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Derivation of Conversion Factors for the Calculation  
of Dissolved Freshwater Aquatic Life Criteria for Metals

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## ABSTRACT

This document presents information concerning the percent of the total recoverable metal that was dissolved in toxicity tests with freshwater organisms. Under contract with the U.S. EPA, the University of Wisconsin at Superior simulated conditions that existed in important aquatic toxicity tests with metals. The final report by UWS presents a detailed description of the methodology used, the raw data, and some summary statistics. The data from these simulation tests are analyzed and interpreted herein. For the metals for which simulation tests were conducted, the recommended conversion factors range from 0.316 for the CMC for chromium(III) to 1.000 for the CMC and the CCC for arsenic(III). The results of these simulation tests should provide a basis for making reasonable decisions concerning conversion factors for deriving dissolved criteria from total recoverable criteria for aquatic life in fresh water.

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## 1. INTRODUCTION

On October 1, 1993, it became the policy of the Office of Water of the U.S. EPA that "the use of dissolved metal to set and measure compliance with water quality standards is the recommended approach, because dissolved metal more closely approximates the bioavailable fraction of metal in the water column than does total recoverable metal" (Prothro 1993). Previously, aquatic life criteria for metals had been expressed only in terms of the total recoverable measurement or the acid-soluble measurement. These measurements had been used because the results of most aquatic toxicity tests on metals were expressed either in terms of nominal concentrations (i.e., intended concentrations) or in terms of concentrations that had been measured using the total recoverable method or other methods that are expected to give equivalent concentrations in toxicity tests. (When the concentrations were not measured, the initial total recoverable concentration was assumed to equal the nominal concentration.) This expected equivalency between a variety of methods when used to measure concentrations in many toxicity tests is the rationale for the decision that a factor of 1.0 should be used to convert aquatic life criteria for metals that are expressed on the basis of the acid-soluble measurement to criteria expressed on the basis of the total recoverable measurement (Attachment #2 of Prothro 1993).

The new policy recommending use of dissolved metal created the problem of deciding how aquatic life criteria should be derived for dissolved metal. (The term "metal" is used herein to include both "metals" and "metalloids".) The concentration of dissolved metal has been measured in very few toxicity tests with aquatic organisms, and so dissolved criteria for aquatic life cannot be derived directly from the results of toxicity tests on metals. One alternative was to assume that all of the metal was dissolved in the toxicity tests that were most important in the derivation of the aquatic life criteria. Although this is likely to be true for some metals, application of this assumption to some other metals would result in criteria that would be underprotective by an unknown amount. In addition, this approach probably would discourage generation of data concerning the percent of metal in toxicity tests that is dissolved and discourage measurement of dissolved metal in future toxicity tests.

Another alternative was to repeat all of the toxicity tests that were most important in the derivation of the criteria and measure the metal using both the total recoverable and dissolved methods (and possibly other methods). This approach would allow derivation of dissolved criteria and would increase confidence in the resulting criteria. Substantial resources would be required, however, to conduct the tests, especially to obtain the necessary test organisms. Also, this approach might make it difficult to

use the Recalculation Procedure based on species that "occur at the site" if dissolved data are not available for the most sensitive species in the site-specific dataset.

As a third alternative, EPA decided to use the following equation to convert total recoverable criteria to dissolved criteria:

$$\text{Dissolved criterion} = (\text{CF})(\text{Total recoverable criterion})$$

where CF = Conversion Factor. The CF would correspond to the percent of the total recoverable metal that is dissolved. A literature search was conducted and the data that were found and considered possibly useful were presented in Attachment #2 of Prothro (1993). It was concluded that these data were not sufficient to allow derivation of the CFs and that pertinent high quality data should be generated for the metals of concern. One option was to determine the percent dissolved at the concentrations that are specified in the criteria; a second option was to determine the percent dissolved at the concentrations that occurred in the toxicity tests that were most important in the derivation of the criteria.

Because the criteria are derived from the results of toxicity tests and the percent dissolved for a metal might depend on such factors as the type of the test, it was decided that "simulation tests" should be conducted using solutions simulating those used in the toxicity tests that were most important in the derivation of aquatic life criteria for each metal. The intent was to mimic the way criteria would have been derived if dissolved metal had been measured in each of the toxicity tests.

The CF would be the fraction corresponding to the percent of the total recoverable metal that was dissolved in the toxicity tests that were most important in the derivation of the criterion for the metal. It would probably be appropriate to apply these CFs not only to national criteria but also to recalculated criteria if the recalculated criteria were not too different from the national criteria to which the factors were derived to apply. It was possible, of course, that the simulation tests might show that it is not appropriate to use the same CF for both acute and chronic tests or even for all acute tests. Similarly, separate CFs might be necessary for fresh and salt water for some or all metals; only freshwater simulations are discussed herein.

A work assignment was written to have the University of Wisconsin at Superior conduct freshwater "simulation tests" that were designed by the U.S. EPA. The purpose of this report is to describe the design of the project and present the analysis and interpretation of the data resulting from these simulation tests. The results presented herein are based on the report titled "Results of Freshwater Simulation Tests concerning Dissolved Metal" (Brooke 1995).



## 2. DESIGN OF THE PROJECT

The metals that were selected to be the subjects of freshwater simulation tests were arsenic(III), cadmium, chromium(III), chromium(VI), copper, lead, mercury(II), nickel, selenium(IV), silver, and zinc, but they were not tested in alphabetical order. Except for minor refinements, the initial methodology was used with copper, zinc, chromium(III), lead, arsenic(III), and chromium(VI). Some major refinements affected the planned simulations for selenium(IV), nickel, cadmium, mercury, and silver. Due to resource limitations, no simulations were conducted with either mercury or silver. The metals are addressed in the appendices in the order in which they were tested to facilitate the description of the impact of the refinements on the individual metals.

### Selection of Simulations to be Performed

The following decisions were made concerning the work to be done:

- A. The conditions most likely to substantially affect the percent of the total recoverable metal that is dissolved in toxicity tests are:
  - 1. the test species;
  - 2. the presence or absence of food, and if present, the kind and amount of food;
  - 3. the technique used (static, renewal, or flow-through);
  - 4. the duration of the test;
  - 5. the hardness, pH, alkalinity, and temperature of the dilution water;
  - 6. the tested concentrations of the metal; and
  - 7. the acidity of the stock solution used.
- B. The two processes that are most likely to cause the percent dissolved to be less than 100 percent are precipitation and sorption.
  - 1. Both the probability and the amount of precipitation increase as the concentration of metal increases; thus precipitation might cause the percent dissolved to be lower in acute tests than in chronic tests with the same test organisms.
  - 2. The amount of sorption increases as the concentration of total suspended solids increases; also the importance of sorption increases as the concentration of metal decreases. Thus sorption might cause the percent dissolved to be lower in chronic tests than in acute tests.
- C. Both precipitation and sorption might take several hours to reach equilibrium for some metals.
- D. It is not necessary to simulate all pertinent conditions; it is only necessary to determine whether the percent dissolved is sufficiently similar for a metal under sufficient relevant test conditions that it is reasonable to derive a freshwater

- dissolved Criterion Maximum Concentration (CMC) and/or Criterion Continuous Concentration (CCC) for a metal by multiplying the freshwater total recoverable CMC and/or CCC by conversion factors that are appropriate for the metal.
- E. The purpose of simulation tests should be to determine whether food, organisms, hardness, duration, and concentration of the metal make a substantial difference in the percent of the metal that is dissolved.
  - F. Simulations should be conducted at hardnesses of 50 and 200 mg/L for each metal whose criterion is hardness-dependent; alkalinity and pH should be consistent with hardness.
  - G. Only 48- and 96-hr static simulations should be performed initially; other simulations should be performed later if necessary.
  - H. Both dissolved and total recoverable metal should be measured at 1 and 48 hours in 48-hour static simulations and at 1, 48, and 96 hours in 96-hour static simulations.
  - I. Selection of the kinds of simulations and the concentrations with which simulations should be performed with a metal should be based on the toxicity tests that were most important in the derivation of the criterion for that metal. In freshwater criteria documents, all chronic tests are fed tests, and virtually all acute tests are unfed tests. Thus in fresh water, the range of concentrations of metal in unfed simulations should usually cover both the lowest four GMAVs and the acute values used in the derivation of freshwater acute-chronic ratios (ACRs) that were used in the derivation of the criterion. Similarly, the range of concentrations of metal in fed simulations should cover the chronic values used in the derivation of the Final Chronic Value; these will usually be the chronic values used in the derivation of the Final Acute-Chronic Ratio (FACR).
  - J. At least two concentrations should be used for each type of simulation performed with each metal to determine whether the percent dissolved depends on the concentration of the metal. Simulations should be performed with three concentrations if the range is greater than a factor of ten.
  - K. The same concentrations should not necessarily be used for different simulations. The goal should be to determine whether the same percent dissolved can be used across a variety of conditions, not to determine the impact of specific conditions on the percent dissolved. If two test concentrations are similar, however, it is desirable to have them be the same.
  - L. Extrapolation of the results can be based on the following assumptions:
    - 1. The percent dissolved at one hour in an unfed static simulation is a useful approximation of the percent dissolved in a comparable unfed flow-through simulation for the same metal; the same is assumed for fed simulations.
    - 2. The percent dissolved in a flow-through chronic test does not change substantially from two days to, for example,

thirty days if the flow rate is acceptably high, the tanks are cleaned regularly, etc.

3. The percent of a metal that is dissolved is similar in comparable static, renewal, and flow-through toxicity tests if duration does not make a substantial difference in a static simulation.
  4. The percent of a metal that is dissolved is similar in unfed tests with all species if the percent dissolved is sufficiently similar in unfed simulations with Daphnia magna and fathead minnows; this is even more likely to be true if the percent dissolved is also similar in a fed simulation test.
  5. The percent of a metal that is dissolved is similar in a daphnid chronic test and in chronic tests in which fish are fed trout chow, if the daphnid food contains trout chow.
- Although these five assumptions seem reasonable, it would have been desirable to have had time and resources to test them.

An examination of the tests that were most important in the derivation of the freshwater criterion for each individual metal resulted in the following:

#### Arsenic(III)

1. The FACR was based on three freshwater ACRs and a saltwater ACR for mysids.
2. Freshwater simulations should cover the following:  
Unfed: 874 to 14,400 ug/L.  
Fed: 914 to 3,026 ug/L.
3. The freshwater criterion is not hardness-dependent.

#### Cadmium

1. A freshwater FACR was not used; the freshwater CCC was based on the four lowest chronic values.
2. Freshwater simulations should cover the following:  
Unfed: 2.4 to 30.0 ug/L.  
Fed: 0.13 to 3.9 ug/L.
3. The freshwater criterion is hardness-dependent.

#### Chromium(III)

1. A freshwater FACR was not used. The freshwater CCC was based on a chronic test with rainbow trout by Stevens and Chapman (1984), for which they reported that 83 to 89 percent of the metal was dissolved at the CCC.
2. Freshwater simulations should cover the following:  
Unfed: 2221 to 8684 ug/L.
3. The freshwater criterion is hardness-dependent.

#### Chromium(VI)

1. The freshwater FACR was based on four chronic tests with daphnids.
2. Freshwater simulations should cover the following:  
Unfed: 29 to 67 ug/L.  
Fed: 6 to 40 ug/L.
3. The freshwater criterion is not hardness-dependent.

#### Copper

1. The FACR was based on four freshwater chronic tests, three of which were daphnid tests for which Chapman (1993a) reported values for percent dissolved.
2. Freshwater simulations should cover the following:  
Unfed: 17 to 69 ug/L.  
Fed: 6 to 29 ug/L.
3. The freshwater criterion is hardness-dependent.

#### Lead

1. The FACR was based on five freshwater ACRs and a saltwater ACR for mysids; three of the five freshwater chronic tests were daphnid tests for which Chapman (1993a) reported values for percent dissolved.
2. Freshwater simulations should cover the following:  
Unfed: 143 to 4100 ug/L.  
Fed: 12 to 128 ug/L.
3. The freshwater criterion is hardness-dependent.

#### Mercury

1. The national freshwater CCC is based on bioaccumulation and an FDA action level. A conversion factor for the CCC is probably inappropriate because both particulate and dissolved mercury might be methylated.
2. The GLI CCC is based on the chronic toxicity of mercury to aquatic organisms.
3. Freshwater simulations should cover the following:  
Unfed: 2.6 to 20 ug/L.
4. The freshwater criterion is not hardness-dependent.

#### Nickel

1. The FACR was based on five freshwater ACRs and a saltwater ACR for mysids; three of the five freshwater chronic tests were daphnid tests for which Chapman (1993a) reported values for percent dissolved.
2. Freshwater simulations should cover the following:  
Unfed: 1500 to 28,000 ug/L.  
Fed: 15 to 526 ug/L.
3. The freshwater criterion is hardness-dependent.

### Selenium

1. The freshwater criterion was based on data concerning Belews Lake and on a FACR of 8.314. A conversion factor for the freshwater CCC should be based on the percent of total recoverable selenium that was dissolved in Belews Lake.
2. The FACR was based on a variety of freshwater and saltwater chronics. The major purpose of the freshwater simulations should be to determine whether the dissolved FACR should be the same as the total recoverable FACR. It would also be desirable for the simulations to cover the four lowest GMAVs.
3. Freshwater simulations should cover the following:  
Unfed: 776 to 47,000 ug/L.  
Fed: 92 to 2,891 ug/L.
4. The freshwater criterion is not hardness-dependent.

### Silver

1. Freshwater simulations should cover the following:  
Unfed: 0.39 to 30 ug/L (hardness = 30 to 100 mg/L)  
Unfed: 4.8 to 270 ug/L (hardness = 150 to 280 mg/L)

### Zinc

1. The freshwater FACR was based on five freshwater ACRs and a saltwater ACR for mysids. Three of the five freshwater chronic tests were daphnid tests for which Chapman (1993a) reported values for percent dissolved.
2. Freshwater simulations should cover the following:  
Unfed: 94 to 701 ug/L.  
Fed: 47 to 371 ug/L.
3. The freshwater criterion is hardness-dependent.

It was decided that the following five types of simulation tests would be used to obtain data concerning the percent dissolved metal in freshwater toxicity tests:

1. A 96-hr static unfed exposure of fathead minnows in Lake Superior water, with samples taken at 1, 48, and 96 hours for metal analyses.
2. A 48-hr static fed exposure of daphnids in Lake Superior water, with samples taken at 1 and 48 hours for metal analyses. The daphnids would be fed a yeast-trout chow-cereal leaf (YTC) mixture at a concentration that is typically used in life-cycle tests with daphnids.
3. A 48-hr static fed exposure of fathead minnows in Lake Superior water, with samples taken at 1 and 48 hours for metal analyses. The fish would be fed live newly hatched brine shrimp nauplii at a concentration that is typically used in early life-stage tests. (This simulation would to be

performed with two representative metals to which fish are sensitive.)

4. A 48-hr static fed exposure of daphnids in Lake Superior water to which sufficient calcium sulfate had been added to raise the hardness to  $200 \pm 10$  mg/L, with samples taken at 1 and 48 hours for metal analyses.
5. A 96-hr static unfed exposure of fathead minnows in Lake Superior water to which sufficient calcium sulfate had been added to raise the hardness to  $200 \pm 10$  mg/L, with samples taken at 1, 48, and 96 hours for metal analyses.

When the simulation tests were conducted, the solutions containing fathead minnows (i.e., simulation types 3 and 5) were aerated, but the others were not.

For each metal whose criterion was hardness-dependent, either the fourth or fifth type of simulation would be used to determine whether hardness (and the related alkalinity and/or pH) caused a measurable difference in the percent dissolved. The fourth type of simulation would be performed if daphnids were more sensitive to the metal, whereas the fifth type of simulation would be performed if fish were more sensitive to the metal. (Except for simulations with copper, whenever calcium sulfate was added to increase hardness to  $200 \pm 10$  mg/L, sufficient sodium bicarbonate was also added to increase alkalinity to  $130 \pm 10$  mg/L.)

Each type of simulation would be performed only with the metals for which it was relevant; the simulations deemed necessary were:

<u>Metal</u>	<u>Type of Simulation</u>	<u>Concentration (ug/L)</u>
Arsenic(III)	1	1,000; 10,000
	2	1,000; 3,000
Cadmium	1	3; 30
	2	0.3; 3
	3	0.3; 3
	4	0.3; 3
Chromium(III)	1	2,000; 9,000
	5	2,000; 9,000
Chromium(VI)	1	30; 70
	2	5; 30
Copper	1	20; 70
	2	5; 20
	3	5; 20
	4	5; 20

Lead	1	130; 1,000; 4,100
	2	10; 130
	4	10; 130
Mercury	1	2.5; 20
Nickel	1	2,000; 20,000
	2	15; 100; 500
	4	15; 100; 500
Selenium(IV)	1	700; 7,000; 47,000
	2	90; 700; 2,900
Silver	1	0.3; 3; 30
	5	3; 30; 300
Zinc	1	90; 700
	2	40; 400
	4	40; 400

The above plans were based on the national aquatic life criteria, but the GLI CCC for mercury is quite different from the national CCC for mercury. Whereas the national CCC is based on bioaccumulation and an FDA action level, the GLI CCC is based on chronic toxicity to aquatic life. In addition, it is stated on page 5 of Attachment #2 of Prothro (1993) that it is not appropriate to adjust the CCC for mercury or the CMC and CCC for selenium because these are bioaccumulative chemicals. Regardless of whether these metals bioaccumulate, the important consideration is the exposure that relates to the effect on which the CMC or CCC is based. The CMC is based on acute toxicity and so a relevant consideration is the bioavailability of the metal in the water column; therefore, a total recoverable CMC may be converted to a dissolved CMC if an appropriate CF is used and if there are no overriding risk management considerations. For a CCC, the exposure can be from the water column and from the food, with the food chain consisting of some organisms whose primary exposure is to pollutants in the water column and other organisms whose primary exposure is to pollutants in the sediment. It appears that exposure to the sediment contributes substantially to the concentration of mercury in the food chain, but does not contribute substantially to the concentration of selenium in the food chain. Therefore, it is as acceptable to convert the total recoverable CCC for selenium to a dissolved CCC as it is to convert, for example, the total recoverable CCC for copper to a dissolved CCC. In contrast, it is not acceptable to convert a total recoverable CCC for mercury to a dissolved CCC if the CCC for mercury is based on mercury residues in aquatic organisms. It can, however, be acceptable to convert a CCC for mercury to a dissolved CCC if the CCC is based on the toxicity of mercury to aquatic organisms because the important exposure is through the water column; as before, a dissolved criterion may be derived if

an appropriate CF is used and if there are no overriding risk management considerations.

### Design of the Simulations

For each type of simulation to be conducted with a metal, simulations were conducted in three test chambers at each of the desired concentrations; i.e., there were three experimental units for each treatment. Except for selenium(IV), nickel, and cadmium, the test chambers were conditioned before the simulations began. Each test chamber contained the organisms specified at a density recommended in ASTM Standard E729 concerning acute toxicity tests. If oversized chambers were used, they contained more than ten organisms per test chamber. When placed in the test solutions, the fathead minnows were between 20 and 35 days old, whereas the daphnids were between 1 and 48 hours old. When fed simulations were conducted, the organisms were fed as described above. Test chambers containing fathead minnows were maintained in a temperature-controlled water bath, whereas those containing daphnids were maintained in a temperature-controlled chamber.

The solutions used in the simulations and in the checks of the analytical methods (see below) were made using stock solutions that were prepared by dissolving one of the following salts in metal-free deionized and/or distilled water:

Arsenic(III):	sodium arsenite
Cadmium:	cadmium chloride or sulfate
Chromium(III):	chromic chloride or nitrate or chromium potassium sulfate
Chromium(VI):	potassium chromate or dichromate or sodium chromate or dichromate
Copper:	cupric chloride, nitrate, or sulfate
Lead:	lead chloride or nitrate
Mercury:	mercuric chloride or sulfate
Nickel:	nickelous chloride, nitrate, or sulfate
Selenium(IV):	sodium selenite
Silver:	silver nitrate
Zinc:	zinc chloride, nitrate, or sulfate

If the pH of Lake Superior water (LSW) was changed by more than 0.1 pH unit when it was spiked at the highest concentration specified for a simulation with that metal, the pH of the stock solution was adjusted using the minimum necessary amount of either sodium hydroxide or sulfuric acid. The exact concentration of metal used in a simulation was not considered critical, but each concentration was to be within  $\pm 30$  percent of that specified.

For some metals, a plastic screen was used to exclude daphnids, brine shrimp nauplii, and brine shrimp cases from samples taken



from simulation solutions. For two simulations containing copper, this was accomplished by pouring the solution through the plastic screen, with the initial solution being used to condition the screen. For some other simulations containing copper and for several subsequent metals, the samples were sucked through a sampling apparatus that contained the plastic screen. When this sampling apparatus was used for metals other than copper, it was used to obtain samples from all solutions used in pre-simulation tests and all simulation solutions. For selenium(IV), nickel, and cadmium, neither the plastic screen nor the sampling apparatus was used; daphnids were kept out of the samples by careful sampling, but some samples might have contained brine shrimp nauplii and/or cases.

