Water Quality Criteria Development for Iron

Technical Report

Water Quality Criteria Development for Iron

1008466

Final Report, December 2004

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CITATIONS

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This report describes research sponsored by EPRI.

The report is a corporate document that should be cited in the literature in the following manner:

Water Quality Criteria Development for Iron, EPRI, Palo Alto, CA: 2004. 1008466.

PRODUCT DESCRIPTION

The current national water quality criterion for iron – a criterion continuous concentration of 1 mg Fe/L – was derived 25 years ago. Such ambient water quality criteria are typically derived from toxicity tests in which the reagent grade chemical is dissolved in clean laboratory water. However, due to the complexity of iron speciation in freshwater, adverse effects of iron precipitates on habitat quality, and access of organisms to food, standard toxicity assays may not adequately assess the full impact of iron on aquatic organisms. This project resulted in new protective levels for iron in freshwater, using available bioassessment data.

Results & Findings

Protective water quality values for several insect families in a West Virginia data set were calculated using bioassessment data. Limiting functions for insect families were fit using nonlinear quantile regression analysis (sigmoid model). Mayflies in the family Leptophlebiidae appeared to be the most sensitive macroinvertebrates affected by ambient total iron concentrations, with a calculated field-based effect concentration (FEC) of 0.21 mg Fe/L. None are found at total iron concentrations greater than 4.5 mg/L. This value was used to achieve the Clean Water Act (CWA) biological integrity objective for total iron.

A second protective threshold for total iron strives to ensure propagation of aquatic life, which is the national aquatic life criteria objective, or the CWA 101(a) objective. A value of 1.74 mg/L should allow for biological conditions consistent this goal. At this and lower concentrations – marked by a relative decrease in the populations of several sensitive ubiquitous taxa as well as a relative increase in the abundance of intermediate and tolerant taxa – a remnant population of Leptophlebiids is likely to persist, providing the habitat is adequate. The analysis indicates that the preliminary benchmark of 1.74 mg/L for the CWA 101(a) objective is potentially more conservative than the concentration that normally prompts regulatory action in the State of West Virginia. It does, however, coincide with the concentration normally required to maintain a high level of biological condition.

Challenges & Objective(s)

The protective thresholds derived in this project are intended to represent maximum acceptable total iron concentrations in freshwater consistent with the CWA goal and objective. Because they are computed from field observations, only an association between increasing concentrations of iron and decline in the number of organisms can be detected. It is important to recognize that an association between total iron concentration and the maximum number of organisms in samples does not prove that iron is the stressor causing the decrease in organism abundance. The association does, however, define the limiting constraints of organism populations in the field in relation to total iron concentration and may help derive more appropriate levels of protection for aquatic life.

Applications, Values & Use

With this methodology, a possibility exists for the first time to establish meaningful protective values for total iron to meet the CWA goal and objective. The values not only take into account the direct toxic effects of iron but also its indirect (physical) effects. The cornerstone of the approach is the use of regression quantiles to estimate the limitation imposed by iron on the biological response variable (i.e., maximum numbers of organisms in samples). The advantage to this approach is that it greatly minimizes impacts of confounding factors (both chemical and nonchemical), which have precluded the use of field data in deriving aquatic life criteria. Focusing on changes in maximum number of organisms in response to increasing iron concentrations reduces potential biases that co-contaminants and other confounding factors may have on the results of regression analyses. If accepted, the method could be used to establish nationally recommended guidance for deriving protective thresholds for total iron. Environmental benefits of this application potentially are broad, since the method can be used to derive protective thresholds for other chemicals known to constrain aquatic organisms through indirect (physical) toxicity, such as aluminum.

EPRI Perspective

Currently, no guidance exists for implementing tiered aquatic life criteria such as the one established here for total iron. It is a state's responsibility to develop tiered aquatic life uses and define state reference conditions as part of development of a statewide bioassessment program. The long-term goal of the Environmental Protection Agency (EPA) is for all states to have refined tiered aquatic life uses and biological criteria in their water quality standards.

This report presents a new approach for deriving criteria "benchmarks" to protect aquatic life from the limiting constraints imposed by total iron, using endpoints (maximum abundance) that adequately represent regulatory targets. The approach is fundamentally sound and statistically rigorous for developing updated freshwater criteria (guidelines) for iron and other physical contaminants, based on relevant field bioassessment data. Application of this method for iron is not only novel but also represents a substantial improvement over the method used to derive the existing criterion.

Approach

The decision to derive an updated freshwater criterion for total iron using bioassessment data was based on a consensus reached by the EPA, EPRI, and investigator representatives at a 2001 meeting in Baltimore, Maryland. Investigators reached this consensus after a review of the effects literature for iron revealed that negative effects associated with the precipitation of iron colloids – which dominate the total iron pool at circumneutral pH in most natural freshwaters – would be extremely difficult to simulate in laboratory experiments.

Keywords

Iron
Aquatic Toxicity
Tiered Ambient Water Quality Life Criteria

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1

OVERVIEW OF ENVIRONMENTAL CHEMISTRY AND TOXICOLOGY OF IRON

Environmental Chemistry

The ferrous, or bivalent (Fe^{2+}), and the ferric, or trivalent (Fe^{3+}) forms of iron are the primary species of concern in the aquatic environment, and these two species can convert readily from one form to the other. The two forms have contrasting characteristics in that +2 ferrous iron predominates under anaerobic conditions, and has a higher water solubility, while +3 ferric iron predominates under aerobic conditions and has a much lower water solubility. Variables of principal importance that influence iron speciation include pH, redox potential (Eh), sulphur species and the concentrations of dissolved oxygen and carbon dioxide; relatively small shifts in pH or Eh can cause significant changes in iron speciation.

The most common form of iron in solution in anoxic groundwater is the ferrous ion. Soluble ferrous iron typically enters surface waters from ground waters or mines (or waste deposits and tailings) when they are pumped or drained. Ferrous iron can also be found in the deep waters of stratified lakes with anaerobic hypolimnia. A significant source of ferrous iron is from the chemical weathering of iron sulfide (pyrite; FeS_2), which involves the oxidation of reduced sulfur anions to sulfate and subsequent production of hydrogen ions, which lowers the pH in the water (a problem commonly associated with acid mine drainage). In aerobic waters at typical pH ranges, ferrous iron is rapidly oxidized to ferric iron. The half-life for the oxidation of ferrous iron has been estimated in rivers to be from 1.2 to 9.0 hours. In aerobic soft waters, the half-life for the oxidation of ferrous iron increases exponentially from 2.34 min to 234 min, to 390 h, and to 1625 days as pH decreases respectively from 8 to 7 to 6, then to 5 at 10°C (Davison and DeVitre 1992).

For all practical purposes, the ferric (Fe^{3+}) form of iron is insoluble, the result of a very low dissociation constant (approximately 10^{-36}) for $Fe(OH)_3$. Hence, in aerobic waters any freely dissolved ferric ion that is formed or introduced will hydrolyze with water to form insoluble $Fe(OH)_3$. The amorphous ferric hydroxide produced forms gels or flocs, which eventually result in precipitated $Fe(OH)_3$ and/or Fe_2O_3, and in colloidal iron oxyhydroxide particles that are suspended. The suspended colloidal iron particles can pass through a 0.45 µm filter, and therefore become classified as "dissolved," when in fact they are not truly in chemical solution.

The percent of dissolved (passing through a 0.45 µm Gelman filter) to total iron in a Danish low-land river system (River Vidaa) based on annual averages ranged from 1.2 to 84.3 (Rasmussen and Lindegaard 1988). From field investigations on five streams in West Virginia (Cherry 1983a,b; Loeffelman et al. 1986), the freely dissolved ferrous component was found to comprise an average of 11 percent of the total iron pool. Some additional data from the West Virginia study suggests that the total ferrous iron content was not highly correlated with total iron concentration.

Relationship of Iron to Key Water Quality Parameters

Iron, both ferric and ferrous, may be associated with macromolecular natural organic matter (NOM). High concentrations of NOM frequently are associated with high concentrations of "soluble" iron in natural waters. Both organic and inorganic complexation of iron increases the measured "dissolved" concentration of iron. Much of this measured "soluble" iron, however, is in fact colloidal. Published evidence shows that a large portion of "dissolved" iron in freshwater exists as colloidal particles stabilized by organic materials, especially colloidal humic acid. There is some evidence to suggest that dissolved or colloidal organic material may stabilize ferrous iron and retard its oxidation (Theis and Singer 1974). Esteves Da Silva and co-authors (1998) have shown that fulvic acid forms stable soluble complexes with ferric iron in the acid pH range (3, 4, 5 and 6), although ferric iron in this ferruginous complex can be photoreduced by UV light to the ferrous state (Francko and Heath 1982, Sulzberger et al. 1990). This latter is thought to contribute to some of the large diurnal fluctuations in the speciation and concentration observed for ferrous and total iron in streams (Vuori 1995).

In the pH range of 5 to 8, the oxidation of ferrous iron leading to the formation of amorphous iron (ferric) hydroxides proceeds with a second order dependence on hydroxyl ions according to the rate law:

$$-d[Fe^{2+}]/dt = k[Fe^{2+}]P_{O2}[OH\text{-}]^2$$

and, is highly sensitive to small changes in pH in the presence of oxygen (Vuori 1995). The oxidation process is accelerated by the presence of trace metals, phosphate, fluoride and particles, including autocatalysis by fresh iron oxides (Vuori 1995). Coprecipitates of heavy metals (cadmium, copper, lead, and zinc) incorporate into the iron oxides that are formed during the oxidation of ferrous iron, effectively reducing heavy metal mobility, solubility, and bioavailability, and rendering the heavy metals less toxic (Martinez and McBride 2001).

The metabolic activity of aerobic bacteria such as Crenothrix and Leptothrix also significantly influence the speciation and concentration of iron in water, largely by the oxidation of ferrous iron to ferric hydroxide (Faust and Aly 1980). Thiobacilis ferroxidans, in particular, is known to greatly enhance the oxidation of ferrous iron to ferric hydroxide in acid streams (Vuori 1995).

A typical profile of iron physicochemical speciation in river waters was found when eight Japanese rivers were studied using size fractionation to separate the iron species (Tanizaki et al. 1992). On the average, only 7.6 percent of the iron was "dissolved" (<0.45 µm) in the rivers, with a range from 0.3 to 9 percent. In this study, it was notable that the small molecular weight (MW<500) fraction of iron, which would contain the freely dissolved ions and labile complexes that would provide any bioavailable iron, represented on the average only 9.3 percent of the dissolved iron (range 4.8 to 20 percent), and only 0.7 percent of the total iron.

Iron as a Nutrient and Iron Uptake

Iron is one of the most abundant elements in the earth's crust, and it is an essential micronutrient for microflora, plants and animals. Nearly all cells require iron in the enzymatic pathways of chlorophyll and protein synthesis. Moreover, iron is a key constituent of cytochrome, ferredoxin, nitrogenase and some of the photosynthetic pigments in cyanobacteria (Pany and Mitra 1998), and of hemoglobin in higher animals. Some of the key physiological processes modulated by iron include: DNA and RNA synthesis; cellular respiration; oxygen metabolism, transport, and binding; steroid production; and xenobiotic metabolism (Locke and Nichol 1992).

Iron quantitatively is the most important trace metal. Cells normally require between 220 and 22,000 µg Fe/L for growth (Weinberg 1989). In oxic waters, concentrations of iron typically are below this level because iron exists primarily as ferric phosphates and hydroxides, which are essentially insoluble in the absence of organic chelation, as noted above. This low solubility of iron in many aquatic environments, coupled with the obligate requirement for iron, has led to the evolution of mechanisms for iron acquisition (Hutchins et al. 1991). Cyanobacteria, for example, release siderophores, which are extracellular iron (ferric) chelating agents that aid in the solubilization and assimilation of iron in environments where iron is growth limiting (Wilhelm et al. 1996).

Iron ions generally are assimilated into cells by specialized membrane transport proteins designed for such acquisition. Reduction-oxidation transformations control the speciation and biological availability of iron. For example, photochemical or biological reduction of the more stable ferric iron results in an increase in the more soluble ferrous iron, which has more rapid ligand exchange kinetics, and which forms much weaker complexes than ferric iron (Sunda and Huntsman 1998). Recent evidence indicates that in addition to the cellular uptake of the free metal ion (Fe^{2+}), iron uptake by saltwater algae may also be related to the diffusion of kinetically labile dissolved inorganic ferric iron species [$Fe(OH)^{2+}$, $Fe(OH)_3$, and $Fe(OH)_4^-$] binding to membrane transport sites, because the exchange rates of these kinetically labile species are sufficiently rapid to permit donation to them (Hudson and Morel 1990). Benderliev et al. (1997) examined iron uptake in the freshwater green alga, *Scenedesmus incrassatulus*, which has no membrane-bound chelators, and showed that inorganic iron cannot be directly assimilated, but instead, only the organic humic acid-stable iron chelates were selectively assimilated. Organic chelates and most bound particulate forms of metals (e.g., colloidal metal oxides) previously were discounted as being assimilated, since their dissociation and ligand exchange kinetics were thought to be too slow to permit rapid donation of the metal ion to membrane transport sites (Morel et al. 1991). Fargasova et al. (2000) recently has shown that the concentrations of iron accumulated in algal cells were 2.7 to 19.6 times higher when iron was supplied in the form of complexes than was the case from the inorganic ferric chloride.

Mechanisms of Toxicity

Direct Effects (physiological)

Ferrous iron is considered to be the most toxic form of iron to aquatic animals, in part, because it is the most readily bioavailable form of iron under most circumstances (see above). Damage caused by ferrous iron is most closely associated with DNA and membrane damage (Dandapat et al. 1999; Payne et al. 1998, 2001; Sinha et al. 1997). An increase in lipid peroxidation product and subsequent potassium leakage may be one of the primary responses of iron toxicity associated with membrane damage (Sinha et al. 1997). Alternatively, ferrous iron may act to mechanically destroy cell membranes by ulceration and formation of precipitates on the membrane, thus preventing absorption of nutrients (Gerhardt 1992). Locke and Nichol (1992) point out that bypiridillium herbicides, such as paraquat, act to release iron from the iron storage protein ferritin; thus, liberating ionic iron, which catalyzes the production of damaging free radicals and enhances lipid peroxidation in membranes (also see Saito et al. 1985 and Samokyszyn et al. 1988).

Evidence that formation of iron precipitates on membranes impede uptake of food and nutrients is provided by Gerhardt (1992; also see Gerhardt 1995), who examined the effects of subacute concentrations of iron on the mayfly, *Leptophlebia marginata*, at pH 4.5 and 7. Survival, motility, and feeding behavior of this acid-tolerant mayfly (non-North American species) virtually were unaffected by concentrations as high 50,000 µg Fe/L at pH 7, but at pH 4.5, where ferrous iron was approximately 50 to 90 percent of the total iron concentration, the larvae experienced a decrease in food consumption due to constipation, which ultimately led to mortality (20 percent) after 30 days of exposure to only 10,000 µg Fe^{2+}/L. At the same time, the iron content in and on these animals rose to significantly higher concentrations, due to the surface adsorption from precipitation of the iron compounds on the animals.

Despite causing histopathologic gill damage in fish (Lehtinen and Klingstedt 1983), iron (predominantly as ferric hydroxide) does not seem to exert any direct effects on the osmo-regulatory processes of aquatic animals, at least in the conventional sense. For example, sodium balance in brook trout (*Salvelinus fontinalis*) was not disrupted by dissolved (0.1 µm filtration) iron at the 96-hr LC50 test concentration of 1,005 µg Fe/L. There was a 15 percent loss in body sodium, but plasma sodium was unaffected (Gonzales et al. 1990). Also, total iron at twice the LC50 concentration (approximately 2,513 µg Fe/L) did not escalate the loss of body sodium. The authors attributed the steady plasma Na levels to shifts of water from the extracellular fluid compartment into the intracellular compartment due to the loss of body sodium, and suggest that other factors, such as respiratory distress, may have contributed significantly to mortality.

Direct Effects (physical)

The mechanism of acute toxicity for iron in fingerling brown trout (*Salmo trutta*) by respiratory disruption, resulting from gill clogging and damage, has recently been affirmed by Dalzell and McFarlane (1999). In their study, the authors showed that plasma iron remained constant after 14 days of exposure to sublethal (7,000 µg Fe/L) iron. At this same concentration, the gill iron concentration of juvenile brown trout did not increase over time, supporting the supposition that iron accumulates only at the gill surface, without absorption. There is also no available evidence to suggest that ferric iron was transported across the gill epithelium. Iron removal mechanisms at the gills, such as the excessive mucus production and sloughing of metals, were not readily apparent.

Indirect Effects

In general, the effects of iron, which can act to limit or enhance an organism's access to resources or the quality of those resources, may ultimately be of greater consequence to freshwater aquatic animals than direct physiological or physical effects. These "indirect effects," as defined by Vuori (1995), are manifested by impacts on the periphyton and benthic assemblages, especially in oxic environments. Numerous field and laboratory studies indicate that ferric hydroxides and iron-humus precipitates on both biological and other surfaces can affect organisms by changing the structure and quality of benthic habitats and food resources (Amelung 1982; McKnight and Feder 1984, Rasmussen and Lindegaard 1988; Smith et al. 1973; Sode 1983; Sykora et al. 1972). Moverover, iron deposition and/or re-suspension may interfere with the ability of aquatic life to acquire food resources (Gerhardt 1992; Randall et al. 1999). Typical observations include the formation of thick iron hydroxide layers on eggs and substrata, appendages and mouth parts of benthic invertebrates, and on respiratory surfaces of fish (gills). Vuori (1995) provides an excellent review of many kinds of indirect physical effects attributed to iron in lotic systems. Many of these effects are included in the summaries of available data, provided below.

Assessment and Summary of Available Data for Formulation of Freshwater Criteria Based on Total and Ferrous Iron

A literature search to obtain the relevant literature on the toxic effects of the various iron species and total iron to freshwater aquatic organisms was conducted using the Chemical Abstracts Service (CAS), Biological Abstracts (BIOSIS) and ACQUIRE databases for the years 1987 to 2001 (a literature search for iron that included the years 1986 and before was conducted in April 1987 by the University of Wisconsin - Superior and Battelle to assist in the development of an Ambient Water Quality Advisory for Iron).

The literature review included direct (acute and chronic toxicity) and indirect (physical toxicity) effects data for freshwater organisms and communities, and was conducted in accordance with the procedures used by the U.S. EPA for the derivation of water quality criteria for aquatic life (Stephan et al. 1985). Based upon this review, it was determined which of the existing data can be used to derive an updated criterion. Descriptions and assessments of the studies are provided in the section below, followed by a brief summary. Effects data which meet the requirements set forth in the guidelines (Stephan et al. 1985) for use in developing ambient aquatic life criteria are separated according to acute and chronic (Table 1-2), and within the tables, according to invertebrate and vertebrate, and ferrous and total iron (predominantly ferric iron). Results from other tests and unused studies (studies which cannot be used to derive a criterion) are also included as a basis for comparison and contrast. These other and unused studies are summarized in tabular form in Tables 1-6 and 1-7.

Acute Toxicity of Ferrous Iron to Freshwater Aquatic Invertebrates

Information concerning the acute toxicity of ferrous iron (Fe^{2+}) to invertebrates is sparse (Table 1-1). There is only one acute toxicity test with invertebrates where ferrous iron concentrations were measured in laboratory test water. Rousch et al. (1997) assessed the acute effects of ferrous iron on hatching and survival of the water mite (*Arrenurus manubriator*) and the midge (*Chironomous riparius*) by adding ferrous sulfate to soft water (41.6 mg/L as $CaCO_3$) at pH 4. The fate of ferrous sulfate under these test conditions was determined in a separate experiment that was identical to the toxicity experiments, but without organisms. In the iron experiment without organisms, dissolved (the material passing through a 0.45 µm filter) ferrous and total iron concentrations differed by only 4 percent, and dissolved ferrous concentrations differed from nominal levels by only 2.7 percent. Nevertheless, an orange floc still appeared in the test beakers. Subsequent measurements of total suspended solids (range: 0.0 to 111.2 mg/L) indicated that the loss of iron through precipitation was probably no more than 5 percent.

