-	140	49.88	40
					SMCV	gm 26.66	
Moina macrocopa							
	-	S	<5(?)	-	353.6	2488	68
M. irrasa							
	-	Cl	<5	-	26.30	182.9	72
					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							
	LC	Cl	31		>5243	>7861	EPA87
Physa gyrina							
	21d	Cl	20	-	570	1219	43
Erpobdella octulata							
	70d LC-50	S	-	-	100	-	67
	70d LC-50	S	-	-	60	-	67
				SMCV	gm 77.46		

Species	Test	Chem	H	Limit	CV/ MATC (µg/l)	corrected for H	Reference
Vertebrates							
Pimephales promelas							
	LC	S		46	78-145	106.3	EPA87
	terato	S		100	600	333.5	14
		S		100	800	444.7	14
	gro	-		159	1039	389.9	24
		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	438.1!	47
		S		46.5	405	430.7!	47
					SMCV	gm 254.6	
Poecilia reticulata							
	LC	S		30	<173	<266.7	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

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Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*

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Key words: Copper, zinc, lead, mercury, *Hyalella azteca*, toxicity, accumulation, regulation

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 μg Zn g^{-1}, 7.1/16 μg Pb g^{-1} and 56/90 μg Hg g^{-1} dry weight. Concentrations of lead and mercury in control animals were substantially lower (1.3 μg Pb g^{-1} and 0.4 μg Hg g^{-1}), but concentrations of zinc in controls (74 μg 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 μg 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 $\mu g\ g^{-1}$ 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 $mg\ l^{-1}$, alkalinity 90 $mg\ l^{-1}$, 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 μl of 70% nitric acid was added to

1 to 4 amphipods (0.5 to 2 mg dry weight), and allowed to sit for 1 week. Then 17 µl 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 (± S.D.) by week 6 and 10, and copper accumulated ($\mu g\,g^{-1}$ dry weight ± S.D.) by week 10. Lowest concentration with significantly reduced survival (chi-square, $P<0.01$) indicated with **. Copper acumulated at each concentration was not significantly different from control.

Nominal copper concentration:	Control	5.6 $\mu g\,l^{-1}$	10 $\mu g\,l^{-1}$	18 $\mu g\,l^{-1}$	32 $\mu g\,l^{-1}$	56 $\mu g\,l^{-1}$	100 $\mu g\,l^{-1}$
Measured concentration							
$\mu g\,l^{-1}$	3.5 ± 1.4	7.7 ± 1.8	10.7 ± 1.3	16.7 ± 1.6	25.4 ± 2.8	43.8 ± 8.2	81.3 ± 9.0
n	20	20	20	20	20	20	16
Week 6 ($n=4$)							
Survival (%)	71 ± 14	68 ± 3	69 ± 13	63 ± 9	41 ± 23**	36 ± 22	3 ± 3
Weight	2.1 ± 0.6	2.1 ± 0.9	2.1 ± 0.9	2.0 ± 0.4	1.8 ± 0.4	1.4 ± 0.3	0.1 ± 0
Young	0.3 ± 0.4	0.6 ± 0.4	0.6 ± 1.0	0.2 ± 0.3	0.1 ± 0.3	0.04 ± 0.10	0
Week 10 ($n=4$)							
Survival	54 ± 18	54 ± 13	50 ± 4	40 ± 14	29 ± 25**	6 ± 13	0
Weight	4.0 ± 0.7	3.5 ± 1.1	4.0 ± 0.6	3.6 ± 0.7	4.3 ± 0.5	3.4 ± 0	–
Young	3.4 ± 3.0	3.9 ± 1.3	3.7 ± 3.0	1.9 ± 1.3	1.3 ± 1.2	0.8 ± 0	–
Cu in *Hyalella*	79 ± 20	91 ± 11	92 ± 14	95 ± 26	88 ± 13	80 ± 5	–
n	8	8	8	8	8	4	–

Table 2. Percent Survival, wet weight (mg) and total number of young produced per initial animal added (± S.D.) by week 6 and 10, and zinc accumulated ($\mu g\, g^{-1}$ dry weight ± 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 **.

