ENVIRONMENTAL EFFECTS OF MANGANESE AND PROPOSED GUIDELINES TO PROTECT FRESHWATER LIFE IN BRITISH COLUMBIA

By

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ABSTRACT

Manganese is a naturally occurring substances that is present in surface waters and biota. Aquatic organisms have exhibited toxic responses to manganese in surface waters and regulatory bodies in some jurisdictions have established guidelines for levels of manganese in surface water to protect aquatic life. In British Columbia, a guideline of 0.1 mg/L was established by the Ministry of Environment, Lands and Parks, although it was recognized that the scientific data on which this guideline was based were weak. Toxicity tests applicable to aquatic life in B.C. waters were commissioned to strengthen the relevant data base and to apply the British Columbia procedures for deriving water quality criteria in an effort to establish more defensible guidelines for the protection of aquatic life in B.C. Acute and chronic toxicity tests were conducted on fish, invertebrates and freshwater algae. Acute tests included 48 and 96 hour LC50's, while chronic tests included reproduction, growth and survival endpoints. A range of organisms was chosen in order to evaluate the range of sensitivities to manganese.. The possible relationship between water hardness and toxicity to manganese was also investigated at water hardnesses of 25, 100 and 250 mg/L $CaCO_3$.

Data were also gathered from literature sources in support of the new toxicity information. Both acute and chronic studies were identified for fish species resident in B.C. fresh waters. The collective data were evaluated for suitability with respect to the B.C. water quality guideline derivation process. Toxicity test data that met the requirements for use in guideline derivation were screened for sensitivity in order to fulfill the objective of developing a guideline protective of the most sensitive aquatic organisms.

A pattern emerged whereby the concentrations of manganese at which adverse effects were observed increased with increasing water hardness. This pattern was identified in both the literature data and in all but one of the new toxicity tests commissioned by the Ministry of Environment, Lands and Parks. Acute and chronic regression equations were developed using the most sensitive data for various (in both cases six) water hardness values. The acute equation was $Y = 0.0441X + 1.81$ and the chronic equation was $Y = 0.0176 + 2.42$, where X = water hardness in mg/L $CaCO_3$ and Y = Mn concentration in mg/L. The equations were used to predict manganese concentrations at water hardness increments of 25 mg/L $CaCO_3$ over the hardness range of 25-325 mg/L $CaCO_3$, a range that encompasses the vast majority of B.C. surface waters. A factor of safety of 0.25 was applied to the predicted concentrations to account for uncertainty and was based on scientific judgement and the strength of the data set used in the derivation process. The resulting acute manganese concentrations ranged from 0.6 to 3.8 mg/L and are proposed as guidelines for exposure of ≤96 hours. The resulting chronic manganese concentrations ranged from 0.6 to 1.9 mg/L and are proposed as guidelines for exposure exceeding 96 hours. While B.C. and other surface water data indicate that manganese rarely exceeds concentrations of 1 mg/L, it is recognized that natural events may result in periodic increases. The application of guidelines intended to protect aquatic life from anthropogenic sources of manganese should reflect this in the sampling methodology requirements.

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1.0 INTRODUCTION

Manganese is a metallic element that occurs naturally in rock and soils/sediments weathered from rock. It is most abundant in areas of metamorphic and sedimentary rock. Dissolution from rock and soils/sediments into ground and surface waters has resulted in the presence of varying levels of manganese in natural waters. The Canadian Water Quality Guidelines (CCME, 1987) lists a range of manganese concentrations of 0.01-1.70 mg/L for Pacific Region surface waters. High concentrations of dissolved manganese have been observed in many coastal areas, including the Lower Mainland, Vancouver Island and the Queen Charlotte Islands (SEACOR, 1998).

Adverse effects of manganese on freshwater aquatic organisms have been reported in a number of studies, although the cause-effect evidence is not extensive. In order to lay the foundation for the establishment of scientifically based guidelines for the protection of aquatic life in British Columbia, a number of toxicity tests were initiated using representative freshwater and marine organisms present in B.C. waters. The studies were commissioned by the Water Management Branch of the B.C. Ministry of Environment, Lands and Parks and were conducted by Environment Canada at their Pacific Environmental Science Center aquatic toxicity laboratory in North Vancouver, B.C.

1.1 OBJECTIVES

The primary purpose of the toxicity testing was to provide new ecological toxicity data for British Columbia freshwater and marine organisms and to use the data to develop scientifically based, defensible guidelines for the protection of aquatic life. Use of species native to British Columbia waters should result in guidelines that are more applicable to B.C. waters. Evaluation and interpretation of this data has provided new research information on the concentration/effects relationship of manganese on aquatic organisms that are present in B.C. waters. This thesis research concentrates on freshwater organisms rather than marine organisms.

The objectives of the research presented in this thesis are as follows:

1. To review the existing freshwater aquatic life guideline for manganese;
2. To evaluate the practicality of the existing guideline;
3. To review the information available in the literature on manganese toxicity in aquatic environments; and
4. To use new toxicity test data generated by the B.C. Ministry of Environment, Lands and Parks for native B.C. species and information gathered in Step 3 in order to improve the existing freshwater aquatic life guideline.

1.2 REGULATORY BASIS OF EXISTING GUIDELINE

The B.C. Ministry of Environment, Lands and Parks has a mandate (under the Environment Management Act and the Guideline and Standard Procedure Policy) to establish water quality guidelines to protect water quality in B.C. The Canadian Council of Ministers of Environment (CCME) develops water quality guidelines at the national level to protect the Canadian environment and publishes Canadian Water Quality Guidelines (CCME, 1987 with updates) for inorganic and organic parameters based on various water uses including drinking water, irrigation, and aquatic life support. BCMELP will adopt CCME guidelines as working values for parameters for which no B.C. guidelines exist.

The B.C. Ministry of Environment, Lands and Parks published the document *Approved and Working Criteria for Water Quality – 1995* (BCMELP 1995) which compiled water quality criteria for various substances and several water uses, including aquatic life. For manganese, values were recommended for drinking water, food processing, fresh water aquatic life, marine water aquatic life, irrigation water and industrial uses including boilers, textiles, pulp and paper, tanning, chemical production and cooling. Recommended values of 0.1 to 1 mg/L were provided for fresh water aquatic life and dissolved manganese and manganese precipitates were the important forms to consider.