The results of this study indicated that ferrous iron at concentrations as high as 1,000,000 µg Fe^{2+}/L did not affect hatching of either mite and midge eggs. After 48 hrs of exposure, however, midge larvae showed a decrease in survival compared to controls (based on ANOVA) starting at 400,000 µg Fe^{2+}/L, while water mite deutonymphs showed a marked decrease in survival at 200,000 µg Fe^{2+}/L. Interestingly, the survival of mite larvae was not affected by high concentrations of ferrous iron, nor was the survival of adult females, but adult male mites exhibited significant mortality at 1,000,000 µg Fe^{2+}/L. The apparent sensitivity of midge larvae to ferrous iron could be attributed, in part, to their lack of tolerance to the low test pH (approximately 50 percent of the larvae survived at pH 4.0). The survival of mite larvae, deutonymph, and adults, however, was unaffected by the low test pH.

Unused Acute Ferrous Toxicity Data From Tests With Freshwater Aquatic Invertebrates

Maltby et al. (1987) investigated the acute toxicity of ferrous iron to a non-North American freshwater isopod, *Asellus aquaticus* in a static test (Table 1-4). The isopods were collected from two sites upstream and downstream of effluent input from an abandoned coal mine on the River Don, U.K. Analar ferrous sulfate was added to artificial pond water (calcium hardness approximately 50 mg/L as $CaCO_3$) to produce a series of test concentrations in which the pH of solutions was either adjusted to approximately 4.5, or buffered to approximately 6.0 using a tartrate buffer system (0.0025 M sodium potassium tartrate). For the low pH test solutions, most of the soluble (dissolved) iron was ferrous, which remained constant in solution for up to 6 days. There was about a 30 percent reduction in the initial concentration of ferrous iron in the buffered system after 4 days. The 50-hr LC50 values for *A. aquaticus* from the upstream sites (299,800 and 419,200 µg Fe^{2+}/L) in which the test organisms were exposed to the low pH and tartrate buffer system, respectively, were lower than those observed in isopods collected downstream of the coal mine effluent (428,500 and 466,700 µg Fe^{2+}/L, respectively), indicating a greater tolerance of those animals previously exposed to ferrous iron and low pH. The buffer appeared to have rendered the ferrous iron less toxic, which the authors attributed to complexation, and they also acknowledged the possibility that low pH and ferrous iron could be acting synergistically.

Acute tests with another non-North American species, the mayfly (*Leptophlebia marginata*) indicated that ferrous iron is likely more toxic at low pH (Gerhardt 1994). Mayfly nymphs were collected from a small, softwater stream (calcium hardness approximately 18 mg/l as $CaCO_3$; pH 6.5) with high humic acid content (dissolved organic carbon 21.6 mg C/l). Mayflies were exposed to the stream water in aquaria dosed with ferrous sulfate at pH conditions of 4.5 and 7. Ferrous iron in the various test concentrations constituted greater than 90 percent of the total iron content at both test pH levels. The 96-hr LC50 values for *L. marginata* nymphs were 106,300 and 89,500 µg Fe^{2+}/L at pH values of 7 and 4.5, respectively (Table 1-4). The 96-hr EC50 values based on escape behavior were similar, at 70,000 and 63,900 µg Fe^{2+}/L, respectively.

Acute Toxicity of Total and Ferric Iron to Freshwater Aquatic Invertebrates

There is a large range (315-fold) in the acute toxicity to invertebrates reported for total and ferric iron based upon laboratory exposures conducted with dilution water at circumneutral pH (6 to 8.5). Khangarot (1991) examined the acute toxicity of total iron to the freshwater tubificid worm, *Tubifex tubifex* (Muller), and reported a 96-hr LC50 for this species of 101,840 µg Fe/L (Table 1-1). Test water was renewed every 24 hrs, but the concentrations of iron in the water were not measured. Mean pH, dissolved oxygen, and hardness of the tubewell (i.e., well) water used for the toxicity tests were, 7.6, 5.8 mg/L, and 245 mg/L as $CaCO_3$, respectively. The test solutions produced precipitation 2 to 3 hrs after the addition of the metal salt (ferric chloride).

Conversely, the toxicity of iron associated with a ferrous salt (ferrous sulfate) was found to be approximately 5.5 times lower to another freshwater worm, *Branchiura sowerbyi,* under similar test conditions (Mukopadhyay and Konar 1984). The 96-hr LC50 values at test pH values of 6.5, 7.0, and 8.5 were 580,000, 560,000 and 446,000 µg Fe/L, respectively (Table 1-1), using unchlorinated borehole (i.e. well) water (initial pH 7.0; DO 7.5 mg/L; alkalinity 115 mg/L as $CaCO_3$; hardness 268 mg/L as $CaCO_3$). In the same study, the authors reported 96-hr LC50 values of 35,200, 33,200, and 36,000 µg Fe/L for the copepod, *Cyclops viridis*, at the respective test pH values (Table 1-1). Apparently, planktonic copepods are considerably more sensitive (approximately an order of magnitude) to iron than the benthic worms.

Cladocerans also are more acutely sensitive to iron than worms. A 48-hr LC50 value of 12,600 µg Fe/L was reported for *Ceriodaphnia dubia* in a static, non-renewal test conducted with moderately hard (hardness 90 mg/L as $CaCO_3$) reconstituted water (Fort and Stover 1995). In this particular study, the pH of the ferric chloride (Fe^{3+}) dilutions ranged from 7.0 to 7.3 throughout the study, and the dissolved oxygen levels ranged from 7.8 to 8.2 mg/L. Likewise, Khangarot and Ray (1989) investigated the acute toxicity of iron associated with the exposure of *Daphnia magna* to varying concentrations of ferrous sulfate (Fe^{2+}). A 48-hr EC50 value of 7,200 µg Fe/L was reported based on the nominal amount of metal ion added (Table 1-1). At the beginning of the experiments, the pH values ranged from 7.2 to 7.8, and dissolved oxygen concentrations ranged from 5.2 to 6.5 mg/L. Total hardness of the dilution water (well water) was 240 mg/L as $CaCO_3$. The test solutions began to precipitate iron after only 3 to 5 hrs.

The most acutely sensitive freshwater invertebrate tested was the mayfly, *Ephemerella subvaria*, in a static exposure (Warnick and Bell 1969). A 96-hr LC50 value of 320 µg Fe/L was obtained for this species, based on the initial concentration of metal added (as ferrous sulfate) to the dilution water (soft: hardness 48 mg/L as $CaCO_3$, pH 8.2, and dissolved oxygen 8.2 mg/L in the test water after 15 days). Concentrations of the iron in the test dilutions were measured at test initiation by a polarograph and were found to be within 5 percent of the calculated (nominal) values.

The only other applicable acute tests with invertebrates that were obtained were for species in the family Gammaridae (amphipods) and Astacidae (crayfish). Martin and Holdich (1986) tested the toxicity of ferrous sulfate (added metal was Fe^{2+}) and ferric chloride (added metal was Fe^{3+}) to the amphipod *Crangonyx pseudogracilis*. The tests were static, and the test solutions were renewed every 24 hours for up to 96 hours. The actual percentage of Fe^{2+} and Fe^{3+} present in the test solutions was not measured; the test concentrations were calculated on the basis of metal ion in the respective salts added. Significant precipitation of hydrated oxides was observed in both tests. The initial pH and oxygen content of the soft (50 mg/L as $CaCO_3$) dilution water for the two tests was 6.75 and 9.6 mg/L, respectively. The reported 96 hr LC50 value for *Crangonyx* exposed to iron added in the reduced form (as ferrous sulfate) was 95,000 µg Fe/L, while the LC50 value for ferric chloride was 120,000 µg Fe/L (Table 1-1). The authors reported that in tests with ferric chloride, the pH in the test chambers of the four highest iron concentrations (which exceeded 100,000 µg Fe/L), was between 2.8 and 2.4. In a separate pilot study, the authors determined that *Crangonyx* could tolerate pH values between 3.2 and 3.6 for at least 96 hours, but that mortality (approaching 20 percent after 96 hours) ensued between 2.7 to 3.3.

The 96-hr LC50 value for crayfish, *Orconectes limosus*, exposed to iron as ferric chloride was 32,000 µg Fe/L as reported by Boutet and Chaisemartin (1973) [Table 1-1]. Thirty-day LC50 values, in which crayfish were and were not fed, were 22,000 and 21,000 µg Fe/L, respectively for the two forms of iron. Dissolved oxygen was maintained at saturation during the tests, but it is uncertain whether addition of the ferric chloride salt modified the pH from an initial value of 7. No further information was provided regarding the buffer capacity (hardness) of the dilution water.

Other Acute Data from Tests with Freshwater Aquatic Invertebrates

Tatara et al. (1998) reported a 24-hr LC50 value of 17,900 µg Fe/L for the free-living soil nematode, *Caenorhabditis elegans*, in an artificially prepared aqueous medium (Table 1-3). The test solutions were prepared with a ferric nitrate salt, and animals were fed to reduce control mortality. The LC50 value for the free ion species was predicted with metal speciation software (PC MINTEQA2 Version 3.10), and was considerably lower, 3,351 µg Fe^{2+}/L (presumably all ferrous iron).

Twenty-four hour EC50 values of 5,250 and 36,800 µg Fe/L (based on calculated metal ion concentrations) were reported for the cladocerans *D. magna* (Lilius et al. 1994) and *D. pulex* (Lilius et al. 1995), respectively, exposed to ferrous sulfate in a standard hard (approx. 400 mg/L as $CaCO_3$; pH 7.6) reconstituted water mix (Table 1-3). When exposed to ferric chloride in a natural surface water (Lake Baikal, Russia), the 24-hour No Observed Adverse Effect Concentration (NOAEC) for *D. magna* was reported to be 5,585 µg Fe/L (Stom and Zubareva 1994).

Sorvari and Sillanpää (1996) recently showed a 6 to 700 percent decrease in the toxicity (increase in the EC50 value) of ferric chloride to *D. magna* with and without complexation to ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). The organic complexation of metal to EDTA/DTPA decreased the toxicity of ferric chloride to this species. The 24-hr EC50 values for ferric iron and the Fe^{3+}+EDTA and Fe^{3+}+DTPA complexes in the study were 16,000, 17,000 and 110,000 µg Fe/L, respectively. The relationship between the conditional stability constant and the relative toxicity reduction accomplished by EDTA and DPTA complexation could not be established in the study because of the precipitation of iron during the experiments.

At least one study suggests an increased advantage of living in an iron polluted environment. Postma et al. (1995) investigated the site-specific differentiation in tolerance of populations of the midge, *C. riparius*, in iron contaminated streams. First generation larvae from parents sampled in the most contaminated field sites exhibited an increase in growth rate when cultured at iron (as ferric chloride) concentrations as high as 10,250 µg Fe/L in clean environments (laboratory), relative to the larvae of parents from reference populations (Table 1-3).

Unused Acute Data From Tests With Freshwater Aquatic Invertebrates

A 50-hr LC50 value of 7,230 µg Fe/L for *D. magna* exposed to ferric chloride (Dowden and Bennett 1965) was not used because the test procedures were not adequately described (Table 1-4). Likewise, the 48-hr LC50 value of 9,600 µg Fe/L for *D. magna* exposed to ferric chloride was not used because the daphnids were fed a mixture of dried grass and enriched trout-fry granules during the exposure (Biesinger and Christensen 1972). This latter study is the only study for which an acute:chronic ratio (ACR) could be calculated (see section on Chronic Toxicity to Freshwater Invertebrates below).

Acute Toxicity of Ferrous Iron to Freshwater Aquatic Vertebrates

Few results are available for acute toxicity tests exposing freshwater vertebrate species to ferrous iron. The only applicable values for fish were published in a report prepared for the American Electric Power Service Corporation (Cherry 1983a,b), and later summarized in a special technical publication (No. 891) for the American Society of Testing and Materials (see Loeffelman et al. 1986). A 96-hr LC50 value of 4,400 µg Fe^{2+}/L based on bioavailable ferrous iron (as determined with the bathophenanthroline or BAP-reactive method) was reported for static tests with juvenile rainbow trout, *Oncorhynchus mykiss* (Cherry 1983a). The test solutions were continually renewed with iron salt (ferrous sulfate), and iron measurements were made twice daily throughout the experiment in an attempt to maintain the nominal test concentrations at the desired level. The corresponding 96-hr LC50 value for this species based on total recoverable iron was approximately 4 times higher (18,300 µg Fe/L) than for ferrous iron (Table 1-1).

Similar tests were conducted by Cherry (see 1983b) with adult fathead minnows, *Pimephales promelas*. The 96-hr LC50 values for BPA-reactive ferrous iron and total recoverable iron were 3,700 µg Fe^{2+}/L and 14,400 µg Fe/L, respectively (Table 1-1). A second test with fathead minnows produced 96-hr LC50 values of 9,200 µg Fe^{2+}/L for BAP- reactive ferrous iron, 9,900 µg Fe^{2+}/L for hydrochloric acid (HCL)-reactive ferrous iron (total ferrous iron), and 24,200 µg Fe/L for total recoverable iron. A nominal pH of 6 was the test pH goal for all exposures, although actual pH measurements indicated a variable range, especially in the higher test concentrations during the rainbow trout exposure (e.g., 4.1 to 6.3; control 7.2). Substantial fluctuation in BPA-reactive ferrous iron concentrations were also oberved throughout the exposures, and concentrations of total iron in the static test chambers were greater on the bottom of the chambers than in the middle of the chambers, indicating oxidation and subsequent precipitation of the ferric species.

Acute Toxicity of Total and Ferric Iron to Freshwater Aquatic Vertebrates

The acute toxicity of iron (ferrous sulfate) to brown trout fingerlings(*Salmo tratta*) was investigated by Dalzell and MacFarlane (1999) in a flow-through system using de-chlorinated City of Nottingham, England tapwater (pH 7.62; total hardness 287 mg/L as $CaCO_3$). Samples were collected and analyzed for total and dissolved (0.45 µm filtered) iron throughout the exposure. The mean pH in the test water decreased from 6.23 in controls (190 µg total Fe/L) to 5.74 in the highest test concentration (42,400 µg Fe/L). The effect of the relatively modest decrease in pH to brown trout was not assessed. The reported 96-hr LC50 values (interpolated by an unspecified methdology) for total recoverable and total dissolved iron were 47,000 and 240 µg Fe/L, respectively. The reported LC50 values exceeded the highest test concentrations by 112 and 150 percent, respectively. Examination of surviving trout showed significant accumulation of iron on the gills of exposed fish, resulting in epithelial lifting and cell hypertrophy, but there was no evidence of excessive mucus formation.

The acute toxicity of iron to yearling brook trout, *Salvelinus fontinalis*, in the form of ferrous sulfate at pH values of 7, 6, and 5.5 was examined by Decker and Menedez (1974). The dilution water for the flow-through experiments was dechlorinated City of Elkins, West Virginia tapwater from the Tygart River (water quality characteristics not reported). The respective 96-hr LC50 values, based on measurements of total recoverable iron, were: pH 7.0- 1,750 µg Fe/L; pH 6.0- 480 µg Fe/L; and pH 5.5- 410 µg Fe/L (Table 1-1). No mortalities were recorded for control fish at any of the pH levels tested. Gill hyperemia was consistently observed in fish exposed to the highest test concentrations (range: 1,700 to 5,200 µg Fe/L).

The acute toxicity of iron to two different size classes (3 and 6 cm) of common carp, *Cyprinus carpio*, was assessed by Alam and Maughan (1992). Ferrous sulfate was added to a dilution water of unspecified hardness. The arithmetic mean of two 96-hr LC50 values reported for the two size classes of carp were 1,500 and 3,035 µg Fe/L, respectively, based on the amount of metal ion added (Table 1-1). The dissolved oxygen concentration and pH reported for tests with other metals in the study were 6.4 mg/L and 7.1, respectively.

The fish species least sensitive to iron on an acute basis was the tilapia, *Tilapia mossambica* (Mukhopadhyay and Konar 1984). The 96-hr LC50 values determined in static tests with this fish species at pH levels of 6.5, 7.0, and 8.5 were 119,600, 83,200, and 118,000 µg Fe/L, respectively (Table 1-1). The dilution water was unchlorinated well water, with a reported pH of 7, dissolved oxygen of 7.5 mg/L, and hardness of 268 mg/L as $CaCO_3$.

Acute Effects Data Summary

Genus mean acute values (GMAVs) were calculated for a total of 16 freshwater species, including 4 based on ferrous iron, and 14 based on total iron (Table 1-5). The total iron GMAVs for the 14 freshwater species tested range from a low of 320 µg Fe/L for the mayfly (*E. subvaria*), to 446,000 µg Fe/L for the tubificid worm (*B. sowerbyi*), exhibiting a range of over three orders of magnitude. Two of the four most sensitive genera are freshwater fish. A similar range in species sensitivity was demonstrated by freshwater fish and invertebrates exposed to ferrous iron. Based on very limited aquatic vertebrate (fish) data, it appears that the acute toxicity threshold to the more bioavailable ferrous iron may be higher than for total iron, which is composed almost entirely of ferric iron under standard laboratory conditions. These latter results, however, are often compromised by the study design (static renewal), which can reduce pH at the highest test concentrations and cause excessive fluctuation of ferrous and total iron concentrations in the test chambers.

The minimum studies reviewed above do not meet the number of acceptable acute tests that would be required by the Guidelines (Stephan et al. 1985) derive an acute criterion for total iron for freshwater organisms. There are no data for a species from a representative family in a different order of aquatic insect, or are data available for any other species from a different phylum (e.g., mollusca, rotifera). The present data are also insufficient to derive an acute criterion for ferrous iron. Data from only two acute studies are available to generate a ferrous iron acute criterion, and these data are questionable based upon the Guidelines methodology.

Chronic Toxicity of Total Iron to Freshwater Invertebrates

Only one chronic toxicity test with iron meets the requirements set forth in the U.S. EPA's criterion derivation guidelines. Biesinger and Christensen (1972) examined the survival and reproduction of *D. magna* (12 hrs old) to iron after a 3 week exposure to various concentrations of ferric chloride in Lake Superior dilution water (mean pH 7.74, total hardness 45.3 mg/L as $CaCO_3$). The test water was renewed at weekly intervals, and a water sample was collected for analysis of total iron. A chronic value of 5,900 µg Fe/L for survival was inferred by graphical interpolation, while a 16 percent reproduction impairment at 4,380 µg Fe/L was interpolated using the same methodology (Table 1-2). Ferric chloride stock solutions were equilibrated (aged) for 4 days prior to testing. Upon addition to the dilution water, the pH in the higher test concentrations (e.g., > 10,000 µg Fe/L) dropped to as low as 6.4, but then rose back up to 7.6 prior to next scheduled renewal (one week later). The dissolved oxygen concentration remained close to saturation (approximately 9 mg/L) throughout the study. The acute:chronic ratio (ACR) calculated based opon these data was approximately 2.19 (9,600 ÷ 4,380 µg Fe/L). The 48-hr acute value of 9,600 µg Fe/L, however, which was used to calculate the ACR was relegated to other data because the test organisms were fed during the acute test. This is the only known ACR available for iron at the present time.

Other Chronic Freshwater Invertebrate Data

Dave (1984) investigated the chronic (21-day) effects of iron (as ferric chloride) on the survival, growth, reproduction, and hemoglobin content in D. magna in hard (250 mg/L as $CaCO_3$) reconstituted water using static renewal tests Table 1-3. The pH was adjusted in the highest test concentration prior to dilution in order to maintain a nominal pH range of 8. Measured values for pH and dissolved oxygen in the chronic tests (with food) were 7 to 8, and 50 - 100 percent air saturation, respectively. After 21 days, body length was generally unaffected by iron concentrations as high as 2,048 µg Fe/L (nominal). At nominal concentrations greater than or equal to 256 µg Fe/L, reproduction was reduced to between 0 and 12 percent of the control reproduction. Significant mortality (56 percent) was not observed until concentrations exceeded 512 µg Fe/L. Hemoglobin was stimulated above the control level in all concentrations of iron tested (> 0.0625 µg Fe/L).