Nominal zinc concentration:	Control	32 $\mu g\, l^{-1}$	56 $\mu g\, l^{-1}$	100 $\mu g\, l^{-1}$	180 $\mu g\, l^{-1}$	320 $\mu g\, l^{-1}$	560 $\mu g\, l^{-1}$
Measured concentration							
$\mu g\, l^{-1}$	5.6 ± 3.8	13.0 ± 8.9	21.2 ± 8.9	42.3 ± 16.6	108 ± 32	185 ± 67	316 ± 129
n	20	20	20	20	20	20	20
Week 6 ($n=4$)							
Survival (%)	75 ± 5	65 ± 9.1	69 ± 14	72 ± 10	68 ± 10	32 ± 17**	8 ± 12
Weight	1.4 ± 0.3	1.8 ± 0.6	1.5 ± 0.4	1.9 ± 0.2	1.7 ± 0.3	1.7 ± 0.3	1.8 ± 0.6
Young	0.1 ± 0.1	0.2 ± 0.4	0.3 ± 0.4	0.5 ± 0.2	0	0	0
Week 10 ($n=4$)							
Survival	63 ± 8	50 ± 8	56 ± 23	51 ± 11	35 ± 17**	6 ± 5	3 ± 3
Weight	3.7 ± 0.2	3.6 ± 0.9	3.7 ± 1.1	3.1 ± 0.5	3.0 ± 0.9	4.2 ± 1.8	3.6 ± 2.3
Young	1.9 ± 1.0	2.2 ± 1.2	2.0 ± 1.1	2.7 ± 0.9	1.0 ± 0.4	0	0
Zn in *Hyalella*	74 ± 27	66 ± 7	85 ± 14	126 ± 46**	136 ± 39	167 ± 22	167 ± 53
n	15	15	15	28	19	4	2

Table 3. Percent Survival, wet weight (mg) and total number of young produced per initial animal added (± S.D.) by week 6 and 10, and lead accumulated ($\mu g\, g^{-1}$ dry weight ± 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 $\mu g\, l^{-1}$	56 $\mu g\, l^{-1}$	100 $\mu g\, l^{-1}$	180 $\mu g\, l^{-1}$	320 $\mu g\, l^{-1}$
Measured concentration							
$\mu g\, l^{-1}$	0.4 ± 0.6	3.3 ± 1.9	2.6 ± 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 ± 12	69 ± 27	74 ± 8	68 ± 3	35 ± 8**	13 ± 6	4 ± 5
Weight	1.7 ± 0.3	2.2 ± 0.6	2.0 ± 0.8	2.6 ± 1.0	0.9 ± 1.0	2.0 ± 0.7	1.1 ± 1.4
Young	0.4 ± 0.4	0.5 ± 0.3	0.1 ± 0.2	0.4 ± 0.6	0.1 ± 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 ± 0.7	4.0 ± 0.3	3.3 ± 1.4	4.4 ± 0.7	3.4 ± 0.9	4.5 ± 1.0	2.1 ± 2.8
Young	4.2 ± 3.0	5.8 ± 1.5	4.0 ± 1.9	3.6 ± 3.0	1.8 ± 0.7	0.4 ± 0.6	0
Pb in *Hyalella*	1.3 ± 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 $\mu g\, l^{-1}$ before acidification, and 96.7 $\mu g\, l^{-1}$ after acidification of the entire flask. The difference between measured and nominal concentrations is, therefore, due to adsorption 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

Table 4. Percent Survival, wet weight (mg) and total number of young produced per initial animal added (± S.D.) by week 6 and 10, and mercury accumulated ($\mu g\, g^{-1}$ dry weight ± 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 **.