Immediately before any samples were taken from a test chamber, the solution in the chamber was stirred to ensure that settling of particles did not result in a gradient in the concentration of the metal at the time of sampling. For most metals, the sampling procedure consisted of stirring the simulation solution, sucking the solution through the sampling apparatus to condition the apparatus and the plastic screen, taking a sample for analysis using the acidification method, taking a sample to be used to condition the membrane filter and the filter holder, and taking a sample for analysis using the dissolved method. For selenium(IV), nickel, and cadmium, however, the simulation solution was stirred before each individual sample was taken, i.e., the solution was stirred before a sample was taken for analysis using the acidification method and it was stirred again before a sample was taken for analysis using the dissolved method. (As stated above, samples for these three metals were not put through the plastic screen.) Each time a set of three test chambers was sampled, two were sampled once for each analytical method and the third was sampled twice for each analytical method. When the duplicate sampling was performed, the whole sampling procedure was repeated beginning with the stirring of the simulation solution and using a sampling apparatus that was freshly acid-washed. Duplicate filtrations of the same solution were performed using different filters just as if two different solutions were being filtered.

In addition to the specified metal measurements, the following were also completed:

- a. Hardness, alkalinity, and total suspended solids were measured once during the simulation in one test chamber in each set of three.
- b. Temperature and pH were measured at 1 hour and at the end of the simulation in each test chamber.
- c. A sample of at least 50 mL for measurement of total organic carbon (TOC) was collected from one test chamber in each set of three, acidified within one hour to pH  $\leq$  2 with HCl or H<sub>2</sub>SO<sub>4</sub>, and delivered to the U.S. EPA.

During simulations with some of the metals, the concentrations measured by the acidification and dissolved methods decreased substantially from 1 to 48 and/or 96 hours. In an attempt to determine whether this might have been due to sorption onto the test chamber, after the last sample was taken from simulations for selenium(IV), nickel, and cadmium, the solution remaining in the test chamber was acidified, mixed, and sampled after 15 to 20 minutes for analysis for the metal. When solutions were to be acidified at the end of the simulations, the test chambers were not conditioned before the beginning of the simulations.

### Analytical methodology

To help ensure adequate accuracy and precision, both (a) the detection limit for each metal and (b) the concentrations of metals in blanks were required to be less than 10 % of the lowest concentration with which a simulation was to be performed with the metal; 33 % was used for silver because of the existing analytical capabilities of the contractor. Clean techniques, solvent extraction, ion exchange columns, etc., would have been used if necessary to satisfy these requirements. The detection limit for each metal was determined using the procedure described in the Federal Register (1984). (Because the metal was concentrated by a factor of two during the total recoverable sample preparation procedure, the detection limit of the total recoverable method was a factor of two lower than those of the acidification and dissolved methods. For convenience, the detection limit reported herein is that of the acidification and dissolved methods.)

The procedures described by U.S. EPA (1991) were used for preparing and analyzing samples for the total recoverable and dissolved methods, except that any necessary procedures that were not described in U.S. EPA (1991) were obtained from U.S. EPA (1983). Although the purpose of this project concerned the total recoverable and dissolved methods, it was considered desirable to also use a method that consisted only of acidification of the sample (with no digestion and no filtration) for three reasons:

1. Samples obtained during many of the toxicity tests used in criteria documents for metals were analyzed using various versions of the "acidification method".
2. For routine analyses, the acidification method is easier to perform than the total recoverable method.
3. It is better to check the dissolved method by comparing it with the acidification method than by comparing it with the total recoverable method because the acidification method is less subject to contamination and to loss of metal.

The "acidification method" was defined to consist of (a) acidification of the sample the same as the filtrate is acidified in the dissolved method, followed by (b) mixing the acidified

sample and allowing it to equilibrate for at least 4 hours before analysis. No filtration was performed before or after acidification.

Sample preparation was the only difference between the three analytical methods. Whenever data were to be compared for different sample preparation methods, quantification for all of the methods was performed using the same analytical technique. To account for possible matrix effects on standard curves for copper, three sets of standards were prepared: the first contained 1 % nitric acid and was used with acidified LSW; the second contained 2 % nitric acid and 1 % hydrochloric acid and was used with solutions resulting from the total recoverable digestion; the third contained 0.05 N sodium bicarbonate and 1 % nitric acid and was used with acidified bicarbonate solutions.

Filtrations for determining dissolved metal were performed with polycarbonate filters that were (a) effective to 0.40  $\mu\text{m}$ , (b) 47 mm in diameter, and (c) held in an all-plastic filtration apparatus. The polycarbonate membrane filters had individual holes of relatively uniform diameter of 0.40  $\mu\text{m}$  ( $\sim \pm 20\%$ ) and retained particles only on the surface (Horowitz et al. 1992). When the concentration of dissolved metal in a solution was measured more than once, each filtration was performed using a different 0.40- $\mu\text{m}$  polycarbonate membrane filter and the filter apparatus was cleaned and conditioned as it would have been between two different solutions.

To minimize contamination and loss, the filter apparatus and membrane filter were cleaned by rinsing with and/or dipping in metal-free acidified (to  $\text{pH} \leq 2$ ) distilled and/or deionized water and then rinsing with metal-free distilled and/or deionized water; they were then conditioned by filtering and discarding about 100 mL of the solution to be filtered. The concentration of the metal of concern in the acidified solution used for the acid washing was less than 10 % (33 % for silver) of the lowest concentration with which a simulation was to be performed with that metal; the concentration of metal in the acidified solution was measured using either the acidification method or the total recoverable method.

Regarding silver, U.S. EPA (1991) says "Store in amber container" on page 135; this was interpreted to mean that "all standards and samples for silver must be in amber containers, except that samples may be in clear containers if they are analyzed within eight hours and are not in bright light during that time".

Before any simulations were conducted with a metal, the following comparison of the acidification and total recoverable methods was performed to determine whether these two methods would give comparable results if used to analyze simulation solutions:

- A. Sufficiently large volumes of the following solutions were prepared as necessary for each metal:
11. Metal-free distilled or deionized water. "Metal-free water" was defined as "water that contains less than 10 % (33 % for silver) of the lowest concentration of the metal with which a simulation is to be performed with that metal, as measured by either the acidification method or the total recoverable method".
  12. Lake Superior water (with no added food) spiked with the lowest concentration ( $\pm 30$  %) of the metal with which a simulation was to be performed with that metal.
  13. Lake Superior water with food added the same as it would be added in fed simulations with daphnids (simulation type 2 above, but without daphnids). The food used was a yeast-trout chow-cereal leaf (YTC) mixture and was added at a rate of 6.5 mL per 800 mL; the change in volume was not taken into account in the calculation of the results.
  14. Lake Superior water with daphnid food added the same as for solution 13 above, and spiked with the metal the same as for solution 12 above.
  15. Lake Superior water with food added the same as it would be added in fed simulations with fathead minnows (simulation type 3 above, but without fathead minnows).
  16. Lake Superior water with fathead minnow food added the same as for solution 15 above, and spiked with the metal the same as for solution 12 above.
  17. Lake Superior water with no added food and no added metal. (Solution 17 was used only with selenium(IV), nickel, and cadmium.)

When such solutions were used, the same amount of metal was added to solutions 12, 14, 16; the same amount of daphnid food was added to solutions 13 and 14; and the same amount of fathead minnow food was added to solutions 15 and 16. The Lake Superior water used to prepare solutions 12, 13, 14, 15, 16, and/or 17 (as needed for a particular metal) was from the same batch so that the background concentration of the metal would be the same for each solution. The Lake Superior water was obtained from a pipe that extended about 1000 feet into Lake Superior at Superior, Wisconsin. It was expected that the concentrations in unspiked solutions (i.e., solutions 11, 13, 15, and 17) would be below the detection limit for most metals.

- B. Solutions 11 and 12 were used for all metals. Solutions 13 and 14 were used only for metals for which fed simulations were to be performed with daphnids (simulation types 2 and 4 above). Solutions 15 and 16 were used only for metals for which fed simulations were to be performed with fathead minnows (simulation type 3 above). Solution 17 was used with selenium(IV), nickel, cadmium, mercury; it would have been used with silver if the comparison had been conducted with silver. Thus for each metal the solutions used in the

comparison of the acidification and total recoverable methods were:

Arsenic(III)	11,12,13,14
Cadmium	11,12,13,14,15,16,17
Chromium(III)	11,12
Chromium(VI)	11,12,13,14
Copper	11,12,13,14,15,16
Lead	11,12,13,14
Mercury	11,12,17
Nickel	11,12,13,14,17
Selenium(IV)	11,12,13,14,17
Silver	11,12,17
Zinc	11,12,13,14

This comparison was performed with mercury, but not silver.

- C. Each solution was mixed and then equilibrated at least overnight.
- D. The following twelve analyses were performed on the first solution, and then on the second solution, and then on the third solution, etc., until the twelve analyses had been performed on each solution prepared for the metal:

A sample was prepared for analysis using the total recoverable method and then a sample was prepared for analysis using the acidification method. This sequence was repeated six times so that six samples were prepared alternately for analysis by the total recoverable method and six samples for the acidification method.

For some metals, each solution was stirred just before each total recoverable sample was taken, but was not stirred just before a sample was taken for the acidification method. For the last few metals (i.e., selenium, nickel, and cadmium), however, the solution was stirred just before each total recoverable sample was taken and was stirred again just before each acidification sample was taken.

Until use of solution 17 was begun, the concentration of the metal was measured in one or more separate batches of Lake Superior water for all metals except chromium(VI). The value reported for chromium(III) would have included any chromium(VI) that was in the water.

Also before any simulations were conducted with a metal, the following evaluation of the dissolved method was performed to determine whether the dissolved methodology worked with that metal:

- A. Sufficiently large volumes of the following four solutions were prepared for each metal:
21. Metal-free distilled or deionized water.
  22. A 0.05 N sodium bicarbonate solution (prepared by dissolving metal-free sodium bicarbonate in the same metal-free water used as solution 21 above). "Metal-free sodium bicarbonate" was defined as sodium bicarbonate that contains a sufficiently low amount of the metal that at least 90 percent of the analyses of a 0.05 N sodium

bicarbonate solution using the acidification method will be less than 10 % (33 % for silver) of the lowest concentration with which a simulation was to be performed with that metal. (For cadmium, a 0.001 N sodium bicarbonate solution with added calcium sulfate was also used.)

23. A 0.05 N sodium bicarbonate solution (prepared the same way as solution 22 above) and spiked with the lowest concentration ( $\pm 30$  %) of the metal with which a simulation was to be performed with that metal. (For cadmium, a 0.001 N sodium bicarbonate solution with added calcium sulfate was also used.)
24. Lake Superior water spiked with the metal the same as for solution 23 above.

Each of these four solutions was used only to compare results obtained using the two analytical methods on each solution individually. These solutions were not used to check recovery, and so it was not necessary that solutions 23 and 24 be spiked identically or that the concentration of metal be measured by each method in unspiked LSW.

- B. Each solution was mixed and then equilibrated at least overnight.
- C. The following twenty analyses were performed on the first solution, and then on the second solution, and then on the third solution, and then on the fourth solution:

A sample was prepared for analysis using the acidification method and then a sample was prepared for analysis using the dissolved method. This sequence was repeated ten times so that ten samples were prepared alternately for analysis by the acidification method and ten samples for the dissolved method.

For some metals, each solution was stirred just before each sample was taken for the acidification method, but was not stirred just before the sample was taken for the dissolved method. For the last few metals (i.e., selenium, nickel, and cadmium), however, the solution was stirred just before each acidification sample was taken and was stirred again just before each dissolved sample was taken.
- D. Simulations were not performed with a metal unless the following requirements were satisfied:
  1. For the metal-free water (solution 21), at least 90 percent of the results obtained using the acidification method and at least 90 percent of the results obtained using the dissolved method were required to be less than 10 % (33 % for silver) of the lowest concentration with which a simulation was to be performed with that metal.
  2. For the unspiked sodium bicarbonate solution (solution 22), at least 90 percent of the results obtained using the acidification method and at least 90 percent of the results obtained using the dissolved method were required to be less than 10 % (33 % for silver) of the lowest

concentration with which a simulation was to be performed with that metal.

3. For the spiked sodium bicarbonate solution (solution 23), the quotient of the dissolved mean divided by the acidification mean was required to be between 0.9 and 1.1. [At the concentrations at which this comparison was to be performed, 100 percent of each metal was expected to be dissolved in metal-free bicarbonate solution (MFBS) and so the mean results obtained by the two methods were expected to agree well.]
4. For the spiked Lake Superior water (solution 24) the quotient of the mean obtained using the dissolved method divided by the mean obtained using the acidification method was required to be less than 1.1. (In LSW, some of the metal might not be dissolved and so the quotient might be less than 0.9, but it should not be greater than 1.1.)
5. For the four sets of results obtained by analyzing the two spiked solutions (solutions 23 and 24) using the two analytical methods, all four coefficients of variation were required to be less than 20 percent.

As a check on the analyses performed for the simulations, the following two solutions were prepared and then analyzed using both the acidification and dissolved methods at the beginning and end of each group of samples, and in the middle of any group containing more than 12 samples:

- a. A 0.05 N sodium bicarbonate solution (prepared by dissolving metal-free sodium bicarbonate in metal-free deionized and/or distilled water, letting the solution equilibrate overnight or longer after dissolution of the sodium bicarbonate, and maintaining it in a closed bottle). For cadmium, a 0.001 N sodium bicarbonate solution with added calcium sulfate was used.
- b. A 0.05 N sodium bicarbonate solution (prepared using sodium bicarbonate and water from the same batches as above, spiked with the metal of interest to the lowest concentration with which a simulation was to be performed with that metal, equilibrated as above, and maintained in a closed bottle). For cadmium, the spike was added to a 0.001 N sodium bicarbonate solution to which calcium sulfate had been added.

In addition, recoveries were performed regularly during the analyses on samples from simulation solutions and/or bicarbonate solutions.

The labware, apparatus, and procedures used for collecting, handling, preparing, storing, and analyzing samples for measurements using the acidification and dissolved methods were the same for all such analyses performed during the comparisons of the methods and for the analyses of the simulation solutions.

Special attention was given to conditioning of apparatus, labware, and sample containers to prevent bias of the results:

1. Acid-cleaned plastic, such as polyethylene or a fluoroplastic, was the only material that ever contacted a sample, except during the digestions used in the total recoverable method.
2. Because acid-cleaned plastic and glass sorb some metals from unacidified solutions, but not from acidified solutions, all sampling apparatus, etc., was conditioned if it contacted a sample only before the sample was acidified, but it was not conditioned if it contacted the sample after the sample was acidified:
  - a. If the sample was acidified in the sample container, the sample container was not conditioned.
  - b. If the sample was not acidified in the sample container, the sample container was conditioned.
3. Because samples for the dissolved method are not acidified until after filtration, all sampling apparatus, sample containers, labware, filter holders, membrane filters, etc., that contacted the sample before or during filtration were conditioned by rinsing with a portion of the sample and discarding the portion.
4. For the total recoverable method:
  - a. The sampling apparatus was conditioned because the sample was not acidified until it was in a sample container.
  - b. Sample containers were not conditioned because the samples were acidified in the sample containers.
5. If the total recoverable and dissolved measurements were performed on the same sample (rather than on two different samples from the same solution), all of the apparatus and labware, including the sample container, was conditioned before the sample was placed in the sample container; then an aliquot was removed for analysis using the total recoverable method (and acidified, digested, etc.) and an aliquot was removed for analysis using the dissolved method (and filtered, acidified, etc.)
  - a. If a different procedure had been used, bias of the results could have occurred when working with metals that sorb from unacidified solutions and then desorb when the sample is acidified. For example, if a container had been conditioned and filled with sample and an aliquot was removed for the dissolved measurement and then the solution in the container was acidified before removal of an aliquot for the total recoverable measurement, the resulting measured total recoverable concentration might have been biased high because the acidification might have desorbed metal that had been sorbed onto the walls of the sample container. The amount of bias would have depended on the relative volumes involved and the amount of sorption and desorption.

For selenium(IV), nickel, and cadmium, some procedures were changed so that conditioning of apparatus, measurement of pH, etc., were performed in such a way that they could not cause changes in pH of solutions or in the concentrations of metals in



solutions. Specifically, if apparatus or labware was conditioned in simulation solution, it was conditioned in a sample of the simulation solution that had been poured into another container; it was not conditioned in the simulation solution in the test chamber. For selenium(IV), nickel, and cadmium, samples were taken from test chambers using a rigid tube that remained in the chamber throughout the simulation; this tube was also used to mix the solution just before each individual sample was taken.

### 3. RESULTS

#### Analytical methods

Data concerning several important properties of the three analytical methods can be compared across metals. One such property is the mean COV, using data obtained in the pre-simulation comparisons of the methods:

Metal	Mean COV			Dissolved
	Total Recoverable	Acidification (T.R.)	(Diss.)	
Copper	4.18 %	1.38 %	1.55 %	3.00 %
Zinc	5.45 %	3.35 %	4.40 %	4.65 %
Chromium(III)	1.00 %	0.90 %	2.00 %	10.85 %*
Lead	3.60 %	6.65 %**	6.05 %	6.70 %
Arsenic(III)	1.85 %	1.35 %	1.90 %	2.10 %
Chromium(VI)	4.75 %	5.05 %	4.35 %	6.25 %
Selenium(IV)	3.50 %	1.95 %	2.40 %	2.10 %
Selenium(IV)	5.45 %	2.70 %	-----	-----
Nickel	2.15 %	4.75 %	5.30 %	4.70 %
Cadmium	2.77 %	4.40 %	3.05 %	3.80 %
Cadmium	-----	-----	2.75 %	2.65 %

\* This value is the average of 2.5 % (which was determined using spiked MFBS) and 19.2 % (which was determined using spiked LSW). The MFBS and the LSW were spiked at about the same concentration, but all of the chromium(III) was dissolved in the spiked MFBS, whereas only about one-half was dissolved in the spiked LSW.

\*\* This value is the mean of 10.7 and 2.6 %.

These data do not allow a direct comparison the means COVs for the three methods because data from which COVs can be calculated were not generated using all three methods to analyze the same solutions. Mean COVs for the total recoverable and acidification methods can be directly compared, as can mean COVs for the dissolved and acidification methods. In addition, the two COVs determined for the acidification method using different solutions can be compared.

The duplicate mean COVs obtained with selenium and cadmium in replicate solutions show considerable variation for the total recoverable and dissolved methods, but much better agreement for the acidification method. In addition, the pair of mean COVs obtained for the acidification method show reasonable agreement for most metals. The acidification method definitely gave the lowest COVs with copper and zinc and the highest with nickel; the results obtained with the other metals are mixed.

The agreement between replicate analyses of a simulation solution can also be compared across metals. The data used in this comparison are the results of the duplicate analyses that were performed on the simulation solution in one of the three test chambers for each treatment in the simulation tests. These are analyses of duplicate samples, not duplicate analyses of one sample. Each datum is the quotient of the higher of the duplicate values divided by the lower of the two values; "N" is the number of quotients (i.e., the number of solutions that were analyzed twice). Except for one solution containing chromium(VI), the same solutions were analyzed using both the acidification and dissolved methods. Such data are not available for the total recoverable method because it was not used to analyze simulation solutions.

<u>Metal</u>	<u>Acidification Method</u>			<u>Dissolved Method</u>		
	<u>N</u>	<u>Range</u>	<u>Mean</u>	<u>N</u>	<u>Range</u>	<u>Mean</u>
Copper	18	0.91-1.00	0.96	18	0.87-1.00	0.95
Zinc	14	0.90-1.00	0.97	14	0.91-1.00	0.97
Chromium(III)	12	0.82-0.99	0.94	12	0.38-0.84	0.63
Lead	17	0.87-1.00	0.95	17	0.36-1.00	0.86
Arsenic(III)	10	0.93-1.00	0.97	10	0.95-1.00	0.98
Chromium(VI)	9	0.96-1.00	0.98	10	0.90-1.00	0.98
Selenium(IV)	15	0.90-1.00	0.98	15	0.93-1.00	0.98
Nickel	18	0.97-1.00	0.99	18	0.96-1.00	0.98
Cadmium	14	0.91-1.00	0.98	14	0.95-1.00	0.99

The reproducibility was good for all of the analyses except for the measurement of dissolved chromium(III) and lead; these two also gave the poorest reproducibility with the acidification method. Interestingly, all of the conversion factors that are less than 0.85 are for chromium(III) and lead.

A third property that can be compared is the percent recovery. Most of the data concerning recovery was obtained during analyses of simulation solutions, and such recovery data are only available for the acidification and dissolved methods. These recoveries were performed by spiking into solutions to which acid had been added; the spikes were added after the solutions had been acidified for the acidification method and after the filtrate had been acidified for the dissolved method.