Chronic Toxicity of Iron to Freshwater Aquatic Vertebrates

The chronic effects of a ferric hydroxide suspension on juvenile brook trout were first investigated by Sykora et al. (1972). The ferric hydroxide was obtained by neutralization of ferrous sulfate using calcium hydroxide. The pH of the suspension was maintained slightly above 7 throughout the study (6 months). The percent of total ferrous iron to total iron ranged from 1.1 in the lowest treatment group (7,800 µg Fe/L) to approximately 0.3 in the highest treatment groups (50,180 µg Fe/L), which illustrates that ferric iron was dominant. After 31 weeks, the mean wet weight of brook trout exposed to 7,800 µg Fe^{3+}/L was slightly elevated compared to controls (no observed adverse effect concentration), while juvenile brook trout exposed to 13,420 µg Fe^{3+}/L exhibited an approximate 20 percent reduction in wet weight (lowest observed adverse effect concentration). Survival of juvenile brook trout exposed to the ferric hydroxide suspensions was not adversely affected at concentrations as high as 50,180 µg Fe^{3+}/L. The reduced growth observed in brook trout at the higher ferric hydroxide concentrations (13,420 µg Fe^{3+}/L) was thought to be a result of impaired visibility, which ultimately reduced food consumption, or possibly a result of inefficient energy utilization caused by long-term stress. The chronic value estimated for juvenile brook trout in this study was 10,230 µg Fe^{3+}/L (geometric mean of 7,800 and 13,420 µg Fe^{3+}/L i see Table 1-2).

Using a similar experimental design, Smith and Sykora (1976) examined the early developmental effects of lime-neutralized ferric hydroxide suspensions on brook trout and coho salmon, *Oncorhynchus kisutch*, where the mean pH was 8.1 in the test chamber. The mean percentage of total ferrous iron to total iron in the respective treatment groups was 2.8 (range: 0.5 to 9). Ferric hydroxide suspensions at high as 10,500 µg Fe^{3+}/L had no effect on hatching success of brook trout or coho salmon, and hatching times of control and test groups of brook trout and coho salmon eggs did not differ (Table 1-2). Brook trout alevin survival and length after 90 days of exposure were also unaffected by exposure to ferric hydroxide. Coho salmon alevin survival declined sharply, however, when exposed to concentrations greater than 4,360 µg Fe^{3+}/L after only 30 days of exposure. There was no apparent decrease in the length of coho salmon alevins relative to controls after 90 days. A chronic value could not be determined for brook trout in the study, because the no-observed adverse effect concentration (NOAEC) for brook trout was the highest concentration tested (10,500 µg Fe^{3+}/L). The estimated chronic value for coho salmon based on survival at 30 days was the geometric mean of 4,360 and 10,500 µg Fe^{3+}/L or 6,766 µg Fe^{3+}/L (Table 1-2).

Other Chronic Freshwater Aquatic Vertebrate Data

Amelung (1982) examined the effects of dissolved iron compounds on the development of eggs and larvae of rainbow trout, *Oncorhynchus mykiss* (Table 1-3). Hatching was delayed at iron concentrations of 5,700 µg Fe/L, although no significant increase in mortality was observed up to 11,800 µg Fe/L. Subsequent morphometric measurements indicated that newly-hatched larvae exposed to 5,700 µg Fe/L were significantly shorter than controls. After hatching, rainbow trout alevins exposed to 1,300 µg Fe/L died within 3 days.

Conversely, there was no hatching of brown trout eggs (*Salmo trutta*) reported when the ferrous and total iron concentrations were 230 µg Fe^{2+}/L and 460 µg Fe/L, respectively (Table 1-3), in three Danish water courses (Geertz-Hansen and Mortensen 1983). Subsequent observations of alevins and eyed eggs exposed to iron showed increased mortality at an average of 2,910 µg Fe^{2+}/L (ferrous) and 5,170 µg Fe/L (total iron); the highest average concentrations of ferrous and total iron concentrations recorded in this neutral to slightly acidic (6.55 to 7.05) ambient surface water.

Chronic Effects Data Summary

Chronic values exist for one freshwater invertebrate species (*D. magna*) and 3 freshwater fish species (Table 1-2). The fish species include two salmonids (coho salmon and brook trout) and the fathead minnow. The test results with the fish were based almost entirely on the toxicity of a ferric hydroxide suspension, which was achieved by liming with calcium hydroxide (see Smith et al. 1973; Smith and Sykora 1976; Sykora et al. 1972). Other data suggest that the chronic effects threshold for ferrous iron may be as low as 230 µg Fe/L for the brown trout (see Geertz-Hansen and Mortensen 1983; Table 1-3). This is in stark contrast to the rainbow trout, brook trout, and fathead minnow data, where chronic effect threshold values were shown to greatly exceed 2,000 µg Fe/L (Amelung 1982; Smith et al. 1973; Smith and Sykora 1976; Sykora et al. 1972).

The Guidelines (Stephan et al. 1985), studies reviewed here do not meet the minimum number of acceptable chronic tests required to derive a chronic criterion for protection of freshwater organisms against the adverse effects of total iron for freshwater organisms. There currently are no chronic values for a benthic crustacean, an aquatic insect, a family in a phylum other than Arthropoda or Chordata, and any species from a representative family in a different order of aquatic insect, or any other species from a different phylum (e.g., mollusca, rotifera). The data are also insufficient to calculate a chronic criterion based on an acute-chronic ratio. Data from only one acute and chronic study are available for such a calculation (see Biesinger and Christensen 1972), and the acute value reported in this study is questionable based upon the conventional usage of the Guidelines. There is not enough data to suggest that plants are exceptionally sensitive to iron give range in value for each species, and there is little indication that iron accumulates to deleterious levels in aquatic organisms (Table 1-6).

Table 1-1
Acute Toxicity of Total (Fe) and Ferrous (Fe) Iron to FreshWater Animals.

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	pH	LC50 or EC50 (µg Fe/L)[b]	LC50 or EC50 (µg Fe+2/L)	GMAV (µg Fe/L)[c]	Reference
Tubificid worm, *Branchiura sowerbyi*	S,U	Ferrous sulfate	268	6.5	580,000	-	-	Mukhopadhyay and Konar 1984
Tubificid worm, *Branchiura sowerbyi*	S,U	Ferrous sulfate	268	7.0	560,000	-	-	Mukhopadhyay and Konar 1984
Tubificid worm, *Branchiura sowerbyi*	S,U	Ferrous sulfate	268	8.5	446,000	-	525,200	Mukhopadhyay and Konar 1984
Tubificid worm, *Tubifex tubifex (Muller)*	R,U	Ferric chloride	245	7.5-7.7	101,840	-	101,800	Khangarot 1991
Copepod, *Cyclops viridis*	S,U	Ferrous sulfate	268	6.5	35,200	-	-	Mukhopadhyay and Konar 1984
Copepod, *Cyclops viridis*	S,U	Ferrous sulfate	268	7.0	33,200	-	-	Mukhopadhyay and Konar 1984
Copepod, *Cyclops viridis*	S,U	Ferrous sulfate	268	8.5	36,000	-	34,780	Mukhopadhyay and Konar 1984
Cladoceran (<24h), *Ceriodaphnia dubia*	S, U	Ferric chloride	90	7.0-7.3	12,600	-	12,600	Fort and Stover 1995
Cladoceran, *Daphnia magna*		Ferrous sulfate	240	7.2-7.8	7,200	-	7,200	Khangarot and Ray, 1989
Midge (3rd Instar), *Chironomus riparius*	R,M	Ferrous sulfate	41.6	4	-	400,000	-	Rousch et al. 1997

Table 1-1 (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	pH	LC50 or EC50 (µg Fe/L)[b]	LC50 or EC50 (µg Fe+2/L)	GMAV (µg Fe/L)[c]	Reference
Mite, *Arrenurus manubriator*	R,M	Ferrous sulfate	41.6	4	-	200,000	-	Rousch et al. 1997
Amphipod (4 mm), *Crangonyx pseudogracilis*	R,U	Ferric chloride	50	6.7-6.8	120,000	-	-	Martin and Holdich 1986
Amphipod (4 mm), *Crangonyx pseudogracilis*	R,U	Ferrous sulfate	50	6.7-6.8	95,000	-	106,800	Martin and Holdich 1986
Crayfish (19-32 mm), *Orconectes limosus*	S,U	Ferric chloride	-	7.0	32,000	-	32,000	Boutet and Chaisemartin 1973
May fly (nymph), *Ephemerella subvaria*	S,U	Ferrous sulfate	48	8.2	320	-	320	Warnick and Bell 1969
Rainbow trout (Juvenile), *Oncorhynchus mykiss*	R,M	Ferrous sulfate	70-110	4.1-7.2	18,300	4,400	18,300	Cherry 1983; Loeffelman et al. 1986
Brown trout (Fingerlings), *Salmo trutta*	F,M	Ferrous sulfate	287	7.39-7.85	47,000 (240 are diss. Fe)	-	47,000	Dalzell and Macfarlane 1999
Brook trout (Juvenile), *Salvelinus fontinalis*	F,M	Ferrous sulfate	-	5.5	410[d]	-	-	Decker and Menendez 1974
Brook trout (Juvenile), *Salvelinus fontinalis*	F,M	Ferrous sulfate	-	6.0	480	-	917	Decker and Menendez 1974
Brook trout (Juvenile), *Salvelinus fontinalis*	F,M	Ferrous sulfate	-	7.0	1,750	-	-	Decker and Menendez 1974
Common carp (3.2 cm), Cyprinus carpio	R,U	Ferrous sulfate	-	-	1,800	-	-	Alam and Maughan 1992

Table 1-1 (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	pH	LC50 or EC50 (µg Fe/L)[b]	LC50 or EC50 (µg Fe+2/L)	GMAV (µg Fe/L)[c]	Reference
Common carp (3.2 cm), *Cyprinus carpio*	R,U	Ferrous sulfate	-	-	1,200	-	-	Alam and Maughan 1992
Common carp (6.0 cm), *Cyprinus carpio*	R,U	Ferrous sulfate	-	-	3,700	-	-	Alam and Maughan 1992
Common carp (6.0 cm), *Cyprinus carpio*	R,U	Ferrous sulfate	-	-	2,370	-	2,086	Alam and Maughan 1992
Fathead minnow, *Pimephales promelas*	R,M	Ferrous sulfate	70-140	5.7-6.2	14,400	3,700		Cherry 1983; Loeffelman et al. 1986
Fathead minnow, *Pimephales promelas*	R,M	Ferrous sulfate	70-130	5.1-7.3	24,200	9,200	18,670	Cherry 1983; Loeffelman et al. 1986
Tilapia, *Tilapia mossambica*	S,U	Ferrous sulfate	268	6.5	119,600	-	-	Mukhopadhyay and Konar 1984
Tilapia, *Tilapia mossambica*	S,U	Ferrous sulfate	268	7.0	83,200	-	-	Mukhopadhyay and Konar 1984
Tilapia, *Tilapia mossambica*	S,U	Ferrous sulfate	268	8.5	118,000	-	105,500	Mukhopadhyay and Konar 1984

[a] S= static; R = renewal; F = flow-through; M = measured; U = unmeasured;

[b] Results are expressed as iron, not as the chemical.

[c] Freshwater Genus Mean Acute Values are calculated as the geometric mean of the associated underlined number(s) in the preceding column.

[d] Not used in calculations because possible sublethal stress due to low test pH.

Table 1-2
Chronic Toxicity of Iron to Aquatic Animals.

Species	Test[a]	Chemical	pH	Hardness (mg/L as $CaCO_3$)	Chronic Limits (µg Fe/L)	Chronic Value (µg Fe/L)	Reference
Cladoceran, Daphnia magna	LC	Ferric Chloride	7.74	45.3	-	5,900	Biesinger and Christensen 1972
Cladoceran, Daphnia magna	LC	Ferric Chloride	7.74	45.3	-	4,380	Biesinger and Christensen 1972
Coho salmon, Oncorhynchus kisutch	PLC	Ferrous Sulfate	8.1	168-180	4,360 - 10,500	6,766*	Smith and Sykora 1976
Brook trout, Salvelinus fontinalis	PLC	Ferrous Sulfate	8.1	168-180	>10,500	>10,500*	Smith and Sykora 1976
Brook trout, Salvelinus fontinalis	PLC	Ferrous Sulfate	>7	141-160	7800-13420	10,230*	Sykora 1972; Sykora et al. 1972; Sykora et al. 1975
Fathead minnow, Pimephales promelas	PLC	Ferrous Sulfate	6.9-7.2	146	240 - 2,000	693*	Smith et al. 1973; Sykora 1972

[a] LC = life cycle; PLC = partial life cycle.

[b] Results are expressed as iron, not as the chemical.

Table 1-3
Other Data on Effects of Iron on Freshwater Aquatic Organisms.

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	pH	Duration	Effect	Conc. (µg Fe/L)[b]	Conc. (µg Fe+2/L)	Reference
Cyanobacteria, *Anabaena variabilis*	S,U	-	-	-	10 d	LOEC (Biomass)	50,000	-	Pany and Mitra 1998
Cyanobacteria, *Anabaena variabilis*	S,U	-	-	-	10 d	LOEC (Chlorophyll)	50,000	-	Pany and Mitra 1998
Cyanobacteria, *Aphanizomenon flosaquae*	S,U	Ferric Chloride	-	8.00	20 h	NOEC (N fixation)	8,600	-	Peterson et al. 1995
Algae, *Chlorella vulgaris*	S,U	Ferric Chloride		4.00	21 d	NOEC (Cell count)	10,000	-	Keller et al. 1978
Algae, *Chlorella vulgaris*	S,U	Ferric Chloride		6.00	21 d	NOEC (Cell count)	10,000	-	Keller et al. 1978
Algae, *Chlorella vulgaris*	S,U	Ferric Chloride		8.00	21 d	NOEC (Cell count)	10,000	-	Keller et al. 1978
Algae, Scenedesmus quadricauda	S,U	Ferric Chloride	-	7.20	16 d	Oxygen production	55,730	-	Fargasova et al. 2000
Common duckweed, *Lemna minor*	S,U	-	-	7.50	96 h	EC50 (Frond Number)	3,700	-	Wang 1986
Cattail, *Typha latifolia*	R,U	-	-	5.00	20 d	NOEC (Leaf Elongation)	100,000	-	Taylor and Crowder 1983
Nematode, *Caenorhabditis elegans*	S,M	Ferric Nitrate	-	-	24 h	LC50	17,900	3,351	Tatara et al. 1998
Cladoceran (< 24 h), *Daphnia magna*	R,U	Ferrous Sulfate	250	8	21 d	LOEC (progeny/ female)	256	-	Dave 1984

Table 1-3 (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	pH	Duration	Effect	Conc. (µg Fe/L)[b]	Conc. (µg Fe+2/L)	Reference
Cladoceran (< 24 h), *Daphnia magna*	R,U	Ferrous Sulfate	250	8	21 d	LOEC (Survival)	512	-	Dave 1984
Cladoceran (< 24 h), *Daphnia magna*	S,U	Ferric Sulphate	-	7.60	24 h	EC50	5,250	-	Lilius et al. 1994; Lilius et al. 1995
Cladoceran (2-3 d), *Daphnia magna*	S,U	Ferrous chloride	-	7.60	24 h	NOEC (Survival)	5,585	-	Stom and Zubareva 1994
Cladoceran, *Daphnia magna*	S,U	Ferric Chloride	-	-	24 h	EC50	16,000	-	Sorvari and Sillanpaa, 1996
Cladoceran (< 24 h), *Daphnia pulex*	S,U	Ferric Sulphate	-	7.60	24 h	EC50	36,800	-	Lilius et al. 1995
Amphipod, *Gammurus minus*	S,U	-	-	-	15 min	LOEC (Increased oxygen cons.)	2,000	-	Keller et al. 1978
Midge (1st Instar), *Chironomus riparius*	S,M	Ferric Chloride	-	7.80	96 h	LOEC (Growth Inhibition)	10,250	-	Postma et al. 1995
Stonefly (nymph), *Acroneuria carolinensis*	S,M,D	Ferrous Sulfate	-	3.30	69.4 h	Mean Life Expectancy	22,222	-	Whipple and Dunson 1993
Stonefly (nymph), *Acroneuria carolinensis*	S,M,D	Ferrous Sulfate	-	3.50	97.8 h	Mean Life Expectancy	18,709	-	Whipple and Dunson 1993
Coho salmon, *Oncorhynchus kisutch*	F,T,M	Ferric hydroxide	75	7.50	90 d	NOEC (Hatchability)	3,000	-	Brenner and Cooper, 1978
Rainbow trout (ovum), *Oncorhynchus mykiss*	S.M,D	Ferric Chloride	-	9.00	40 min	Fertilization	730	-	Billard and Roubaud, 1985
Rainbow trout (sperm), *Oncorhynchus mykiss*	S.M,D	Ferric Chloride	-	9.00	40 min	Fertilization	730	-	Billard and Roubaud, 1985
Rainbow trout, *Oncorhynchus mykiss*	S.M,D	Ferric Chloride	-	9.00	40 min	Insemination	80		Billard and Roubaud, 1985

Table 1-3 (continued)

Species	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	pH	Duration	Effect	Conc. (µg Fe/L)[b]	Conc. (µg Fe+2/L)	Reference
Rainbow trout, *Oncorhynchus mykiss*	M	-	-	-	-	NOEC (Length)	5,700	-	Amelung 1982
Brook trout (yearling), *Salvenilus fontinalis*	F,M,D	Ferric Sulphate	25	5.8-6	48 h	15% decrease in body Na conc	1,005	-	Gonzalez et al. 1990
Brown trout, *Salmo trutta*	M	Natural stream		6.55-7.05	-	LOEC (Hatchability)	460	230	Geertz-Hansen and Mortensen 1983
Brown trout (fry), *Salmo trutta*	F,M,T	Ferric Chloride	2.3-3.4	5.78-6.64	8 h	LOEC (Ca efflux)	36.86	-	Sayer et al. 1991
Common shiner, *Notropus cornutus*	F,T	Ferric hydroxide	112-114	7.1-7.3	2-8 wk	LOEC (Blood ions, etc.)	3,000	-	Brenner et al. 1976

[a] S= static; R = renewal; F = flow-through; M = measured; U = unmeasured;

[b] Results are expressed as iron, not as the chemical.

Table 1-4
Unused Iron Effects (Direct and Indirect) Data on Freshwater Aquatic Organisms.