Nominal mercury concentration:	Control	3.2 $\mu g\, l^{-1}$	5.6 $\mu g\, l^{-1}$	10 $\mu g\, l^{-1}$	18 $\mu g\, l^{-1}$
Measured concentration					
$\mu g\, l^{-1}$	0.05 ± 0.48	0.62 ± 0.52	1.12 ± 0.57	2.42 ± 1.46	3.96 ± 1.48
n	10	10	10	10	5
Week 6 ($n=2$)					
Survival (%)	88 ± 11	58 ± 4	70 ± 14	25 ± 0**	0
Weight	1.8 ± 0.1	1.3 ± 0.3	2.1 ± 0.4	1.9 ± 0.7	–
Young	0.2 ± 0.1	0.02 ± 0.04	0	0	0
Week 10 ($n=2$)					
Survival	72 ± 4	58 ± 4	65 ± 7	20 ± 0**	0
Weight	4.0 ± 0.1	3.2 ± 0.2	4.3 ± 0.3	5.0 ± 1.2	–
Young	2.4 ± 1.1	2.9 ± 0.8	4.4 ± 1.9	0.9 ± 1.3	0
Hg in *Hyalella*	0.42 ± 0.06	25 ± 6**	56 ± 14	90 ± 32	–
n	4	4	4	4	

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 $\mu g\, l^{-1}$ respectively ($P<0.01$). Zinc was significantly toxic at 320 $\mu g\, l^{-1}$ after week 6 and at 180 $\mu g\, l^{-1}$ by week 10. The lowest nominal concentration of lead which was toxic was 100 $\mu g\, l^{-1}$ after 6 weeks, but by week 10 significant mortality occurred at 56 $\mu g\, l^{-1}$ 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

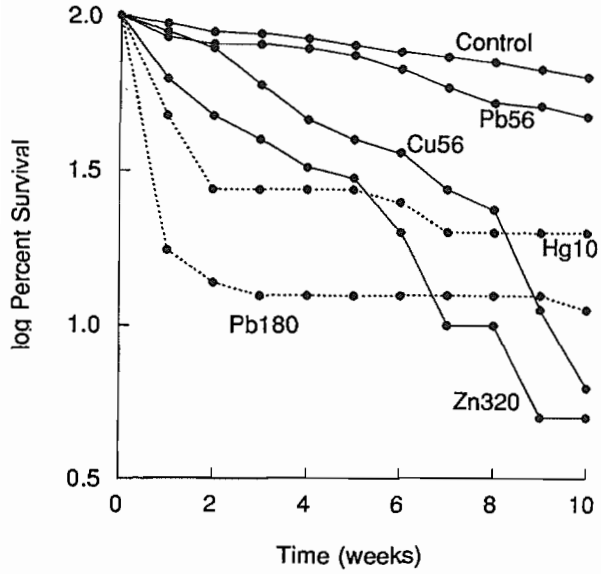

Fig. 1. Time course of mortality at selected metal concentrations. Numbers following the metal symbol represent nominal concentrations in $\mu g\ l^{-1}$.

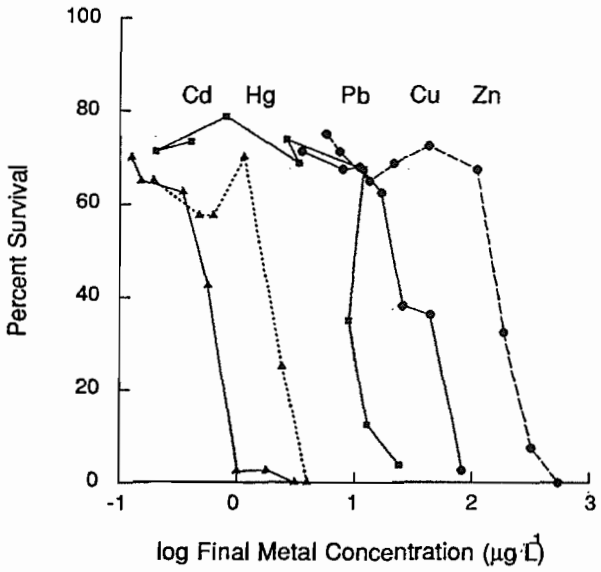

Fig. 2. The relationship between survival after 6 weeks and final measured metal concentrations at the end of each week before water and metal renewal. Data for cadmium are from Borgmann *et al.* (1991).

was unable to regulate zinc as effectively. Although zinc accumulation increased with increasing exposure, the difference between metal