At present, B.C. guidelines for manganese are tentative and under review. CCME guidelines for manganese exist for water used for human consumption and for irrigation watering but no guidelines exist for the protection of aquatic life. The drinking water guideline of 0.05 mg/L was not toxicologically based; it was established to address aesthetic considerations such as staining of plumbing and laundry and undesirable taste. The irrigation water guideline of 0.2 mg/L was applied to continuous watering on all soil types specifically to protect possible toxic responses by plants growing in acidic soils. A guideline of 10 mg/L was recommended for neutral and alkaline soils for water use of up to twenty years. Data regarding toxic effects on aquatic life were not considered sufficient to recommend a guideline.

1.3 SCIENTIFIC BASIS OF EXISTING GUIDELINE

The source for the recommended manganese fresh water aquatic life values of 0.1 to 1 mg/L was the United States Environmental Protection Agency (National Academy of Sciences, 1973). A document titled *A Review of the EPA Red Book: Quality Criteria for Water* (Thurston et. al., 1979) reviewed many of the existing water quality criteria, including manganese. The chapter on manganese raised several questions regarding the scientific basis for the EPA guidelines and stated that the *Red Book's* description of the effects of manganese on aquatic life "is inadequate, of little value to aquatic biologists, generally out of date, lacks completeness, and seldom cites the available literature." This suggests that the existing water quality criteria for aquatic life protection are not soundly based.

Review of data generated from research conducted since 1979 in conjunction with the BC.MELP toxicity tests, is expected to provide new information to enhance the data used by EPA to establish the Red Book guidelines on which the BCMELP criterion was based. Review of new literature information is one of the objectives of this thesis research and is presented in the Literature Review section.

2.0 LITERATURE REVIEW

2.1 NATURAL OCCURRENCE OF MANGANESE

Manganese comprises approximately 0.085% to 0.095% of the earth's crust and is a component of many rock types, particularly those of metamorphic and sedimentary origin (CCME, 1987). It is associated with iron ores of submarginal concentration; the predominant ores of manganese include pyrosulite (MnO_2), manganite ($Mn_2O_3 \cdot H_2O$), hausmannite (Mn_3O_4), psilomelane and rhodochrosite ($MnCO_3$) (CCME, 1987; Moore, 1991). Ferromanganese minerals such as biotite mica and amphiboles contain large amounts of manganese and manganese-rich nodules have been identified on the sea floor in conjunction with cobalt, nickel and copper (CCME, 1987; Moore, 1991). Important natural sources of manganese include soils, sediments and metamorphic and sedimentary rocks.

Manganese occurs in soil as a result of weathering of rock containing manganese during the process of pedogenesis. A broad range of naturally occurring manganese concentrations in soil has been observed. The B.C. Ministry of Environment, Lands and Parks (1998a) has collected data on uncontaminated British Columbia soils for various regions of the province. A summary of this data is presented in Table 2.1, as follows:

TABLE 2.1: MANGANESE CONCENTRATIONS IN BRITISH COLUMBIA SOILS - 0 to 60 cm

Region	Sample Size	Concentration (µg/g)			
		Minimum	Maximum	Mean	Median
Vancouver Island	72	38	8620	1359	660
Lower Mainland	64	4.4	679	284	272
Greater Vancouver I	56	12	2220	400	289
Greater Vancouver II	80	3.8	2044	436	320
Southern Interior	72	280	1380	618	544
Kootenays	56	102	1710	428	342
Omineca Peace	56	28	2610	447	336
Skeena	48	2.2	2306	570	482
Cariboo	24	274	690	461	456

The data in Table 2.1 illustrate the broad range of concentrations of manganese that occur in British Columbia soils. Notable regional differences are apparent in the data, with concentrations in Vancouver Island soils significantly higher than those in other regions. Regional mean concentrations varied from 284 µg/g to 1359 µg/g while median concentrations (50th percentile) varied from 272 µg/g to 660 µg/g. Although the samples were obtained from a variety of locations within each region, samples were typically collected in or near areas of settlement and from native rather than fill soils. The size of and geologic variability within each region may limit the degree to which the data are representative on a region-wide basis. However, the data do provide valuable information regarding the range of manganese concentrations that occur in British Columbia soils.

The natural presence of manganese in rock and soil provides a source of manganese that may dissolve in ground and surface waters or may erode and deposit as sediment, with the subsequent potential for dissolution. Manganese accumulated in plant material will also provide a source for dissolution during decomposition. Manganese solubility increases at low pH and under reducing conditions and is most commonly in the 2+ and 4+ oxidation states in aquatic systems (Clement Associates, 1985). The presence of high concentrations of chlorides, nitrates and sulphates may increase manganese solubility, increasing both aqueous mobility and uptake by plants (Clement Associates, 1985). Manganese precipitates out in sediment mainly as Mn4+ and re-solubilizes in the water column mainly as Mn2+ (Moore, 1991).

Dissolved concentrations of manganese in natural waters that are essentially free of anthropogenic sources/influences range from <0.01mg/L to >10 mg/L (McNeely et. al., 1979). Manganese concentrations in natural surface waters seldom reach 1.0 mg/L and are usually less than 0.2 mg/L, while seawater typically contains approximately 2 µg/L of manganese (McNeely et. al., 1979). Environment Canada data for the period of 1980- 1985 for the Pacific Region (CCME, 1991) and data from the B.C. Ministry of Environment, Lands and Parks (1998b) are summarized in Table 2.2:

TABLE 2.2: TOTAL MANGANESE IN B.C. SURFACE WATERS

Region	Total Mn Concentration (mg/L)	No. of Samples
Pacific Region	0.01-1.70	155 samples
Cariboo/Omineca/Peace	0.002 - 1.53	1000 samples ±
Thompson	<0.001 - 0.56	500 samples ±

Total manganese concentrations in surface water showed a typical seasonal trend, with the highest annual manganese concentrations observed during high runoff periods (e.g. spring snow melt period for B.C. Interior streams) and lower concentrations observed during periods of stable stream flow. Concentrations in stream waters were higher than concentrations in lakes and concentrations in streams downstream of lakes were lower than concentrations in other streams. These trends are in keeping with expected results as higher suspended sediment (and consequent higher manganese) loads typically occur during higher runoff periods and in flowing water. Concentrations in excess of 1.0 mg/L were rare in the BCMELP data set.

2.2 MAN-MADE SOURCES OF MANGANESE

Manganese is used in industrial processes and in various consumer products. The major man-made sources of environmental manganese include municipal wastewater discharge, sewage sludge, emissions generated during alloy, steel and iron production, and to a lesser extent emissions from the combustion of fuel additives (Moore, 1991; Jaques, 1987). Worldwide anthropogenic input of manganese to freshwater is summarized in the following table (Nriagu et. al., 1988).