<u>Metal</u>	<u>Acidification Method</u>			<u>Dissolved Method</u>		
	<u>N</u>	<u>Range</u>	<u>Mean</u>	<u>N</u>	<u>Range</u>	<u>Mean</u>
Copper	9	98-114 %	103 %	7	90-102 %	98 %
Zinc	8	92-106 %	97 %	6	90-101 %	97 %
Chromium(III)	6	78-107 %	95 %	6	91-111 %	100 %
Lead	7	97-119 %	108 %	10	81-121 %	101 %
Arsenic(III)	4	88-105 %	98 %	5	101-111 %	106 %

Chromium(VI)	5	80- 98 %	90 %	5	83-102 %	95 %
Selenium(IV)	7	95-122 %	99 %	7	76-113 %	96 %
Nickel	11	83-118 %	99 %	9	88-124 %	101 %
Cadmium	6	91-112 %	98 %	8	82-124 %	98 %

Because the spikes were added to solutions that contained acid, it is surprising that so many of the recoveries were below 95 % or above 105 %; it is even more surprising that some of the means are outside this range.

Another useful property is the mean blanks that were obtained in the pre-simulation comparisons of the methods. Two means are available for the acidification method because it was separately compared with the total recoverable and dissolved methods.

Metal	Mean Blank (ug/L)			
	Total Recoverable	Acidification (T.R.)      (Diss.)		Dissolved
Copper	< 0.37	< 0.31	< 0.31	< 0.31
Zinc	< 3.0	< 3.3	< 3.0	< 3.1
Chromium(III)	< 100	< 100	< 100	< 100
Lead	< 1.0	< 1.0	< 1.0	< 1.0
Arsenic(III)	< 2.0	< 2.0	< 2.0	< 2.0
Chromium(VI)	< 0.14	< 0.14	< 0.15	< 0.15
Selenium(IV)	< 6.0	< 6.0	< 6.0	< 6.0
Nickel	< 1.0	< 1.0	< 1.0	< 1.0
Cadmium	< 0.034	< 0.03	< 0.03	< 0.03

There were only a few small differences between the mean blanks.

The easiest comparison of the total recoverable and acidification methods is the comparison of the mean concentrations obtained by replicate analyses of a sample of Lake Superior water that was spiked with the metal (i.e., "solution 12" used in the pre-simulation comparisons of the methods).

Metal	Mean Concentration (ug/L)		Ratio
	Total Recoverable	Acidification	
Copper	4.81	4.46	1.08
Zinc	36.04	40.53	0.89
Chromium(III)	2062	2070	1.00
Lead	9.291	9.639	0.96
Arsenic(III)	1040	1061	0.98
Chromium(VI)	5.601	5.154	1.09
Selenium(IV)	64.98	88.92	0.73
Selenium(IV)	55.20	69.31	0.80
Nickel	14.10	12.54	1.12
Cadmium	0.280	0.195	1.43

The ratio given is the total recoverable mean divided by the acidification mean. Each mean is the result of six measurements, and each ratio is expected to be 1. The differences between the methods cannot be explained on the basis of the differences in the blanks given above. The total recoverable method is expected to be more subject to contamination and loss than the acidification method; many of the ratios are greater than 1, but the ratio for zinc seems low, whereas those for selenium(VI) are very low. Volatilization of selenium during the digestion step in the total recoverable procedure is a possibility.

The best comparison of the dissolved and acidification methods is the comparison of the mean concentrations obtained by replicate analyses of MFBS that was spiked with the metal (i.e., "solution 23" used in the pre-simulation comparisons of the methods).

Metal	Mean Concentration (ug/L)		Ratio
	<u>Dissolved</u>	<u>Acidification</u>	
Copper	3.866	3.982	0.97
Zinc	40.24	38.80	1.04
Chromium(III)	1997	2008	0.99
Lead	8.397	9.025	0.93
Arsenic(III)	997.4	975.6	1.02
Chromium(VI)	4.483	4.314	1.04
Selenium(IV)	88.61	85.73	1.03
Nickel	11.70	11.66	1.00
Cadmium	0.3107	0.3591	0.87
Cadmium	0.3072	0.3057	1.00

The ratio given is the dissolved mean divided by the acidification mean, and each mean is the result of ten measurements. At the concentrations used, all of each metal is expected to be dissolved in MFBS, and so each ratio is expected to be 1. All of the ratios are close to 1, except for the first ratio for cadmium. The differences between the methods cannot be explained on the basis of the differences in the blanks given above.

### Simulation tests

The results of measurements of water quality characteristics are given for each simulation by Brooke (1995). The measured concentration of dissolved oxygen was acceptable in all simulations. The measured value of pH was acceptable, except that the values measured at the end of two simulations with zinc were unreasonably low; the results of these simulations were not used. All except two of the measured concentrations of TSS were below 5 mg/L. Some of the measured concentrations of TOC were

below 5 mg/L; the concentration of TOC was not consistently higher in simulation solutions containing YTC.

The raw data from each simulation are also given by Brooke (1994), but the calculated results for each metal are presented in the appendices to this document. All of the planned tests were completed except for mercury and silver. For mercury, the pre-simulation tests were completed, but no simulation tests were completed. For silver, neither pre-simulation tests nor simulation tests were completed. No results are presented herein for mercury or silver.

Because the methodology was refined at various times during the project, the individual metals are addressed in the appendices in the order in which the simulation tests were performed. To clearly identify how the various refinements affected the work with each individual metal, the following items are addressed in the appendix for each individual metal:

- a. How was the concentration of metal determined in Lake Superior water (LSW)?
- b. Was alkalinity increased if hardness was increased?
- c. What sodium bicarbonate solution was used?
- d. Were the test chambers conditioned with simulation solution before the simulations were begun?
- e. If samples were passed through plastic screen, how was this accomplished and how was the plastic screen conditioned?
- f. When samples were taken from a solution, was the solution stirred only once before a pair of samples was taken or was the solution stirred just before the first sample was taken and stirred again just before the second sample was taken.
- g. Were the solutions remaining in the test chambers at the end of the simulations acidified to see if there was an indication of sorption of metal onto the test chambers?

The appendices consider three kinds of results for each metal:

- A. Pre-simulation test results.
  1. The detection limit for each metal was required to be less than 10 % (except 33 % for silver) of the lowest concentration with which a simulation test was to be performed with that metal.
  2. Use of the acidification method with each metal was evaluated by comparison with the total recoverable method based on replicate analyses of specified solutions. The number and compositions of the solutions specified for this comparison depended on the simulation tests that were to be performed with that particular metal. Each of two or more specified solutions was analyzed six times alternately by the two methods. For each metal, one of the specified solutions was metal-free water so that blanks for the two methods could be compared. The other solutions were prepared by adding the metal, calcium sulfate, sodium bicarbonate, daphnid food, and/or fathead minnow food to

LSW, depending on the simulations that were to be conducted with that metal. For a particular metal, if any of these was added to more than one solution, the same amount was added to each solution to which it was added.

3. Use of the dissolved method was evaluated by comparison with the acidification method based on replicate analyses of the following four solutions:

Solution 21: Metal-free water.

Solution 22: Metal-free bicarbonate solution (MFBS).

Solution 23: MFBS spiked with the metal.

Solution 24: LSW spiked with the metal.

Each of the four solutions was analyzed ten times alternately by the dissolved and acidification methods.

- a. For each of the four sets of ten blanks that were obtained by analyzing solutions 21 and 22 by both of the methods, at least 90 percent of the values in each set of ten blanks was required to be less than 10 % (33 % for silver) of the lowest concentration with which a simulation was to be performed with that metal.
- b. For the replicate analyses of solution 23 by the two methods, the quotient of the dissolved mean divided by the acidification mean was required to be between 0.9 and 1.1.
- c. For the replicate analyses of solution 24 by the two methods, the quotient of the dissolved mean divided by the acidification mean was required to be less than 1.1.
- d. For the replicate analyses of solutions 23 and 24 by the two methods, each of the four coefficients of variation (COVs) was required to be less than 20 percent.

- B. Results of checks of the acidification and dissolved methods during analyses of the samples from the simulation tests.

These included blanks, replicate analyses of a standard (MFBS spiked with the metal), duplicate analyses of simulation solutions, and spike recoveries in MFBS and/or in simulation solutions.

- C. Results of the simulation tests.

The mean of the results obtained using the acidification method and the mean of the results obtained using the dissolved method, as well as the resulting values for percent dissolved, were calculated for each sampling time in each simulation.

For selenium(IV), nickel, and cadmium (i.e., the last three metals), some data are given concerning whether there might have been sorption of metal onto the walls of the test chamber.

## Conversion factors

The purpose of the simulation tests was to obtain data that could be used to derive conversion factors (CFs). The way that CFs are derived from the data must take into account the data on which the existing total recoverable (or acid soluble) criteria are based and the way that dissolved criteria would be derived if all the toxicity tests were repeated and the concentrations of dissolved metal were measured. This is not simply a matter of determining the percent dissolved at one or more points in time during a simulation test.

The simulations were intended to mimic things that happened during the toxicity tests, such as sorption onto test chambers, uptake by test organisms, precipitation, and conversion of one oxidation state to another for arsenic, chromium, and selenium. The concept was that these would have occurred during toxicity tests if they occurred during simulation tests.

Interpretation of the results of simulation tests should take into account how results of toxicity tests should be calculated for the purposes of the derivation of water quality criteria.

- a. The '85 guidelines specified that results of static and renewal tests should be based on initial concentrations, but that nominal concentrations are acceptable for most test materials if measured concentrations are not available (page 30 of U.S. EPA 1985). Unless otherwise noted, all concentrations reported in criteria documents for metals are expected to be essentially equivalent to acid-soluble concentrations and to total recoverable concentrations.
- b. It is the consensus of the Aquatic Life Criteria Guideline Committee (Chapman, Delos, Erickson, Hansen, and Stephan) that if a dissolved LC50 is calculated from a static or renewal toxicity test, it should be calculated on the basis of the time-weighted average (TWA) of the measured dissolved concentrations of the metal; calculation of a TWA is explained in Appendix J. This is how LC50s and EC50s would have been calculated if the toxicity tests had been repeated and the dissolved concentrations had been measured.

The "TWA percent dissolved" obtained from a static simulation test can be calculated either by (1) calculating the TWA of the measured dissolved concentrations and then dividing by the initial concentration measured by the acidification method or (2) dividing each dissolved concentration by the initial concentration measured by the acidification method and then calculating the TWA of the quotients. These two procedures will give the same value for the "TWA percent dissolved".

In the appendix for each metal, as many of the following three kinds of TWAs as possible were calculated from the results of each simulation:



1. For use with flow-through tests, the 1-hour percent dissolved was calculated from all simulations.
2. For use with 48-hr static tests and tests renewed at 48 hours, the 48-hr TWA percent dissolved was calculated from all simulations.
3. For use with 96-hr static tests, the 96-hr TWA percent dissolved was calculated from all simulations that lasted 96 hours.

The equations used to calculate the TWAs are given in Appendix K. The average value of each of the three TWAs was calculated for each metal. How these three TWAs were used to calculate the recommended Conversion Factors (CFs) for the CMC and/or CCC depended on the data that were important in the derivation of the CMC, CCC, and/or FACR for that metal, as explained in each appendix. The equations used to calculate the CFs are also given in Appendix K.

The following recommended conversion factors are derived in the appendices:

<u>Metal</u>	<u>Recommended Conversion Factors</u>	
	<u>CMC</u>	<u>CCC</u>
Arsenic(III)	1.000	1.000
Cadmium <sup>a</sup>		
Hardness = 50 mg/L	0.973	0.938
Hardness = 100 mg/L	0.944	0.909
Hardness = 200 mg/L	0.915	0.880
Chromium(III)	0.316	0.860 <sup>b</sup>
Chromium(VI)	0.982	0.962
Copper	0.960	0.960
Lead <sup>a</sup>		
Hardness = 50 mg/L	0.892	0.892
Hardness = 100 mg/L	0.791	0.791
Hardness = 200 mg/L	0.690	0.690
Nickel	0.998	0.997
Selenium	0.922	0.922
Zinc	0.978	0.986

<sup>a</sup> The recommended conversion factors (CFs) for any hardness can be calculated using the following equations:

Cadmium

$$\text{CMC: } CF = 1.136672 - [(\ln \text{ hardness}) (0.041838)]$$

$$\text{CCC: } CF = 1.101672 - [(\ln \text{ hardness}) (0.041838)]$$

$$\text{Lead (CMC and CCC): } CF = 1.46203 - [(\ln \text{ Hardness}) (0.145712)]$$

where:

(ln hardness) = natural logarithm of the hardness.

- <sup>b</sup> This CF applies only if the CCC is based on the test by Stevens and Chapman (1984). If the CCC is based on other chronic tests, it is likely that the CF should be 0.590, 0.376, or the average of these two values (see Appendix G).

The recommended CFs are given to three decimal places because they are intermediate values in the calculation of dissolved criteria.

#### 4. DISCUSSION

##### Analytical methods

The "acidification method" was used here merely to facilitate the project. This is not an official EPA method and there are no plans to consider it for adoption as an official EPA method. This project certainly could have been completed without using the acidification method, but it was decided by EPA that it would be advantageous to use the acidification method because it is less complex and less subject to contamination and loss of metal than the total recoverable method. In addition, versions of the acidification method have been used to measure metals in many toxicity tests with aquatic organisms.

It is not necessary that only EPA approved methods be used in this kind of project. The "acidification method" is somewhat similar to the "acid-soluble method" that was addressed by EPA in several publications. The two methods differ mostly in the kind and amount of acid used, the minimum duration between the adding of acid and the analyzing of the solution, and whether the sample is filtered. Use of the acidification method in this project should not be interpreted to constitute a change in EPA policy as to how metals should be measured for regulatory purposes such as permit monitoring, ambient monitoring, establishing water effect ratios, etc.

For each metal, it was expected that the COV for the acidification method would be lower than the COVs for the total recoverable and dissolved methods because the acidification method is less complex and less subject to contamination and loss of metal. Although the acidification method gave the lowest COVs for copper and zinc, it gave the highest for nickel; the results were mixed for the other metals. The COV was especially high (19.2 %) for the measurement of dissolved chromium(III) in Lake Superior water; this was not a sampling problem because the COV obtained using the acidification method to analyze the same solution was only 1.3 %.

These COVs were calculated to evaluate the acceptability of the methodology. Additional COVs could have been calculated from duplicate analyses of the solution in one test chamber and from the analyses of the triplicate test chambers. These additional COVs could have been compared with the COVs that were calculated, but this would not have aided in the interpretation and use of the data.

The variation between replicate analyses of simulation solutions was low, except that it was higher for the measurement of dissolved lead and was quite high for the measurement of dissolved chromium(III). A number of the recoveries were

surprisingly high or low, considering that they were performed by spiking into acidified solution. Almost all of the blanks were very low for all three methods. The acidification and total recoverable methods produced similar results for all metals except selenium when they were added to Lake Superior water.

All of the results discussed above, except for the data concerning reproducibility of duplicate analyses, were obtained during studies of the analytical methods that were performed before the simulations were begun. As expected, QA/QC checks during the analyses of simulation solutions were not as good and produced some high blanks and low recoveries.

Two observations seem appropriate:

1. The COV of an analytical method should be less than 10 %.
2. If possible, the COV of the dissolved method should be checked using a solution in which about 50 % of the metal of concern is dissolved.

### Simulation tests

Although it would have been desirable to conduct many more simulation tests and to test some or all of the five assumptions given in item L on page 4, the additional cost would have made the project prohibitively expensive. A regulatory authority might decide, therefore, to continue using total recoverable criteria until additional questions are answered concerning appropriate conversion factors.

After the simulations were begun, it was decided that some refinements in the methodology would be beneficial.

- a. Solution 17 was added to the comparison of the total recoverable and acidification methods to provide a comparison at a low concentration and to aid in the interpretation of the analyses of spiked Lake Superior water.
- b. Alkalinity was increased whenever hardness was increased to maintain a reasonable relationship between the two in simulations in which the hardness was 200 mg/L.
- c. The intent was for the sodium bicarbonate solution to have about the same alkalinity as Lake Superior water. The solution to be used, however, was initially specified to be 0.05 N sodium bicarbonate, rather than 0.001 N sodium bicarbonate that has the appropriate alkalinity.
- d. After the concentrations measured using the acidification method were observed to decrease during simulation tests with several metals, the addition of acid to the test chambers at the end of the test was instituted to determine whether the decrease was likely to be due to sorption onto the walls of the test chamber. This also made it inappropriate to condition the test chambers before the beginning of a simulation.

- e. A sampling apparatus was used during several simulations to keep daphnids, brine shrimp nauplii, and brine shrimp cases out of the samples and was conditioned in the test chambers before samples were taken. This might have been the cause of the low pHs in two of the simulations with zinc. This apparatus was not used for the last few metals.
- f. Stirring of the solution before each sample was taken, rather than stirring before each pair of samples, was deemed desirable to reduce the possibility of a gradient in precipitate when the second sample was taken.

Although these refinements were considered desirable, no studies were conducted to determine whether they actually made a difference in the results.

Although it might be argued that loss of metal to the walls of the test chamber should not be taken into account the calculation of conversion factors, it was taken into account here because it probably also occurs in actual toxicity tests.

Two observations seem appropriate:

1. Equipment to sample test solutions or make measurements on test solutions should not be placed in the solutions during the test; one alternative is to leave a sampling tube, for example, in the test chamber from the beginning to the end of the test.
2. Measurement of such water quality characteristics as pH at the end of a test not only characterize the test solution but also might detect problems that occurred during the test.

### Conversion factors

Determination and use of a CF for the CMC is relatively straightforward because the CMC is calculated from the results of acute toxicity tests. Determination and use of a CF for the CCC is not as straightforward for some metals because the CCC is calculated by dividing the FAV by the FACR. The following example illustrates, however, that conversion of the FAV and FACR results in the same dissolved CCC as does conversion of the CCC.

Assume:

CF for the CMC = 0.70

CF for the CCC = 0.90

Based on total recoverable measurements:

FAV = 84 ug/L

FACR = (90 ug/L) / (30 ug/L) = 3

One calculation approach is to calculate the total recoverable CMC and CCC and then convert them to dissolved:

$$\text{CMC} = \text{FAV}/2 = (84 \text{ ug/L})/2 = 42 \text{ ug/L}$$

$$\text{CCC} = \text{FAV}/\text{FACR} = (84 \text{ ug/L})/3 = 28 \text{ ug/L}$$

Conversion to dissolved:

$$\text{CMC} = (0.70)(42 \text{ ug/L}) = 29.4 \text{ ug/L}$$

$$\text{CCC} = (0.90)(28 \text{ ug/L}) = 25.2 \text{ ug/L}$$

A second calculation approach is to convert the total recoverable FAV and FACR to dissolved and then calculate the CMC and CCC:

$$\text{FAV} = (0.70)(84 \text{ ug/L}) = 58.8 \text{ ug/L}$$

$$\text{FACR} = \frac{(0.70)(90 \text{ ug/L})}{(0.90)(30 \text{ ug/L})} = \frac{63 \text{ ug/L}}{27 \text{ ug/L}} = 2.33333$$

$$\text{CMC} = \text{FAV}/2 = (58.8 \text{ ug/L})/2 = 29.4 \text{ ug/L}$$

$$\text{CCC} = \text{FAV}/\text{FACR} = (58.8 \text{ ug/L})/2.33333 = 25.2 \text{ ug/L}$$

The two approaches give the same results.

The situation is more complicated when the CFs are hardness dependent and the FACR was calculated from ACRs that were determined at different hardnesses. An even more difficult situation might involve metals for which the FACR was based on both freshwater and saltwater ACRs (i.e., arsenic, lead, nickel, and zinc). These are complicated if the CFs for the CMC and the CCC are not the same in fresh and salt water.

Although additional testing is probably desirable, the data contained herein should be sufficient to justify deriving and using CFs to convert total recoverable criteria to dissolved criteria when use of dissolved criteria are otherwise desirable. One of the reasons a regulatory authority might decide to continue using total recoverable criteria is to wait until additional questions are answered concerning appropriate conversion factors.

Some of the values for percent dissolved in Attachment 2 of Prothro (1993) are substantially lower than the values obtained in this project, possibly because some of the values in Attachment 2 were determined at concentrations that are substantially different from the relevant CMC or CCC. Some of the differences might be due to differences in such water quality

characteristics as pH. Few of the values contained in Attachment 2 are supported by the validation and QA studies that were conducted as part of the work performed by UWS and some reports do not present such information as the type of filter used. A particular source of concern is sorption of metal by the filter and the filter holder, which could result in the reported dissolved concentrations being inappropriately low.

Some values for percent dissolved have been reported for toxicity tests that were important in the derivation of criteria; in particular, Chapman (1993) reported percent dissolved for acute and chronic tests on copper, lead, nickel, and zinc. One of the problems with these data is the lack of QA/QC data for the dissolved measurements; another is the inability to use these data in the determination of time-weighted averages. These same issues can be raised concerning the percent dissolved data reported by Stevens and Chapman (1984) for the chronic toxicity test on chromium(III); one difference is that the CCC for chromium(III) was based on this test, whereas the CCCs for copper, lead, nickel, and zinc were based on several ACRs for each metal. Also, the test on chromium(III) was a flow-through test and so the percent dissolved probably did not change substantially during the test. In contrast, the life-cycle tests with daphnids were renewal tests and the percent dissolved might have changed from the beginning to the end of the test.