Species	Exposure	Method[a]	Chemical	Hardness (mg/L as $CaCO_3$)	pH	Duration	Effect	Conc. (µg Fe/L)[b]	Conc. (µg Fe+2/L)	Reference
Cladoceran, *Daphnia longispina*	Lab	S,M,T,D	Ferric sulfate in River water	-	-	48 h	LOEC (survival)	10,000	-	Randall et al. 1999
Cladoceran, *Daphnia longispina*	Lab	S,M,T,D	Ferric sulfate in River water	-	-	21 d	LOEC (survival)	2,000	-	Randall et al. 1999
Cladoceran, *Daphnia magna*	Lab	S,U	Feric chloride	45	-	96 h	EC50	9,600	-	Biesinger and Christensen 1972
Cladoceran, *Daphnia magna*	Lab	S,U	Feric chloride	-	-	96 h	EC50	7,230	-	Dowden and Bennett 1965
Isopod (adult), *Asellus aquaticus*	Lab	S,M,D	Ferrous sulfate	50	4.5	50 h	EC50	299,800	-	Maltby et al. 1987
Isopod (adult), *Asellus aquaticus*	Lab	S,M,D	Ferrous sulfate	50	4.5	50 h	EC50	428,500	-	Maltby et al. 1987
Isopod (adult), *Asellus aquaticus*	Lab	S,M,D	Ferrous sulfate	50	6	50 h	EC50	419,200	-	Maltby et al. 1987
Isopod (adult), *Asellus aquaticus*	Lab	S,M,D	Ferrous sulfate	50	6	50 h	EC50	466,700	-	Maltby et al. 1987
Mayfly, *Leptophlebia marginata*	Lab	R,M,T	Ferrous sulfate	-	4.5	30 d	LOEC (feeding and motility)	10,000	10,000	Gerhardt 1992
Mayfly, *Leptophlebia marginata*	Lab	R,M,T	Ferrous sulfate	-	7	30 d	NOEC (feeding, survival, motility)	50,000	<1,000	Gerhardt 1992

Table 1-4 (continued)

Mayfly (nymph), *Leptophlebia marginata*	Lab	R,M,T	Ferrous sulfate	18	4.5	96 h	LC50	89,500	-	Gerhardt 1994
Mayfly (nymph), *Leptophlebia marginata*	Lab	R,M,T	Ferrous sulfate	18	7	96 h	LC50	106,300	97,539	Gerhardt 1994
Mayfly, *Leptophlebia marginata*	Lab	R,M,T	Natural	-	4.5	24 d	EC50 (survival)	73,070	-	Gerhardt 1995
Mayfly, *Leptophlebia marginata*	Lab	R,M,T	Natural	-	4.5	24 d	EC50 (food consump.)	50,120	-	Gerhardt 1995
Mayfly, *Leptophlebia marginata*	Lab	R,M,T	Natural	-	6-7.4	24 d	40 percent body encrustation	1,640	-	Gerhardt 1995
Mayfly, *Leptophlebia marginata*	Field	R,M,T	Natural	-	6-7.5	12 wk	80 percent body encrustation	1,460	-	Gerhardt 1995
Mayfly, *Leptophlebia marginata*	Field	R,M,T	Natural	-	4.5	12 wk	EC50 (Escape behavior)	23,470	-	Gerhardt 1995
Zooplankton, Benthic invertebrate, and warm water fish communities	Field	Stream in Central Missouri	Acid Strip-mine Effluent	343	6	Natural	Stable community	600	400	Parsons et al. 1968

Table 1-4 (continued)

Benthic macroinvertebrate community	Field	Turtle Creek, PA	Mixed organic waste and acid mine drainage		3.80	Natural	Low productivity; low overall species diversity; but elevated numbers of certain types	2,800	-	Koryak et al. 1972
Benthic macroinvertebrate community	Field	Turkey Run, PA	Limestone strip mining		6.5-7.3	Natural	No effect on species diversity; sig. reduced abundance of certain species	510-710	-	Osborne and Davies 1979
Benthic macroinvertebrate community	Field	Taff Bargoed, S. Wales, U.K.	Coal industry pollution and old mine workings		6.3-7.9	Natural	Reduction in abundance and diversity	2,390	-	Scullion and Edwards 1980
Benthic macroinvertebrate community	Field	Rocky Mountain Stream; Snake R.	Acid conditions (weathering of pyritic rocks) and precipitation of hydrous metal oxides		5.5-6.5	Natural	Reduced abundance due to flocculent of hydrous A and Fe hydroxides cop	300		McKnight and Feeder 1984

[a] S= static; R = renewal; F = flow-through; M = measured; U = unmeasured;

[b] Results are expressed as iron, not as the chemical.

Table 1-5
Ranked Freshwater Genus Mean Acute Values for Total (Fe) and Ferrous (Fe+2) Iron With Acute-Chronic Ratios (If Available).

Ranka	Species	GMAV (µg Fe/L)	GMAV (µg Fe+2/L)	Acute-Chronic Ratio
16	Tubificid worm, *Branchiura sowerbyi*	525,200		-
15	Midge, *Chironomous riparius*		400,000	
14	Mite, *Arrenurus manubriator*		200,000	
13	Tilapia, *Tilapia mossambica*	105,500		-
12	Tubificid worm, *Tubifex tubifex (Muller)*	101,800		-
11	Amphipod, *Crangonyx pseudogracilis*	106,800		-
10	Brown trout, *Salmo trutta*	47,000		-
9	Copepod, *Cyclops viridis*	34,780		-
8	Crayfish, *Orconectes limosus*	32,000		-
7	Fathead minnow, *Pimephales promelas*	18,670	9,200	-
6	Rainbow trout, *Oncorhynchus mykiss*	18,300	4,400	-
5	Cladoceran, *Ceriodaphnia dubia*	12,600		-
4	Cladoceran, *Daphnia magna*	7,200		2.91b
3	Common carp, *Cyprinus carpio*	2,086		-
2	Brook trout, *Salvelinus fontinalis*	917		-
1	May fly, *Ephemerella subvaria*	320		-

[a] Ranked from most resistant to most sensitive based on Genus Mean Acute Value

[b] Acute-chronic ratio based on chronic value (4,380 µg Fe/L) in Table 2 and acute value (fed test; 9,600 µg Fe/L) in Appendix A. Data are from Biesinger and Christensen (1972).

Table 1-6
Bioaccumulation of Iron by Aquatic Organisms.

Species	Tissue	Chemical	Hardness (mg/L as $CaCO_3$)	Concentration water (µg Fe/L)a	pH	Duration (days)	BCF or BAF	Reference
Algae, *Spirodela polyrrhiza*	-	Field	-	1719	-	-	2305	Sinha et al. 1994
Sea lamprey(larvae), *Petromyzon marinus*	Whole body	Field	-	180000	7.36	-	5	Holmes and Youson, 1996
Sea lamprey(larvae), *Petromyzon marinus*	Whole body	Field	-	106000	7.53	-	6.8	Holmes and Youson, 1996
Sea lamprey(larvae), *Petromyzon marinus*	Whole body	Field	-	31000	7.54	-	22.6	Holmes and Youson, 1996
Sea lamprey(larvae), *Petromyzon marinus*	Whole body	Field	-	70000	7.49	-	10.3	Holmes and Youson, 1996
Sea lamprey(larvae), *Petromyzon marinus*	Whole body	Field	-	24000	7.42	-	38	Holmes and Youson, 1996
Sea lamprey(larvae), *Petromyzon marinus*	Whole body	Field	-	69000	7.69	-	12.5	Holmes and Youson, 1996
Common carp (juvenile), *Cyprinus carpio*	Whole body	Ferrous Sulfate	-	500	-	15	0.66	Alam and Maughan, 1992

[a] Results are expressed as iron, not as the chemical.

Assessment of Available Field Data Regarding Ecological/Habitat Impacts

The field aquatic threshold effect level for iron may be key to defining an acceptable level of protection for aquatic life. There are numerous accounts of direct and indirect effects of iron precipitates on macrofauna. Most of the studies have examined the effects of iron in association with acid (coal) mine drainage, where iron typically is a dominant pollutant of concern, but where other pollutants are also present. The pollutants that commonly are present with iron include highly toxic metals, such as Cu, Cd, and Zn; these waters are also very acidic. Among the studies selected for review here, a few are notable because the effects observed were purely physical, and thought to be attributable predominantly to elevated levels of iron (dissolved and total). These studies are described below, and summarized in Table 1-7. Additionally, results of other related, but unused studies (see Table 1-4), are also discussed.

Parsons (1968) examined the effects of acid strip-mine discharges on the ecology of a stream in central Missouri (Table 1-4). Ferrous and ferric iron were among the ions analyzed in samples collected upstream, within, and downstream of the strip-mine areas. The acid mine discharges mainly contained sulfates of ferrous iron, ferric iron, aluminum, calcium and magnesium, along with other ions including sulfate, zinc, copper, and lead. Rainfall initiated excessive acid flows by dissolving the various sulfate salts from the strip-mine areas. The consequences were marked physical effects on the previously unpolluted downstream sites, which were caused by the precipitation of a silt load that was accentuated by a whitish flocculent deposited on the bottom substrate. With the excessive acidic flow, some ions were increased substantially at the downstream sites, except for ferrous and ferric iron, which decreased in concentration rapidly via oxidation of the ferrous iron and subsequent precipitation as ferric hydroxide. Species composition in sites continuously affected by acid mine effluent (pH 3.7; Fe^{3+} = 6,800 µg/L; Fe^{2+}= 2,900 µg/L; Cu = 400 µg/L; Pb = 500 µg/L) under normal flow conditions included four species of zooplankton (all rotifers) and five benthic species, but no fish species. Feshwater clam populations in the downstream area subjected to occasional pollution from the acid mine discharge appeared to be the most sensitive group overall. The authors state that under such conditions no single indicator species can be used to assess water quality, but rather the response of the whole community is the best indicator of water quality. According to the authors, stable communities of zooplankton, benthic invertebrates, and warmwater fishes have been documented at a mean pH of 6.0, ferric iron 600 µg/L, ferrous iron 400 µg/L, and lead and copper at 100 µg/L.

Koryak et al. (1972) investigated the effects of mixed organic waste and acid mine drainage from coal mining activity on the bottom fauna of streams in western Pennsylvania (Table 1-4). Species assemblages and abundance were assessed along with measurements of total acidity, total iron concentration, dissolved oxygen, and pH. At pH values in the range of 2.6 - 3.0 and total iron concentration ranges of 22,000 to 89,000 µg/L, the benthic community primarily was composed of midge larvae of the genus *Chironomus* (mostly *Chironomus riparius*); a few Neuroptera and Coleoptera were also present in the less affected area. In regions of neutralization corresponding with heavy iron hydroxide precipitation (pH range from 3.3 to 3.8; total dissolved and suspended iron 2,800 µg/L) very little benthic plant life developed, but the first Dipterans, Ephemeroptera, Plecoptera, Amphipods, and Oligocheates were present. Population densities increased progressively downstream as pH increased to an average pH range of 5.6 to 6.4. Threshold values associated with an increased number of genera appeared to be approximately a pH of 3 and 25,000 µg Fe/L. The authors noted the conspicuous absence of crayfish, blackflies, mayflies, most species of caddisflies, and especially stoneflies.

Letterman and Mitsch (1978) investigated the effects of coal mine drainage on benthic communities and fish populations upstream and downstream of the discharge in a relatively non-polluted stream in western Pennsylvania where the pH below the mine discharges remained between 6.5 and 8.0. The stream received typical acid mine drainage (pH 2.7, total iron 493,000 µg/L, acidity 1290 mg/L as $CaCO_3$), and also discharge through a limestone rock tunnel (pH 6.0, total iron 5,000 µg/L, acidity 69 mg/L as $CaCO_3$). Therefore, in this instance the instream hydrogen ion concentration and dissolved oxygen levels remained relatively unchanged downstream of the mine discharges. Total iron concentrations upstream of the mine discharges generally were less than 200 µg Fe/L, and abundance and diversity of both fish and invertebrates was relatively rich. Effects of the altered water chemistry and ferric hydroxide deposits downstream of the discharge (as high as 3,600 µg Fe/l, but gradually decreasing to near upstream levels after 3 miles) on biomass, numbers, and diversity of fish and invertebrates were clearly evident (Table 1-7). While benthic organisms seemed to recover in numbers further downstream, biomass remained low, suggesting the average size of invertebrates was significantly reduced . Odonata in general, and Gomphidae in particular, were intolerant of the ferric hydroxide precipitate, as was the caddisfly *Rhyacophila*. The distribution of Trichoptera was the best indicator of ecosystem damage and recovery in the stream, while chironomids were the most tolerant of mine drainage. Benthic fish were the best indicators of mine drainage pollution among the fish species present in the steam. Mottled sculpin, white sucker, and hog sucker, which were dominant upstream (but are sedentary, poor swimmers, which rely heavily on the benthic invertebrates as a food source), were among the fish species most affected by the ferric hydroxide precipitate. Brown trout appeared to be less sensitive, possibly due to their mobility and ability to feed on drifting organisms in the water column. The most tolerant fish species was the creek chub; large populations of this species were observed downstream of the mine discharges.

The effects of limestone strip mining on the composition and structure of the benthic macroinvertebrate communities of Turkey Run, Clarion County, Pennsylvania, were investigated by Osborne et al. (1979). Turkey Run was sampled for macroinvertebrates and conductivity, sulfate, total iron, alkalinity, total acidity, and pH at sites upstream and downstream of a first order tributary, which receives the discharges from three limestone strip mines; the tributary was also sampled. The pH remained relatively stable between 6.5 and 7.3 among sites throughout the study. Total acidity, alkalinity, conductivity, and sulfate were elevated in the tributary, but total iron (range: 510 to 710 µg Fe/L) was not significantly different among sites on Turkey run (Table 1-4). The abundance of individual species was affected in the tributary, which the authors attributed to either the higher sulfate concentrations, or to the deposition of ferric hydroxides, which they concluded reduced the availability of the food resource. There were, however, no significant differences found in the calculated evenness and equitability biological indices in the mainstream sites.

Scullion and Edwards (1980) conducted an excellent study on the effects of coal industry pollutants on the macroinvertebrate fauna of a small stream (Taff Bargoed) in South Wales, England. Particularly meaningful was the examination of a ferruginous drainage into a tributary (Nant Caeach) at approximately neutral pH (pH 6.3 to 7.9) from old mine workings, which caused siltation in an area receiving periodic discharges of storm sewage. The mean total iron concentration in the Nant Caeach in a relatively unaffected site upstream of the ferruginous drainage was 710 µg Fe/L (range 240 to 1060 µg Fe/L). Below the drainage, the total iron concentration averaged 2,390 µg Fe/L, and ranged from 500 to 3,320 µg Fe/L. Mean downstream copper, zinc and manganese concentrations were 60, 50, and 190 µg/L, respectively. The site subjected to heavy ferric hydroxide deposits supported a dominance of oligochaetes and chironomid larvae; however, generally lacked such species as Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Mollusca, Crustacea, and Acari. There were a few notable exceptions, one of which included the mayfly *Baetis rhodani*. The authors concluded that, at near neutral pH and relatively low ferric hydroxide concentration in the water column, the reduction in invertebrate abundance and diversity likely was attributable to the indirect toxic effects of deposited ferric hydroxide through ingestion, interference with respiration, and with attachment and feeding mechanisms, or to secondary effects on plant growth. It is worth noting here, however, that the area below the ferruginous drainage on the Nant Caeach is also subject to the discharge of storm sewage, especially in the autumn when the highest densities of chironomid larvae and oligochaetes was found to occur.

The distribution of macroinvertebrates and fish in the River Don, U.K., and several of the river's tributaries was investigated by Greenfield and Ireland (1978). Discharges from coal mine spoils adjacent to the river was highly acidic and ferruginous in nature during periods of heavy rainfall, and is treated using a hydrated lime process. At stations immediately below the discharge from the treatment plant, and for several miles down stream of the plant (where pH ranged from 6.2 to 7.8 and concentrations of suspended iron were high: 4,540 to 6,420 µg Fe/L), there was a distinct change in the composition of the benthic community and fish populations. Plecopterans, ephemeropterans, and trichopterans found at stations upstream of the treatment plant were not found immediately below the plant at downstream stations, presumably because of the precipitation of ferric compounds at these sites. The species of macroinvertebrates most common in the downstream waters were oligochaetes and the larvae of ceratopognids and chironomids. Two of the four fish species (the stickleback, *Gasterostreus aculeatus*, and stoneloach, *Noemacheilus barbatulus L.*) were present immediately below the plant, but in reduced numbers relative to the upstream sites. Caging experiments with the minnow Phoxinus phoxinus and B. barbatulus indicated that the percentage survival of minnows was reduced to 25 percent after 96 hours at one of the downstream sites (total iron 6,420 µg Fe/L), whereas B. barbatulus survival remained relatively high (87.5 percent).

McKnight and Feder's (1984) study on the ecological effects of acid conditions and precipitation of hydrous metal oxides in a Rocky Mountain stream showed that metal precipitants had more of an adverse impact on stream communities than low pH and high concentrations of dissolved metal ions (Table 1-4). The metal precipitant (predominantly ferric and aluminum hydroxides) effects were observed in the Snake River for several miles downstream of its confluence with Deer Creek. Upstream of the confluence, the Snake River is acidic and rich in dissolved metals from the weathering of pyritic rocks in the watershed (pH 3.8; DOC 0.6 mg C/L; dissolved metals: Al 2,360 µg/L; Fe 660 µg/L; Cu, Mn, and Zn 18 to 93 µg/L). Deer Creek, on the other hand, had a mean pH of 7.3, a DOC concentration of 0.9 mg C/L, and dissolved metal concentrations of: Al 40 µg/L; Fe 60 µg/L; Cu, Mn, and Zn <10 µg/L, respectively. Note that some of the Fe passing through the 0.45 µm Nucleopore™ membrane actually is colloidal and not truly dissolved. Upstream of the confluence, periphyton in Deer Creek were much more abundant than in the Snake River, but both sites were similar in terms of diversity; a total of fifteen species were identified at each site with seven species in common. None of the major species in the Snake River, however, were found in Deer Creek, indicating that these species are adapted to the acidic, metal-enriched conditions of the Snake River. Downstream of the confluence, with a pH between 5.5 and 6.5 and dissolved aluminum and iron of about 200 to 300 µg/L, respectively, the streambed was covered (up to 1 cm thick) with a flocculent of hydrous Al and Fe oxides coprecipitated with aquatic humic substances. There were only diatom fragments at the most upstream site, with no intact cells in two of the three samples. Similarly, upstream of the confluence of the two rivers the benthic assemblages of each river were distinctly different, but the abundance was similar. There were significantly fewer benthic invertebrates downstream of the confluence, where the dissolved metal concentrations were much lower than upstream in the Snake River, but the thick covering of the rock substrate by metal precipitates appeared to have prevented the development of a stable stream community.

The effects of iron-rich drainage on the macroinvertebrate community in a lowland stream with neutral or slightly acidic pH, and low sulfate and heavy metal concentrations (River Vidaa, Denmark) was examined by Rasmussen and Lindegaard (1988). Both total and dissolved (0.45 µm Gelman® filter) iron concentrations were measured in water samples from numerous sites (n=28). The authors reported that the dissolved iron component consisted almost entirely of ferrous iron, and the pH among the sites ranged from 6.7 to 8.8. Dissolved iron concentrations generally were higher during winter under high-flow conditions, and average total iron concentrations showed no correlation to concentrations of dissolved iron, except at the highest iron concentrations. The greatest number of taxa collected were at concentrations of dissolved iron less than 200 µg Fe (reported as Fe^{2+})/L. There was a 20 percent decrease in total taxa between 200 and 300 µg Fe^{2+}/L (approximately 800 to 7,800 µg total iron (Fe)/L); the reduction was primarily attributable to grazers (including: Naididae, Ephemeroptera, Plecoptera, and Chironimidae) which feed on biofilm, as a consequence of the precipitation of ferric hydroxide (Table 1-7). Numbers of all groups of taxa progressively decreased as the dissolved iron concentration increased up to 10,000 µg Fe^{2+}/L. The most tolerant species (up to 30,000 µg Fe^{2+}/L) were Tubificids and Chironomids, and also included a snail (*Lymnaea peregra*), a megalopteran (*Sialis lutaria*), and the plecopteran (*Nemoura cinerea*). Food limitation combined with substrate destabilization were thought to be responsible for the reduced number of taxa at the lower concentrations of dissolved iron.

Wellnitz et al. (1994) showed that blooms of iron-depositing bacteria, *Leptothrix ochracea*, can also contribute to food limitation and substrate destablization, as well as to toxicity (Table 1-7). Studies were carried out primarily in the Unnamed Brook, a small second order stream in northern Vermont, but also included a survey of five other Vermont streams displaying zones of iron deposition. The mean annual pH of Unnamed Brook was 6.2, and ferromanganese flocs from Unnamed Brook contained sheathed, iron-depositing bacteria characteristic of *L. ochracea*. Metal oxides and *L. ochracea* displaced the algal community and covered substrates below the entry of high iron and manganese concentrations from groundwater input via a culvert. The iron-bacteria bloom began just below the culvert, and extended about 250 meters downstream. Concentrations of bacteria and total iron and manganese in the bloom were variable seasonally, and were highest in mid-summer; maximum of 540 - 3,460 µg/L for iron, and 470 - 2,270 µg/L for manganese, in 1989 and 1990, respectively (Note: the annual variation in this study was for total iron). Microscopic examination of the substrate revealed L. ochracea sheaths and oxide precipitates, but very few diatom frustules. Macroinvertebrate diversity was lowest inside the L. ochracea bloom during summer. Similar trends were noted for four of the other five Vermont streams surveyed. Experimental studies showed species-dependent responses to the conditions in the blooms through feeding choice, growth, and toxicity trials, which explains, in part, how these changes may act to influence benthic abundance and distribution in streams. The authors noted that elevated concentrations of both iron (approximately 190 µg Fe/L) and manganese (approximately 90 µg Mn/L) are necessary for *L. ochracea* blooms to persist. Neither elevated iron or manganese concentrations alone can sustain bacterial blooms.