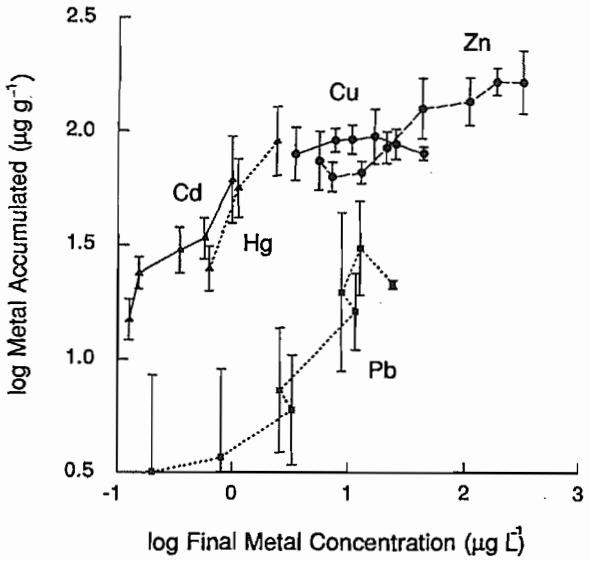

Fig. 3. The relationship between metal accumulation (dry weight basis) and final measured metal concentrations. Data for cadmium are from Borgmann *et al.* (1991). Bars represent ± 1 standard deviation.

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.

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 *et al.* (1991).

Metal	Concentration ($\mu g\ g^{-1}$ dry wt.)			Regression coefficients		
	Control	Highest non-toxic	Lowest toxic	a	b	R^2
Cu	79	95[a]	88[a]	(1.94)	0	–
Zn	74	126	136	1.46	0.35	0.82
Pb	1.3	7.1	16	0.40	0.77	0.44
Hg	0.4	56	90	1.62	0.90	0.76
Cd	2.4	23	30	1.71	0.52	0.65

[a] Not significantly different from control.

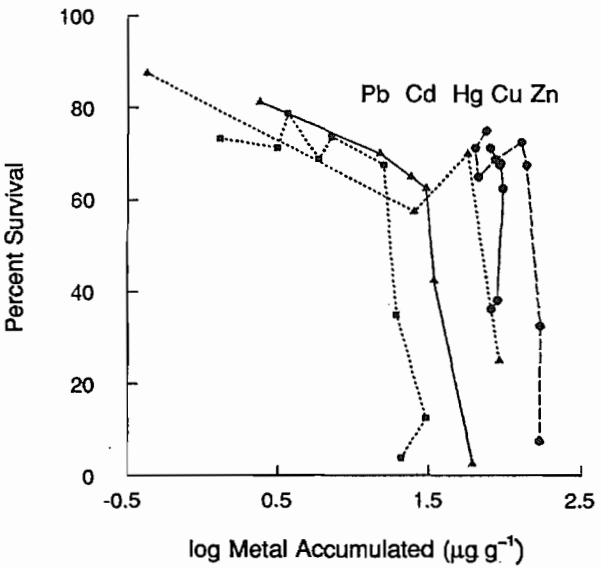

Fig. 4. The relationship between survival after 6 weeks and metal concentrations accumulated by *Hyalella azteca*. Data for cadmium are from Borgmann *et al.* (1991).

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.

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 $\mu g\ l^{-1}$ (Johnson & Jones, 1989), but *Gammarus pulex* demonstrated no zinc regulatory ability (Bascombe *et al.*, 1990). The apparent copper and zinc regulatory abilities of

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 amphipods to adapt to a copper stress, after which regulation is possible.

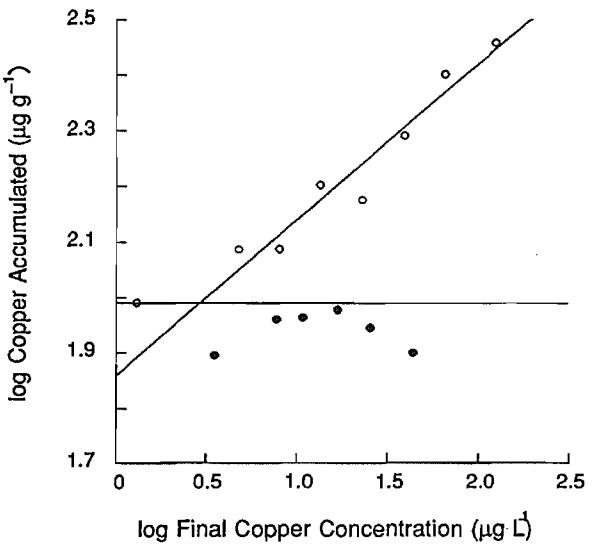

Fig. 5. Comparison of copper accumulation during 10 week chronic (solid symbols) and 1 week (open symbols) exposure to various final copper concentrations. The horizontal line represents the concentration of copper in control amphipods in the 1 week exposure experiment.