For cadmium, chromium(III), and lead (i.e., for the metals for which the concentrations measured by the acidification method decreased by more than 20 percent in more than one simulation test), pertinent references in the criteria documents were searched for information concerning whether the test concentrations decreased during freshwater static and renewal tests. The references considered pertinent were those concerning either renewal chronic tests or static and renewal acute tests in which the test concentrations were measured and the species was in one of the four genera that were most sensitive to the metal. Only two of the references provided relevant information. Canton and Slooff (1982) reported substantial declines in the concentration of cadmium, but the methodology used was not described in detail. Chapman (1993b) reported that total copper concentrations decreased about 25 to 30 percent in 2 or 3 days, but the measurements were of total copper in the water column; dissolved concentrations appeared to be more stable than total concentrations.

The COVs that were calculated and the additional COVs that could be calculated could have been used in a hypothesis test to determine whether the conversion factors are significantly different from 1. This might be used as an rationale for setting any CF that is not significantly different from 1 to be equal to 1. This was not done because there is no rationale for believing that the CF should be 1. An alternative would be to determine

whether the CF is significantly different from 0.90 or 0.80, and set the CF equal to the lowest value from which it is not significantly different.

Some people might feel that conversion factors should be 1.0 because conservatism is built into other portions of the process used to derive permit limits. It is inappropriate to be unnecessarily underprotective (or overprotective) in one portion of a program just because another portion of the program is or is assumed to be overprotective (or underprotective). The correct approach is to try to make each portion of the program as appropriate as possible.

Conversion factors were derived here for selenium even though Prothro (1993) stated that it is not appropriate to adjust the CMC and CCC for selenium or the CCC for mercury because these are bioaccumulative chemicals. Regardless of whether these metals bioaccumulate, the important consideration is the exposure that relates to the effect on which the CMC or CCC is based. As explained earlier, if an appropriate CF is used and if there are no overriding management considerations, dissolved CMCs may be derived for selenium and mercury and a dissolved CCC may be derived for selenium. Under the same circumstances, a dissolved CCC may be derived for mercury if the total recoverable CCC is based on toxicity to aquatic organisms, but not if the total recoverable CCC is based on mercury residues in aquatic organisms. Although a conversion factor cannot be used to derive a dissolved CCC for mercury if the CCC is based on mercury residues in aquatic organisms, this does not necessarily mean that losses due to fate and transport processes, such as volatility, cannot be taken into account in the derivation of a permit limit.



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## Appendix A: Copper

The three kinds of results described in Section 3 are presented below for copper; the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with copper as follows:

- a. The concentration of copper in Lake Superior water (LSW) was measured in the three batches of LSW that were used in these tests with copper.
- b. Although the hardness of LSW was increased for some simulations with copper, alkalinity was not increased for any simulation with copper.
- c. The sodium bicarbonate solution was 0.05 N.
- d. Each test chamber was conditioned with simulation solution before the simulation was begun.
- e. Samples from solutions 11, 12, 13, and 14 were not passed through the plastic screen. Samples from solutions 15 and 16 were poured through the screen; the screen was conditioned by pouring a portion of the solution through the screen and discarding this portion of the solution. Samples from solutions 21, 22, 23, and 24 and from all of the simulation solutions were passed through the plastic screen by using the sampling apparatus that was described in Section 2; the apparatus was conditioned in the solution to be sampled.
- f. Each solution that was sampled was stirred just before a pair of samples was taken; solutions were not stirred just before each individual sample was taken. If a total recoverable sample was in the pair, it was taken before the acidification sample; if a dissolved sample was in the pair, it was taken after the acidification sample.
- g. Solutions remaining in the test chambers at the end of the simulations were not acidified to see if there was an indication of sorption onto the test chambers.

### Pre-simulation test results

1. The detection limit for copper was determined to be 0.31 ug/L. The lowest concentration with which a simulation was to be performed with copper was 5 ug/L, and so the detection limit was less than ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus copper.
  - Soln 13: LSW plus daphnid food.
  - Soln 14: LSW plus daphnid food and copper.

Soln 15: LSW plus fathead minnow food.

Soln 16: LSW plus fathead minnow food and copper.

The mean measured concentrations (in ug/L, with the standard deviations in parentheses) were:

Method	Solution					
	11	12	13	14	15	16
Acidification	<0.31	4.46 (0.08)	2.36 (0.04)	4.62 (0.06)	2.40 (0.03)	4.64 (0.05)
Total Recoverable	<0.37	4.81 (0.14)	2.60 (0.16)	4.64 (0.25)	2.53 (0.09)	4.65 (0.14)

Analyses of three batches of LSW (to which food had not been added) using the acidification method indicated that the concentration of copper was 2.3, 2.4, and 2.5 ug/L. The mean of these three concentrations is about the same as the mean concentrations (2.36 and 2.40 ug/L) that were obtained when LSW plus food was analyzed using the acidification method, but is lower than the two mean concentrations (2.60 and 2.53 ug/L) that were obtained when LSW plus food was analyzed using the total recoverable method.

- Solution 11 was used to determine method blanks. All six blanks for the acidification method were less than 0.31 ug/L; of the six blanks for the total recoverable method, three were less than 0.31 ug/L and the other three ranged from 0.35 to 0.58 ug/L.
- The concentrations measured in solution 13 and in solution 15 were higher using the total recoverable method than the acidification method.
- Taking into account the blanks and standard deviations, the agreement between the results obtained using the two methods was reasonably good for solutions 12, 14, and 16.
- For all six of the solutions, the total recoverable method gave higher mean concentrations than did the acidification method, although the difference was very small for solutions 14 and 16.
- Comparison of the results for solutions 14 and 16 with those for solution 12 indicates that both foods possibly increased the concentration of copper measured using the acidification method, but possibly decreased the concentration measured using the total recoverable method.
- The following differences should all be the same if there was no effect of food or analytical method:

<u>Method</u>	<u>#14 - #13</u>	<u>#16 - #15</u>
Acidification	2.26 ug/L	2.24 ug/L
Total recoverable	2.04 ug/L	2.12 ug/L

The results might indicate a difference between the two methods, but the number of comparisons is small and the differences are comparable to the standard deviations.

- g. The five coefficients of variation (COVs) obtained using the acidification method to analyze solutions 12 through 16 ranged from 1.0 to 1.7 %, whereas the five COVs obtained using the total recoverable method to analyze the same solutions ranged from 2.8 to 6.2 %.
3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
    - Soln 21: Metal-free water.
    - Soln 22: Metal-free bicarbonate solution (MFBS).
    - Soln 23: MFBS plus copper.
    - Soln 24: LSW plus copper.
    - a. For the replicate analyses of solutions 21 and 22 using both methods, all forty blanks were less than 0.31 ug/L. The blanks were sufficiently low.
    - b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 0.97, which was acceptable.
    - c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 0.98, which was acceptable.
    - d. For the replicate analyses of solutions 23 and 24, the COVs were 1.8 and 1.3 % for the acidification method and 3.3 and 2.7 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.
  4. The COVs obtained with the three methods were:

<u>Method</u>	<u>Number of COVs</u>	<u>Range (%)</u>	<u>Mean (%)</u>
Total Recoverable	5	2.8 to 6.2	4.18
Dissolved	2	2.7 to 3.3	3.00
Acidification	7	1.0 to 1.8	1.43

All of the COVs for the acidification method were lower than all of the COVs for the other two methods.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun.

Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Two sets of QA samples were analyzed because the samples from the simulation tests were analyzed in two batches.
  - a. All blanks were less than 0.31 ug/L for both methods and were therefore acceptable.
  - b. The results of replicate analyses of MFBS spiked with copper were:

<u>Method</u>	<u>Number</u>	<u>Range</u> <u>(ug/L)</u>	<u>Mean</u> <u>(ug/L)</u>	<u>COV</u> <u>(%)</u>
Acidification	8	3.6 to 4.5	4.19	7.3
	4	3.7 to 4.3	4.02	6.5
Dissolved	8	3.8 to 4.3	3.97	4.8
	4	3.6 to 4.0	3.80	4.2

The quotients of the dissolved mean divided by the acidification mean were 0.95 and 0.94. A quotient of 0.97 was reported above for spiked MFBS, and a quotient of 0.98 was reported above for spiked LSW. The dissolved method gave lower COVs than did the acidification method, and the COVs for both methods were higher than the pre-simulation test mean COVs.

- c. No spike recoveries were performed with the QA samples.
2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	18	0.91 to 1.00	0.96
Dissolved	18	0.87 to 1.00	0.95

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	9	98 to 114 %	103 %
Dissolved	7	90 to 102 %	98 %

These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions.

# Results of the simulation tests

Type of Sim.	Time (hr)	Hard (a)	Sp. (b)	Food (c)	Acidifica. Method (d)	Dissolved Method (d)	% Diss. (e)	% Diss. (f)
1	1	50	FM	No	19.34	19.10	98.8	98.8
1	48	50	FM	No	17.90 (-7%)	17.20 (-10%)	96.1	88.9
1	96	50	FM	No	15.95 (-18%)	14.88 (-22%)	93.3	76.9
1	1	50	FM	No	70.32	69.65	99.0	99.0
1	48	50	FM	No	64.50 (-8%)	61.53 (-12%)	95.4	87.5
1	96	50	FM	No	59.85 (-15%)	61.28* (-12%)	102.4	87.1
2	1	50	DM	YTC	4.968	4.841	97.4	97.4
2	48	50	DM	YTC	4.908 (-1%)	4.965 (+3%)	101.2	99.9
2	1	50	DM	YTC	19.77	17.91	90.6	90.6
2	48	50	DM	YTC	18.49 (-6%)	18.64 (+4%)	100.8	94.3
3	1	50	FM	BS	4.793	4.728	98.6	98.6
3	48	50	FM	BS	4.536 (-5%)	4.022 (-15%)	88.7	83.9
3	1	50	FM	BS	18.59	19.44	104.6	104.6
3	48	50	FM	BS	17.23 (-7%)	15.43 (-21%)	89.5	83.0
4	1	200	DM	YTC	5.000	4.947	98.9	98.9
4	48	200	DM	YTC	4.743 (-5%)	4.549 (-8%)	95.9	91.0
4	1	200	DM	YTC	18.76	18.06	96.3	96.3
4	48	200	DM	YTC	17.16 (-9%)	16.95 (-6%)	98.8	90.4

\* = One value was considered an outlier and was not used.

a = mg/L.

b = Species (FM = fathead minnow; DM = Daphnia magna).

c = Food (YTC = yeast-trout chow-cereal leaf; BS = brine shrimp nauplii).

d = ug/L; the numbers in parentheses are percent change from the concentrations at one hour.

e = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.

f = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

The concentrations at 48 and 96 hours were lower than those at 1 hour for all ten cases for the acidification method and for eight of ten cases for the dissolved method. The decrease in the concentrations from 1 hour to 48 and 96 hours might have

been due to precipitation, uptake by test organisms, and/or sorption onto test chambers. Precipitation could cause the dissolved measurements to be lower if equilibrium had not been achieved within the first hour and could cause the acidification measurements to be lower if the stirring did not resuspend all of the precipitate and/or did not keep it suspended during the sampling. Uptake is not likely important because fishes and daphnids do not bioconcentrate substantial amounts of copper. The chambers were conditioned before the simulation tests began, but this conditioning might not have been sufficient to prevent sorption. Data obtained with nickel and cadmium indicate that sorption onto the test chambers was probably substantial and was probably greater in simulations containing daphnids and YTC.

The 36 values for percent dissolved ranged from 76.9 to 104.6 percent.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

<u>Type of Simulation</u>	<u>Hard. (mg/L)</u>	<u>Species (a)</u>	<u>Food (b)</u>	<u>Initial Conc. c</u>	<u>TWA % dissolved</u>		
					<u>1-hr<sup>d</sup></u>	<u>48-hr<sup>e</sup></u>	<u>96-hr<sup>f</sup></u>
1	50	FM	No	19.34	98.8	93.8	88.4
1	50	FM	No	70.32	99.0	93.2	90.3
2	50	DM	YTC	4.968	97.4	98.6	----
2	50	DM	YTC	19.77	90.6	92.4	----
3	50	FM	BS	4.793	98.6	91.2	----
3	50	FM	BS	18.59	104.6	93.8	----
4	200	DM	YTC	5.000	98.9	95.0	----
4	200	DM	YTC	18.76	96.3	93.4	----
Mean TWA					98.0	93.9	89.4

<sup>a</sup> Species (FM = fathead minnow; DM = Daphnia magna).

<sup>b</sup> Food (YTC = yeast-trout chow-cereal leaf; BS = brine shrimp nauplii).

<sup>c</sup> Initial concentration (ug/L) measured by the acidification method.

<sup>d</sup> This is the percent dissolved at 1 hour.

<sup>e</sup> This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).

<sup>f</sup> This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

The 18 TWAs ranged from 88.4 to 104.6 percent. The value of 104.6 is obviously high, but the replicates are similar and the other TWA from that simulation seems reasonable. The one-hour mean TWA would be 97.1 if the TWA of 104.6 was not used.

The mean TWA decreased as the duration of the simulation increased. For each duration, the range of the TWAs was relatively small and did not seem to depend on species, food, or hardness.

The acute toxicity tests that were important in the derivation of the freshwater criterion for copper were flow-through tests and 48-hr static tests. Therefore, the recommended conversion factor for the CMC is 0.960, which is the average of 0.980 and 0.939.

Of the four freshwater chronic toxicity tests that were used in the derivation of the criterion, three were renewal tests and one was a flow-through test. Therefore, the recommended conversion factor for the CCC is 0.960, which is the average of 0.980 and 0.939.

Many of the percent dissolved values given for copper in Attachment 2 of Prothro (1993) are reasonably close to 96.0 percent, but many are substantially lower.



## Appendix B: Zinc

The three kinds of results described in Section 3 are presented below for zinc; the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with zinc as follows:

- a. The concentration of zinc in Lake Superior water (LSW) was not measured in the batch of LSW used to obtain the results reported below; the concentration was measured in a different batch of LSW.
- b. Whenever the hardness of LSW was increased for a simulation with zinc, the alkalinity was also increased.
- c. The sodium bicarbonate solution was 0.05 N.
- d. Each test chamber was conditioned with simulation solution before the simulation was begun.
- e. Samples for measurement of zinc were passed through a plastic screen by using the sampling apparatus that was described in Section 2; the apparatus was conditioned in the solution to be sampled.
- f. Each solution that was sampled was stirred just before a pair of samples was taken; solutions were not stirred just before each individual sample was taken. If a total recoverable sample was in the pair, it was taken before the acidification sample; if a dissolved sample was in the pair, it was taken after the acidification sample.
- g. The solutions remaining in the test chambers at the end of the simulations were not acidified to see if there was an indication of sorption onto the test chambers.

### Pre-simulation test results

1. The detection limit for zinc was determined to be 3.0 ug/L. The lowest concentration with which a simulation was to be performed with zinc was 40 ug/L, and so the detection limit was less than ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus zinc.
  - Soln 13: LSW plus daphnid food.
  - Soln 14: LSW plus daphnid food and zinc.

The mean measured concentrations (in ug/L, with the standard deviations in parentheses) were:

Method	Solution			
	11	12	13	14
Acidification	< 3.3	40.53 (1.1)	< 3.7	36.14 (1.4)
Total Recoverable	< 3.0	36.04 (1.5)	< 3.0	37.48 (2.5)

Analysis of a different batch of LSW (without daphnid food) using the acidification method found that the concentration of zinc was less than 3.0 ug/L.

- a. Solution 11 was used to determine method blanks. The six blanks for the acidification method ranged from < 3.0 to 4.3 ug/L; all six blanks for the total recoverable method were less than 3.0 ug/L.
  - b. The total recoverable method did not detect zinc in solution 13, whereas the acidification method did.
  - c. Taking into account the blanks and standard deviations, the agreement between the results obtained using the two methods was reasonably good for solution 12 and very good for solution 14.
  - d. The results obtained with solutions 12 and 14 indicate that the daphnid food had negligible effect on results obtained using both analytical methods. Surprisingly, the results of the analyses of solution 12 using the acidification method are higher than the other three results.
  - e. The two coefficients of variation (COVs) obtained using the acidification method to analyze solutions 12 and 14 were 2.7 and 4.0 %, whereas the two COVs obtained using the total recoverable method to analyze the same solutions were 4.2 and 6.7 %.
3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
- Soln 21: Metal-free water.
  - Soln 22: Metal-free bicarbonate solution (MFBS).
  - Soln 23: MFBS plus zinc.
  - Soln 24: LSW plus zinc.
- a. For the replicate analyses of solutions 21 and 22, all twenty blanks for the acidification method and nineteen of the twenty blanks for the dissolved method were less than 3.0 ug/L; the other one was 5 ug/L. The blanks were sufficiently low.
  - b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 1.04, which was acceptable.

- c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 1.05, which was acceptable.
- d. For the replicate analyses of solutions 23 and 24, the COVs were 4.5 and 4.3 % for the acidification method and 4.6 and 4.7 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.

4. The COVs obtained with the three methods were:

<u>Method</u>	<u>Number of COVs</u>	<u>Range (%)</u>	<u>Mean (%)</u>
Total Recoverable	2	4.2 to 6.7	5.45
Dissolved	2	4.6 to 4.7	4.65
Acidification	4	2.7 to 4.5	3.88

The COVs for the three methods were similar.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun.

Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Two sets of QA samples were analyzed because the samples from the simulation tests were analyzed in two batches.
  - a. All blanks were less than 3.0 ug/L for both methods and were therefore acceptable.
  - b. The results of replicate analyses of MFBS spiked with zinc were:

<u>Method</u>	<u>Number</u>	<u>Range (ug/L)</u>	<u>Mean (ug/L)</u>	<u>COV (%)</u>
Acidification	8	35.6 to 42.4	37.6	5.4
	3*	29.5 to 34.0	32.5	5.0
Dissolved	8	33.4 to 43.6	36.8	6.3
	4	29.1 to 34.0	31.6	6.4

\* = One value was considered an outlier and was not used.

The quotients of the dissolved mean divided by the acidification mean were 0.98 and 0.97. A quotient of 1.03 was reported above for spiked MFBS and a quotient of 1.05 was reported above for spiked LSW. The acidification method gave slightly lower COVs than did the dissolved method, and the COVs for both methods were higher than the pre-simulation test mean COVs.

- c. Recoveries in MFBS for the acidification method gave 96.6, 88.4, and 103.3 %; recoveries in MFBS for the dissolved method gave 100.0 and 110.4 %. These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.
2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	14	0.90 to 1.00	0.97
Dissolved	14	0.91 to 1.00	0.97

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	8	92 to 106 %	97 %
Dissolved	6	90 to 101 %	97 %

These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions.

#### Results of the simulation tests

<u>Type of Sim.</u>	<u>Time (hr)</u>	<u>Hard (a)</u>	<u>Sp. (b)</u>	<u>Food (c)</u>	<u>Acidifica. Method (d)</u>	<u>Dissolved Method (d)</u>	<u>% Diss. (e)</u>	<u>% Diss. (f)</u>
1	1	50	FM	No	92.66	91.06	98.3	98.3
1	48	50	FM	No	92.76 (0%)	91.07 (0%)	98.2	98.3
1	96	50	FM	No	84.97 (-8%)	85.60 (-6%)	100.7	92.4
1	1	50	FM	No	674.8	673.3	99.8	99.8
1	48	50	FM	No	645.2 (-4%)	636.6 (-5%)	98.7	94.3
1	96	50	FM	No	625.2 (-7%)	623.0 (-7%)	99.6	92.3
2	1	50	DM	YTC	38.79	38.97	100.5	100.5
2	48	50	DM	YTC	38.22* (-1%)	39.47 (+1%)	103.3#	101.8#
2	1	50	DM	YTC	364.3	368.5	101.2	101.2
2	48	50	DM	YTC	368.4 (+1%)	375.6 (+2%)	102.0#	103.1#

4	1	200	DM	YTC	36.60	36.03	98.5	98.5
4	48	200	DM	YTC	35.20*(-4%)	34.68 (-4%)	98.5	94.8
4	1	200	DM	YTC	323.6	318.7	98.5	98.5
4	48	200	DM	YTC	313.0*(-3%)	317.4*(0%)	101.4	98.1

\* = One value was considered an outlier and was not used.  
 # = This value was not used because the pH of the simulation solution was unreasonably low at the end of the simulation.

a = mg/L.

b = Species (FM = fathead minnow; DM = Daphnia magna).

c = Food (YTC = yeast-trout chow-cereal leaf).

d = ug/L; the numbers in parentheses are percent change from the concentrations at one hour.

e = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.

f = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

The concentrations at 48 and 96 hours were lower than those at 1 hour for six of eight cases for the acidification method, but they were lower for only four of eight cases for the dissolved method. The decrease in the concentrations from 1 hour to 48 and 96 hours might have been due to precipitation, uptake by test organisms, and/or sorption onto test chambers. Precipitation could cause the dissolved measurements to be lower if equilibrium had not been achieved within the first hour and could cause the acidification measurements to be lower if the stirring did not resuspend all of the precipitate and/or did not keep it suspended during the sampling. Uptake is not likely important because fishes and daphnids do not bioconcentrate substantial amounts of zinc. The chambers were conditioned before the simulation tests began, but this conditioning might have not been sufficient to prevent sorption. Data obtained with nickel and cadmium indicate that sorption onto the test chambers was probably substantial and was probably greater in simulations containing daphnids and YTC.