Summary of Iron Effects Data From the Field

Iron generally is a dominant metal associated with mining effluents, but probably the least toxic of the other metals typically increasing in bioavailability because of the acidic conditions. Runoff from ferruginous (e.g., pyritic) soils can also lead to temporary decreases in pH, which may increase the toxicity and bioavailability of iron-associated metals temporarily, but oxidation of ferrous to ferric iron causes coprecipitation of iron with many of these metals, potentially rendering them less toxic (Martinez and McBride 2001).

Despite the obvious effects of acidity and the associated toxic metals described in some of the cited field studies, other data show that losses of certain “iron-sensitive species” at dissolved iron concentrations in the 200 - 300 µg Fe/L range (total iron Table 1-4). Nevertheless, stable communities have been reported at dissolved iron concentrations exceeding 600 µg Fe/L (see Table 1-4). The decrease in species diversity due to ferric hydroxide precipitates appears to be the result of decreased species abundance, rather than composition (Letterman and Mitsch 1978; Osborne et al. 1979), though somewhat conflicting information was found (see Rasmussen and Lindegaard 1988 and Wellnitz et al. 1994). Grazers (primarily Ephemeroptera and Plecoptera) seem to be among the most sensitive to iron, but even some chironomids are highly sensitive, so there is no one group which is always affected. Species presence or absence is highly site-specific. An interesting observation is that many of the same invertebrates tolerating high iron concentrations are those that thrive in areas with organic contamination (i.e., Tubificidae, some Chironimidae and Tipulidae).

It is worth noting here that a number of factors other than iron can affect interpretation of these field results, including substrate type, water velocity and organic pollution (Rasmussen and Lindegaard 1988).

Table 1-7
Field Data on Effects of Iron on Freshwater Aquatic Organisms.

Species	Exposure	Location	Acidity (mg/L as $CaCO_3$)	pH	Main Effects	Concentration (µg Fe /L)[a]	Reference
Benthic macroinvertebrates	Field	Unnamed Brook, VT	-	6.2	Increase in *Leptothrix ochracea*; significantly lower chlorophyll-a (diatoms); significantly lower diversity	540 - 3,460	Wellnitz et al. 1994
Benthic macroinvertebrates	Field	River Vidaa, Denmark	-	6.7-8.8	20 percent reduction in taxa	800-7,800	Rasmussen and Lindegaard 1988
Fish and benthic macroinvertebrate community	Field	Ben's Creek Watershed, PA	2-15	6.0 - 8.5	Order of magnitude decrease in biomass within area of ferric hydroxide precipitation. Invertebrate diversity not as sensitive of indicator as biomass. Standing crop of fish was reduced by 20 times.	3,600 (max)	Letterman and Mitsch 1978

[a] Results are expressed as iron, not as the chemical.

Implications for Criteria

Due to the inherent solubility/toxicity characteristics associated with the ferrous and ferric forms of iron in freshwater systems, a single criterion based on standard EPA Guideline derivation methodology will not adequately describe the actual bioavailability, toxicity and ecological effects of iron on aquatic organisms. According to the Guidelines, data for highly oxidizable materials, such as iron, should not be used for derivation of a CMC or CCC if the data were not generated using "flow-through tests in which the concentrations of test material in the test solutions were measured often enough using acceptable analytical methods." This requirement restricts the published acute and chronic iron toxicity data set to a limited number of studies. Also, the Guidelines state that data obtained from tests where the organisms were exposed to concentrations of test material that exceed the solubility of the material in water usually should be considered qualitative rather than quantitative. This requirement also has implications for the majority of the studies that have been published.

Based on the current state of the science in aquatic toxicology, each different oxidation state of a metal should be considered a separate material. Insufficient detail, however, regarding the actual measurements of iron (ferrous and total) in the test chambers, as well as of other important water quality parameters (e.g., pH, dissolved oxygen) precludes determining the oxidation state responsible for the observed effect in most of the studies previously discussed. Arguably, most of the existing freshwater acute data could be considered to be toxicity associated with ferric iron, regardless of the iron salt used, since these tests typically were conducted in laboratory water at circumneutral pH (pH 6 to 8) with low organic carbon (less than 5 mg C/L), when ferrous iron oxidizes rapidly (see Environmental Chemistry of Iron above). Examples include studies by Dalzell and MacFarlane (1999), Khangarot and Ray (1989), Mukhopadhyay and Konar 1984, and Warnick and Bell (1969) (Table 1-1). Conversely, any tests conducted at low pH (less than 6.0) in poorly buffered water (softwater) may or may not represent the acute toxicity of the soluble ferrous iron species. Iron speciation in reconstituted laboratory water is highly dependent on pH, DO, DOC, the concentration and type of salt added, as well as the experimental design utilized, i.e., flow-through, renewal or static test conditions.

Approaches for Formulating Criteria for Iron

Additional Laboratory Toxicity Testing of Ferrous Iron

Limited evidence suggests that bioavailable (ferrous) iron may be more toxic to aquatic organisms than total (ferric) iron, although occurrences of significant levels of ferrous iron in the natural environment are rare. The derivation of a freshwater criterion based on this form of iron, which would be consistent with current EPA guidance, requires the development and optimization of acceptable laboratory flow-through testing procedures specific to the species being tested. Each test must be designed to maximize the level of the soluble ferrous ion relative to the insoluble ferric ion under oxygenated exposure conditions, without compromising the health of the test organisms. Analytical methods for determining the level of both ferrous and ferric species in the test water would also require refinement and validation.

At a minimum, acute toxicity tests would be required using a third family in the phylum Chordata (e.g., fish, amphibian, etc.), a planktonic crustacean (e.g., cladoceran, copepod, etc.), a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish), an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.), a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.), and a family in any order of insect or a phylum not already represented. It would also be important to conduct flow-through toxicity tests with rainbow trout and the fathead minnow to confirm the earlier findings from the static tests conducted by Cherry (1983 a,b).

Chronic flow-through measured animal toxicity testing would also be required with three of the same species tested for acute effects to obtain the necessary acute-to-chronic ratios. The species selected for the chronic tests would be from three different families, and would include at least one fish species, at least one invertebrate species, and at least one acutely sensitive freshwater species (e.g., the mayfly or brook trout).

One test would also be required with either a freshwater algal or vascular plant species to provide additional toxicity information on plants. Bioaccumulation testing is not recommended at this time because iron is an essential element for most living organisms, and because there is no evidence of the biomagnification of iron (Table 1-6). In addition, the guidelines state that "data concerning bioaccumulation by aquatic organisms are only required if relevant data are available concerning the significance of residues in aquatic organisms"; the investigators are not aware of any such data for iron.

Modeling Approaches for Predicting Site-Specific Bioavailable Iron Concentrations in Fresh Water Receiving Waters

The relationship between total iron concentration and the bioavailable ferrous ion in natural receiving water and receiving water/discharge mixtures will vary from site-to-site due to a number of site- and condition-specific variables. Therefore, a modeling approach using mathematical models for the various physicochemical processes, combined with a limited number of water quality measurements for model input and confirmation, may provide the best, most cost-effective, and workable, option for NPDES permitting purposes. A modeling approach for predicting site-specific bioavailable iron concentrations requires consideration of some (or all) of the following processes: (1) equilibrium metal speciation, (2) inorganic and organic complexation, (3) sorption to particulate and dissolved organic matter, and to ferric hydroxide flocs, (4) precipitation and coagulation, and (5) oxidation kinetics. For site-specific application, these processes generally must be modeled in a transport and fate (mass balance) framework. These frameworks may vary in complexity, from static or probabilistic dilution models, to spatially-resolved steady state or even time-variable models, depending upon the complexity of the site, whether waste allocation among multiple sources is necessary, and the adverse cost associated with making overly-conservative assumptions. While models for each of these processes exist and are available at various levels of complexity, their utility and predictive capabilities are (to the best of the investigators' knowledge) essentially unknown in the context of real-world applications for iron. Based upon this recognition, some effort should be devoted to identifying and developing an appropriate model for use in determining the expected iron speciation at any given site, which will ultimately be needed for deriving suitable iron NPDES permit limits. Speciation modeling may prove invaluable in distinguishing the truly bioavailable form of iron (ferrous iron) from the insoluble suspended form (ferric iron) under most field conditions, and provide the basis for protecting aquatic communities from the impacts of suspended iron flocs, as well as from ferrous iron. A modeling approach has already been utilized for evaluating the speciation of iron in the U.S. Steel coke plant effluent, U.S. Steel Gary Coke Works, Gary, IN (The Advent Group 1997).

In-Stream Biological Condition Based on Indirect Effects of Total (Ferric) Iron

The field data for iron, which have been published, suggest that the in-stream ecological effects of iron may be the most important factor to consider in protecting the aquatic environment. Although an ecological effects-based criterion has yet to be implemented at the national level, several states, including Ohio, Kentucky, Pennsylvania, Tennessee and West Virginia may collectively possess sufficient data to develop a sensitive ecological metric relative to the total iron concentration found in several different waterbody types. In fact, a similar ecological metric such as the one proposed here has already been developed by the Ohio Environmental Protection Agency (OEPA 1998). The development of an ecological-effects based criterion for ferric iron would require the acquisition and compilation of all pertinent data for these and other states, followed by the derivation of a new threshold level for total recoverable iron that would be protective of biological impairment. Most deleterious effects to freshwater biotic communities are thought to be closely associated with the indirect effects of elevated total iron concentrations, which consists primarily of the ferric hydroxide suspension. The acquisition of a very large database is required because the concentration of total iron in natural surface water is expected to fluctuate substantially on a diurnal and seasonal basis (Vuori 1995), and because in any given natural water body contaminated with high iron levels, iron is often the main, but may not be the sole component of the toxicants present.

2
TIERED FRESHWATER AQUATIC LIFE CRITERIA FOR IRON

Introduction

Current regulatory policy is designed to protect aquatic life against the adverse environmental impacts of iron and other toxic chemicals. Under section 304(a)(1) of the Clean Water Act (CWA) 1977 (P.L. 95-217), the United States Environmental Protection Agency (EPA) is required to publish maximum acceptable pollutant concentrations in ambient waters that accurately reflect the latest scientific knowledge of the kind and extent of all identifiable contaminant effects on human health and aquatic life (EPA 2000). If these water quality criteria are adopted by a state or authorized tribe as water quality standards under section 303 of the CWA, they become enforceable regulations. Their purpose is to achieve a level of water quality that protects the designated use of the waterbody and ensures protection and propagation of aquatic species, human recreation, as well as maintenance and restoration of biological integrity (EPA 1994).

Excess concentrations of total iron can have a wide range of negative effects on aquatic organisms. Iron can disrupt cell membranes and damage the DNA of aquatic plants and animals (Danapat et al. 1999; Payne et al. 1998; Sinha et al. 1997). In addition, precipitation of ferric hydroxides and iron-humus colloids may accumulate on fish gills, limiting respiration (Dalzell and McFarlane 1999; Lehtinen and Klingstedt 1983; Peuranen et al. 1994; Steffens et al. 1993), constrain food access by invertebrates (Gerhardt 1992; Randall et al. 1999), and alter the quality and structure of benthic habitats (McKnight and Feder 1984; Letterman and Mitsch 1978; Scullion and Edwards 1980). Reduced abundance and species diversity of periphyton, benthic invertebrates, and fishes are often reported in water bodies with high iron concentrations (Greenfield and Ireland 1978; Koryak et al. 1972; McKnight and Feder 1984; Letterman and Mitsch 1978; Parsons 1968; Rasmussen and Lindegaard 1988; Scullion and Edwards 1980).

The physico-chemical processes affecting the various chemical forms of iron in an ambient surface water matrix are extremely complex. The relationship between total iron (Fe) concentration and the bioavailable ferrous ion (Fe^{2+}) varies from site-to-site due to a number of abiotic and biotic variables: pH, temperature, redox, humic content, chloride (Davison and DeVitre 1992; Stumm and Morgan 1982). Given this variability, it is apparent that any numerical value based on a single ionic species of iron is difficult to defend.

EPA's current "Metals Policy" recommends derivation of aquatic life criteria for dissolved metals (Prothro 1993). Since ferrous iron is soluble and ferric iron (Fe^{3+}) is essentially insoluble (<1 pg/L at pH 7), as it first hydrolyzes then polymerizes to insoluble colloidal iron oxy-hydroxide polymer in natural systems, iron criteria should be based upon the toxicity of the bioavailable Fe^{2+} to aquatic test organisms. Unfortunately, few studies have determined the toxicity of Fe^{2+} to aquatic test organisms. What little data exist suggests this form of iron is neither highly toxic to aquatic organisms, nor does it usually occur at high levels in most natural waters.

The current water quality criterion of 1 mg/L for total iron is based primarily on field observations (EPA 1976). This approach was taken, in part, because effects of total iron on freshwater biota can be both direct and indirect (physical), and the latter is difficult to measure in laboratory assays. The bulk of available evidence suggests that indirect effects of dissolved iron colloids, the form of iron that eventually precipitates out of solution and coats substrates and aquatic organisms, are more detrimental than direct toxic impacts of ferrous iron (Vuori 1995). Negative effects of iron colloids on respiration and food consumption, however, are not easily determined in laboratory experiments, and confound any interpretation of conventional toxicity test results (Cherry 1983 a,b). For example, laboratory tests of the direct toxicity of ferrous and ferric iron on freshwater fish and invertebrates generally exceed 2 to 5 mg/L, and can be as high as 118 (*Tilapia mossambica*) to 446 mg/L (Tubificid worm) (Mukhopadhyay and Konar 1984). Field surveys, on the other hand, report adverse environmental impacts of iron in concentrations that are, in general, lower than those associated laboratory toxicity tests (Vuori 1995). Unfortunately, the lack of guidance for deriving values protective of aquatic life based on field observations leaves the current standard susceptible to criticism.

Two independent analyses have raised doubts about the validity of the current iron criterion. First, the basis for the existing standard is weakly supported (OEPA 1998; Thurston et al. 1979). The value was derived primarily from a single field study where trout and other fish species, in a polluted Colorado stream affected by acid mine drainage were found to be absent at concentrations exceeding 1 mg/L total iron (EPA 1976). Further justification of this criterion relied on acute laboratory toxicity tests for only a few freshwater fish species, and a sensitive aquatic invertebrate. Second, an extensive study by the State of Pennsylvania, also based on field surveys, established a higher total iron standard of 1.5 mg/L. Pennsylvania's standard was originally derived based upon a correlation between total iron (predominantly ferric oxyhydroxide) and streambed fouling. This value was subsequently corroborated by qualitative benthic macroinvertebrate sampling performed in conjunction with physical and chemical measurements (Sheaffer and Schoener, 1973). The latter assessment involved observations on the degree of streambed staining at 68 chemical sampling stations in 30 watersheds in Pennsylvania where benthic macroinvertebrate were also collected. Results were consistent with results obtained in similar biosurveys performed in Pennsylvania in the early to mid-1970s (Gale et al. 1976; Weed and Rutschky 1972).

The use of field or bioassessment information to derive water quality criteria has not been clearly addressed by EPA. It is fundamentally different than the *Guidelines* approach (Stephan *et al.* 1985), which has the strength of establishing causation of toxic effects and the limitations associated with the assumptions underscoring laboratory to field extrapolations. The *Guidelines* approach also has the premise that it protects at least 95 percent of all species. Besides the inability of determining causation, an acceptable level of effect has not been established for field-derived criteria. The biological condition of streams and other surface waters is a continuum ranging from natural conditions, where there is little or no anthropogenic influence, to severely degraded, where human-caused physical and/or chemical disturbances have severely altered the ecosystem. The amount of acceptable change in the biological condition of an aquatic community due to a stressor such as iron is a premise that should be established independent of any statistical treatment of the data. Both of these new challenges to bioassessment-based criteria, (1) relating biological effects to iron concentrations and (2) establishing acceptable levels of change in the biological community, have been addressed in this document and are introduced below.

In this chapter the investigators employ a novel statistical and interpretative approach to define two levels of protection for total iron based on field bioassessment data. The proposed method uses data from biological monitoring programs to fit models that estimate changes in maximum numbers of sensitive organisms along a gradient of iron concentrations. The best model is then applied to project the iron concentration that causes a significant decline in the maximum number of organisms established at reference sites. The statistical approach used for analyzing these data, which employs regression quantile analysis (Cade and Noon 2003, Cade et al. 1999, Koenker and Hollock 2003), is described in detail in Methods below. The investigators selected the number of organisms in field samples because it is believed this metric is likely to be a more reliable indicator of abundance than survival in laboratory experiments, even if non-random sampling (such as through the use of rapid bioassessment protocols) prevents estimates of population size. This approach provides a robust method for the challenge of relating bioassessment data to total iron concentration in streams.

The two new proposed protective values for total iron are designed to protect the health and welfare of aquatic life at concentrations consistent with the CWA goal and objective. Results of the regression quantile analysis of field bioassessment data are interpreted using EPA's Tiered Aquatic Life Use (TALU) model. Briefly, EPA's TALU Model is one expressed as a scale of deteriorating biological condition with increasing human disturbance, e.g., increasing ambient total iron concentration (Figure 2-1). The TALU model was used in the development of proposed protective of total iron by establishing acceptable levels of biological change in the aquatic community independently of the statistical analysis of the data. According to the model, the upper tier (tier 1) describes the structural and functional integrity of a biological community in its natural state. Few such systems currently meet this description in the United States. Tier 2 is represented by aquatic systems having minimal changes in structure and function, manifested by slight increases in taxa and biomass, most likely the result of nutrient enrichment (though other anthropogenic factors may be involved). At this level of biological condition, there are no incidental anomalies and ecosystem level functions are fully maintained. It is the investigators' consensus, as well as that of EPA's tiered aquatic life workgroup, that tiers 1 and 2 meet the biological integrity objective of the CWA (Figure 2-2).

Tiers 3 and 4 are marked by slight to moderate changes in structure due to loss of some rare native taxa (tier 3) and/or replacement of sensitive ubiquitous taxa with more tolerant taxa (tier 4). Ecosystem functions are either fully or largely maintained in these tiers, and there is an overall balance in distribution of all expected taxa (Figure 2-2). The investigators and EPA's tiered aquatic life workgroup agree that tiers 3 and 4 meet the protection and propagation of fish, shellfish, and wildlife objective of the CWA, referred to in this report as the CWA 101(a) objective.

Tiers 5 and 6 are marked by more extreme changes in structure (Figure 2-1). At tier 5, sensitive ubiquitous taxa (e.g., most mayflies, and many stoneflies and caddisflies) are markedly diminished or absent, and the distribution of major groups is conspicuously unbalanced.

Organisms also begin to show signs of physiological stress (reduced growth and reproduction), and ecosystem function shows reduced complexity and redundancy. In tier 6, all the indicators listed above in tier 5 are further degraded. Conditions at tiers 5 and 6 do not meet the CWA 101(a) uses for the protection and propagation of aquatic life, and constitute a threshold for needed restoration actions (Figure 2-2).