TABLE 2.3: ANTHROPEGENIC SOURCES OF MANAGNESE TO FRESHWATER			
Source	Estimated Input (10^3 tonnes/year)	Source	Estimated Input (10^3 tonnes/year)
Domestic Wastewater	58-171	Metals Manufacturing	2.5-20
Sewage Sludge Disposal	32-106	Chemicals Manufacturing	2-15
Iron/Steel Refining	14-36	Pulp and Paper Production	<0.1-1.5
Non-ferrous Metal Refining	2-15	Steam Electric Production	5-18
Base Metal Mining/Dressing	0.8-12	Atmospheric Fallout	3.2-20

The primary manmade sources of atmospheric manganese worldwide are secondary non-ferrous metal production, coal burning and municipal waste incineration (Moore, 1991). Incineration of sewage sludge was estimated to be the third largest worldwide anthropogenic source of manganese emissions to the atmosphere in 1983 (Moore, 1991). Environment Canada estimated that 1984 emissions of manganese in Canada totaled 1225 tonnes, of which 47% resulted from ferromanganese and silico-manganese production (all in Quebec), 28% resulted from iron and steel production (mainly in Ontario and to a lesser extent Quebec) and 17% resulted from gasoline-powered motor vehicle emissions (Jaques, 1987). In British Columbia, total emissions were estimated at 31 tonnes, with 27 tonnes originating from gasoline powered vehicles (Jaques, 1987).

Although it is not known whether manganese emissions from sources other than gasoline powered vehicles have increased significantly in British Columbia since 1984, it seems probable that vehicle emissions continue to be the major source of manganese emissions in the province. Manganese additives in gasoline are the source of manganese in vehicle emissions. Methylcyclopentadienyl manganese tricarbonyl, or MMT, is the main additive containing manganese (approx. 24.4% by weight); the additives LP62 (containing 62% MMT) and LP 46 (containing 46% MMT) are also common (Jaques, 1987). The main benefits of MMT addition to gasoline are octane enhancement and suppression of smoke during combustion. The recommended Canadian limit for MMT in gasoline is 18 mg Mn/L. Based on the emissions information provided by Environment Canada, it would not appear that MMT is a significant source of environmental manganese. This may be borne out by the soils data for Greater Vancouver and the Lower Mainland (see Table 2.1), the area with the greatest urban population and concentration of automobiles. Manganese concentrations in the upper 60 cm of soil from these areas had the lowest median concentrations in the province and maximum individual sample concentrations were low as compared to many other regions.

2.3 FUNCTIONS/ESSENTIALITY OF MANGANESE IN BIOTA

CCME (1987) reports that manganese is an essential trace element for microorganisms, plants and animals and is present in almost all organisms. Manganese in plant tissues mainly occurs in nuts, seeds, whole grains (particularly the bran and germ), legumes, dark leafy green vegetables and alfalfa; egg yolks, black tea and coffee beans also contain significant manganese (Haas, 1998; Klassen, 1996). Manganese content in plant tissue is largely dependent on sufficient manganese content in the soils in which the plants grow.

Manganese activates an essential part of enzyme systems that metabolize proteins and energy in all animals; manganese is also involved in the formation of mucopolysaccharides needed for healthy joint membranes (Haas, 1998). It concentrates in the mitochondria and is present in higher concentrations in tissues rich in mitochondria. Manganese concentrations in fish tissue were found to be higher in liver and gill tissue than in muscle tissue (Legoburu et. al., 1988). In humans, manganese is involved in the digestion and absorption of food through peptidase activity, in the synthesis of cholesterol and fatty acids, in glucose metabolism and in the use of biotin, thiamine, vitamin C and choline (Haas, 1998). In the divalent state (Mn++), it also appears to provide protection against oxygen free radicals as part of the enzyme superoxide dismutase (Haas, 1998). A daily allowance of 1.2 mg of manganese has been recommended for humans and information appears to indicate that insufficient manganese may result in inhibited carbohydrate metabolism and impaired insulin production, while excess manganese may inhibit iron absorption (Moore, 1991).

2.4 FRESHWATER AQUATIC TOXICITY DATA IN LITERATURE

Studies pertaining to the toxicity of manganese to various fresh water organisms were researched to determine the breadth and applicability of existing data. Although the number of studies that evaluated manganese toxicity to aquatic organisms was not extensive, a few studies provided important information to supplement the new information generated by BCMELP and presented in Section 4 of this thesis. For ease of presentation, studies that are applicable to species that exist in B.C. waters have been separated from species not present in B.C. waters.

2.4.1. Studies on Species Present in B.C. Waters

A summary of aquatic toxicity literature data for species present in B.C. fresh water is provided in Table 2.4.

TABLE 2.4: LITERATURE DATA SUMMARY

Organism	Toxicity Test	pH	Temperature (°C)	Dissolved Oxygen (mg/L)	Hardness (mg/L $CaCO_3$)	Mn Conc. (mg/L)
D. magna[1]	48 Hour LC50	OECD	OECD	OECD	ASTM Hard Water	4.7-56.1
Rainbow Trout[2]	96 Hour LC50	7.53	14.3	7.73	34.0	4.83
Brown Trout[2]	96 Hour LC50	7.54	14.4	7.63	38.0	3.77
Rainbow Trout[2]	4 Month Chronic	7.53	14.3	7.73	34.0	0.79
Brown Trout[2]	4 Month Chronic	7.54	14.4	7.63	38.0	2.7
Brown Trout[3]	62 day Chronic	7.6 7.9 7.8	12±1 12±1 12±1	7.8 8.7 9.0	30 150 450	4.67 (IC25) 5.59 (IC25) 8.68 (IC25)

Note: OECD – Organization of Economic Cooperation and Development, 1981
ASTM – American Society for Testing and Materials, 1980
1 – Baird et. al., 1991
2 – Davies and Brinkman, 1994
3 - Stubblefield et. al., 1997
IC25 – Statistically derived concentrations at which 25% of organisms are inhibited for the exposure endpoint in the study (e.g. growth, reproduction, hatching success) vs. controls

Baird et. al. (1991) evaluated six clones of Daphnia magna to determine the differences in acute toxic response between genotypes to nine different chemicals, including manganese. Measured ionic concentrations used in the studies ranged from 1-100 mg/L for Mn 2+ as manganese chloride. Daphnia species 14 day reproduction testing methodology outlined by OECD (1981), which included an acute immobilization test, was employed and the measured effect was lethality as evidenced by immobility. Hard water, as defined by the ASTM (1980), was used to culture the organisms. No hardness value was reported.