The 28 values for percent dissolved only ranged from 92.3 to 103.3 percent. For the percent dissolved based on the acidification measurement made at the same time, in four of the cases, the highest percent dissolved occurred at the end of the simulation and in all four of these cases, the percent dissolved at the end was greater than 100 percent; the other two cases gave very similar results. For two of these, the percent dissolved was greater than 100 percent at the end of

the simulation even when the percentage was calculated based on the initial concentration measured by the acidification method.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

<u>Type of Simulation</u>	<u>Hard. (mg/L)</u>	<u>Species (a)</u>	<u>Food (b)</u>	<u>Initial Conc. c</u>	<u>TWA % dissolved</u>		
					<u>1-hr d</u>	<u>48-hr e</u>	<u>96-hr f</u>
1	50	FM	No	92.66	98.3	98.3	96.8
1	50	FM	No	674.8	99.8	97.0	95.2
2	50	DM	YTC	38.79	100.5	----	----
2	50	DM	YTC	364.3	101.2	----	----
4	200	DM	YTC	36.60	98.5	96.6	----
4	200	DM	YTC	323.6	98.5	98.3	----
Mean TWA					99.5	97.8	96.0

<sup>a</sup> Species (FM = fathead minnow; DM = Daphnia magna).

<sup>b</sup> Food (YTC = yeast-trout chow-cereal leaf).

<sup>c</sup> Initial concentration (ug/L) measured by the acidification method.

<sup>d</sup> This is the percent dissolved at 1 hour.

<sup>e</sup> This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).

<sup>f</sup> This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

The 14 TWAs ranged from 95.2 to 102.2 percent. The mean TWA decreased slightly as the duration of the simulation increased. For each duration, the range of the TWAs was small and did not seem to depend on species, food, or hardness.

The acute toxicity tests that were important in the derivation of the freshwater criterion for zinc were flow-through, renewal, 48-hr static, and 96-hr static tests. Therefore, the recommended conversion factor for the CMC is 0.978, which is the average of 0.995, 0.978, and 0.960.

Of the five freshwater chronic toxicity tests that were used in the derivation of the criterion, three were renewal tests and two were flow-through tests. Therefore, the recommended

conversion factor for the CCC is 0.986, which is the average of 0.995 and 0.978.

Many of the percent dissolved values given for zinc in Attachment 2 of Prothro (1993) are reasonably close to 98.1 and 99.2 percent, but many are substantially lower.

## Appendix C: Chromium(III)

The three kinds of results described in Section 3 are presented below for chromium(III); the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with chromium(III) as follows:

- a. The concentration of chromium(III) in Lake Superior water (LSW) was not measured in the batch of LSW used to obtain the results reported below; the concentration was measured in a different batch of LSW.
- b. Whenever the hardness of LSW was increased for a simulation with chromium(III), the alkalinity was also increased.
- c. The sodium bicarbonate solution was 0.05 N.
- d. Each test chamber was conditioned with simulation solution before the simulation was begun.
- e. Samples for measurement of chromium(III) were passed through a plastic screen by using the sampling apparatus that was described in Section 2; the apparatus was conditioned in the solution to be sampled.
- f. Each solution that was sampled was stirred just before a pair of samples was taken; solutions were not stirred just before each individual sample was taken. If a total recoverable sample was in the pair, it was taken before the acidification sample; if a dissolved sample was in the pair, it was taken after the acidification sample.
- g. The solutions remaining in the test chambers at the end of the simulations were not acidified to see if there was an indication of sorption onto the test chambers.

Because the analytical method used to measure chromium(III) could not differentiate between chromium(III) and chromium(VI), it is appropriate to report some information in terms of chromium rather than chromium(III).

### Pre-simulation test results

1. The detection limit for chromium(III) was determined to be 100 ug/L. The lowest concentration with which a simulation was to be performed with chromium(III) was 2000 ug/L, and so the detection limit was less than ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus chromium(III).

The mean measured concentrations (in ug/L, with the standard deviations in parentheses) were:



<u>Method</u>	<u>Soln 11</u>	<u>Soln 12</u>
Acidification	< 100	2070 (19)
Total Recoverable	< 100	2062 (21)

Analysis of a different batch of LSW using the acidification method found that the concentration of chromium was less than 100 ug/L.

- a. Solution 11 was used to determine method blanks. All blanks for both methods were less than 100 ug/L.
  - b. Taking into account the blanks and standard deviations, the agreement between the results obtained using the two methods was very good for solution 12.
  - c. The coefficient of variation (COV) obtained using the acidification method to analyze solution 12 was 0.9 %, whereas the COV obtained using the total recoverable method to analyze the same solution was 1.0 %.
3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
- Soln 21: Metal-free water.
  - Soln 22: Metal-free bicarbonate solution (MFBS).
  - Soln 23: MFBS plus chromium(III).
  - Soln 24: LSW plus chromium(III).
- a. For the replicate analyses of solutions 21 and 22 using both methods, all forty blanks were less than 100 ug/L. The blanks were sufficiently low.
  - b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 0.99, which was acceptable.
  - c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 0.45, which was acceptable.
  - d. For the replicate analyses of solutions 23 and 24, the COVs were 2.7 and 1.3 % for the acidification method and 2.5 and 19.2 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable, but the COV for analysis of solution 24 (i.e., spiked LSW) using the dissolved method was borderline. The COV for the dissolved method was low for spiked MFBS, in which all of the chromium(III) was dissolved, but was high for spiked LSW, in which about one-half of the chromium(III) was dissolved. The low COV for the acidification method for solution 24 indicated that the high COV for the dissolved method was not due to sampling variation.

4. The COVs obtained with the three methods were:

<u>Method</u>	<u>Number of COVs</u>	<u>Range (%)</u>	<u>Mean (%)</u>
Total Recoverable	1	---	1.00
Dissolved	2	2.5 to 19.2	10.85
Acidification	3	0.9 to 2.7	1.63

The high COV obtained for the dissolved method with spiked LSW (i.e., solution 24) is a concern.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun, although one COV for the dissolved method is high.

Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Only one set of QA samples was analyzed because all of the samples from the simulation tests were analyzed at the same time.
  - a. All blanks were less than 100 ug/L for both methods and were therefore acceptable.
  - b. The results of replicate analyses of MFBS spiked with chromium(III) were:

<u>Method</u>	<u>Number</u>	<u>Range (ug/L)</u>	<u>Mean (ug/L)</u>	<u>COV (%)</u>
Acidification	9	1902 to 2092	1981	3.1
Dissolved	9	1902 to 2017	1946	2.3

The quotient of the dissolved mean divided by the acidification mean was 0.98. A quotient of 0.99 was reported above for spiked MFBS, and a quotient of 0.45 was reported above for spiked LSW. The COVs were similar to the pre-simulation test COVs for spiked MFBS.

- c. Recoveries in MFBS for the acidification method gave 92.6 and 98.9 %; the only recovery in MFBS for the dissolved method gave 95.5 %. These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.
2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	12	0.82 to 0.99	0.94
Dissolved	12	0.38 to 0.84	0.63

The values for the acidification method are acceptably high; for the dissolved method, however, both the highest value of 0.84 and the mean of 0.63 are low values for duplicates. These low values are consistent with the high pre-simulation test COV of 19.2 % that was obtained when spiked LSW (i.e., solution 24) was analyzed using the dissolved method.

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	6	78 to 107 %	95 %
Dissolved	6	91 to 111 %	100 %

It is surprising that the range of these recoveries is so great considering that they were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions. The agreement between the duplicate analyses was not very good for the dissolved method, which agrees with the high COV reported in the pre-simulation test results.

#### Results of the simulation tests

<u>Type of Sim.</u>	<u>Time (hr)</u>	<u>Hard (a)</u>	<u>Sp. (b)</u>	<u>Food</u>	<u>Acidifica. Method (c)</u>	<u>Dissolved Method (c)</u>	<u>% Diss. (d)</u>	<u>% Diss. (e)</u>
1	1	50	FM	No	2166	1540	71.1	71.1
1	48	50	FM	No	1468 (-32%)	384 (-75%)	26.2	17.7
1	96	50	FM	No	1066 (-51%)	274 (-82%)	25.7	12.7
1	1	50	FM	No	9866	5874	59.5	59.5
1	48	50	FM	No	9258 (-6%)	598 (-90%)	6.5	6.1
1	96	50	FM	No	7089 (-28%)	961 (-84%)	14.6	9.7
5	1	200	FM	No	2331	1467	62.9	62.9
5	48	200	FM	No	1234 (-47%)	403 (-73%)	32.7	17.3
5	96	200	FM	No	960 (-59%)	345 (-76%)	35.9	14.8

5	1	200	FM	No	9647	4105	42.6	42.6
5	48	200	FM	No	6528 (-32%)	2253 (-45%)	34.5	23.4
5	96	200	FM	No	5289 (-45%)	848 (-79%)	16.0	8.8

a = mg/L.

b = Species (FM = fathead minnow).

c = ug/L; the numbers in parentheses are percent change from the concentrations at one hour within each simulation.

d = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.

e = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

For both analytical methods, all concentrations at 48 and 96 hours were substantially lower than those at 1 hour. The decrease in the concentrations from 1 hour to 48 and 96 hours might have been due to precipitation, uptake by test organisms, and/or sorption onto test chambers. Precipitation could cause the dissolved measurements to be lower if equilibrium had not been achieved within the first hour and could cause the acidification measurements to be lower if the stirring did not resuspend all of the precipitate and/or did not keep it suspended during the sampling. Uptake is not likely important because fishes and daphnids do not bioconcentrate substantial amounts of chromium(III). The chambers were conditioned before the simulation tests began, but this conditioning might have not been sufficient to prevent sorption. Data obtained with nickel and cadmium indicate that sorption onto the test chambers was probably substantial and was probably greater in simulations containing daphnids and YTC.

The range of the values for percent dissolved was 6 to 71.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

<u>Type of Simulation</u>	<u>Hard. (mg/L)</u>	<u>Species (a)</u>	<u>Food</u>	<u>Initial Conc. b</u>	<u>TWA % dissolved</u>		
					<u>1-hr c</u>	<u>48-hr d</u>	<u>96-hr e</u>
1	50	FM	No	2166	71.1	44.4	29.8
1	50	FM	No	9866	59.5	32.8	20.4
5	200	FM	No	2331	62.9	40.1	28.1
5	200	FM	No	9647	42.6	33.0	24.6

Mean TWA

Hard. = 50	65.3	38.6	25.1
Hard. = 200	52.8	36.6	26.4
All	59.0	37.6	25.7

- <sup>a</sup> Species (FM = fathead minnow).
- <sup>b</sup> Initial concentration (ug/L) measured by the acidification method.
- <sup>c</sup> This is the percent dissolved at 1 hour.
- <sup>d</sup> This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).
- <sup>e</sup> This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

The 12 TWAs ranged from 20.4 to 71.1 percent. The percent dissolved decreased as the initial concentration of chromium(III) increased, probably because the percent dissolved was controlled more by precipitation than sorption. The mean TWA decreased as the duration of the simulation increased. The relation between percent dissolved and hardness was much different at 96 hours than it was at one hour.

The acute toxicity tests that were important in the derivation of the freshwater criterion for chromium(III) were 48-hr and 96-hr static tests. Therefore, the recommended conversion factor for the CMC is 0.316, which is the average of 0.376 and 0.257.

The CCC for chromium(III) was based on a test by Stevens and Chapman (1984), who reported the percent dissolved to be in the range of 83 to 89 percent. Therefore, the recommended conversion factor for the CCC for chromium(III) is 0.860. If, however, the CCC for chromium(III) is based on other chronic tests, it is likely that the CF should be 0.590 or 0.376 or the average of the two.

The percent dissolved values given for chromium(III) in Attachment 2 of Prothro (1993) tend to show a lower percent dissolved at higher concentrations, which is consistent with a precipitation mechanism.

## Appendix D: Lead

The three kinds of results described in Section 3 are presented below for lead; the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with lead as follows:

- a. The concentration of lead in Lake Superior water (LSW) was not measured in the batch of LSW used to obtain the results reported below; the concentration was measured in a different batch of LSW.
- b. Whenever the hardness of LSW was increased for a simulation with lead, the alkalinity was also increased.
- c. The sodium bicarbonate solution was 0.05 N.
- d. Each test chamber was conditioned with simulation solution before the simulation was begun.
- e. Samples for measurement of lead were passed through a plastic screen by using the sampling apparatus that was described in Section 2; the apparatus was conditioned in the solution to be sampled.
- f. Each solution that was sampled was stirred just before a pair of samples was taken; solutions were not stirred just before each individual sample was taken. If a total recoverable sample was in the pair, it was taken before the acidification sample; if a dissolved sample was in the pair, it was taken after the acidification sample.
- g. The solutions remaining in the test chambers at the end of the simulations were not acidified to see if there was an indication of sorption onto the test chambers.

### Pre-simulation test results

1. The detection limit for lead was determined to be 1.0 ug/L. The lowest concentration with which a simulation was to be performed with lead was 10 ug/L, and so the detection limit was ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus lead.
  - Soln 13: LSW plus daphnid food.
  - Soln 14: LSW plus daphnid food and lead.

The mean measured concentrations (in ug/L, with the standard deviations in parentheses) were:

Method	Solution			
	11	12	13	14
Acidification	< 1.0	9.639 (1.03)	< 1.0	7.875 (0.21)
Total Recoverable	< 1.0	9.291 (0.23)	< 1.0	8.552 (0.40)

Analysis of a different batch of LSW (without daphnid food) using the acidification method found that the concentration of lead was less than 1.0 ug/L.

- a. Solution 11 was used to determine method blanks. All blanks for both methods were less than 1.0 ug/L.
  - b. Neither method detected lead in solution 13.
  - c. Taking into account the blanks and standard deviations, the agreement between the results obtained using the two methods was reasonably good for solution 12 and for solution 14.
  - d. The results obtained with solutions 12 and 14 indicate that the food might have reduced the concentration of lead or caused an interference with both methods.
  - e. The two coefficients of variation (COVs) obtained using the acidification method to analyze solutions 12 and 14 were 10.7 and 2.6 %, whereas the two COVs obtained using the total recoverable method to analyze the same solutions were 2.5 and 4.7 %.
3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
- Soln 21: Metal-free water.
  - Soln 22: Metal-free bicarbonate solution (MFBS).
  - Soln 23: MFBS plus lead.
  - Soln 24: LSW plus lead.
- a. For the replicate analyses of solutions 21 and 22 using both methods, all forty blanks were less than 1.0 ug/L. The blanks were sufficiently low.
  - b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 0.93, which was acceptable.
  - c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 0.90, which was acceptable.
  - d. For the replicate analyses of solutions 23 and 24, the COVs were 7.7 and 4.4 % for the acidification method and 9.2 and 4.2 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.

4. The COVs obtained with the three methods were:

<u>Method</u>	<u>Number of COVs</u>	<u>Range (%)</u>	<u>Mean (%)</u>
Total Recoverable	2	2.5 to 4.7	3.60
Dissolved	2	4.2 to 9.2	6.70
Acidification	4	2.6 to 10.7	6.35

The COVs for the three methods overlapped, but the mean for the total recoverable method was lower than the other two means.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun.

Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Two set of QA samples were analyzed because the samples from the simulation tests were analyzed in two batches.
  - a. All blanks were less than 1.0 ug/L for both methods and were therefore acceptable.
  - b. The results of replicate analyses of MFBS spiked with lead were:

<u>Method</u>	<u>Number</u>	<u>Range (ug/L)</u>	<u>Mean (ug/L)</u>	<u>COV (%)</u>
Acidification	8	9.1 to 10.5	9.889	4.7
	4	8.4 to 9.6	9.136	5.0
Dissolved	8	9.3 to 10.3	9.706	3.4
	4	8.2 to 9.3	8.829	5.0

The quotients of the dissolved mean divided by the acidification mean were 0.98 and 0.97. A quotient of 0.93 was reported above for spiked MFBS, and a quotient of 0.90 was reported above for spiked LSW. The COVs were similar and were lower than the pre-simulation test mean COVs.

- c. Recoveries in MFBS for the acidification method gave 83.9, 90.1, and 77.9 %, which are low; recoveries in MFBS for the dissolved method gave 98.8 and 97.9 %. It is surprising that some of these recoveries are so low considering that they were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.



2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	17	0.87 to 1.00	0.95
Dissolved	17	0.36 to 1.00	0.86

For the dissolved method, 4 of the 17 values ranged from 0.36 to 0.77, which seems very low.

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	7	97 to 119 %	108 %
Dissolved	10	81 to 121 %	101 %

It is surprising that the range of the recoveries was so large and that 5 of the 17 were above 113 %, considering that these recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions.

#### Results of the simulation tests

<u>Type of Sim.</u>	<u>Time (hr)</u>	<u>Hard (a)</u>	<u>Sp. (b)</u>	<u>Food (c)</u>	<u>Acidifica. Method (d)</u>	<u>Dissolved Method (d)</u>	<u>% Diss. (e)</u>	<u>% Diss. (f)</u>
1	1	50	FM	No	139.7	140.6	100.6	100.6
1	48	50	FM	No	117.2 (-16%)	109.1 (-22%)	93.1	78.1
1	96	50	FM	No	102.0 (-27%)	92.2 (-34%)	90.4	66.0
1	1	50	FM	No	1093	1141	104.4	104.4
1	48	50	FM	No	799.4 (-27%)	664.9 (-42%)	83.2	60.8
1	96	50	FM	No	856.0 (-22%)	759.3 (-33%)	88.7	69.5
1	1	50	FM	No	4417	4556	103.1	103.1
1	48	50	FM	No	3595 (-19%)	3139 (-31%)	87.3	71.1
1	96	50	FM	No	3630 (-18%)	3102 (-32%)	85.5	70.2
2	1	50	DM	YTC	10.44	9.717	93.1	93.1
2	48	50	DM	YTC	8.70 (-17%)	7.220 (-26%)	83.0	69.2

2	1	50	DM	YTC	136.7	131.6	96.3	96.3
2	48	50	DM	YTC	115.5 (-16%)	107.5 (-18%)	93.0	78.6
4	1	200	DM	YTC	11.26	7.852	69.7	69.7
4	48	200	DM	YTC	9.54 (-15%)	5.569 (-29%)	58.3	49.5
4	1	200	DM	YTC	133.0	101.7	76.5	76.5
4	48	200	DM	YTC	112.5 (-15%)	84.7 (-17%)	75.3	63.7

a = mg/L.

b = Species (FM = fathead minnow; DM = Daphnia magna).

c = Food (YTC = yeast-trout chow-cereal leaf).

d = ug/L; the numbers in parentheses are percent change from the concentrations at one hour.

e = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.

f = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

For both analytical methods, all concentrations at 48 and 96 hours were substantially lower than those at 1 hour. The decrease in the concentrations from 1 hour to 48 and 96 hours might have been due to precipitation, uptake by test organisms, and/or sorption onto test chambers. Precipitation could cause the dissolved measurements to be lower if equilibrium had not been achieved within the first hour and could cause the acidification measurements to be lower if the stirring did not resuspend all of the precipitate and/or did not keep it suspended during the sampling. Uptake is not likely important because fishes and daphnids do not bioconcentrate substantial amounts of lead. The chambers were conditioned before the simulation tests began, but this conditioning might have not been sufficient to prevent sorption. Data obtained with nickel and cadmium indicate that sorption onto the test chambers was probably substantial and was probably greater in simulations containing daphnids and YTC.

The 34 values for percent dissolved ranged from 49.5 to 104.4 percent.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

Type of Simulation	Hard. (mg/L)	Species (a)	Food (b)	Initial Conc. <sup>c</sup>	TWA % dissolved		
					1-hr <sup>d</sup>	48-hr <sup>e</sup>	96-hr <sup>f</sup>
1	50	FM	No	139.7	100.6	89.4	80.7
1	50	FM	No	1093	104.4	82.6	73.9
1	50	FM	No	4417	103.1	87.1	78.9
2	50	DM	YTC	10.44	93.1	81.2	----
2	50	DM	YTC	136.7	96.3	87.4	----
4	200	DM	YTC	11.26	69.7	59.6	----
4	200	DM	YTC	133.0	76.5	70.1	----

#### Mean TWA

All		92.0	79.6	77.8
No food				
Hard = 50 mg/L		102.7	86.4	77.8
YTC				
Hard = 50 mg/L		94.7	84.3	----
Hard = 200 mg/L		73.1	64.8	----

<sup>a</sup> Species (FM = fathead minnow; DM = Daphnia magna).

<sup>b</sup> Food (YTC = yeast-trout chow-cereal leaf).

<sup>c</sup> Initial concentration (ug/L) measured by the acidification method.

<sup>d</sup> This is the percent dissolved at 1 hour.

<sup>e</sup> This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).

<sup>f</sup> This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

The 17 TWAs ranged from 59.6 to 104.4 percent. The mean TWA decreased substantially as the duration of the simulation increased. The 1-hr TWA depended on food and hardness, whereas the 48-hr TWA depended on hardness, but not food. All three of the 1-hr TWAs for simulations with no food were greater than 100 percent.