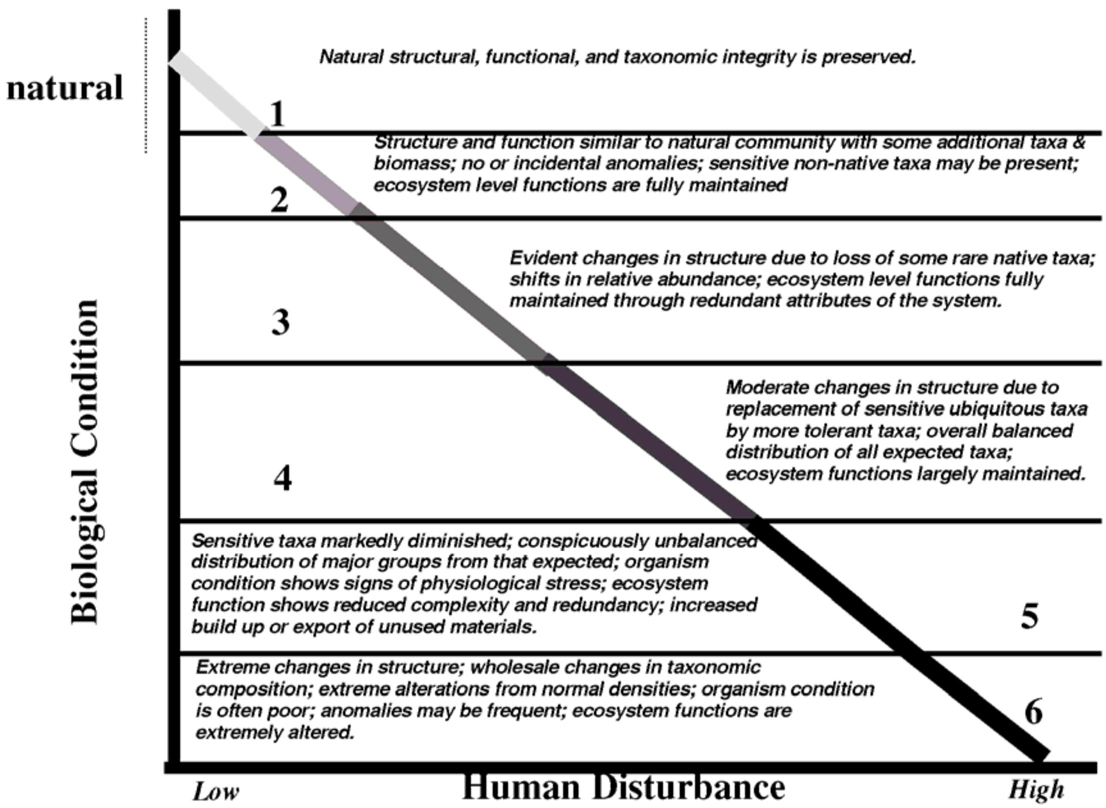

Figure 2-1
EPA's Tiered Aquatic Life Use (TALU) Model: Conceptual Framework.

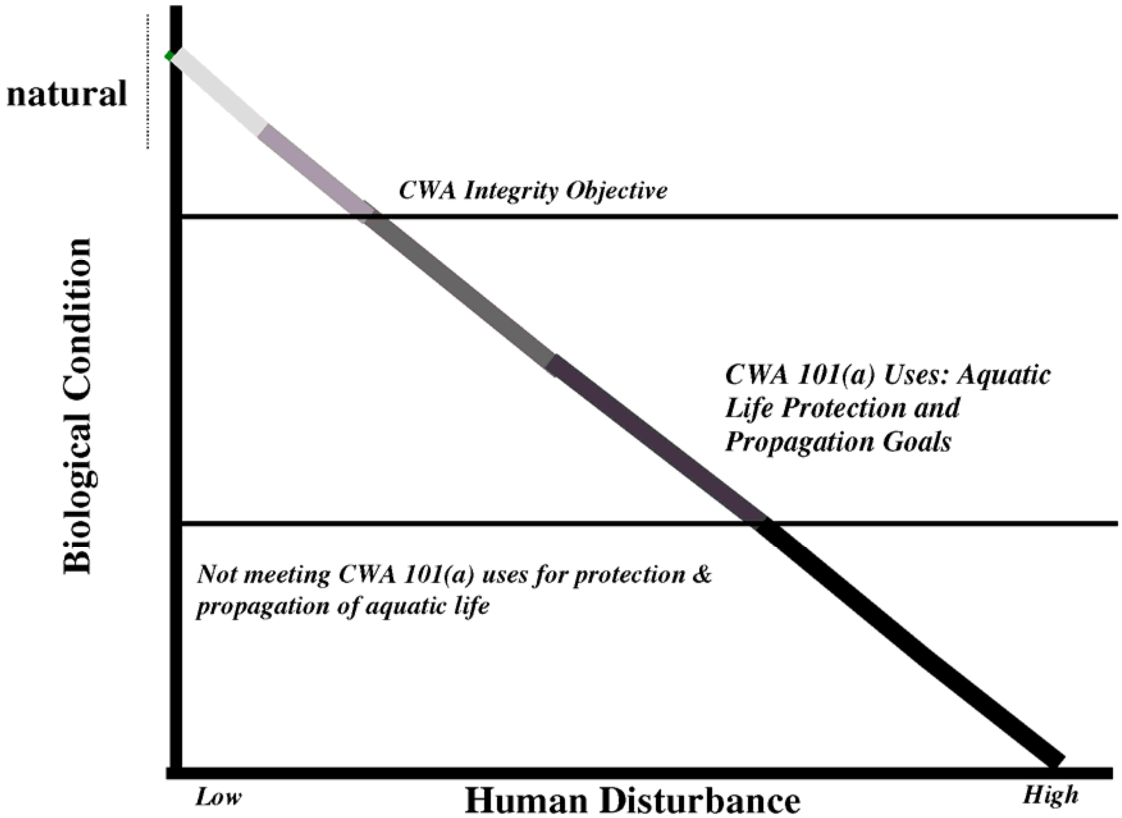

Figure 2-2
Tiered Aquatic Life Criteria for Total Iron: Conceptual Framework.

The protective thresholds (or biological benchmarks) derived by the system proposed here are intended to represent maximum acceptable concentrations consistent with the CWA goal and objective (EPA 1994). As they are computed from field observations, only an association between increasing concentrations of the contaminant and declines in the number of organisms can be established. It is important to recognize that an association between total iron concentration and the maximum number of organisms in samples does not prove that iron is toxic. The association does, however, quantify the decline in maximum number organisms in the field as a function of total iron concentration, and thus provides the possibility of deriving appropriate benchmarks of contaminant concentration for protection of aquatic life.

Methods

Iron as a Constraining Factor: Quantile Regression Analysis

The greatest challenge for estimating effect levels from field observations is to discern effects of the contaminant of concern from the impact of numerous sources of stress: toxic chemicals, habitat degradation, invasive species, etc. In laboratory toxicological experiments, the response of organisms to increasing concentrations of a toxic substance is measured in the absence of additional stressors. In such controlled conditions, variability in the selected endpoint is greatly reduced, and it is possible to infer that negative effects are caused by the contaminant. Field observations, in contrast, record the dynamic (e.g., growth, survival) or structure (e.g., abundance, diversity) of populations and communities, as well as concentrations of one or more uncontrolled toxic chemicals. Not surprisingly, the expected association between contaminant concentration, an estimate of dose, and the magnitude of deviation in the selected endpoint from levels at reference conditions, an estimate of response, is often obscured by excessive variation in endpoint values due to effects of factors that are not related to the contaminant of concern.

Since it is not feasible to eliminate all factors that bias estimates of organism abundance or diversity in the field, it is proposed to analyze effects of contaminants of concern on the number of organisms in samples using quantile regression. Focus is on changes in the maximum number of organisms in samples along a gradient in total iron concentration. As the estimates of extreme quantiles are very uncertain, the investigators focused on changes in the 90th quantile. A quantile expresses the fraction of samples, sorted in ascending order, with equal or lower values. The median is the 0.5 quantile, often called the 50th quantile. For instance, use of a number of insects in a sample as the endpoint and plot these values against their respective iron concentrations (Figure 2-3), the progressive decline in the maximum number of insects with increasing iron concentrations reveals the greater role of iron in limiting abundance of these animals. Such effects can be quantified with quantile regression (Cade et al. 1999, Koenker and Hallock 2001).

Quantile regression estimates a function (ξ) that projects a selected proportion τ (the quantile) of the dependent variable (y) as a function of the independent factor (x) (Koenker and Bassett 1978). For a sample of size n, estimates of model parameters minimize the sum of absolute deviations, weighted by the selected quantile or its complementary fraction (1-τ),

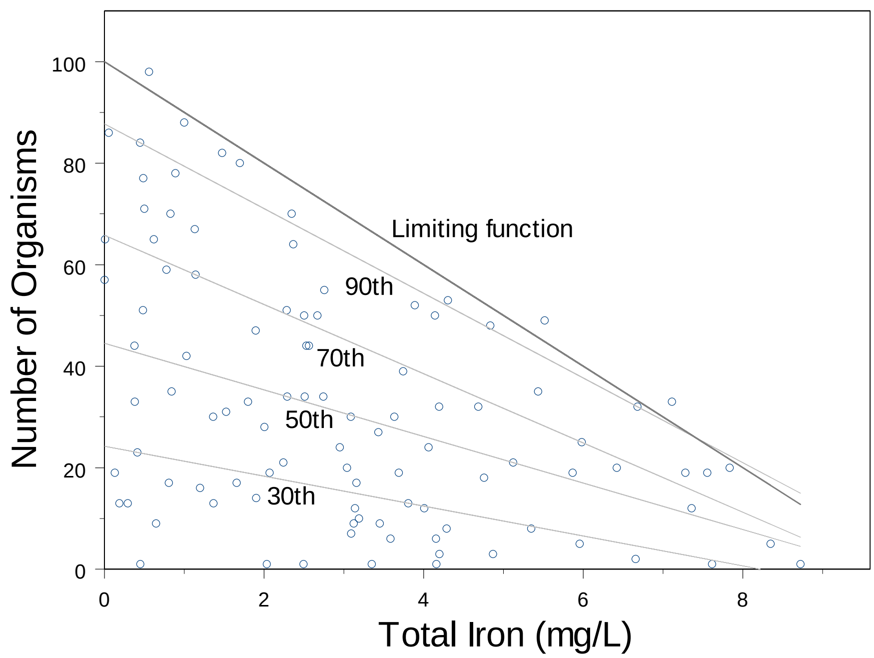

Figure 2-3
The regression lines displayed project the 30th, 50th, 70th, and 90th quantiles (τ = 0.3, 0.5, 0.7, 0.9, respectively) of the number of organisms collected in samples as a function of iron concentration. Simulated data (n=100) were generated from random uniform distributions (range: 0-10 for iron concentrations, 1-100 for number of organisms). The decline in number of organisms with increasing iron concentrations was imposed by discarding points where number of organisms > 100 – (10*[Fe]), the limiting function. Natural variability in the number of organisms was simulated by the addition of a random normal deviate with mean = 0 and standard deviation = 5.

$$\min\left[\sum_{i=1}^{n} \rho_\tau \left| y_i - \xi(x_i) \right|\right]$$ **Equation 2-1**

where

$$\begin{cases} \rho_\tau = \tau & \text{for} \quad y_i \geq \xi(x_i) \\ \rho_\tau = 1 - \tau & \text{for} \quad y_i < \xi(x_i) \end{cases}$$

Thus, except for $\tau = 0.5$, positive and negative residuals are differentially weighted, and the difference in weights ascribed to them increases as τ departs from 0.5 and approaches 0 or 1. Approximately $n\tau$ observations are below and $n(1-\tau)$ observations are above the fitted line (curve). Parameters of the linear model that result in the least absolute deviation were computed with a simplex linear algorithm (Koenker and D'Orey 1987), which builds on the median regression code written by Barrodale and Roberts (1974). Parameters of nonlinear models that minimize absolute deviations were estimated with an interior point algorithm (Koenker and Park 1996).

All quantile regression analyses were performed with packages Quantreg and Nlrq of R, a language and environment for statistical computing and graphics (Ihaka and Gentleman, 1996; http://www.r-project.org/).

The objective of using quantile regression in the analysis of field observations is to estimate the "limiting function," an expression that projects the maximum number of organisms in samples along a gradient of iron concentrations (see Figure 2-3). A focus on a high quantile of the number of organisms in samples represents a conceptual departure from the traditional emphasis on the mean response, as in ordinary least squares regression. The great advantage of using the maximal response is that it is less likely to be influenced by other factors. For instance, consider the hypothetical example where mayflies along a gradient of iron concentrations are also exposed to copper. The maximum number of these insects in samples is reduced, and such reduction is of greater magnitude where copper concentrations are higher (Figure 2-4). Co-contaminants, as well as limiting abiotic and biotic factors decrease the number of organisms in samples, and thus bias the average or any other response level below the limiting function.

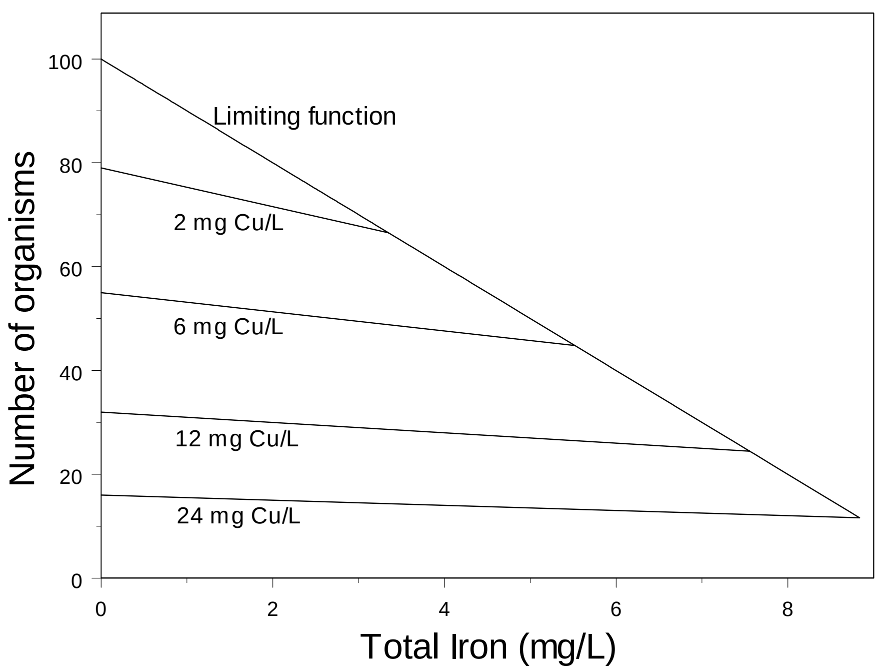

Figure 2-4
Hypothetical plot illustrating how a co-contaminant (copper) would further reduce the maximum number of organisms in samples. All lines define upper bounds of the distribution of number of organisms in samples. Values are hypothetical and build on the simulated data of Figure 2-1.

The focus on variation of a selected quantile(s) of the dependent variable, instead of its mean, is not the only difference between quantile and ordinary least squares (OLS) regressions. Quantile regression is less sensitive to outliers than OLS, and it generates estimates of model parameters that are unbiased for linear and nonlinear transformations of the response variable (Koenker and Bassett 1978, Birkes and Dodge 1993). Such desired property permits, for example, computation of unbiased estimates of parameters a and b in the linear function $y = a + bx$ from estimates of β_0 and β_1 in the transformed function $\log(y) = \beta_0 + \beta_1\log(x)$. In least squares regression, estimates of a and b are biased when computed from β_0 and β_1 (Beauchamp and Olson 1973). Furthermore, with rank-score statistics it is possible to test hypotheses and build confidence intervals for parameters of linear models with heterogeneous distribution of errors (Koenker 1994, Koenker and Machado 1999). Logarithmic transformation of variables, as in the example above, is often done to achieve a homogeneous error distribution, which is a fundamental assumption of OLS. It is not yet possible to test hypotheses or build confidence intervals for parameters in nonlinear models.

Selection of the appropriate quantile is an important step in the process of estimating the limiting function. Estimates of model parameters often vary with quantile (e.g., Figure 2-3). Since the limiting function, by definition, sets an upper bound for values of the response variable, it is naturally tempting to use the highest quantile that is feasible to compute. Estimates of model parameters for extreme quantiles, though, have greater uncertainty than most other estimates (Figure 2-5), and in general are more sensitive to outliers. For instance, alteration of only one of the 100 simulated points in Figure 2-3, a three-fold increase in the number of organisms (from 20 to 60) associated with an iron concentration of 7.84 mg/L reduced slopes of the 90th, 95th, and 99th quantiles by 7.0%, 6.6%, and 47.2%, respectively. Selection of the 90th quantile was based on visual analysis of confidence intervals for estimates of slopes of quantile regression lines, which project the number of organisms in samples as a function of iron concentration (Figure 2-6).

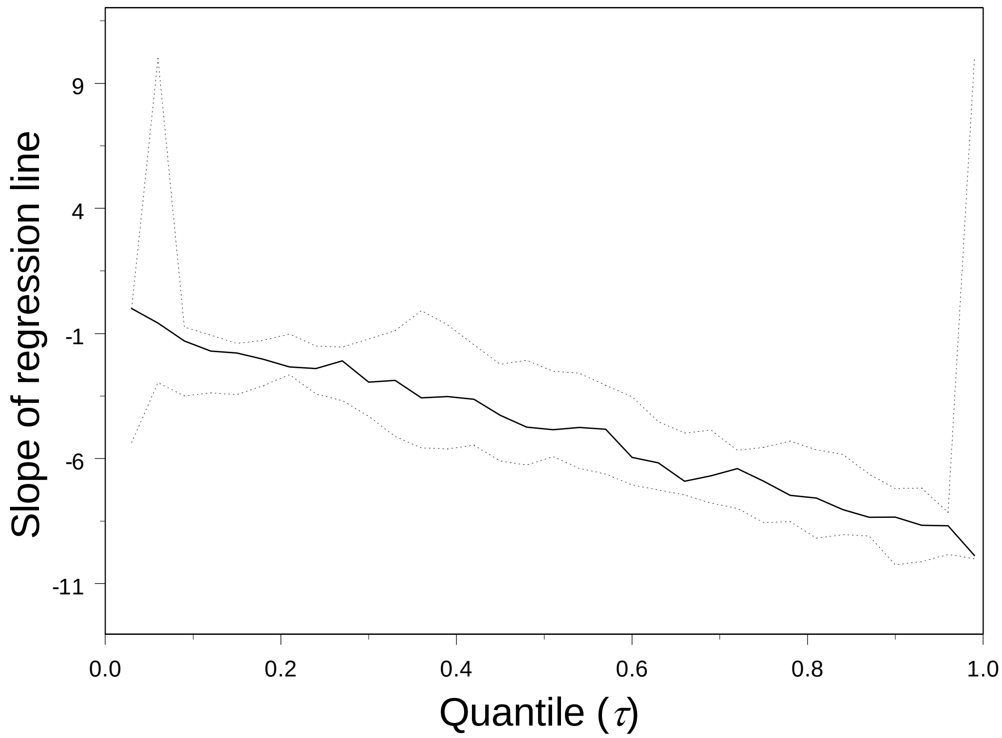

Figure 2-5
Estimates of slopes of quantile regression lines, which project the number of organisms in samples as a function of iron concentration, for the full range of quantiles. Dashed lines represent the 95 % confidence interval of slope estimates. Hypothetic values computed from the simulated data in Figure 2-3.

Here the investigators use quantile regression to fit models that project the 90th quantile ($\tau = 0.9$) of the number of benthic macroinvertebrates in samples (m_τ) as a function of iron concentration ([Fe]). As the exact form of the limiting function is not known, the investigators compared four candidate models that project the τ quantile of the number of benthic macroinvertebrates in samples as a function of iron concentration:

I)	$m_\tau = a$	Constant
II)	$m_\tau = a + b\,[Fe]$	Linear
III)	$m_\tau = \exp(a + b\,[Fe])$	Exponential
IV)	$m_\tau = \frac{1}{\left(c + a[Fe]^b\right)}$	Sigmoid

where *a*, *b* and *c* are parameters that the investigators wish to estimate. Model (I) implicitly assumes that numbers of organisms in samples are independent of contaminant concentrations. It corresponds to the standard null hypothesis in regression analyses: slope of the regression line is not significantly different from zero. Models (II), (III) and (IV) project declines in number of organisms as linear, exponential and sigmoid functions, respectively, of increasing contaminant concentrations in the water.