The resulting EC_{50} (concentrations at which effects were observed in 50% of organisms vs. controls) data were converted to normal density functions with relative frequency (i.e. fraction or percentage of occurrence) plotted against concentration. The EC_{50} values represented the midpoints of the density functions. The EC_{50} values presented in the report for the six genotype clones ranged from a minimum of 4.7 mg/L to a maximum of 56.1 mg/L. In the absence of raw data, the lowest concentration for which a toxic response was observed was extrapolated from the probability density plots. A value of approximately 3 mg/L resulted and this value was associated with the plot having an EC50 of 4.7 mg/L. In the context of a freshwater aquatic life guideline, the relevance of particular genotypes may be little more than recognition of the most sensitive genotype and the associated EC_{50} concentration, thus ensuring a conservative and ecologically protective approach.

Baird et. al. (1991) concluded that genotypes of Daphnia magna exhibited a considerable range of EC_{50} concentrations with no concordance between genotype response for the different chemicals. Genotypes that were the most sensitive to one chemical may have been the least sensitive to a second chemical and lie near the middle of the response results for a third chemical, with no pattern emerging.

The recent study by Stubblefield et. al. (1997) focussed on brown trout, a species that is present in British Columbia in localized waters. The objectives of the study were to "determine the toxicity of manganese to early life stages of brown trout, to evaluate the hardness-toxicity relationships and to provide data useful in developing a protective manganese criterion." The hardness-toxicity relationship was evaluated by testing several manganese concentrations at water hardness values of 30, 150 and 450 mg/L $CaCO_3$. The life stages utilized in the study included fertilized eggs and larvae/fry. A summary of the materials and methods applied during this study follows.

Measured amounts of manganese chloride (Mn $CL_2 \cdot 4H_20$) were dissolved in de-ionized water to prepare the test solutions. Reservoir water with a hardness of 30 mg/L $CaCO_3$, well water with a hardness of 450 mg/L $CaCO_3$, and a mixture of the two water sources to obtain a hardness value of 150 mg/l $CaCO_3$ were used. Seven nominal manganese concentrations were tested at each of the three hardness values, with dissolved concentrations analyzed weekly. The toxicity testing methodology was based on ASTM Method E1241-92

(ASTM, 1993). Mean dissolved concentration ranges to which organisms were exposed were 0.43 to 15.15 mg/L for a hardness of 30, 2.84 to 71.95 mg/L for a hardness of 150 and 2.41 to 93.36 mg/L for a hardness of 450. The dissolved manganese concentrations used for the control groups were all <0.02 mg/L.

For each test, fifteen randomly chosen embryos were placed in 2.2 litres of test solution contained in a glass aquarium. Each test was repeated four times for a total of sixty organisms per treatment. Temperature was maintained at 12±1°C and the total duration of the tests was sixty-two days. Mean organism wet weights were measured and statistically evaluated to compare hatching success, survival and growth versus controls for each of the tests. The lowest observable effect concentration or LOEC was established as the lowest concentration for which a statistically significant effect was observed versus controls. The no observable effect concentration or NOEC was established as the highest concentration for which no statistically significant effect was observed. Although some discussion regarding statistical testing applied during the study was provided in the text, it was not clear what constituted "statistically significant."

The main findings reported by Stubblefield et. al. (1997) were as follows:

1. Hatching success varied from 86.6% to 98.2% and was not generally affected by exposure to manganese at the test concentrations used. The mean time to hatch decreased for the highest manganese concentrations at hardness values of 150 and 450 mg/L $CaCO_3$.
2. Survival of larvae decreased with increasing manganese concentrations for each of the test hardness values. Dissolved manganese LOEC values for organism survival (not growth) were determined to be 7.38 mg/L for a hardness of 30 mg/L $CaCO_3$, 8.81 mg/L for a hardness of 150 mg/L $CaCO_3$ and 16.21 mg/L for a hardness of 450 mg/L $CaCO_3$
3. For each water hardness tested, organism mortality was observed sooner at higher dissolved manganese concentrations and in general, increased manganese concentration equated to increased mortality.
4. Reductions in growth, as indicated by decreased body weights, were observed at significantly lower dissolved manganese concentrations than the concentrations affecting survival and thus growth was determined to be a more sensitive exposure endpoint. Dissolved manganese LOEC values based on organism body weight (not survival) were 4.41 mg/L for a hardness of 150 mg/L $CaCO_3$ and 8.68 mg/L for a hardness of 450 mg/L $CaCO_3$.
5. IC25 values (interpolated concentrations at which a measurable biological response would be anticipated in 25% of organisms) for dissolved manganese were determined to be 4.67 mg/L at a hardness of 30 mg/L $CaCO_3$, 5.59 mg/L at a hardness of 150 mg/L $CaCO_3$ and 8.68 mg/L at a hardness of 450 mg/L $CaCO_3$.

In the discussion section of the paper, the authors stated that the current study results confirmed previous results that indicated a relationship between water hardness and manganese toxicity. Brown trout embryos were found to be tolerant of dissolved manganese at the concentrations analyzed. Although some decreases in mean time to hatching were observed, the ecological importance of this observation was not clear to the authors and hardness did not appear to have affected hatching success.

IC25 concentrations were found to increase with increasing water hardness and were greater than the statistically derived NOEC values for all three water hardnesses and less than the LOEC values for water hardnesses of 150 and 450 mg/L $CaCO_3$. A LOEC value was not determined for a hardness of 30 mg/L $CaCO_3$ due to a statistically insignificant difference between the test organisms and the control groups. Stubblefield et. al. (1997) recommended the use of IC25 values over NOECs and LOECs. They based this recommendation on the fact that, by definition, the NOEC and LOEC values must be two of the test solution concentrations and the values are dependent on statistical testing which may or may not determine a biological response to be significant. Use of interpolated values such as an IC25 provides a means of evaluating concentration response data based on an acceptable level of effect without the constraints of pre-set concentrations where the effect concentration is determined by the initial test concentrations.

An equation to calculate hardness-based IC25 values is provided by the study. The equation, which was determined by plotting the IC25 values from the study against the natural logarithms of the water hardness values, is shown below:

$$IC25_{(\text{at specified hardness})} = e^{0.2064(\ln \text{hardness}) + 7.7092}$$

The regression analysis used to develop the equation had a reported positive correlation of $r^2 = 0.88$. The authors concluded that "the data presented here provide a basis upon which to estimate the potential adverse effects of chronic manganese exposure to salmonid species" and "in conjunction with acute and chronic data from other species, can be used to derive standards protective of aquatic organisms."