The acute toxicity tests that were important in the derivation of the freshwater criterion for lead were flow-through, renewal, 48-hr static, and 96-hr static tests. Because all of the acute tests used in the derivation of the criterion were unfed tests and the percent dissolved is higher in unfed tests, the recommended conversion factor (CF) for the CMC is 0.890, which is the average of 102.7, 86.4, and 77.8. All of these values were determined at hardness = 50 mg/L.

Of the five freshwater chronic toxicity tests that were used in the derivation of the criterion, three were renewal tests and two were flow-through tests; all of these were fed tests. Therefore, the recommended CF for the CCC at hardness = 50 mg/L is 0.895, which is the average of 0.947 and 0.84, and

0.690 at hardness = 200 mg/L, which is the average of 0.731 and 0.648.

Because the CFs for the CMC and CCC are similar at hardness = 50 mg/L, but the CF for the CCC at hardness = 200 mg/L is quite different, the CFs for the CMC and CCC will be assumed to be 0.892 at hardness = 50 mg/L, and will be assumed to be 0.690 at hardness = 200 mg/L. In addition, it will be assumed that the following equation can be used to calculate the CF at any hardness:

$$CF = 1.46203 - [(\ln \text{Hardness})(0.145712)]$$

where:

(ln hardness) = the natural logarithm of the hardness.

Many of the percent dissolved values given for lead in Attachment 2 of Prothro (1993) are reasonably close to 89.2 and 69.0 percent, but many are substantially lower.

## Appendix E: Arsenic(III)

The three kinds of results described in Section 3 are presented below for arsenic(III); the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with arsenic(III) as follows:

- a. The concentration of arsenic in Lake Superior water (LSW) was not measured in the batch of LSW used to obtain the results reported below; the concentration was measured in a different batch of LSW.
- b. Neither the hardness nor the alkalinity of LSW was increased for any simulation with arsenic(III) because the criterion for arsenic is not hardness-dependent.
- c. The sodium bicarbonate solution was 0.05 N.
- d. Each test chamber was conditioned with simulation solution before the simulation was begun.
- e. Samples for measurement of arsenic(III) were passed through a plastic screen by using the sampling apparatus that was described in Section 2; the apparatus was conditioned in the solution to be sampled.
- f. Each solution that was sampled was stirred just before a pair of samples was taken; solutions were not stirred just before each individual sample was taken. If a total recoverable sample was in the pair, it was taken before the acidification sample; if a dissolved sample was in the pair, it was taken after the acidification sample.
- g. The solutions remaining in the test chambers at the end of the simulations were not acidified to see if there was an indication of sorption onto the test chambers.

Because the analytical method used to measure arsenic(III) could not differentiate between arsenic(III) and arsenic(V), it is appropriate to report some information in terms of arsenic rather than arsenic(III).

### Pre-simulation test results

1. The detection limit for arsenic(III) was determined to be 2 ug/L. The lowest concentration with which a simulation was to be performed with arsenic(III) was 1.000 ug/L, and so the detection limit was less than ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus arsenic(III).
  - Soln 13: LSW plus daphnid food.
  - Soln 14: LSW plus daphnid food and arsenic(III).

The mean measured concentrations (in ug/L, with the standard deviations in parentheses) were:

Method	Solution			
	11	12	13	14
Acidification	< 2.0	1061 (10)	< 2.0	1025 (19)
Total Recoverable	< 2.0	1040 (19)	< 2.0	1033 (19)

Analysis of a different batch of LSW (without daphnid food) using the acidification method found that the concentration of arsenic was less than 2.0 ug/L.

- a. Solution 11 was used to determine method blanks. All blanks for both methods were less than 2.0 ug/L.
  - b. Neither method detected arsenic in solution 13.
  - c. Taking into account the blanks and standard deviations, the agreement between the results obtained using the two methods was good for solution 12 and very good for solution 14.
  - d. Both methods gave lower concentrations of arsenic in solution 14 than in solution 12; the reduction was greater for the acidification method than for the total recoverable method, because the acidification method measured more arsenic in solution 12 than did the total recoverable method.
  - e. The two coefficients of variation (COVs) obtained using the acidification method to analyze solutions 12 and 14 were 0.9 and 1.8 %, whereas the two COVs obtained using the total recoverable method to analyze the same solutions were 1.9 and 1.8 %.
3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
- Soln 21: Metal-free water.
  - Soln 22: Metal-free bicarbonate solution (MFBS).
  - Soln 23: MFBS plus arsenic(III).
  - Soln 24: LSW plus arsenic(III).
- a. For replicate analyses of solutions 21 and 22 using both methods, all forty blanks were less than 2.0 ug/L. The blanks were sufficiently low.
  - b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 1.02, which was acceptable.
  - c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 1.00, which was acceptable.

- d. For the replicate analyses of solutions 23 and 24, the COVs were 2.1 and 1.7 % for the acidification method and 3.1 and 1.1 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.

4. The COVs obtained with the three methods were:

<u>Method</u>	<u>Number of COVs</u>	<u>Range (%)</u>	<u>Mean (%)</u>
Total Recoverable	2	1.8 to 1.9	1.85
Dissolved	2	1.1 to 3.1	2.10
Acidification	4	0.9 to 2.1	1.62

The COVs for the three methods were all low and similar.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun.

Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Only one set of QA samples was analyzed because all of the samples from the simulation tests were analyzed at the same time.
  - a. All blanks were less than 2.0 ug/L for both methods and were therefore acceptable.
  - b. The results of replicate analyses of MFBS spiked with arsenic(III) were:

<u>Method</u>	<u>Number</u>	<u>Range (ug/L)</u>	<u>Mean (ug/L)</u>	<u>COV (%)</u>
Acidification	8	932 to 1007	970	2.9
Dissolved	8	934 to 1030	972	3.2

The quotient of the dissolved mean divided by the acidification mean was 1.00. A quotient of 1.02 was reported above for spiked MFBS, and a quotient of 1.00 was reported above for spiked LSW. The COVs for both methods were low, but were higher than the pre-simulation test mean COVs.

- c. The only recovery in MFBS for the acidification method gave 94.6 %; recoveries in MFBS for the dissolved method gave 94.2 and 100.9 %. These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.

2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	10	0.93 to 1.00	0.97
Dissolved	10	0.95 to 1.00	0.98

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	4	88 to 105 %	98 %
Dissolved	5	101 to 111 %	106 %

These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions.

#### Results of the simulation tests

<u>Type of Sim.</u>	<u>Time (hr)</u>	<u>Hard (a)</u>	<u>Sp. (b)</u>	<u>Food (c)</u>	<u>Acidifica. Method (d)</u>	<u>Dissolved Method (d)</u>	<u>% Diss. (e)</u>	<u>% Diss. (f)</u>
1	1	50	FM	No	1006	1009	100.3	100.3
1	48	50	FM	No	1022 (+2%)	1022 (+1%)	100.0	101.6
1	96	50	FM	No	1018 (+1%)	968 (-4%)	95.1	96.2
1	1	50	FM	No	9650	9559	99.1	99.1
1	48	50	FM	No	9943 (+3%)	9886 (+3%)	99.4	102.4
1	96	50	FM	No	9559 (-1%)	9569 (0%)	100.1	99.2
2	1	50	DM	YTC	1033	1038	100.5	100.5
2	48	50	DM	YTC	1065 (+3%)	1041 (0%)	97.7	100.8
2	1	50	DM	YTC	2811	2874	102.2	102.2
2	48	50	DM	YTC	2833 (+1%)	2672 (-7%)	94.3	95.1

a = mg/L.

b = Species (FM = fathead minnow; DM = Daphnia magna).

c = Food (YTC = yeast-trout chow-cereal leaf).

d = ug/L; the numbers in parentheses are percent change from the concentrations at one hour.



- e = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.
- f = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

For both analytical methods, the concentrations at 48 and 96 hours seemed to be randomly higher and lower than those at one hour, with the largest amount of change being 7 percent; the changes for the two methods did not seem to be correlated.

The 20 values for percent dissolved only ranged from 94.3 to 102.4 percent.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

<u>Type of Simulation</u>	<u>Hard. (mg/L)</u>	<u>Species (a)</u>	<u>Food (b)</u>	<u>Initial Conc.<sup>c</sup></u>	<u>TWA % dissolved</u>		
					<u>1-hr<sup>d</sup></u>	<u>48-hr<sup>e</sup></u>	<u>96-hr<sup>f</sup></u>
1	50	FM	No	1006	100.3	101.0	99.9
1	50	FM	No	9650	99.1	100.8	100.8
2	50	DM	YTC	1033	100.5	100.6	-----
2	50	DM	YTC	2811	102.2	98.6	-----
Mean TWA					100.5	100.2	100.4

a Species (FM = fathead minnow; DM = Daphnia magna).

b Food (YTC = yeast-trout chow-cereal leaf).

c Initial concentration (ug/L) measured by the acidification method.

d This is the percent dissolved at 1 hour.

e This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).

f This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

The 10 TWAs ranged from 98.6 to 102.2 percent. The mean TWA did not depend on the duration of the simulation. For each duration, the range of the TWAs was very small and did not seem to depend on species or food. Because arsenic(III) is an oxyion, it is not expected to sorb or precipitate in toxicity tests with aquatic organisms, and the percent dissolved is not

expected to depend on hardness; the criterion for arsenic is not hardness-dependent.

The acute toxicity tests that were important in the derivation of the freshwater criterion for arsenic were flow-through tests and 48-hr static tests. Therefore, the recommended conversion factor for the CMC would be 1.0035, which is the average of 1.005 and 1.002. Because the conversion factor cannot be greater than 1.0, the recommended conversion factor is 1.000.

Of the three freshwater chronic toxicity tests that were used in the derivation of the criterion, one was a renewal test and two were flow-through tests. Therefore, the recommended conversion factor for the CCC would be 1.0035, which is the average of 1.005 and 1.002. Because the conversion factor cannot be greater than 1.0, the recommended conversion factor is 1.000.

The percent dissolved values given for arsenic in Attachment 2 of Prothro (1993) are close to 100 percent.

## Appendix F: Chromium(VI)

The three kinds of results described in Section 3 are presented below for chromium(VI); the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with chromium(VI) as follows:

- a. The concentration of chromium(VI) in Lake Superior water (LSW) was not measured.
- b. Neither the hardness nor the alkalinity of LSW was increased for any simulation with chromium(VI) because the criterion for chromium(VI) is not hardness-dependent.
- c. The sodium bicarbonate solution was 0.05 N.
- d. Each test chamber was conditioned with the simulation solution before the simulation was begun.
- e. Samples for measurement of chromium(VI) were passed through a plastic screen by using the sampling apparatus that was described in Section 2; the apparatus was conditioned in the solution to be sampled.
- f. Each solution that was sampled was stirred just before a pair of samples was taken; solutions were not stirred just before each individual sample was taken. If a total recoverable sample was in the pair, it was taken before the acidification sample; if a dissolved sample was in the pair, it was taken after the acidification sample.
- g. The solutions remaining in the test chambers at the end of the simulations were not acidified to see if there was an indication of sorption onto the test chambers.

Because the analytical method used to measure chromium(VI) could not differentiate between chromium(III) and chromium(VI), it is appropriate to report some information in terms of chromium rather than chromium(VI).

### Pre-simulation test results

1. The detection limit for chromium(VI) was determined to be 0.14 ug/L. The lowest concentration with which a simulation was to be performed with chromium(VI) was 5 ug/L, and so the detection limit was less than ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus chromium(VI).
  - Soln 13: LSW plus daphnid food.
  - Soln 14: LSW plus daphnid food and chromium(VI).

The mean measured concentrations (in ug/L, with the standard deviations in parentheses) were:

Method	Solution			
	11	12	13	14
Acidification	< 0.14	5.154 (0.31)	< 0.14	5.515 (0.23)
Total Recoverable	< 0.14	5.601 (0.30)	< 0.14	5.326 (0.22)

LSW was not analyzed for chromium(VI).

- a. Solution 11 was used to determine method blanks. All blanks for both methods were less than 0.14 ug/L.
  - b. Neither method detected chromium in solution 13.
  - c. Taking into account the blanks and standard deviations, the agreement between the results obtained using the two methods was reasonably good for solution 12 and for solution 14.
  - d. The addition of food to LSW did not result in a measurable amount of chromium in solution 13 using either the acidification method or the total recoverable method. For the acidification method, there was more chromium in solution 14 than in solution 12, but for the total recoverable method, the concentration in solution 14 was less than the concentration in solution 12. For each method, the difference is comparable to the standard deviation.
  - e. The two coefficients of variation (COVs) obtained using the acidification method to analyze solutions 12 and 14 were 6.0 and 4.1 %, whereas the two COVs obtained using the total recoverable method to analyze the same solutions were 5.3 and 4.2 %.
3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
- Soln 21: Metal-free water.
  - Soln 22: Metal-free bicarbonate solution (MFBS).
  - Soln 23: MFBS plus chromium(VI).
  - Soln 24: LSW plus chromium(VI).
- a. For the replicate analyses of solutions 21 and 22, one blank for the acidification method was 0.25 ug/L, and the other 19 blanks for the acidification method were all less than 0.14 ug/L. Three of the blanks for the dissolved method were 0.25, 0.25, and 0.15 ug/L, and the other 17 blanks for the dissolved method were all less than 0.14 ug/L. Because the lowest concentration with which a simulation was to be conducted was 5 ug/L, the

- blanks were sufficiently low, even though four of the blanks were somewhat above the detection limit.
- b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 1.04, which was acceptable.
  - c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 0.99, which was acceptable.
  - d. For the replicate analyses of solutions 23 and 24, the COVs were 3.3 and 5.4 % for the acidification method and 6.6 and 5.9 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.

4. The COVs obtained with the three methods were:

<u>Method</u>	<u>Number of COVs</u>	<u>Range (%)</u>	<u>Mean (%)</u>
Total Recoverable	2	4.2 to 5.3	4.75
Dissolved	2	5.9 to 6.6	6.25
Acidification	4	3.3 to 6.0	4.70

The mean COV for the dissolved method was higher than those for the total recoverable and acidification methods.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun.

#### Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Two sets of QA samples were analyzed because the samples from the simulation tests were analyzed in two batches.
  - a. Of the ten blanks for the acidification method, six were less than 0.14 ug/L, but the other four were 0.34, 9.53, 1.33, and 0.84 ug/L. Of the ten blanks for the dissolved method, eight were less than 0.14 ug/L, but the other two were 0.38 and 0.20 ug/L. Six of the twenty blanks seem high and two of these were very high.
  - b. The results of replicate analyses of MFBS spiked with chromium(VI) were:

<u>Method</u>	<u>Number</u>	<u>Range (ug/L)</u>	<u>Mean (ug/L)</u>	<u>COV (%)</u>
Acidification	4	4.8 to 5.2	4.95	3.0
	6	4.6 to 5.7	5.08	9.2
Dissolved	4	4.8 to 5.3	4.98	4.9
	6	4.6 to 5.2	4.92	6.2

The quotients of the dissolved mean divided by the acidification mean were 1.01 and 0.97. A quotient of 1.04 was reported above for spiked MFBS and a quotient of 0.99 was reported above for spiked LSW. The COVs were similar and were similar to the pre-simulation test mean COVs.

- c. The only recovery in MFBS for the acidification method gave 97.6 %; recoveries in MFBS for the dissolved method gave 108.0 and 102.7 %. These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.
2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	9	0.96 to 1.00	0.98
Dissolved	10	0.90 to 1.00	0.98

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	5	80 to 98 %	90 %
Dissolved	5	83 to 102 %	95 %

The mean recovery for the acidification method seems low considering that the recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified. The recoveries reported in section 1.c above are higher.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions.

#### Results of the simulation tests

<u>Type of Sim.</u>	<u>Time (hr)</u>	<u>Hard (a)</u>	<u>Sp. (b)</u>	<u>Food (c)</u>	<u>Acidifica. Method (d)</u>	<u>Dissolved Method (d)</u>	<u>% Diss. (e)</u>	<u>% Diss. (f)</u>
1	1	50	FM	No	29.71	29.84	100.4	100.4
1	48	50	FM	No	28.72 (-3%)	28.21 (-5%)	98.2	95.0
1	96	50	FM	No	27.59 (-7%)	27.88 (-7%)	101.1	93.8

1	1	50	FM	No	68.97	69.63	101.0	101.0
1	48	50	FM	No	66.18 (-4%)	68.35 (-2%)	103.3	99.1
1	96	50	FM	No	70.10 (+2%)	69.12 (-1%)	98.6	100.2
2	1	50	DM	YTC	4.710	4.825	102.4	102.4
2	48	50	DM	YTC	4.645 (-1%)	4.321 (-10%)	93.0	91.7
2	1	50	DM	YTC	26.90	26.19	97.3	97.3
2	48	50	DM	YTC	22.66 (-16%)	22.40 (-14%)	98.9	83.3

a = mg/L.

b = Species (FM = fathead minnow; DM = Daphnia magna).

c = Food (YTC = yeast-trout chow-cereal leaf).

d = ug/L; the numbers in parentheses are percent change from the concentrations at one hour.

e = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.

f = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

The raw data from the simulation tests did not show the irregularities that occurred in the pre-simulation test results and in the checks during the analyses.

The 20 values for percent dissolved ranged from 83.3 to 103.3 percent.

The concentrations at 48 and 96 hours were lower than those at 1 hour for five of six cases for the acidification method and for all six cases for the dissolved method. The decreases were less than eight percent for the solutions that contained neither organisms nor food. Three of the four cases for solutions containing daphnids and daphnid food showed decreases of 10 to 14 percent, and the fourth showed a decrease of 1 percent.

The decrease in the concentrations from 1 hour to 48 and 96 hours might have been due to precipitation, uptake by test organisms, and/or sorption onto test chambers. Precipitation could cause the dissolved measurements to be lower if equilibrium had not been achieved within the first hour and could cause the acidification measurements to be lower if the stirring did not resuspend all of the precipitate and/or did not keep it suspended during the sampling. Uptake is not likely important because fishes and daphnids do not bioconcentrate substantial amounts of chromium(VI). The chambers were conditioned before the simulation tests began, but this conditioning might not have been sufficient to prevent sorption. Data obtained with nickel and cadmium

indicate that sorption onto the test chambers was probably substantial and was probably greater in simulations containing daphnids and YTC.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

<u>Type of Simulation</u>	<u>Hard. (mg/L)</u>	<u>Species (a)</u>	<u>Food (b)</u>	<u>Initial Conc. c</u>	<u>TWA % dissolved</u>		
					<u>1-hr d</u>	<u>48-hr e</u>	<u>96-hr f</u>
1	50	FM	No	29.71	100.4	97.7	96.0
1	50	FM	No	68.97	101.0	100.0	99.8
2	50	DM	YTC	4.710	102.4	97.0	----
2	50	DM	YTC	26.90	97.3	90.3	----
Mean TWA					100.3	96.2	97.9

<sup>a</sup> Species (FM = fathead minnow; DM = Daphnia magna).

<sup>b</sup> Food (YTC = yeast-trout chow-cereal leaf).

<sup>c</sup> Initial concentration (ug/L) measured by the acidification method.

<sup>d</sup> This is the percent dissolved at 1 hour.

<sup>e</sup> This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).

<sup>f</sup> This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

Nine of the ten TWAs are between 96.0 and 102.4 percent; the other one was 90.3 percent. In each simulation the mean TWA decreased as the duration of the simulation increased. For each duration, the range of the TWAs was small and did not seem to depend on species or food. Because chromium(VI) is an oxyion, it is not expected to sorb or precipitate in toxicity tests with aquatic organisms, and the percent dissolved is not expected to depend on hardness; the criterion for chromium(VI) is not hardness-dependent.

The acute toxicity tests that were important in the derivation of the freshwater criterion for chromium(VI) were flow-through tests and renewal tests. Therefore, the recommended conversion factor for the CMC is 0.982, which is the average of 1.003 and 0.962.

All four of the freshwater chronic toxicity tests that were used in the derivation of the criterion were renewal tests.



Therefore, the recommended conversion factor for the CCC is 0.962.

The percent dissolved values given for chromium(VI) in Attachment 2 of Prothro (1993) are close to 98.2 and 96.2 percent, but were determined at very high concentrations of chromium(VI).

## Appendix G: Selenium

The three kinds of results described in Section 3 are presented below for selenium(IV); the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with selenium(IV) as follows:

- a. The concentration of selenium(IV) in Lake Superior water (LSW) was measured in the batch of LSW used to obtain the results reported below.
- b. Neither the hardness nor the alkalinity of LSW was increased for any simulation with selenium(IV) because the criterion for selenium(IV) is not hardness-dependent.
- c. The sodium bicarbonate solution was 0.05 N.
- d. Test chambers were not conditioned with simulation solution before simulations were begun.
- e. Samples for measurement of selenium(IV) were not passed through a plastic screen.
- f. Each solution that was sampled was stirred just before each individual sample was taken, e.g., the solution was stirred before a sample was taken for analysis using the acidification method and was stirred again before a sample was taken for analysis using the dissolved method.
- g. The solutions remaining in the test chambers at the end of the simulations were acidified to see if there was an indication of sorption onto the test chambers.

Because the analytical method used to measure selenium(IV) could not differentiate between selenium(IV) and selenium(VI), it is appropriate to report some information in terms of selenium rather than selenium(IV).