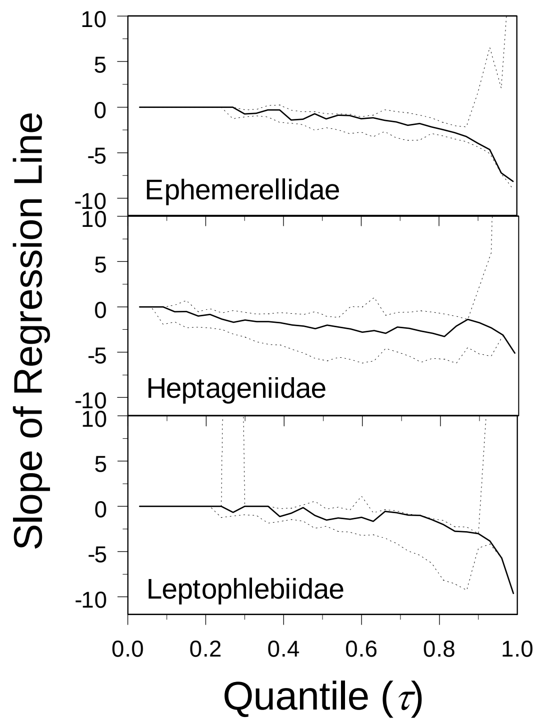

Figure 2-6
Estimates of slopes of quantile regression lines, which project the number of three families of macroinvertebrates as a function of iron concentration, for the full quantile range. Dashed lines represent the 95 % confidence interval of slope estimates.

Model Selection: the Information-Theoretic Approach

Selection of the best model(s) considers both the fit and number of parameters. Models with greater number of parameters generally fit the data better, but such reduction in bias is invariably associated with an increase in variance of parameter estimates (Burnham and Anderson 2002). Model selection methods attempt to find a parsimonious model with the proper tradeoff between bias and variance. The investigators applied the information theoretic approach for model selection. It is based on the Kullback-Leibler information, $I(f,g)$, which expresses the information lost when model g is used to estimate the full reality f. Obviously, the full reality is never known, but an estimate of the relative distance from reality can be estimated by the Akaike Information Criterion (AIC, Akaike 1973).

$$\text{AIC} = -2\ \log(\text{mle}(\text{parameters}|\text{data})) + 2K$$

where K is the number of parameters in the model and mle(parameters|data) is the maximum likelihood of parameter estimates for the available data. The AIC is a poor estimator of $I(f,g)$ when $n/K < 40$ (n is the sample size). In such instances, a second-order version of AIC, AICc, is recommended (Hurvich and Tsai 1989):

$$\text{AIC} = -2\ \log(\text{mle}(\text{parameters}|\text{data})) + 2K\left(\frac{n}{n - K - 1}\right)$$

Hurvich and Tsai (1990) demonstrated that the version of AICc modified for least absolute deviation (L1cAIC) provides an unbiased estimator for the Kullback-Leibler information, but the small sample criterion for normal least squares regression, which is less computationally demanding, performs equally well

$$\mathbf{AIC}_c = n\log(\sigma^2) + 2K\left(\frac{n}{n - K - 1}\right)$$

where ^{2} is estimated as the sum of squared residuals divided by n. For the least absolute deviation regression, ^{2} is estimated as (SWAD/n)^2, thus AICc is computed by the expression

$$\mathbf{AIC}_c = 2n\log(\mathbf{SWAD} / n) + 2K\left(\frac{n}{n - K - 1}\right)$$

The above equation assumes that errors have a double exponential distribution. The AIC and AIC_c are used to rank candidate models. Comparisons among the M ranked candidates are based on the Akaike weight (w), which represents the likelihood of a model given the data

$$w_i = \frac{\exp\left(\frac{-\Delta i}{2}\right)}{\sum_{m=1}^{M}\exp\left(\frac{-\Delta i}{2}\right)}$$

where $_i$ is the difference in AIC (AICc) between model i and the model with the lowest AIC (AICc) value. The best model(s) has the largest weight. Weights for all candidate models add to one. For each of the four models, the investigators computed the sum of weighted absolute deviations (SWAD), AICc and the Akaike weight.

Projection of Protective Criteria

The estimated limiting function provides a quantitative relationship between total iron concentration and the 90th quantile of the number of organisms in samples. If a biologically significant threshold for the number of organisms, or a biologically significant reduction in the 90th quantile of the number of organisms in references sites can be defined, then it is possible to use the estimated limiting function to project the iron concentration associated with such value.

Ideally, biologically significant thresholds would be estimated by analysis of extensive data on temporal variation in abundance and associated iron concentrations, or risk assessment of stochastic models of population dynamics (Burgman *et al.* 1993, chapters 4-7 in Pastorok *et al.* 2001). Instances where adequate information is available to use such approaches, however, are rare. Therefore, to define biologically significant thresholds based on the conceptual framework of tiered aquatic life use (TALU) recently developed by EPA. First, the protective threshold that has as its objective maintenance and restoration of biological integrity is defined as a 20% reduction in the 90th quantile of the number of organisms in a sensitive taxonomic group at reference sites. The second threshold aims to offer a level of protection that only ensures propagation of aquatic species. Therefore, a much higher percent reduction in species abundance is permitted. The second threshold is defined as a 50% reduction in the 90th quantile of the number of organisms in a moderately sensitive taxonomic group at reference sites.

Projection of the iron concentration associated with either threshold is relatively simple. The first step is to adjust the intercept of the quantile regression model. An adjustment is necessary because the 90^{th} quantile of organism numbers at reference sites may be much greater or lower than the 90^{th} quantile of organism numbers at all sites. If the 90^{th} quantile of organism numbers at reference sites is above the projected quantile regression line (curve), then it is not feasible to project a field-based effect concentration. Conversely, if the 90^{th} quantile of organism numbers at reference sites is much lower than the 90^{th} quantile of organism numbers at all sites, then the projected effect concentration will not adequately protect aquatic organisms. The intercept adjustment is just an additional constant to the best model; its magnitude equals the difference between the 90^{th} quantiles of organism numbers at reference and all sites ($m_{\tau=0.9\ \mathrm{Ref}}$ - $m_{\tau=0.9\ \mathrm{All}}$). The second and final step is rearrangement of the best model to solve for the contaminant concentration associated with a 20% reduction in the 90^{th} quantile of the number of organisms at reference sites (Figure 2-7). For example, the selected best model (largest *w*) for the 90^{th} quantile of the hypothetical data in Figure 1 was $m = \exp(4.4936 - (0.14[Fe]))$, and the 90^{th} quantiles of the number of organisms at reference and all sites were 80 and 70, respectively. The projected field-based effect concentration (FEC 20%) for iron was then computed as:

$$\text{FEC } 20\% = \frac{\ln((0.8 \times 80) - (80 - 70)) - 4.4936}{-0.14} = 3.60 \text{ mg/L}$$

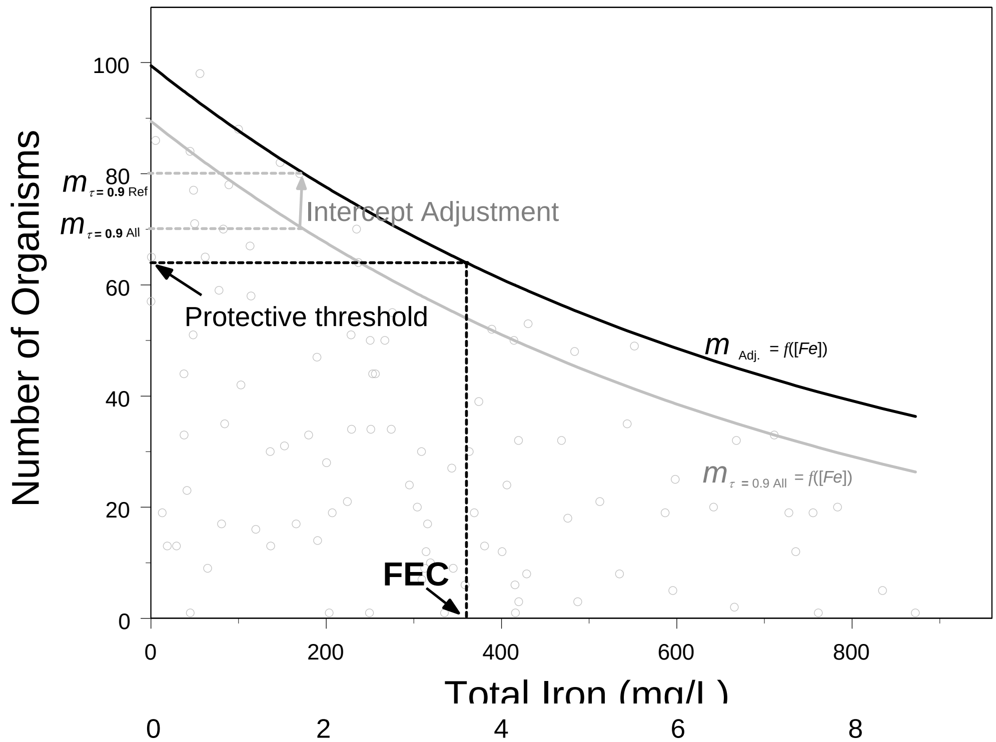

Figure 2-7
Diagram illustrating the proposed method to compute a field-based contaminant effect concentration (FEC). Its value (0.36 mg/L) was derived from the simulated data in Figure 2-3. The selected best model, an exponential curve (in gray), projects the 90th quantile of the number of organisms in samples for any iron concentration ($m_{\tau = 0.9} = f([Fe])$) within the observed range. This approximation of the limiting function was then adjusted for the 90th quantile of organism numbers in samples from reference sites ($m_{\mathrm{Adj.}} = f([Fe])$) and employed to estimate the contaminant concentration associated with a 20% decline in the 90th quantile of organisms in samples from reference sites (protective threshold = 0.8* $m_{\tau = 0.9\ \mathrm{Ref}}$). The adjustment in intercept was simply the difference between the 90th quantiles of the number of organisms at reference and all sites (Adj.= $m_{\tau = 0.9\ \mathrm{Ref}} - m_{\tau = 0.9\ \mathrm{All}}$).

Field Data

We applied the method to derive proposed water quality criteria based upon a large data set of field observations provided by the West Virginia Department of Environmental Protection's (WVDEP) Water Assessment Program (WVDEP data set). The WVDEP data set includes data for benthic macroinvertebrates (years 1996 to 2001), which were used to derive the individual metrics of similar numerical values of biological condition. The data set does not include absent taxa. While absence of a taxon may reflect the toxic effects of the contaminant of concern, it can also result from the impacts of other biological or environmental stressors, seasonal variation in abundance, or a sampling bias. It is the great uncertainty about the cause of taxon absence that justifies exclusion of such data.

The sampling methods used in the WVDEP watershed assessment program are outlined in EPA's Rapid Bioassessment Protocols for Use in Wadeable Rivers and Streams, Second Edition (EPA 841-B-99-002). This protocol has been adopted for use by many states. The procedures call for the use of the single habitat approach when possible, using a rectangular dipnet (0.5 m wide) or smaller (0.3 m wide) d-net with 595 µm mesh size. Collections are generally limited to riffle areas only, and include 8 individual kick samples covering approximately 2 m^2 of stream substrate (four fast riffle/run and four slow riffle/run areas) within a 100-m assessment area. Corresponding water chemistry data were concurrently collected from the exact same sampling locations. All data were carefully scrutinized for data quality prior to analysis. The data for a particular location were not used where data quality or sampling flags indicated either biological or chemical sampling problems.

Reference sites are those which reflect the characteristics of stream segments in watersheds least impaired by human activities, and are used by WVDEP to define attainable biological or habitat conditions (Reference the WVDEP protocol). These sites do not necessarily reflect pristine or undisturbed watersheds, but are representative of the least impaired conditions given the land uses. The criteria used to select reference sites in the WVDEP watershed assessment program included: dissolved oxygen > 5.0mg/l; pH between 6.0 and 9.0; conductivity < 500 µS /cm; fecal coliform < 800 colony/100ml; no violations of State water quality standards; no obvious sources of non-point pollution; epifaunal substrate / available cover score >10; channel alteration score >10; sediment deposition score >10; bank vegetative protection score >5 (right and left banks scored separately); undisturbed vegetation zone width score >5 (right and left bank scored separately); total habitat score ≥ 130 points; evaluation of anthropogenic activities and disturbances; and finally, no known point source discharges upstream and within view of the assessment site (discerned after all of the above conditions are met).

Dependent and Independent Variables

We used the number of benthic macroinvertebrates in each sample as an endpoint because small animals that live on the substrate are likely to be the most affected by precipitation of iron colloids. Benthic macroinvertebrates are also ubiquitous in aquatic ecosystems, and they are good indicators of substrate disturbance in both lotic and lentic benthic habitats (Resh and Jackson 1992). In addition, the sedentary nature of most benthic macroinvertebrates compared to some other animal groups afforded the opportunity to detect the spatial and temporal changes caused by iron precipitates. The wide diversity of benthic macroinvertebrate assemblages also permits analysis for a variety of different responses (relative abundance, species richness, etc.). The investigators found that the number of individuals is a more sensitive indicator of environmental impacts than categories of frequency, or presence/absence data. The focus on animals only reflects the greater availability of data for these taxa than for plants.

Total iron was selected as the independent variable for the regression quantile analyses because it is frequently measured in a majority of chemical monitoring programs, and because total iron avoids the many complexities of iron speciation in surface waters. Dissolved iron is typically not measured in water quality monitoring programs, and is also not consistently related to the measured total iron concentration (Loeffleman et al. 1985, Rasmussen and Lindegaard 1988). The poor correlation between total iron and dissolved iron concentrations in surface waters is a consequence of, among other things, the concentrations of dissolved materials, organic matter, sulfur compounds, carbon dioxide, and the pH and redox potential of the surface water (OEPA 1998). Some confusion exists in the literature regarding the various forms of iron in filtered surface water samples because small iron colloid particles less than 1 µm co-exist with the freely dissolved ferrous iron species in aqueous media (Davison and Seed 1983; Hiraide et al. 1988). Thus, “dissolved” iron consists of the free ferrous form, as well as small iron colloidal particles (principally ferric hydroxides and humates), as is the case for aluminum. Because there have been too few measurements of truly dissolved iron (ferrous) in bioassessment programs, this form of iron cannot be used to derive reliable protective values for this particular chemical species.

The advantage of using bioassessment data for ascertaining the direct and indirect effects of iron precipitate and iron-humus colloids in surface water is clear. This type of data has allowed modelling the constraints imposed by iron at levels that are difficult to determine from existing literature, either because of the gross differences in study design and water quality characteristics, or because sampling procedures and other design factors prohibit estimating an adequate protective threshold from such data.

Results and Discussion

Estimation of Field-Based Effect Concentrations

We estimated protective criteria for 8 families of aquatic macroinvertebrates. The West Virginia data set did not have sufficiently large sample sizes at reference or all sites for many of the other taxa appearing in the database. The investigators’ experience with analysis of these bioassessment data indicated that results were not reliable when numbers of samples at reference and all sites were less than 40 and 200, respectively. Other taxa were excluded because the selected best model was a constant (indicating no relationship between total iron and number of organisms in samples).

Mayflies in the family Leptophlebiidae were the most sensitive taxonomic group. None were found at total iron concentrations greater than 4.5 mg/L (Figure 2-8). The two taxa most tolerant to iron were mayflies belonging to the family Baetidae, and craneflies (Family Tipulidae; Order Diptera). Mayflies in the family Heptageniidae, caddiflies belonging to the family Philopotomidae (Order Trichoptera), and water pennies (Family Psephenidae; Order Coleoptera) are taxa which appeared to be intermediate in tolerance to the limitations imposed by iron (Figure 3).

Using the approach described above to derive ecological-effects based criteria for total iron, preliminary protective values for the eight insect families were calculated (Table 2-1). The response curves for all eight families were fit using nonlinear regression quantile analysis (sigmoid model).

In the process of analyzing these data, several other relationships were examined, including correlations between total iron concentrations and other common constituents in surface waters (Table 2-2). These analyses showed that the negative effects of iron were not likely correlated with any of these other potential co-varying factors, except aluminum and total suspended solids, which are both highly correlated with total iron.

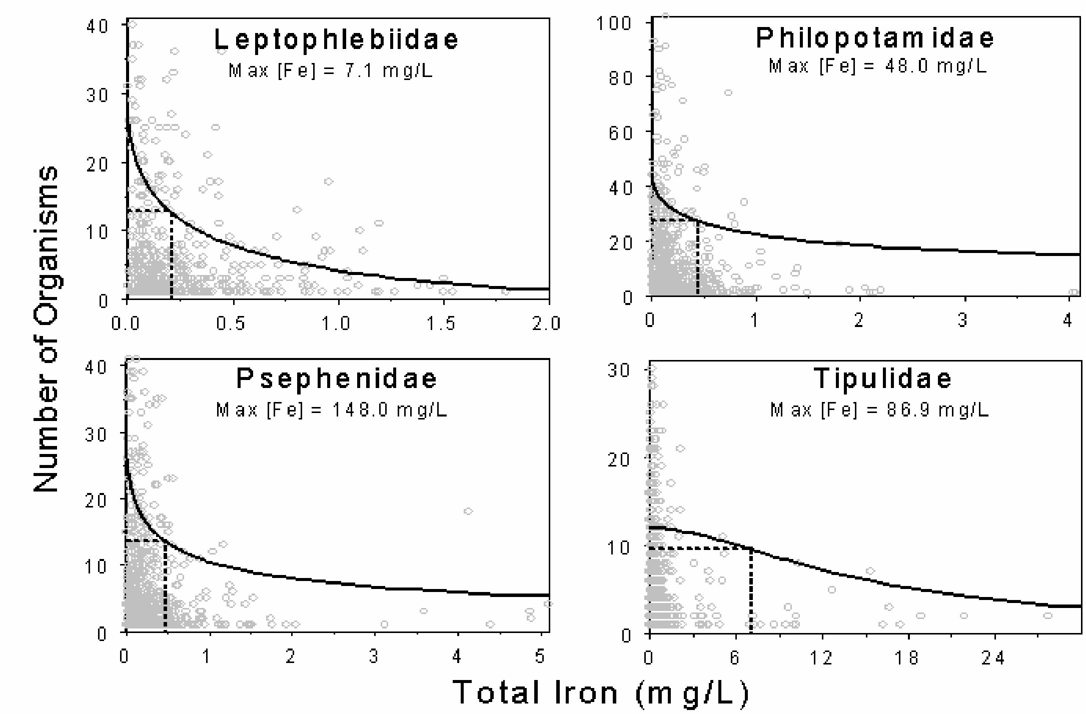

Figure 2-8
Scatter plot of number of organisms in each of eight families and respective total iron concentrations at monitoring sites in streams of West Virginia (WVDEP data set). The points displayed include samples collected over five years (1996 to 2001). Each sample represents a unique site and date. A single habitat approach constrained primarily to riffle/run areas was used for collecting biological samples. All chemical and biological samples were collected from matching locations. Upper dashed lines represent 0.8 times the 90th quantile of insect numbers in reference sites and associated total iron concentration. Lower dashed line represents respective lower bound of the distribution-free confidence interval (or upper bound of the protective value). The X and Y axes are truncated to focus attention on the most relevant portion of each curve. Maximum iron concentrations are provided in each plot. Actual values are provided in Table 2-1.

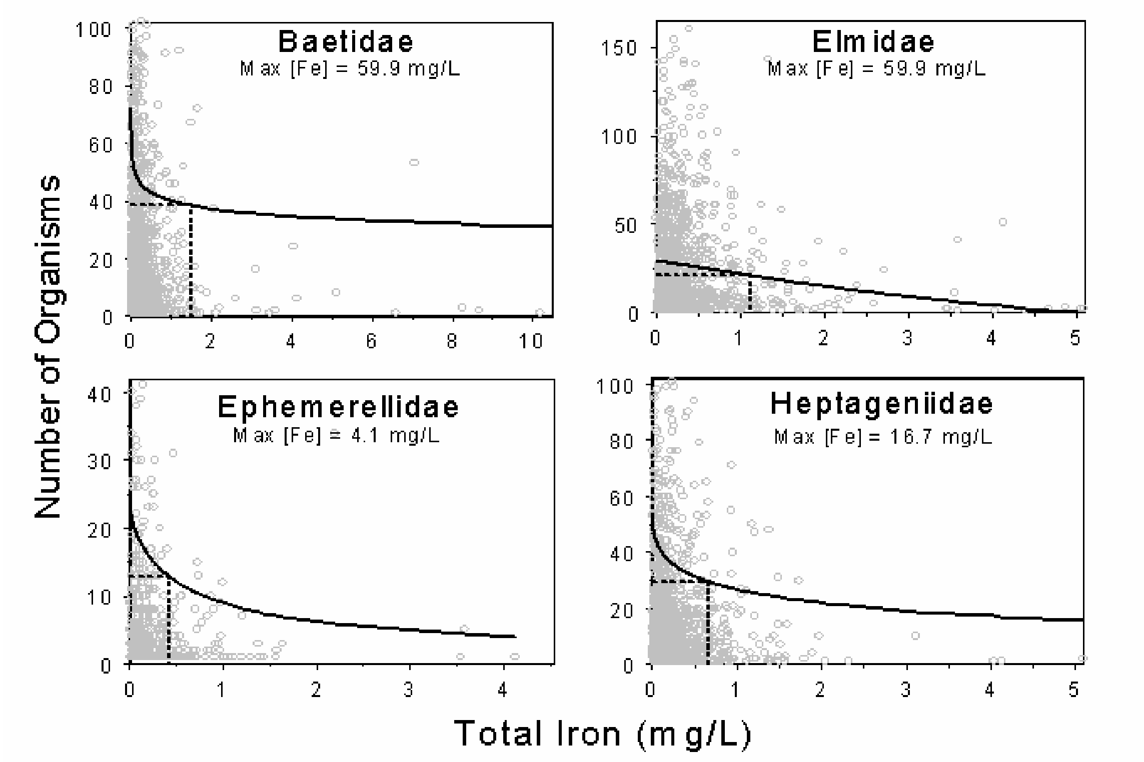

Figure 2-8 (continued)

Table 2-1
Results of the quantile regression analysis used to derive preliminary protective values for eight different families of freshwater benthic macroinvertebrates. The values were estimated as described in Figure 2-3.