The study also quotes unpublished toxicity test data from which IC25 values of 5.71 and 5.15 mg Mn/L were derived for C. dubia at a water hardness of 50 mg/L $CaCO_3$. These values are fairly consistent with the 4.67 mg/L dissolved manganese IC25 concentration determined for brown trout at a hardness of 30 mg/L $CaCO_3$.

Davies and Brinkman (1995) studied the acute toxicity of manganese to brown trout in hard water using 96 hour LC50 tests. Eggs from Colorado's Delaney Butte Reservoir and fingerlings from LaPorte Colorado's Bellevue Research Hatchery were collected for the study. Fish were placed in 92 litre aquaria filled with water sourced from a well, with water quality characteristics determined using American Public Health

Association (1985) methodology. Manganese as $MnSO_4 \cdot H_2O$ was used in the testing, with nominal concentrations of 0.0, 15.0, 27.0, 54.0, 84.4, 112.5 and 150.0 mg Mn/L chosen for analysis. The summarized materials and methods presented in the referenced document indicated that dissolved oxygen was measured using a YSI Model 58 section meter; the number and/or frequency of dissolved oxygen measurements were not identified. Manganese concentrations were measured on a daily basis using grab samples and atomic absorption spectrophotometry. Water hardness was measured in control tanks only, the authors citing interferences from manganese in the other tanks as the reason. Organism mortality was evaluated every second hour during the day (what constitutes "the day" was not defined) during the first 96 hours. Median LC50 concentrations were estimated by applying probit analysis and the Spearmen-Karber method (Hamilton et. al., 1978).

The mean water quality characteristics determined from the control water sampling are summarized below:

Hardness	454 mg/L $CaCO_3$	(1 sample)
Alkalinity	311 mg/L $CaCO_3$	(7 samples)
pH	8.00	(7 samples)
Dissolved Oxygen	7.65 mg/L	(7 samples)
Temperature	16.76 °C	(7 samples)

The average fork tail length and weight of brown trout used in the study were 6 mm and 18.91 gm, respectively. Table 2.5 presents measured manganese concentrations and 96 hour acute mortality data.

TABLE 2.5: BROWN TROUT 96 HOUR LC50

Water Hardness = 454 mg $CaCO_3$/L

Exposure No.	1	2	3	4	5	6	Control
Mn Concentration (mg/L)	166.8	118.9	83.97	47.90	30.25	13.06	<0.02
96 Hour Mortality (%)	100	100	95.0	45.0	5.0	0	0

The median 96 hour LC50 concentration estimated from the experiment was 49.9 mg Mn/L for the probit analysis and the Spearman-Karber method. The 95% confidence intervals about the mean were 43.6-57.4 mg Mn/L for probit analysis and 43.5-57.3 mg Mn/L for the Spearman-Karber method. Very good agreement between the two methods of estimating mean LC50 values was noted by the researchers.

The reported hardness value of 454 mg/L $CaCO_3$ was based on a single measurement. However, seven alkalinity measurements resulted in a mean concentration of 311 mg/L $CaCO_3$, with a standard deviation of 2.60. Based on the low standard deviation value of 2.60, it is probable that water hardness values did not deviate significantly from the measured value.

Davies and Brinkman (1994) also completed acute and chronic studies of the effects of manganese on rainbow trout and brown trout in soft water. Exposed and unexposed test organisms were utilized to determine what effect pre-exposure to low levels of dissolved manganese may have on tolerance during acute and chronic exposures at higher concentrations. Eyed rainbow trout eggs were placed in "relatively soft water" (actual water hardness was not defined by the researchers) at a temperature of 6°C for a four day period to acclimate. Brown trout fingerlings were similarly placed in aquaria containing 6°C soft water and allowed to acclimate for two weeks. The "exposed" test organism groups were subjected to manganese (added as manganous sulphate) concentrations of 0.14 mg/L through Day 2, 0.36 mg/L through Day 5 and 0.80 mg/L for four months. Water quality conditions were the same for the "unexposed" test organisms, with the exception that no manganese was added to the water. Rainbow egg and sac fry mortality were observed daily; the researchers reported no difference in egg and sac fry mortality between the "exposed" and "unexposed" groups. For brown trout, mortality in both groups was reported as negligible. No numerical data (i.e. mortality or survival rates) were presented in the report.

Following the initial exposure period, 96 hour LC50 acute and four month chronic toxicity tests were conducted on surviving organisms; exposure endpoints for the chronic tests included mortality and length/weight of survivors. For "exposed" and "unexposed" rainbow trout, separate aquaria containing water with very similar characteristics were used to conduct the testing. Seven nominal dissolved manganese concentrations, including a control solution containing no detectable manganese, were used in the experiment. For "exposed" and "unexposed" brown trout, sub-groups of twenty fish were placed in each of seven aquaria, with the adipose fin clipped from the "exposed" fish for identification. Each aquarium contained a different dissolved manganese concentration. Fish were not fed during the acute toxicity testing and dissolved manganese concentrations in the aquaria waters were confirmed daily by analyzing samples using atomic absorption spectrophotometry. Temperature, alkalinity, pH, conductivity and dissolved oxygen levels were measured using American Public Health Association (19850 methods. Hardness was measured in the control aquaria only (the researchers cited manganese interference in the other aquaria waters).

For the chronic tests, sub-groups of twenty "exposed" and twenty "unexposed" rainbow trout were placed in separate aquaria for each of the nominal dissolved manganese concentrations evaluated. For brown trout, fish were placed in the same aquarium for each of the manganese test concentrations, with the twenty "exposed" fish having their adipose and right pelvic fins clipped, distinguishing them from the twenty "unexposed" fish. During the initial acute phase of the studies, water quality data were collected as described above. After the 96 hour period had elapsed, samples were collected on Day 7 and weekly thereafter. Hardness was again only measured in the control aquaria. Fish were fed based on weight of control fish and numbers of survivors in each aquarium. The weights of surviving fish were recorded at the end of the four month period.

The 96 hour LC50 concentrations were determined using the Spearman-Karber method (Hamilton et. al., 1978) where 100% mortality occurred and by the probit method where less than 100% mortality occurred. The acute toxicity test results for rainbow trout are summarized in Table 2.6.