### Pre-simulation test results

1. The detection limit for selenium(IV) was determined to be 6.0 ug/L. The lowest concentration with which a simulation was to be performed with selenium(IV) was 90 ug/L, and so the detection limit was less than ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus selenium(IV).
  - Soln 13: LSW plus daphnid food.
  - Soln 14: LSW plus daphnid food and selenium(IV).
  - Soln 17: LSW (with nothing added).

The mean measured concentrations (in ug/L, with the standard deviations in parentheses were:

Method	Solution				
	11	12	13	14	17
Acidification	< 6.0	88.92 (2.2)	< 6.0	88.59 (1.2)	< 6.0
Total Recoverable	< 6.0	64.98 (1.4)	< 6.0	62.18 (3.0)	< 6.0

Because the concentrations obtained using the total recoverable method were so much lower than those obtained using the acidification method, this comparison was repeated after solutions 12, 13, 14, and 17 were prepared again:

Method	Solution				
	11	12	13	14	17
Acidification	< 6.0	69.31 (1.7)	< 6.0	68.37 (2.0)	< 6.0
Total Recoverable	< 6.0	55.20 (4.6)	< 6.0	33.46 (0.9)	< 6.0

The results obtained using the total recoverable method were again much lower than those obtained using the acidification method. It is possible that this was due to volatilization during the digestion step of the total recoverable procedure.

- Solution 11 was used to determine method blanks. All blanks for both methods were less than 6.0 ug/L.
- Neither method detected selenium in solution 13 or in solution 17.
- Taking into account the blanks and standard deviations, the agreement between the results obtained using the two methods was very poor for solution 12 and for solution 14; the total recoverable method gave much lower mean concentrations for solutions 12 and 14 than did the acidification method.
- The results obtained with solutions 12 and 14 indicate that the daphnid food had negligible effect on results obtained using the acidification method. The food might have had a small effect on the results obtained using the total recoverable method the first time; food had a large effect on the results obtained using the total recoverable method the second time.
- The four coefficients of variation (COVs) obtained using the acidification method to analyze solutions 12 and 14 ranged from 1.4 to 2.9 %, whereas the four COVs obtained using the total recoverable method to analyze the same solutions ranged from 2.1 to 8.3 %.

3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
  - Soln 21: Metal-free water.
  - Soln 22: Metal-free bicarbonate solution (MFBS).
  - Soln 23: MFBS plus selenium(IV).
  - Soln 24: LSW plus selenium(IV).
  - a. For the replicate analyses of solutions 21 and 22 using both methods, all forty blanks were less than 6.0 ug/L. The blanks were sufficiently low.
  - b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 1.03, which was acceptable.
  - c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 0.96, which was acceptable.
  - d. For the replicate analyses of solutions 23 and 24, the COVs were 2.2 and 2.6 % for the acidification method and 1.8 and 2.4 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.
4. The COVs obtained with the three methods were:

<u>Method</u>	<u>Number of COVs</u>	<u>Range (%)</u>	<u>Mean (%)</u>
Total Recoverable	4	2.1 to 8.3	4.48
Dissolved	2	1.8 to 2.4	2.10
Acidification	6	1.4 to 2.9	2.35

The COVs for the total recoverable method were somewhat higher than those for the dissolved and acidification methods.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun.

#### Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Two sets of QA samples were analyzed because the samples from the simulation tests were analyzed in two batches.
  - a. All blanks were less than 6.0 ug/L for both methods and were therefore acceptable.
  - b. The results of replicate analyses of MFBS spiked with selenium(IV) were:

<u>Method</u>	<u>Number</u>	<u>Range (ug/L)</u>	<u>Mean (ug/L)</u>	<u>COV (%)</u>
Acidification	6	50.28 to 77.10	59.83	18.9
	4	51.96 to 59.22	54.78	6.2
Dissolved	6	50.77 to 81.07	61.66	21.4
	4	54.41 to 57.84	55.87	2.8

In both sets of six values, two of the values might have been a pair of outliers.

The quotients of the dissolved means divided by the acidification means were 1.03 and 1.02. A quotient of 1.03 was reported above for spiked MFBS, and a quotient of 0.96 was reported above for spiked LSW. The first COV determined for each method was substantially higher than the second; the second COV for each method was similar to the pre-simulation test mean COV for the method.

- c. Recoveries in MFBS for the acidification method gave 78.3, 59.7, and 72.0 %; recoveries in MFBS for the dissolved method gave 55.5, 58.4, 56.0, and 55.1 %. It is surprising that these recoveries are so low considering that they were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified. The recoveries reported below are substantially higher.

2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	15	0.90 to 1.00	0.98
Dissolved	15	0.93 to 1.00	0.98

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	7	95 to 112 %	99 %
Dissolved	7	76 to 113 %	96 %

These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified. These recoveries in simulation solutions were much higher than those reported above for MFBS.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions.

#### Results of the simulation tests

Type of Sim.	Time (hr)	Hard (a)	Sp. (b)	Food (c)	Acidifica. Method (d)	Dissolved Method (d)	% Diss. (e)	% Diss. (f)
1	1	50	FM	No	672.3	674.8	100.4	100.4
1	48	50	FM	No	672.7 (0)	678.1 (0)	100.8	100.9
1	96	50	FM	No	628.4 (-7)	621.5 (-8)	98.9	92.4
1	1	50	FM	No	7432	7469	100.5	100.5
1	48	50	FM	No	7098 (-4)	7296 (-2)	102.8	98.2
1	96	50	FM	No	7466 (0)	7477 (0)	100.2	100.6
1	1	50	FM	No	7180	7110	99.0	99.0
1	48	50	FM	No	7078 (-1)	7034 (-1)	99.4	98.0
1	96	50	FM	No	7395 (+3)	7348 (+3)	99.4	102.3
2	1	50	DM	YTC	60.05	56.18	93.6	93.6
2	48	50	DM	YTC	61.11 (+2)	60.18 (+7)	98.5	100.2
2	1	50	DM	YTC	573.2	587.3 (-2)	102.5	102.5
2	48	50	DM	YTC	579.0 (+1)	578.4 (0)	99.9	100.9
2	1	50	DM	YTC	2779	2859	102.9	102.9
2	48	50	DM	YTC	2709 (-3)	2690 (-6)	99.3	96.8

a = mg/L.

b = Species (FM = fathead minnow; DM = Daphnia magna).

c = Food (YTC = yeast-trout chow-cereal leaf).

d = ug/L; the numbers in parentheses are percent change from the concentrations at one hour.

e = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.

f = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

The 30 values for percent dissolved only ranged from 92.4 to 102.9 percent.

For both analytical methods, the concentrations at 48 and 96 hours seemed to be randomly higher and lower than those at one

hour, with the largest amount of change being 8 percent. The changes for the two methods seemed to be correlated.

To obtain an indication of whether sorption occurred, the test chambers were not conditioned before the simulations began and acid was added to the solutions remaining in the test chambers after the end of the simulations. The acidified solutions were mixed for 15 to 30 minutes and sampled. For each simulation (i.e., for each set of three test chambers), the ratio of the mean concentration of selenium in the acidified solution to the last mean concentration measured using the acidification method was calculated:

<u>Type of Simulation</u>	<u>Food</u>	<u>Mean Concentration<sup>a</sup></u>		<u>Ratio<sup>b</sup></u>
		<u>Before</u>	<u>After</u>	
1	No	628.4	679.5	1.08
1	No	7466.	7365.	0.99
1	No	7395.	7354.	0.99
2	YTC	61.11	56.74*	0.93
2	YTC	579.0	594.0	1.03
2	YTC	2709.	2736.	1.01

\* One value was considered an outlier and was not used.

<sup>a</sup> ug/L; n = 3 for each mean.

<sup>b</sup> The "after" mean concentration divided by the "before" mean concentration.

The volumes of the solutions remaining in the different test chambers were not measured and so it is not known how similar the volumes were. Thus the ratios can only give a qualitative comparison of the metal that was "desorbed" by adding acid to the remaining solution while it was still in the test chamber.

The addition of the acid caused a substantial change in the concentration of selenium in the remaining solution for only one of the six simulations.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

Type of Simulation	Hard. (mg/L)	Species (a)	Food (b)	Initial Conc. <sup>c</sup>	TWA % dissolved		
					1-hr <sup>d</sup>	48-hr <sup>e</sup>	96-hr <sup>f</sup>
1	50	FM	No	672.3	100.4	100.6	98.6
1	50	FM	No	7432	100.5	99.4	99.4
1	50	FM	No	7180	99.0	98.5	99.3
2	50	DM	YTC	60.05	93.6	96.9	----
2	50	DM	YTC	573.2	102.5	101.7	----
2	50	DM	YTC	2779	102.9	99.8	----
Mean TWA					99.8	99.5	99.1

<sup>a</sup> Species (FM = fathead minnow; DM = Daphnia magna).

<sup>b</sup> Food (YTC = yeast-trout chow-cereal leaf).

<sup>c</sup> Initial concentration (ug/L) measured by the acidification method.

<sup>d</sup> This is the percent dissolved at 1 hour.

<sup>e</sup> This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).

<sup>f</sup> This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

The 15 TWAs ranged from 93.6 to 102.9 percent. The mean TWA decreased slightly as the duration of the simulation increased. For each duration, the range of the TWAs was small and did not seem to depend on species or food. Because selenium(IV) is an oxyion, it is not expected to sorb or precipitate in toxicity tests with aquatic organisms and the percent dissolved is not expected to depend on hardness; the criterion for selenium(IV) is not hardness-dependent.

The purpose of the simulations with selenium was to determine whether the dissolved FACR should be the same as the total recoverable FACR, i.e., does the percent dissolved depend on (1) the concentration of selenium and/or (2) whether food is present.

a. The acute toxicity tests that were used in the derivation of the freshwater FACR for selenium(IV) were flow-through tests and 48-hr static tests. Therefore, the recommended conversion factor for the numerator of the FACR is 0.996, which is the average of 0.998 and 0.995.

b. Of the six freshwater chronic toxicity tests that were used in the derivation of the FACR, three were flow-through tests and three were renewal tests. Therefore, the recommended conversion factor for the denominator of the FACR is 0.996, which is the average of 0.998 and 0.995.

Thus the dissolved FACR equals the total recoverable FACR, and the conversion factor for the CMC equals the conversion factor for the CCC.



The freshwater CCC was based on field data from Belews Lake, and so conversion of the CCC from total recoverable to dissolved should also be based on data from Belews Lake. Data for the four samples presented by Cumbie (1978) in Table 11 indicate that an average of 92.2 percent of the selenium in the water column in Belews Lake was dissolved. Thus the recommended conversion factor for the CCC is 0.922. This applies to the selenium in the water column in Belews Lake; it is expected that both selenium(IV) and selenium(VI) were present.

No values for the percent dissolved are given for selenium(IV) in Attachment 2 of Prothro (1993).

## Appendix H: Nickel

The three kinds of results described in Section 3 are presented below for nickel; the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with nickel as follows:

- a. The concentration of nickel in Lake Superior water (LSW) was measured in the batch of LSW used to obtain the results reported below.
- b. Whenever the hardness of LSW was increased for a simulation with nickel, the alkalinity was also increased.
- c. The sodium bicarbonate solution was 0.05 N.
- d. Test chambers were not conditioned with simulation solution before simulations were begun.
- e. Samples for measurement of nickel were not passed through a plastic screen.
- f. Each solution that was sampled was stirred just before each individual sample was taken, e.g., the solution was stirred before a sample was taken for analysis using the acidification method and was stirred again before a sample was taken for analysis using the dissolved method.
- g. The solutions remaining in the test chambers at the end of the simulations were acidified to see if there was an indication of sorption onto the test chambers.

### Pre-simulation test results

1. The detection limit for nickel was determined to be 1.0 ug/L. The lowest concentration with which a simulation was to be performed with nickel was 15 ug/L, and so the detection limit was less than ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus nickel.
  - Soln 13: LSW plus daphnid food.
  - Soln 14: LSW plus daphnid food and nickel.
  - Soln 17: LSW (with nothing added).

The mean measured concentrations (in ug/L, with the standard deviations in parentheses) were:

Method	Solution				
	11	12	13	14	17
Acidification	< 1.0	12.54 (0.50)	< 1.0	12.26 (0.68)	< 1.0
Total Recoverable	< 1.0	14.10 (0.29)	< 1.0	14.42 (0.33)	< 1.0

- a. Solution 11 was used to determine method blanks. All blanks for both methods were less than 1.0 ug/L.
  - b. Neither method detected nickel in solution 13 or in solution 17.
  - c. Taking into account the blanks and standard deviations, the agreement between the results obtained using the two methods was not very good; the total recoverable method gave higher mean concentrations for solutions 12 and 14 than did the acidification method.
  - d. The results obtained with solutions 12 and 14 indicate that the daphnid food had negligible effect on results obtained using both methods.
  - e. The two coefficients of variation (COVs) obtained using the acidification method to analyze solutions 12 and 14 were 4.0 and 5.5 %, whereas the two COVs obtained using the total recoverable method to analyze the same solutions were 2.0 and 2.3 %.
3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
- Soln 21: Metal-free water.
  - Soln 22: Metal-free bicarbonate solution (MFBS).
  - Soln 23: MFBS plus nickel.
  - Soln 24: LSW plus nickel.
- a. For the replicate analyses of solutions 21 and 22 using both methods, all forty blanks were less than 1.0 ug/L. The blanks were sufficiently low.
  - b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 1.00, which was acceptable.
  - c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 0.94, which was acceptable.
  - d. For the replicate analyses of solutions 23 and 24, the COVs were 4.9 and 5.7 % for the acidification method and 6.2 and 3.2 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.
4. The COVs obtained with the three methods were:

Method	Number of COVs	Range (%)	Mean (%)
Total Recoverable	2	2.0 to 2.3	2.15
Dissolved	2	3.2 to 6.2	4.70
Acidification	4	4.0 to 5.7	5.02

Each of the COVs for the total recoverable method was lower than those for the dissolved and acidification methods.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun.

Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Two sets of QA samples were analyzed because the samples from the simulation tests were analyzed in two batches.
  - a. All blanks were less than 1.0 ug/L for both methods and were therefore acceptable.
  - b. The results of replicate analyses of MFBS spiked with nickel were:

Method	Number	Range (ug/L)	Mean (ug/L)	COV (%)
Acidification	8	12.11 to 13.18	12.58	2.9
	4	13.45 to 14.37	13.99	3.2
Dissolved	8	11.18 to 13.18	12.41	5.0
	4	13.29 to 14.69	14.11	7.1

The quotients of the dissolved mean divided by the acidification mean were 0.99 and 1.01. A quotient of 1.00 was reported above for spiked MFBS and a quotient of 0.94 was reported above for spiked LSW. The acidification method gave slightly lower COVs than did the dissolved method, and the COVs were similar to the pre-simulation test mean COVs.

- c. Recoveries in MFBS for the acidification method gave 99.4 and 80.1 %; recoveries in MFBS for the dissolved method gave 95.9, 87.5, 98.7, 110.8, and 104.5 %. These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.
2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	18	0.97 to 1.00	0.99
Dissolved	18	0.96 to 1.00	0.98

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	11	83 to 118 %	99 %
Dissolved	9	88 to 124 %	101 %

It is surprising that the range of these recoveries is so great considering that they were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified. The means, however, are close to 100 %. The range here is greater than that reported in section 1.c above.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions.

#### Results of the simulation tests

<u>Type of Sim.</u>	<u>Time (hr)</u>	<u>Hard (a)</u>	<u>Sp. (b)</u>	<u>Food (c)</u>	<u>Acidifica. Method (d)</u>	<u>Dissolved Method (d)</u>	<u>% Diss. (e)</u>	<u>% Diss. (f)</u>
1	1	50	FM	NO	2146	2152	100.3	100.3
1	48	50	FM	NO	2215 (+3)	2164 (+1)	97.7	100.8
1	96	50	FM	NO	2187 (+2)	2178 (+1)	99.6	101.5
1	1	50	FM	NO	21774	22648	104.0	104.0
1	48	50	FM	NO	21130 (-3)	21207 (-6)	100.4	97.4
1	96	50	FM	NO	22054 (+1)	21511 (-5)	97.5	98.8
2	1	50	DM	YTC	14.96	15.32	102.4	102.4
2	48	50	DM	YTC	12.94 (-14)	13.16 (-14)	101.7	88.0
2	1	50	DM	YTC	101.6	100.1	98.5	98.5
2	48	50	DM	YTC	101.5 (0)	101.0 (+1)	99.5	99.4
2	1	50	DM	YTC	490.4	486.2	99.1	99.1
2	48	50	DM	YTC	520.2 (+6)	518.8 (+7)	99.7	105.8
4	1	200	DM	YTC	17.06	17.00	99.6	99.6
4	48	200	DM	YTC	16.55 (-3)	16.72 (-2)	101.0	98.0

4	1	200	DM	YTC	113.1		112.0	99.0	99.0
4	48	200	DM	YTC	109.2 (-3)		109.9 (-2)	100.6	97.2
4	1	200	DM	YTC	491.4		482.6	98.2	98.2
4	48	200	DM	YTC	500.5 (+2)		498.2 (+3)	99.5	101.4

a = mg/L.

b = Species (FM = fathead minnow; DM = Daphnia magna).

c = Food (YTC = yeast-trout chow-cereal leaf).

d = ug/L; the numbers in parentheses are percent change from the concentrations at one hour.

e = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.

f = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

The 36 values for percent dissolved ranged from 88.0 to 105.8 percent.

For both analytical methods, the concentrations at 48 and 96 hours seemed to be randomly higher and lower than those at one hour, with the largest amount of change being 14 percent; the changes for the two methods seemed to be correlated.

To obtain an indication of whether sorption occurred, the test chambers were not conditioned before the simulations began and acid was added to the solutions remaining in the test chambers after the end of the simulations. The acidified solutions were mixed for 15 to 30 minutes and sampled. For each simulation (i.e., for each set of three test chambers), the ratio of the mean concentration of nickel in the acidified solution to the last mean concentration measured using the acidification method was calculated:

Type of Simulation	Food	Mean Concentration <sup>a</sup>		Ratio <sup>b</sup>
		Before	After	
1	No	2187.	2204.	1.01
1	No	22054.	21630.	0.98
2	YTC	12.94	15.81	1.22
2	YTC	101.5	114.4	1.13
2	YTC	520.2	565.0	1.09
4	YTC	16.55	19.39*	1.17
4	YTC	109.2	117.2	1.07
4	YTC	500.5	523.6	1.05

\* One value was considered an outlier and was not used.

<sup>a</sup> ug/L; n = 3 for each mean.

<sup>b</sup> The "after" mean concentration divided by the "before" mean concentration.

The volumes of the solutions remaining in the different test chambers were not measured and so it is not known how similar the volumes were. Thus the ratios can only give a qualitative comparison of the metal that was "desorbed" by adding acid to the remaining solution while it was still in the test chamber.

The ratios are higher for the solutions containing YTC, and they decrease as the concentration increases. The ratios were 1.09 and 1.05 for the two YTC simulations that contained the highest concentration of nickel, both of which showed an increase, not a decrease, by both analytical methods from 1 to 48 hours. Apparently, sorption occurred within the first hour of the simulation test, desorption occurred between 1 and 48 hours, and additional desorption occurred when acid was added to the solution remaining in the test chamber after the end of the simulation test.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

<u>Type of Simulation</u>	<u>Hard. (mg/L)</u>	<u>Species (a)</u>	<u>Food (b)</u>	<u>Initial Conc. c</u>	<u>TWA % dissolved</u>		
					<u>1-hr d</u>	<u>48-hr e</u>	<u>96-hr f</u>
1	50	FM	No	2146	100.3	100.6	100.8
1	50	FM	No	21774	104.0	100.7	99.4
2	50	DM	YTC	14.96	102.4	95.2	----
2	50	DM	YTC	101.6	98.5	99.0	----
2	50	DM	YTC	490.4	99.1	102.4	----
4	200	DM	YTC	17.06	99.6	98.8	----
4	200	DM	YTC	113.1	99.0	98.1	----
4	200	DM	YTC	491.4	98.2	99.8	----
Mean TWA					100.1	99.3	100.1

<sup>a</sup> Species (FM = fathead minnow; DM = Daphnia magna).

<sup>b</sup> Food (YTC = yeast-trout chow-cereal leaf).

<sup>c</sup> Initial concentration (ug/L) measured by the acidification method.

<sup>d</sup> This is the percent dissolved at 1 hour.

<sup>e</sup> This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).

<sup>f</sup> This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

The 18 TWAs ranged from 95.2 to 104.0 percent. The mean TWA did not seem to depend on the duration of the simulation. For each duration, the range of the TWAs was small and did not seem to depend on hardness, but did seem to depend slightly on food and/or species.

The acute toxicity tests that were important in the derivation of the freshwater criterion for nickel were flow-through, 48-hr static, and 96-hr static tests. Therefore, the recommended conversion factor for the CMC is 0.998, which is the average of 1.001, 0.993, and 1.001.

Of the five freshwater chronic toxicity tests that were used in the derivation of the criterion, three were renewal tests and two were flow-through tests. Therefore, the recommended conversion factor for the CCC is 0.997, which is the average of 1.001 and 0.993.