	Projected	90th Quantile				
	Criterion	Ref.	All	Adj.	N Ref	N All
Leptophlebiidae	0.21	16	23	-7	51	519
Ephemerellidae	0.43	16	16	0	42	485
Philopotamidae	0.44	34	30	4	56	731
Psephenidae	0.48	17	17	0	40	719
Heptageniidae	0.66	37	45	-8	72	1062
Elmidae	1.13	26	61	-35	66	1355
Baetidae	1.48	48	57	-9	73	1252
Tipulidae	7.05	12	12	0	70	1191

Table 2-2
Correlations between concentrations of total iron and concentrations of potential co-contaminants in chemical samples (for suspended solids, entries express the mass of residues per unit volume) collected at the same site and date where animals were collected. For each correlation, the following are presented the Pearson correlation coefficient (r), the Student's t, degrees of freedom (df) and probability (P). Water chemistry data are from the WVDEP set data set and from the Ohio Environmental Protection Agency's bioassessment program (OEPA dataset).

	OEPA data set				WVDEP data set			
Constituent	***r***	***t***	**df**	***P***	***r***	***t***	**df**	***P***
Aluminum	0.85	22.36	184	<0.001	0.86	68.35	1650	<0.001
Arsenic	0.16	2.98	356	0.003	NA	-	-	-
Cadmium	0.08	2.05	625	0.040	NA	-	-	-
Chromium	0.01	0.34	623	0.737	NA	-	-	-
Copper	0.05	1.22	693	0.223	NA	-	-	-
Magnesium	0.08	-2.15	684	0.032	0.18	6.49	1332	<0.001
Manganese	0.33	5.32	230	<0.001	0.33	14.27	1648	<0.001
Nickel	0.01	0.17	590	0.864	NA	-	-	-
Strontium	-0.17	-1.66	95	0.101	NA	-	-	-
Sulfates	NA	-	-	-	0.34	14.09	1563	<0.001
Zinc	0.33	9.21	677	<0.001	0.51	22.35	1395	<0.001
Phosphorus	0.08	2.06	682	0.040	NA	-	-	-
TSS	0.89	45.79	574	<0.001	NA	-	-	-

NA – Data not available.

Derivation of Proposed Protective for Total Iron

The protective thresholds for total iron provided in this document were derived based on the premise that both direct and indirect effects of iron can act to limit the numbers of organisms collected in field samples. Using the conceptual framework established in EPA's TALU model described above, the investigators believe that two separate proposed protective aimed at achieving the goal and objective of the CWA are appropriate for total iron in freshwater. Figure 2-2 depicts where these two proposed protective values lie on a conceptual basis within the context of the biological condition gradient. The basis for deriving preliminary values to meet the two objectives is proposed below.

CWA Biological Integrity Objective

Mayflies in the family Leptophlebiidae appear to be the most sensitive macroinvertebrates affected by ambient total iron concentrations. Using the total iron concentration associated with 0.80 times the 90th percentile of maximum Leptophlebiidae numbers in the reference sites of West Virginia streams (Figure 2-8; Table 2-1), the analysis indicates that concentrations below the estimated protective threshold for Leptophlebiidae (approximately 0.21 mg/L) should adequately protect these and other sensitive taxonomic groups from the direct and indirect effects of iron. The investigators believe that the total iron concentration associated with a 20 percent decline in the 90th quantile of Leptophlebiidae numbers in streams at reference sites of West Virginia protects at the level of tiers 1 and 2 in the aquatic life use model, and therefore, could be used to achieve the biological integrity objective of the CWA.

The application of a protective threshold for total iron based on the single most sensitive taxonomic group alone could, however, result in enforcement actions that are not attainable for many streams in their current state. Therefore, a second more environmentally realistic goal for protection is necessary. For example, based on the distribution of total iron in reference versus all sites in West Virginia, at total iron concentrations less than or equal to 0.25 mg/L, approximately 20 percent of the reference sites (N = 64) could be considered to be impaired by iron, or approximately 50 percent of all the sites (Figure 2-9). The implications of such a low value are profound, especially when considering the number of streams in states with naturally high background levels of iron, e.g., Ohio, Pennsylvania, West Virginia. In this case, a relatively large percentage of the best (i.e., reference) streams in the state of West Virginia would not be in attainment.

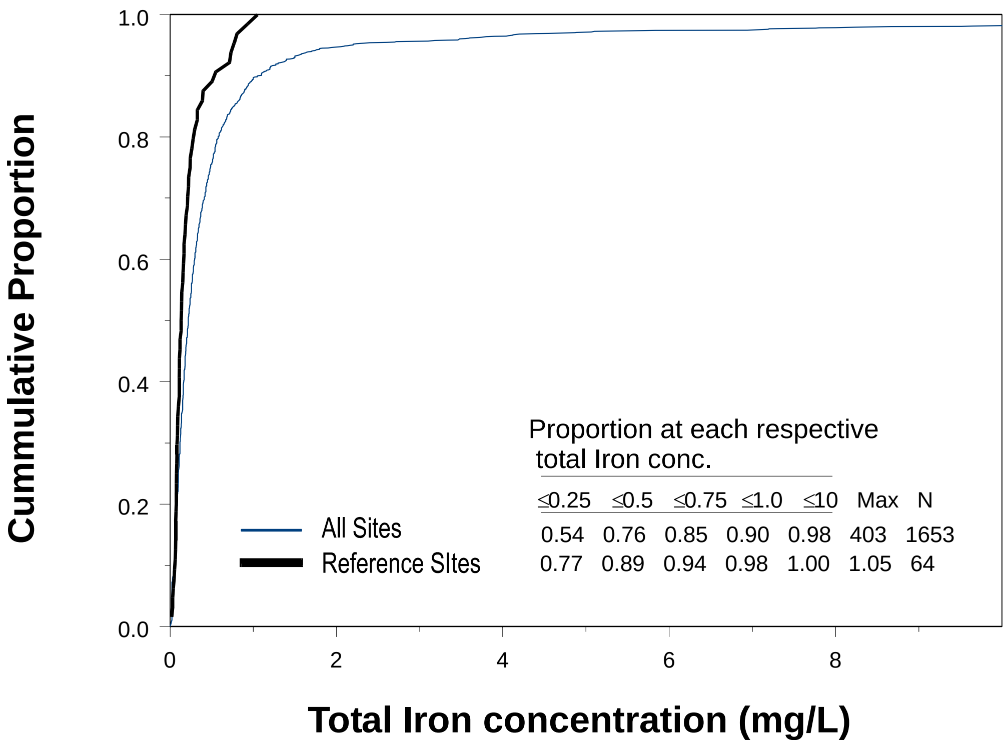

Figure 2-9
Distribution of Total Iron in Sites Where Biological Samples Were Collected in the State of West Virginia.

CWA 101(a) Use Goal

We propose deriving a second protective threshold for total iron using a weight-of-evidence approach which considers, among other things, the relative sensitivities of several other taxonomic groups. The development of such a value would more appropriately assess and protect against the physical (as well as any toxicological) constraints imposed by high iron concentrations at a community or assemblage level, which is consistent with current national aquatic life criteria objectives, or the CWA 101(a) objective (Stephan *et al*. 1985).

By using the point at which a 50 percent reduction in the fourth most sensitive taxonomic group is observed along the iron concentration gradient (Psephenidae), it is found that a value of 1.74 mg/L should allow for biological conditions consistent with tiers 3 and 4. At this and lower concentrations, which is marked by a relative decrease in the populations of several of the sensitive ubiquitous taxa, as well as a relative increase in the abundance of intermediate and tolerant taxa, a remnant population of Leptophlebiids is likely to persist, providing the habitat is adequate.

Figure 2-10 shows an array of sensitivities of different taxonomic groups to increasing ambient total iron concentrations. The magnitude of decrease in several sensitive ubiquitous taxa and the change in ratio between sensitive and tolerant taxa appear to coincide with a second threshold that seems adequate for protecting aquatic life at the CWA 101(a) objective.

In order to compare this second benchmark with the standard used by West Virginia to define biological impairment, the FEC 50% was computed (0.5 times the 90th quantile of maximum number of animals in reference sites) and contrasted it with the corresponding stream condition index (SCI). West Virginia currently uses the SCI as the primary indicator of ecosystem health by identifying biological impairment with respect to a reference (or natural) condition (Tetra Tech 2000). The SCI is calibrated for a long-term biological index period extending from April through October, and consists of the following core metrics: EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa; Total taxa; percent EPT; % Chironomidae; % top 2 dominant taxa; and the Hilsenhoff Biotic Index (determined at the family level). These core metrics provide better discriminatory ability for measures of taxonomic richness and composition, as well as tolerance and intolerance (Tetra Tech 2000).

Our analysis utilized the same data to develop the SCI (WV bioassessment program data collected 1996-1997), as well as additional program data collected prior to 2001. Previous analysis of benthic macroinvertebrates in cobble substrates of Appalachian Mountain rivers and streams indicated that classification by physiographic regions was not required, and so no attempt was made to separate SCI data by region.

SCI values form the basis for the evaluation of biological impairment in West Virginia. A rating of "highly comparable to reference sites" (SCI score of >78 to 100) is applied to sites that score greater than the 25th percentile of reference sites. A "comparable to below-average reference sites" score (>68 to 78) applies to sites scoring greater than the 5th percentile of reference sites (still meeting West Virginia's water quality standards). Any SCI score of 68 or less signifies a biologically stressed condition (impaired sites, not in attainment with West Virginia's water quality standards). By plotting the SCI scores along the gradient of total iron concentration for all sites as before, and using the SCI score of 78 as the protective threshold, the analysis projects an FEC for total iron of 1.25 mg/L (Figure 2-11-thick, gray, dashed line). If an SCI score of 68 is used as the threshold, the FEC is estimated to be 5.28 mg/L (Figure 2-11-black dashed lined). This analysis indicates that the preliminary benchmark of 1.74 mg/L for the CWA 101(a) objective is potentially more conservative than the concentration which normally causes regulatory action in the State of West Virginia, although it does coincide with the concentration normally required to maintain a high level of biological condition, i.e., a rating that is "highly comparable to reference sites."

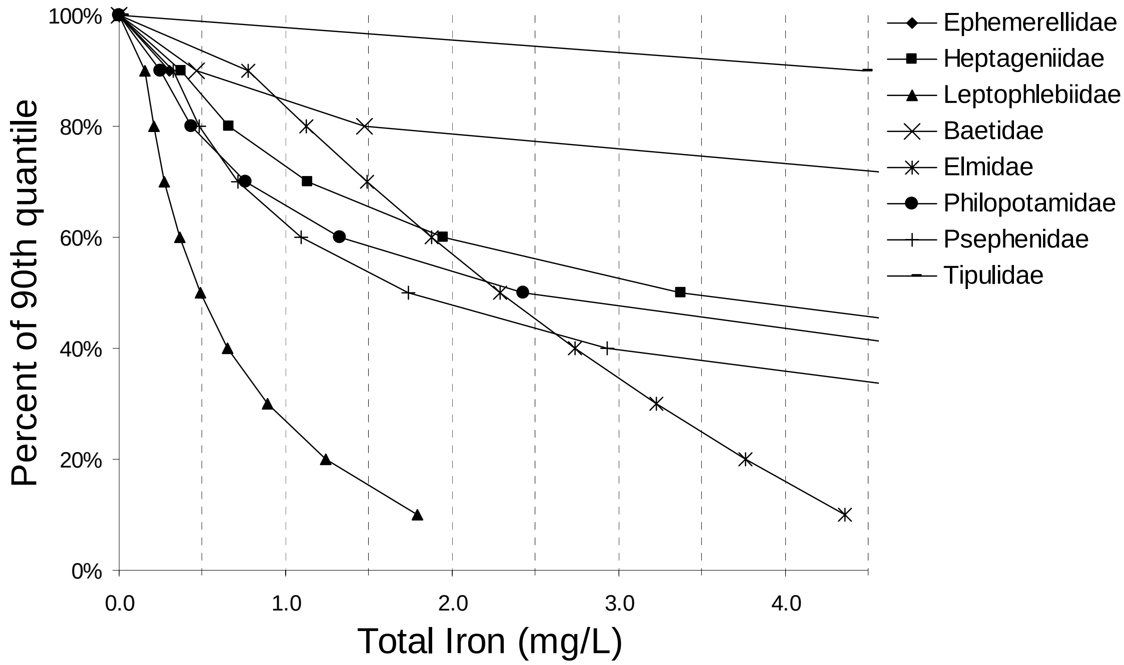

Figure 2-10
Estimated decline in maximum insect numbers in samples from sites in West Virginia with increasing total iron concentration. Relations is based on the 90th regression quantile line (non-linear) fit to the data using a sigmoid function.

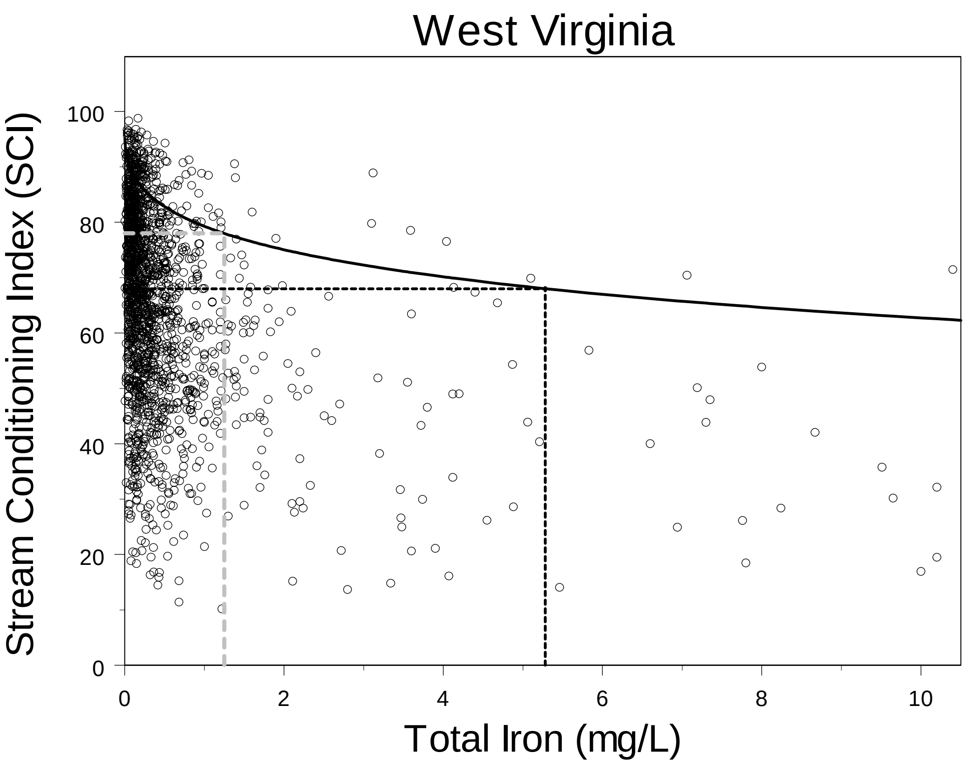

Figure 2-11
Scatter plot of West Virginia stream conditioning index (SCI) and respective iron concentrations. Solid line represents the best model (a sigmoid function) for estimating the 90th quantile of SCI as function of iron concentration. The thick, gray dashed line projects the field-based effect concentration (FEC = 1.25 mg/L) for SCI = 78. The black dashed line projects the FEC (5.28 mg/L) for SCI = 68. These values are adopted by the State of West Virginia as thresholds for identification of reference and impaired sites, respectively.

Implications of Tiered Iron Criteria Within a National Regulatory Framework

With iron, a possibility exists for the first time to establish meaningful protective values to meet the CWA goal and objective. The proposed values not only take into account the direct toxic effects of iron, but also its indirect (physical) effects. The cornerstone of the approach is the use of regression quantiles to estimate the limitation imposed by iron on the biological response variable (i.e., maximum numbers of organisms in samples). The advantage of this approach is that it greatly minimizes impacts of confounding factors (both chemical and non-chemical), which have precluded the use of field data in deriving aquatic life criteria. By using regression quantiles, co-contaminants and other non-related confounding variables can be ignored in the statistical analysis.

Key Issues of Approach

A key issue in the approach is the definition of biologically significant thresholds. The investigators have selected the 20 and 50 percent reduction in maximum numbers of organisms in samples from reference sites (i.e., 0.80 times the 90th percentile) because these thresholds seem consistent with their respective objectives: maintain and restore the biological integrity of waters in the United States of America, and insure propagation of aquatic species. Analysis of spatial and temporal variation in maximum numbers of organisms may eventually lead to more rigorous thresholds, but the use of 20 and 50 % reduction in the 90th quantile of organism numbers in samples from reference sites seems reasonably well justified at this time.

Additional data and analysis are required to separate the potential negative toxicological and physical effects that could be associated with iron in combination with either aluminum or total suspended solids (Table 2-2). At present, the possibility that limitations on maximum organism numbers could be due to the effects of aluminum precipitate can not be ruled out; total suspended solids effect levels are usually at higher concentrations (EIFAC 1964). In terms of conventional toxicity, laboratory toxicity tests have shown aluminum to be more toxic than iron, and recent examination of the acute toxicity of neutral waters located downstream of an acid mine discharge supports this supposition (Soucek et al. 2001). Interestingly; however, the study also shows that the Al-Fe combination is more toxic than either aluminum or iron alone. At the very least, any values recommended using the current approach would be protective of total iron, if not slightly over-protective.

Implementation

There is no guidance currently available for implementing tiered aquatic life criteria. Publishing two proposed protective values for iron as national criteria (or guidelines) may require states and authorized tribes without tiered aquatic life uses to select only one value, that which protects at the level of biological integrity, or that which ensures protection and propagation of aquatic life. It is the state's responsibility to develop tiered aquatic life uses and define reference conditions within that state as part of the development of a program for statewide bioassessment. Only a few states currently have tiered aquatic life use designations in place (e.g.,Vermont, Ohio, and Maine). Many other states are progressing rapidly in establishing the necessary bioassessment information needed to develop tiered aquatic life uses for their water quality standards program. EPA's long-term goal is for all states to have refined tiered aquatic life uses and biological criteria in their water quality standards.

Concluding Remarks

This and the companion report (GLEC 2003) present a new approach to derive criteria "benchmarks" to protect aquatic organisms that promotes protection of aquatic life using endpoints that adequately represent conservation targets. As such, the investigators believe that they have developed a fundamentally sound and statistically rigorous approach for developing updated freshwater criteria (guidelines) for total iron based on relevant field bioassessment data. The method employed to obtain the proposed protective for total iron is not only novel, but represents a substantial improvement over the method used to derive the existing criterion, which is weakly supported by the scientific literature.

3
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