TABLE 2.6: 96 HOUR LC50 ACUTE TOXICITY TEST RESULTS – RAINBOW TROUT

Group	96 Hour LC50 (mg Mn/L)	95% Confidence Interval (mg Mn/L)	Mean Length (mm)	Mean Weight (g)	Water Hardness (mg/L $CaCO_3$)	pH/Temp.	Dissolved Oxygen (mg/L)
Exposed	3.32	2.97 - 3.72	52.4	1.41	34.0	7.53/14.3 °C	7.73
Unexposed	4.83	4.18 - 5.58	42.0	0.65	34.0	7.53/14.3 °C	7.73

Note: Water hardness, pH, temperature and dissolved oxygen values are "corrected" values obtained from an addendum to Davies and Brinkman, 1994, which was appended to Davies and Brinkman, 1995
95% Confidence Intervals based on six to seven Mn concentrations and groups of twenty organisms exposed at each concentration

The 96 hour LC50 value for exposed rainbow trout was 3.32 mg Mn/L, with a 95% confidence interval range of 2.97 to 3.72 mg Mn/L. The 96 hour LC50 concentration for unexposed rainbow trout was 4.83 mg Mn/L and the 95% confidence interval range was 4.18 to 5.58 mg Mn/L. The values for the pre-exposed group were lower than those for the unexposed group, despite the smaller mean length and weight of the unexposed organisms; no explanation as to the cause or significance of these findings was provided and Davies and Brinkman (1994) stated that the "96 hour LC50s were only slightly different in the exposed and unexposed groups."

The 96 hour LC50 results for exposed and unexposed brown trout are presented in Table 2.7.

TABLE 2.7: 96 HOUR LC50 ACUTE TOXICITY TEST RESULTS – BROWN TROUT

Group	96 Hour LC50 (mg Mn/L)	95% Confidence Interval (mg Mn/L)	Mean Length (mm)	Mean Weight (g)	Water Hardness (mg/L $CaCO_3$)	pH/Temp.	Dissolved Oxygen (mg/L)
Exposed	9.06	7.43 - 10.83	138.7	28.87	38.0	7.54/14.4 °C	7.63
Unexposed	3.77	3.17 - 4.41	138.1	28.54	38.0	7.54/14.4 °C	7.63

Note: Water hardness, pH, temperature and dissolved oxygen values are "corrected" values obtained from an addendum to Davies and Brinkman, 1994, which was appended to Davies and Brinkman, 1995
95% Confidence Intervals based on six to seven Mn concentrations and groups of twenty organisms exposed at each concentration

The 96 hour LC50 concentrations for exposed and unexposed brown trout were 9.06 and 3.77 mg Mn/L, respectively, demonstrating a significant difference between the two groups. Surviving organisms mean weights and lengths in each of the groups were very similar. The 95% confidence interval range for the unexposed group was 3.17 to 4.41 mg Mn/L, which fell between the confidence interval ranges for exposed and unexposed rainbow trout.

Chronic toxicity test results were calculated from the geometric means of the effect and no effect concentration data generated from the tests. The values for exposed and unexposed rainbow trout and brown trout are presented in Table 2.8 and Table 2.9, respectively.

TABLE 2.8: FOUR MONTH CHRONIC TOXICITY TEST RESULTS – RAINBOW TROUT

Group	Effect/No Effect Concentration (mg Mn/L)	Chronic Value (mg Mn/L)	Mean Length (mm)	Mean Weight (g)	Water Hardness (mg/L $CaCO_3$)	pH/Temp.	Dissolved Oxygen (mg/L)
Exposed	2.13 /1.15	1.57	89.5	7.44	36.8	7.56/15.2 °C	8.08
Unexposed	1.04/0.60	0.79	87.1	7.02	36.8	7.56/15.0 °C	8.17

The exposed group chronic toxicity test value was 1.57 mg Mn/L while the unexposed group chronic value was 0.79 mg/l. The exposed group chronic value was twice that for the unexposed group and the effect/no effect ranges for the two groups also differed by a factor of about two. Water quality characteristics were very similar and lengths and weights of surviving organisms were also similar. The data suggested an increase in tolerance for the exposed group relative to the unexposed group at a water hardness value of 36.8 mg/L $CaCO_3$.

TABLE 2.9: FOUR MONTH CHRONIC TOXICITY TEST RESULTS – BROWN TROUT

Group	Effect/No Effect Concentration (mg Mn/L)	Chronic Value (mg Mn/L)	Mean Length (mm)	Mean Weight (g)	Water Hardness (mg/L $CaCO_3$)	pH/Temp.	Dissolved Oxygen (mg/L)
Exposed	4.88/3.59	4.19	154.3	39.74	37.5	7.19/15.2 °C	7.07
Unexposed	3.59/2.03	2.70	151.4	39.22	37.5	7.19/15.2 °C	7.07

The test results presented in Table 2.9 for brown trout showed a pattern similar to rainbow trout. The chronic value calculated for the exposed group was 4.19 mg Mn/L while the chronic value for the unexposed group was 2.70 mg Mn/L. Water quality characteristics were identical and mean lengths and weights of surviving fish were very similar. The data suggested that the exposed group exhibited increased tolerance to manganese as a result of pre-exposure versus the unexposed group. This may indicate that organisms inhabiting surface waters may be naturally more tolerant of or acclimated to the manganese levels present in those waters. Such natural tolerance to local water conditions may not be observed in test organisms utilized in toxicity testing and should be given consideration when interpreting data generated from such tests.

Rouleau et. al. (1996) investigated the relationship of manganese uptake in brown trout tissue to pH of water. Groups of five fish (weighing 9.0 ± 1.8 g) were exposed to 0.1 µg Mn/L for 21 days, with ^{54}Mn used as a tracer. Fish were exposed at pH of 7.5 ± 0.2 and at pH 4.9-5.0. The 4.9-5.0 pH rose to 5.3-6.0 after 24 hours and was readjusted daily. The authors attributed this rise to ammonia excretion and determined that the average pH during the experiment was 5.3. Water in the aquaria was changed every three to four days and total Mn concentrations were determined in newly prepared water and in water before replacement; Mn concentrations were found to be constant. At the end of the study, manganese concentrations in tissue were analyzed, the results of which are presented in Table 2.10.

TABLE 2.10: MANGANESE UPTAKE RATIO IN BROWN TROUT	
Organ	Increased Mn Uptake Factor at pH 5.3 vs. pH 7.5
Whole Body	1.7 times
Liver	2.1 times
Viscera without Liver and Kidneys	2.4 times
Brain	2.1 times
Eyes	1.5 times

The study found that manganese concentrations were similar in the rest of the body tissue (excluding viscera, brain and eyes) at both pH values. The authors concluded that "the uptake of $^{54}Mn(II)$ increased significantly at low pH but the mechanisms by which this occurred remain unclear. There was also no indication if this pattern of uptake would occur at much higher manganese concentrations; the reported experimental concentration of 0.1 µg/L would represent a very low concentration of total manganese relative to naturally occurring levels observed in B.C. surface waters.