One of the percent dissolved values given for nickel in Attachment 2 of Prothro (1993) is 100 percent, but the other seven range from 76 to 93 percent.



## Appendix I: Cadmium

The three kinds of results described in Section 3 are presented below for cadmium; the methodology used to obtain these results is described in Section 2. The refinements made in the methodology during the project affected the work with cadmium as follows:

- a. The concentration of cadmium in Lake Superior water (LSW) was measured in the batch of LSW used to obtain the results reported below.
- b. Whenever the hardness of LSW was increased for a simulation with cadmium, the alkalinity was also increased.
- c. Two sodium bicarbonate solutions were used. One was 0.05 N; the other was 0.001 N with sufficient calcium sulfate added to give a hardness of 50 mg/L.
- d. Test chambers were not conditioned with simulation solution before simulations were begun.
- e. Samples for measurement of cadmium were not passed through a plastic screen.
- f. Each solution that was sampled was stirred just before each individual sample was taken, e.g., the solution was stirred before a sample was taken for analysis using the acidification method and was stirred again before a sample was taken for analysis using the dissolved method.
- g. The solutions remaining in the test chambers at the end of the simulations were acidified to see if there was an indication of sorption onto the test chambers.

### Pre-simulation test results

1. The detection limit for cadmium was determined to be 0.03 ug/L. The lowest concentration with which a simulation was to be performed with cadmium was 0.3 ug/L, and so the detection limit was ten percent of the lowest concentration.
2. The acidification method was compared with the total recoverable method by analyzing each of the following solutions six times by each method:
  - Soln 11: Metal-free water.
  - Soln 12: LSW plus cadmium.
  - Soln 13: LSW plus daphnid food.
  - Soln 14: LSW plus daphnid food and cadmium.
  - Soln 15: LSW plus fathead minnow food.
  - Soln 16: LSW plus fathead minnow food and cadmium.
  - Soln 17: LSW (with nothing added).

The mean measured concentrations (in ug/L, with the standard deviations in parentheses) were:

Method	Solution						
	11	12	13	14	15	16	17
Acidifica.	<0.03	0.195 (0.01)	<0.03	0.192 (0.01)	<0.03	0.190 (0.01)	<0.03
Tot. Reco.	<0.03)	0.280 (0.01)	<0.03	0.245 (0.01)	<0.03	0.277 (0.01)	<0.03

- a. Solution 11 was used to determine method blanks. Eight blanks were less than 0.03 ug/L; one acidification blank was 0.06 ug/L, and three total recoverable blanks were between 0.05 and 0.09 ug/L.
  - b. For solutions 13, 15, and 17, three of 18 acidification measurements and two of 18 total recoverable measurements were between 0.03 and 0.09 ug/L; the other 31 measurements were < 0.03 ug/L.
  - c. Taking into account the blanks and standard deviations, the total recoverable method consistently gave higher mean concentrations for solutions 12, 14, and 16 than did the acidification method.
  - d. The results obtained with solutions 12, 14, and 16 indicate that both foods had negligible effect on results obtained using both methods.
  - e. The three coefficients of variation (COVs) obtained using the acidification method to analyze solutions 12, 14, and 16 were 6.5, 3.7, and 3.0 %, whereas the three COVs obtained using the total recoverable method to analyze the same solutions were 3.4, 2.3, and 2.6 %.
3. The dissolved method was compared with the acidification method by analyzing each of the following solutions ten times by each method:
- Soln 21: Metal-free water.
  - Soln 22: Metal-free bicarbonate solution (MFBS).
  - Soln 23: MFBS plus cadmium.
  - Soln 24: LSW plus cadmium.
- The MFBS was 0.05 N sodium bicarbonate.
- a. For the replicate analyses of solutions 21 and 22 using both methods, all forty blanks were less than 0.03 ug/L. The blanks were sufficiently low.
  - b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 0.87, which was too low to be acceptable.
  - c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 0.87, which was acceptable.
  - d. For the replicate analyses of solutions 23 and 24, the COVs were 3.9 and 2.2 % for the acidification method and 3.6 and 4.0 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.

4. The dissolved method was again compared with the acidification method by analyzing each of the following solutions ten times by each method:

Soln 21: Metal-free water.

Soln 22: Metal-free bicarbonate solution (MFBS).

Soln 23: MFBS plus cadmium.

Soln 24: LSW plus cadmium.

This time the MFBS was 0.001 N sodium bicarbonate with sufficient calcium sulfate to give a hardness of 50 mg/L.

- a. For the replicate analyses of solutions 21 and 22 using both methods, all forty blanks were less than 0.03 ug/L. The blanks were sufficiently low.
- b. For the replicate analyses of solution 23, the quotient of the dissolved mean divided by the acidification mean was 1.00, which was acceptable.
- c. For the replicate analyses of solution 24, the quotient of the dissolved mean divided by the acidification mean was 1.00, which was acceptable.
- d. For the replicate analyses of solutions 23 and 24, the COVs were 2.5 and 3.0 % for the acidification method and 2.4 and 2.9 % for the dissolved method. Each COV was less than 20 % and was therefore acceptable.

Although the quotient of the dissolved mean divided by the acidification mean changed from 0.87 for 0.05 N sodium bicarbonate to 1.00 for 0.001 N sodium bicarbonate plus calcium sulfate, the quotient for Lake Superior water changed from 0.87 to 1.00 at the same time.

5. The COVs obtained with the three methods were:

Method	Number of COVs	Range (%)	Mean (%)
Total Recoverable	3	2.3 to 3.4	2.77
Dissolved	4	2.4 to 4.0	3.22
Acidification	7	2.2 to 6.5	3.54

The COVs for the three methods were similar.

These results indicate that the analytical methods were performed acceptably before the simulation tests were begun.

Checks of the acidification and dissolved methods during analyses of samples from the simulation tests

1. Three sets of QA samples were analyzed because the samples from the simulation tests were analyzed in three batches.
  - a. One blank was 0.4 ug/L and the other 33 blanks were less than 0.03 ug/L and were therefore acceptable for both methods.

- b. The results of replicate analyses of MFBS spiked with cadmium were:

<u>Method</u>	<u>Number</u>	<u>Range (ug/L)</u>	<u>Mean (ug/L)</u>	<u>COV (%)</u>
Acidification	8	0.235 to 0.336	0.293	14.4
	4	0.320 to 0.336	0.324	2.6
	5	0.269 to 0.303	0.296	5.0
Dissolved	8	0.235 to 0.320	0.288	11.3
	4	0.286 to 0.320	0.307	5.3
	5	0.269 to 0.332	0.295	8.9

The MFBS used was 0.001 N sodium bicarbonate with sufficient calcium sulfate added to give a hardness of 50 mg/L. The quotients of the dissolved mean divided by the acidification mean were 0.98, 0.94, and 1.00. A quotient of 1.00 was reported above for spiked 0.001 N MFBS and quotients of 0.87 and 1.00 were reported above for spiked LSW. The two methods gave similar COVs, which were slightly higher than the pre-simulation test mean COVs.

- c. Recoveries in MFBS for the acidification method gave 98.2 and 105.9 %; the only recovery in MFBS for the dissolved method gave 112.7 %. These recoveries were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified.

2. For each pair of duplicate analyses of a simulation solution, the lower value was divided by the higher value; the resulting quotients were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	14	0.91 to 1.00	0.98
Dissolved	14	0.95 to 1.00	0.99

3. The results of recoveries in simulation solutions were:

<u>Method</u>	<u>Number</u>	<u>Range</u>	<u>Mean</u>
Acidification	6	91 to 112 %	98 %
Dissolved	8	82 to 124 %	98 %

It is surprising that the range of these recoveries is so great considering that they were performed by spiking into acidified solutions; i.e., for the dissolved method the spike was added after the solution was filtered and acidified. The means, however, are close to 100 %. The

range here is greater than that reported in section 1.c above.

These results indicate that the analytical methods were performed acceptably during the analyses of the simulation solutions.

#### Results of the simulation tests

Type of Sim.	Time (hr)	Hard (a)	Sp. (b)	Food (c)	Acidifica. Method (d)	Dissolved Method (d)	% Diss. (e)	% Diss. (f)
1	1	50	FM	No	3.501	3.534	100.9	100.9
1	48	50	FM	No	3.343 (-5)	3.254* (-8)	97.3	92.9
1	96	50	FM	No	2.722 (-22)	2.647 (-25)	97.2	75.6
1	1	50	FM	No	31.15	31.26	100.3	100.3
1	48	50	FM	No	30.26 (-3)	29.96 (-4)	99.0	96.2
1	96	50	FM	No	30.38 (-2)	29.31 (-6)	96.5	94.1
2	1	50	DM	YTC	0.3280	0.3251	99.1	99.1
2	48	50	DM	YTC	0.2264 (-31)	0.2180 (-33)	96.3	66.5
2	1	50	DM	YTC	2.669	2.467*	92.4	92.4
2	48	50	DM	YTC	2.675 (0)	2.624 (+6)	98.1	98.3
3	1	50	FM	BS	0.3304	0.3202	96.9	96.9
3	48	50	FM	BS	0.3079 (-7)	0.3031 (-5)	98.5	91.7
3	1	50	FM	BS	3.722	3.737	100.4	100.4
3	48	50	FM	BS	2.870 (-22)	2.881 (-23)	100.4	77.4
4	1	200	DM	YTC	0.2724	0.2635*	96.7	96.7
4	48	200	DM	YTC	0.1709 (-37)	0.1449 (-45)	84.8	53.2
4	1	200	DM	YTC	3.340	3.031	90.8	90.8
4	48	200	DM	YTC	2.744 (-18)	2.615 (-14)	95.3	78.3

\* = One value was considered an outlier and was not used.

a = mg/L.

b = Species (FM = fathead minnow; DM = Daphnia magna).

c = Food (YTC = yeast-trout chow-cereal leaf; BS = brine shrimp nauplii).

d = ug/L; the numbers in parentheses are percent change from the concentrations at one hour.

e = These values for percent dissolved were calculated based on the concentrations at the same time measured using the acidification method.

f = These values for percent dissolved were calculated based on the concentrations at one hour measured using the acidification method.

The 36 values for percent dissolved ranged from 53.2 to 100.9 percent. The concentrations at 48 and 96 hours were lower than those at 1 hour for all except one case for both analytical methods. These decreases might have been due to precipitation, uptake by test organisms, and/or sorption onto test chambers. Precipitation could cause the dissolved measurements to be lower if equilibrium had not been achieved within the first hour and could cause the acidification measurements to be lower if the stirring did not resuspend all of the precipitate and/or did not keep it suspended during the sampling. Uptake is not likely important because fishes and daphnids do not bioconcentrate substantial amounts of cadmium.

To obtain an indication of whether sorption occurred, the test chambers were not conditioned before the simulations began and acid was added to the solutions remaining in the test chambers after the end of the simulations. The acidified solutions were mixed for 15 to 30 minutes and sampled. For each simulation (i.e., for each set of three test chambers), the ratio of the mean concentration of cadmium in the acidified solution to the last mean concentration measured using the acidification method was calculated:

<u>Type of Simulation</u>	<u>Food</u>	<u>Mean Concentration<sup>a</sup></u>		<u>Ratio<sup>b</sup></u>
		<u>Before</u>	<u>After</u>	
1	No	2.722	3.596	1.32
1	No	30.38	32.31	1.06
2	YTC	0.2264	0.4675	2.06
2	YTC	2.675	4.099	1.53
3	BS	0.3079	0.3792	1.23
3	BS	2.870	4.019	1.40
4	YTC	0.1709	0.3591*	2.10
4	YTC	2.744	4.200	1.53

\* One value was considered an outlier and was not used.

<sup>a</sup> ug/L; n = 3 for each mean.

<sup>b</sup> The "after" mean concentration divided by the "before" mean concentration.

The volumes of the solutions remaining in the different test chambers were not measured and so it is not known how similar the volumes were. Thus the ratios can only give a qualitative comparison of the metal that was "desorbed" by adding acid to the remaining solution while it was still in the test chamber.

The ratios are higher for the solutions containing daphnids and YTC, and the ratios decrease as the concentration increases for three of the four types of simulations. Both of these trends seem to be related to the loss of cadmium from the beginning to the end of the simulation tests.

### Interpretation of the Results

The interpretation of the results is described in Section 3. Based on the initial concentration measured by the acidification method, the time-weighted averages (TWAs) obtained for percent dissolved for each simulation were:

Type of Simulation	Hard. (mg/L)	Species (a)	Food (b)	Initial Conc. <sup>c</sup>	TWA % dissolved		
					1-hr <sup>d</sup>	48-hr <sup>e</sup>	96-hr <sup>f</sup>
1	50	FM	No	3.501	100.9	96.9	90.6
1	50	FM	No	31.15	100.3	98.2	96.7
2	50	DM	YTC	0.3280	99.1	82.8	----
2	50	DM	YTC	2.669	92.4	95.4	----
3	50	FM	BS	0.3304	96.9	94.3	----
3	50	FM	BS	3.722	100.4	88.9	----
4	200	DM	YTC	0.2724	96.7	75.0	----
4	200	DM	YTC	3.340	90.8	84.6	----
Mean TWA							
All					97.2	89.5	93.6
No food							
Hard = 50 mg/L					100.6	97.6	93.6
BS							
Hard = 50 mg/L					98.6	91.6	----
YTC							
Hard = 50 mg/L					95.8	89.1	----
Hard = 200 mg/L					93.8	79.8	----

<sup>a</sup> Species (FM = fathead minnow; DM = Daphnia magna).

<sup>b</sup> Food (YTC = yeast-trout chow-cereal leaf; BS = brine shrimp nauplii).

<sup>c</sup> Initial concentration (ug/L) measured by the acidification method.

<sup>d</sup> This is the percent dissolved at 1 hour.

<sup>e</sup> This TWA was calculated as the average of the percent dissolved values at 1 and 48 hours (see Section 3).

<sup>f</sup> This TWA was calculated by giving the value for percent dissolved at 48 hours twice the weight as the values for percent dissolved at 1 and 96 hours (see Section 3).

The 18 TWAs ranged from 75.0 to 100.9 percent. For all except one simulation, the TWA decreased as the duration of the simulation increased. The TWA also depended on food, hardness, and the concentration of cadmium. In four out of five comparisons at 48 and 96 hours, the TWA increased with the concentration of cadmium; at 1 hour, the opposite occurred for three out of four comparisons. The two exceptions concerning the concentration of cadmium both occurred with brine shrimp.

The acute toxicity tests that were important in the derivation of the freshwater criterion for cadmium were flow-through, renewal, 48-hr static, and 96-hr static tests. Because all of these are unfed tests and the percent dissolved is higher in unfed tests, the recommended conversion factor (CF) for the CMC is 0.973, which is the average of 1.006, 0.976, and 0.936. All of these were determined at hardness = 50 mg/L.

The chronic toxicity tests that were important in the derivation of the criterion were renewal and flow-through tests. They were all fed tests, but some were fed brine shrimp and some were fed YTC. The percent dissolved depended on the food used and on the hardness:

	<u>Mean TWAs</u>	<u>Average</u>
Hardness = 50 mg/L		
BS	0.986, 0.916	0.951
YTC	0.958, 0.891	0.924
Hardness = 200 mg/L		
YTC	0.938, 0.798	0.868
BS (predicted)		0.893*

$$* [0.951] [(0.868)/(0.924)] = 0.893$$

Therefore, the recommended CF for the CCC at hardness = 50 mg/L is 0.938, which is the average of 0.951 and 0.924, and at hardness = 200 mg/L is 0.880, which is the average of 0.868 and 0.893. It will be assumed that the following equation can be used to calculate the CF for the CCC at any hardness:

$$CF \text{ for the CCC} = 1.101672 - [(\ln \text{ hardness}) (0.041838)]$$

where:

(ln hardness) = the natural logarithm of the hardness.



It will also be assumed that the same slope describes the relationship between the CF for the CMC and hardness, so that the following equation can be used to calculate the CF for the CMC at any hardness:

$$\text{CF for the CMC} = 1.136672 - [(\ln \text{ hardness}) (0.041838)]$$

Two of the values for percent dissolved given for cadmium in Attachment 2 of Prothro (1993) are 41 and 59 percent, but the other ten range from 75 to 96 percent and are within the range of the TWAs reported here.

## Appendix J: Calculation of Time-Weighted Averages

If a sampling plan (e.g., for measuring metal in a treatment in a toxicity test) is designed so that a series of values are obtained over time in such a way that each value contains the same amount of information (i.e., represents the same amount of time), then the most meaningful average is the arithmetic average. In most cases, however, when a series of values is obtained over time, some values contain more information than others; in these cases the most meaningful average is a time-weighted average (TWA). If each value contains the same amount of information, the arithmetic average will equal the TWA.

A TWA is obtained by multiplying each value by a weight and then dividing the sum of the products by the sum of the weights. The simplest approach is to let each weight be the duration of time that the sample represents. Except for the first and last samples, the period of time represented by a sample starts halfway to the previous sample and ends halfway to the next sample. The period of time represented by the first sample starts at the beginning of the test, and the period of time represented by the last sample ends at the end of the test. Thus for a 96-hr toxicity test, the sum of the weights will be 96 hr.

The following are hypothetical examples of grab samples taken from 96-hr flow-through tests for two common sampling regimes:

Sampling time (hr)	Conc. (mg/L)	Weight (hr)	Product (hr) (mg/L)	Time-weighted average (mg/L)
0	12	48	576	
96	14	<u>48</u>	<u>672</u>	
		96	1248	1248/96 = 13.00
0	8	12	96	
24	6	24	144	
48	7	24	168	
72	9	24	216	
96	8	<u>12</u>	<u>96</u>	
		96	720	720/96 = 7.500

When all the weights are the same, the arithmetic average equals the TWA. Similarly, if only one sample is taken, both the arithmetic average and the TWA equal the value of that sample.

The calculations are more complex for composite samples and for samples from renewal tests. In all cases, however, the sampling plan can be designed so that the TWA equals the arithmetic average.

## Appendix K: Calculation of Conversion Factors

The three values for Percent Dissolved (PD) are calculated as:

$$PD(1) = \frac{\text{Dissolved Concentration at 1 hour}}{\text{Acidification Concentration at 1 Hour}}$$

$$PD(48) = \frac{\text{Dissolved Concentration at 48 hours}}{\text{Acidification Concentration at 1 Hour}}$$

$$PD(96) = \frac{\text{Dissolved Concentration at 96 hours}}{\text{Acidification Concentration at 1 Hour}}$$

The three Time-Weighted Averages (TWAs) are calculated as:

$$TWA(1) = PD(1)$$

$$TWA(48) = \frac{PD(1) + PD(48)}{2}$$

$$TWA(96) = \frac{PD(1) + 2 \cdot PD(48) + PD(96)}{4}$$

The equations for TWA(48) and TWA(96) are not exact because they both treat PD(1) as if it were PD(0). In the calculation of TWA(96), twice as much weight is given to PD(48) as to either PD(1) or PD(96).

The Conversion Factors (CFs) are calculated as unweighted means of various combinations of the three TWAs, depending on the kinds of toxicity tests that were important in the derivation of the CMC or CCC in the criteria document:

1. 48-hr static tests only:

$$CF = TWA(48) = \frac{PD(1) + PD(48)}{2}$$

2. 96-hr static tests only:

$$CF = TWA(96) = \frac{PD(1) + 2 \cdot PD(48) + PD(96)}{4}$$

3. Flow-through tests only:

$$CF = TWA(1) = PD(1)$$

4. 48-hr static tests and 96-hr static tests:

$$\begin{aligned}
 CF &= \frac{TWA(48) + TWA(96)}{2} \\
 &= \frac{\frac{PD(1) + PD(48)}{2} + \frac{PD(1) + 2 \cdot PD(48) + PD(96)}{4}}{2} \\
 &= \frac{3 \cdot PD(1) + 4 \cdot PD(48) + PD(96)}{8}
 \end{aligned}$$

5. 48-hr static tests and flow-through tests:

$$\begin{aligned}
 CF &= \frac{TWA(48) + TWA(1)}{2} \\
 &= \frac{\frac{PD(1) + PD(48)}{2} + PD(1)}{2} \\
 &= \frac{6 \cdot PD(1) + 2 \cdot PD(48)}{8}
 \end{aligned}$$

6. 96-hr static tests and flow-through tests:

$$\begin{aligned}
 CF &= \frac{TWA(96) + TWA(1)}{2} \\
 &= \frac{\frac{PD(1) + 2 \cdot PD(48) + PD(96)}{4} + PD(1)}{2} \\
 &= \frac{5 \cdot PD(1) + 2 \cdot PD(48) + PD(96)}{8}
 \end{aligned}$$

7. 48-hr and 96-hr static tests and flow-through tests:

$$\begin{aligned}
 CF &= \frac{TWA(48) + TWA(96) + TWA(1)}{3} \\
 &= \frac{\frac{PD(1) + PD(48)}{2} + \frac{PD(1) + 2 \cdot PD(48) + PD(96)}{4} + PD(1)}{3} \\
 &= \frac{7 \cdot PD(1) + 4 \cdot PD(48) + PD(96)}{12}
 \end{aligned}$$