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 $\mu g\ g^{-1}$ dry weight. At external zinc concentrations above 316 $\mu g\ l^{-1}$ 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 ± S.D.) by 4 wk old *Hyalella azteca* after 1 wk exposure to various copper additions ($\mu g\ l^{-1}$ ± S.D.). Amphipod wet weight averaged 0.94 ± 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 *Hyalella*	*n*
0	1.3 ± 0.4	98 ± 21	16
5.6	4.8 ± 0.5	122 ± 22	16
10	8.0 ± 0.6	123 ± 22	16
18	13.3 ± 2.0	159 ± 41	16
32	22.8 ± 1.1	150 ± 42	14
56	39.2 ± 2.3	196 ± 43	16
100	65.1 ± 7.7	252 ± 38	12
180	124 ± 19	288 ± 140	9

200 $\mu g\ g^{-1}$ (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 $\mu g\ l^{-1}$ 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 ^{65}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 $\mu g\ g^{-1}$ 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 $\mu g\ g^{-1}$ wet weight for the amphipod *Bathyporeia pilosa*. By comparison, our highest non-toxic body burden (56 μg Hg g^{-1} dry weight) is equivalent to approximately 13 $\mu g\ g^{-1}$ wet weight. Accumulation of mercury by *Daphnia magna* exposed to the highest non-toxic and lowest toxic mercuric chloride concentrations were 15 and 23 $\mu g\ g^{-1}$ dry weight respectively. Methyl mercuric chloride was toxic at the lowest methyl mercury concentration tested, which resulted in accumulation of 16 μg Hg g^{-1} (Biesinger *et al.*, 1982). *Hyalella 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 *Artemia salina* ranged from 280 to 920 $\mu g\ g^{-1}$ 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-

Table 7. Cadmium concentrations accumulated by crustacea at or near toxic waterborne cadmium concentrations.

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, 1991
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
Cambarus latimanus	15 22	5 mo	No significant mortality Significant mortality	Thorp *et al.*, 1979

lation relationships has not been investigated in this species.

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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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 MCV Rankings			Revised MCV Rankings		
List of lowest MCV's at 50 mg/L hardness	1	**18.8**	(***Hyalella***)	1	26.66	(*Ceriodaphnia*)
	2	26.66	(*Ceriodaphnia*)	2	**30.08**	(***Hyalella***)
	3	51.97	(*Daphnia*)	3	51.97	(*Daphnia*)
	4	77.46	(*Erpobdella*)	4	77.46	(*Erpobdella*)

Hyalella MCV Normalization
Using Correct MATC of 67.59 µg/L

MATC = 67.59 µg/L
Hardness = 130 mg/L
Slope (B) = 0.8473
MATC = $e^{[\ln 67.59 - (0.8473(\ln 130 - \ln 50))}$
MATC = $e^{3.4039}$
MATC = **30.08 µg/L**

Number of MAV's entered: 4

Existing FCV (µg/L) = 12.16

B (slope) = 0.8473 (original rulemaking)

FCV = $e^{[A + (0.8473(\ln 50))]}$
12.16 = $e^{[A + (0.8473(3.912020))]}$
2.4982 = 3.3147 + A
A = -0.8165

Existing Chronic Zinc Equation = $e^{[A + B\ln(H)]}$
where A = -0.8165, B = 0.8473

Number of MAV's entered: 4

Revised FCV (µg/L) = 17.62

B (slope) = 0.8473 (retained from original rulemaking)

FCV = $e^{[A + (0.8473(\ln 50))]}$
17.62 = $e^{[A + (0.8473(3.912020))]}$
2.8691 = 3.3147 + A
A = -0.4456

Revised Chronic Zinc Equation = $e^{[A + B\ln(H)]}$
where A = -0.4456, B = 0.8473