2.4.2 Other Studies

Wepener et. al. (1992) studied the non-lethal effects of manganese on the banded tilapia (Tilapia sparrmanii) of South Africa. The effects of a manganese chloride concentration of 4.43 mg/L (manganese concentration of 1.93 mg/L) at pH values of 5 and 7.4 in 96 hour flow-through tests were evaluated with respect to red and white blood cell counts, hemoglobin concentrations, mean corpuscular volume and hematocrit. At pH of 5, significant decreases in all the exposure endpoints parameters were observed, while at pH of 7.4, the white blood cell count, hemoglobin concentration and mean corpuscular volume decreased significantly. A slight increase in the activity of delta-aminolevulinic dehydratase was noted at both pH values. Overall, manganese was observed to cause a greater stress at a pH of 7.4 versus a pH of 5 and concentrations were observed to be detrimental to the organisms at non-lethal levels.

Studies on the potential toxic effects of manganese on aquatic plants are not extensive. Unni et. al. (1995) researched the effect of manganese on growth and physiology of rice (Oryza sativa L.). Concentrations studied ranged from 2 to 200 ppm (parts per million - assumed to be mg/L) for a period of 40 days under hydroponic conditions. Exposure endpoints included seed germination, growth retardation, and total chlorophyll, soluble sugar and protein contents. Table 2.11 summarizes the main results of the study.

TABLE 2.11: MANGANESE TOXICITY TO RICE					
Measurement Day	Manganese Concentration (ppm or mg/L)	% Reduction vs. Controls			
		Shoot Length	Chlorophyll Content	Sugar Content	Protein Content
Day 10	2	0	8.6	26.9	2.4
	100	0	6	2.7	0.4
	200	0	61.4	16	7
Day 25	2	-	-	-	-
	100	26.3	-	-	-
	200	45.8	-	-	-
Day 40	2	-	31.9	77.2	52.2
	100	37.8	54.6	35	28.4
	200	52	99	50	36.4

Seed germination was not affected by the presence of manganese at the concentrations used in the study. The results of the study indicated a progressive reduction in chlorophyll, sugar and protein contents with increased exposure time at all three study concentrations. It was not clear why a concentration of 2 ppm (mg/L) resulted in greater reductions in several of the exposure endpoints. For example, reductions in sugar content on Day 10 and Day 40 were greatest at 2 ppm (mg/L) versus 100 or 200 ppm (mg/L). In all cases, however, reductions increased with increasing exposure time.

Wang (1986) conducted 4 day acute and 7 day sub-chronic tests on the effects of manganese on the growth of duckweed (Lemna minor). The study was conducted using tap water at a pH of 7.5 (no hardness or temperature data provided) and the exposure endpoint was growth as indicated by the number of fronds initially and at the end of the exposure period. Twenty colonies of duckweed were studied and an EC50 (reduction in frond growth in 50% of test organisms vs. controls) of 31 mg/L was derived.

Stauber and Florence (1987) demonstrated the ameliorating effect of manganese on copper toxicity to the marine diatom Nitzschia closterium. Copper affects the organism's ability to defend against hydrogen peroxide and oxygen-free radicals, while manganese aids in the complexation of these compounds. Kaitala (1988) determined that the presence of copper ions increased the uptake of manganese in blue mussels (Mytilus edulis) and burrowing clams (Macoma baltica). Concentrations of copper (0.2 mg/L) and manganese (2 mg/L) were evaluated as individual applications and in combination along with zinc (0.4 mg/L). Kaitala (1988) concluded that a 100% increase in manganese accumulation and a 25% increase in zinc accumulation was apparent in mussels when copper was present. For clams, manganese accumulated but zinc did not, suggesting that copper has a significant effect on the accumulation of manganese in these organisms.

Sinha et. al. (1993) studied the effect of chromium and manganese interaction on the aquatic plant Hydrilla verticalla. Manganese uptake was enhanced while chromium uptake was inhibited when the metals were combined versus uptake of the individual metals when tested separately.

3.0 MATERIALS AND METHODS

The materials and methods used in this thesis research are provided in this section. Acute and chronic toxicity testing was conducted on B.C. resident species of fish, invertebrates and algae. Literature data were gathered to support the data collected from toxicity testing on B.C. species. All relevant data were used to improve the freshwater aquatic life guideline for manganese contained in the B.C. Ministry of Environment, Lands and Parks document *Approved And Working Criteria For Water Quality* (BCMELP, 1995).

3.1 B.C. PROTOCOL

The B.C. Ministry of Environment, Lands and Parks has developed procedures for deriving water quality criteria in British Columbia. These procedures are described in the document *Derivation Of Water Quality Criteria To Protect Aquatic Life In British Columbia* (September, 1995 Draft), which is presented in Appendix B of this thesis. This draft document outlines the minimum requirements that need to be met for data to be used in deriving water quality criteria and the minimum numbers of tests for each class of organisms (fish, invertebrates and plants) required to derive full and/or interim guidelines for the protection of aquatic life. The reader is referred to Appendix B for further details. The BCMELP procedures (B.C. Protocol) are similar to the Canadian Council of Ministers of Environment procedures detailed in the document *A Protocol For The Derivation Of Water Quality Guidelines For The Protection Of Aquatic Life* (CCME, 1987), which is presented in Appendix C of this thesis.

Applying the B.C. Protocol will allow the objectives of this thesis research to be fulfilled by addressing the following key components:

1. Reviewing published and unpublished literature data
2. Determining data requirements where literature sources do not provide sufficient data for water quality derivation purposes.
3. Using new toxicity data in the derivation of water quality criteria protective of aquatic life.

The need for additional data on British Columbia species to supplement the data available in the literature was identified during the establishment of the 1995 freshwater aquatic life guideline for manganese. A toxicity testing program was therefore undertaken using species native to B.C. The aquatic toxicity testing procedures and methodologies were based on standard Environment Canada protocols, which also incorporated procedures adopted from organizations such as ASTM. Additional details pertaining to the testing methodologies utilized are provided in Appendix A. The species included in the suite of toxicity tests along with the toxicity endpoints measured are presented in Table 3.1.

TABLE 3.1: AQUATIC TOXICITY TESTING - B.C. FRESHWATER SPECIES		
Type of Test	Organism	Toxicity Endpoint
96 Hour LC50 Fish Bioassay	Rainbow Trout Under-yearlings Coho Salmon Early Life Stage	Survival as measured by lethality
7 Day Early Life Stage	Rainbow Trout	Survival as measured by egg hatching success
48 Hour LC50 Invertebrate Bioassay 96 Hour LC50 Invertebrate Bioassay	Daphnia Magna Chironomid Tentans Larvae (3rd instar)	Survival as measured by immobility and lethality
96 Hour LC50 Amphipod Bioassay	Hyalella Azteca	Survival as measured by lethality
21 Day Chronic Invertebrate Bioassay	Daphnia Magna	Survival and reproduction, including time to brood, survival and mobility
Microtox® IC50 - 5 and 15 Minute	Vibrio Fischeri	Concentrations resulting in 50% decrease in light production after 5 and 15 minutes
72 Hour IC50 Freshwater Algal Bioassay	Selenastrum Capricomutum	50% reduction in growth as measured by cell number/mass

Note: LC50 - interpolated concentration at which 50% lethality occurs in test organisms versus control group
IC50 - interpolated concentration at which 50% inhibition of toxicity endpoint (e.g. light production, plant mass) occurs in test organisms versus control group

Details regarding the sources from which test organisms were obtained, summaries and references for the toxicity tests, and quality assurance/quality control information including the acceptable ranges for water quality criteria (pH, DO, temp.) and the statistical methods applied to each test are presented in Appendix A.

Manganese chloride ($MnCl_2$) was chosen as the chemical form to prepare dissolved manganese test solutions for use in the toxicity testing program. A stock solution of 10 000 mg $MnCl_2$ dissolved in 1 litre of de-ionized water was prepared as required and test concentrations were prepared by placing pre-measured volumes of stock solution in a volumetric flask and filling with de-ionized water to achieve the desired concentration. Test concentrations varied based on the type of test, the organisms under study and observations/test results noted during the testing programs.

Existing information on manganese and other similar metals such as copper and zinc suggested a relationship between aquatic toxicity and water hardness. In order to further explore this relationship, three nominal water hardness values were chosen for evaluation in several of the toxicity tests, specifically 25 mg/L $CaCO_3$, 100 mg/L $CaCO_3$ and 250 mg/L $CaCO_3$. A groundwater well with a water hardness of 100 mg/L was used as the water source for the freshwater testing program. The 25 mg/L softer water was prepared by diluting the well water with de-ionized water while the 250 mg/L hard water was prepared by reconstituting well water. All toxicity tests were conducted using a hardness value of 100 mg/L $CaCO_3$, with a portion of the tests conducted at all three water hardness values.

3.2 APPLICATION OF B.C. PROTOCOL

Classification of toxicity testing data as primary, secondary or unacceptable is required under the B.C. Protocol. The requirements for primary data include preferred partial or full life cycle exposure endpoints such as embryonic development effects, hatching or germination success, survival of juvenile stages, and growth, reproduction and survival of adults. For secondary data, the requirements include those for primary data as well as pathological, behavioural and physiological effects. The detailed requirements for primary and secondary data are described in Table 3.1 of Appendix B. Unacceptable data are those that do not meet the requirements of either primary or secondary data.

The studies under consideration for use in guideline derivation are also classified as acute or chronic and the types of organisms used in the tests are assessed. Minimum numbers of acute and chronic tests on species of fish, invertebrates and aquatic plants are required for full and interim guideline derivation. Details are provided in Appendices B and C, and in Section 4.1.2.

Data that is acceptable for use in guideline derivation is then reviewed to determine the concentrations at which adverse effects were observed. The lowest observed effect concentration or LOEC and the no observed effect concentration or NOEC are reviewed as are statistically derived effects concentrations such as LC50s and IC25s. These concentrations are compared with acceptable data for all organisms to determine the lowest concentrations from acute and chronic studies, which should be indicative of the more sensitive organisms under acute and chronic exposure conditions. As the objective of guideline derivation is the protection of aquatic life, organisms that are less tolerant of a substance in freshwater weigh more heavily in the establishment of a guideline. Once a minimum value (or values) has been established, a safety factor is applied to compensate for uncertainty associated with the data set. The B.C. Protocol suggests a typical range of 0.1 to 0.5 (Section 4.1.1, Appendix B) depending on the quality of data and degree to which the toxicity of the particular substance is understood. The final acute and/or chronic guidelines with the safety factor applied are compared to the available data to ensure there is sufficient protection of sensitive species.

4.0 RESULTS AND DISCUSSION

4.1 TOXICITY TESTING DATA CLASSIFICATION

4.1.1 Primary and Secondary Data Classification

The freshwater toxicity testing conducted by Environment Canada, on behalf of BCMELP, included nine separate test/organism combinations. A summary of the degree to which the studies conducted by Environment Canada/BCMELP met the data requirements for primary and secondary data is presented in Table 4.1.

TABLE 4.1: B.C. FRESHWATER AQUATIC TOXICITY TESTING – DATA CLASSIFICATION

Type of Test	Primary Data Requirements					
	Acceptable Lab Practices Used?	Concentrations Measured at Beginning/End?	Was the Test Flowthrough? (see Note 1)	Partial or Full Life Cycle Endpoints?	Were Controls Responses Measured?	Temp., pH, DO and Hardness Reported?
96 Hour LC50 Rainbow Trout	Yes	Yes	No	Yes	Yes	Yes
96 Hour LC50 Coho Salmon	Yes	Yes	No	Yes	Yes	Yes
7 Day Early Life Stage Rainbow Trout	Yes	Yes	No	Yes	Yes	Yes
48 Hour LC50 Invertebrate Bioassay	Yes	Yes	No	Yes	Yes	Yes
96 Hour LC50 Invertebrate Bioassay	Yes	Yes	No	Yes	Yes	Yes
96 Hour LC50 Amphipod Bioassay	Yes	Yes	No	Yes	Yes	Yes
21 Day Chronic Invertebrate Bioassay	Yes	Yes	No	Yes	Yes	Yes
Microtox® IC50 5 and 15 Minute	Yes	No	No	Yes	Yes	Yes
72 Hour IC50 Freshwater Algal Bioassay	Yes	No	No	Yes	Yes	Yes

Note: Acceptable lab practices are based on standardized test protocols for fish, invertebrates and plants (see Section IX.3, Appendix C)
Preferred toxicity test endpoints for primary classification for partial or full life cycle tests include effects on embryonic development, hatching or germination success, survival of juvenile stages, growth, reproduction and survival of adults
Preferred toxicity endpoints for secondary classification include those listed above for primary as wells as pathological, behavioural and physiological effects (Appendix B and C)
1 – Static test data is acceptable if concentrations did not change during the test and environmental conditions for the test species were maintained, conditions that the laboratory has stated were met (Pacific Environmental Science Center, 1998/1999)

The information presented in the above table confirms that the toxicity testing conducted by Environment Canada on behalf of BCMELP met the requirements for primary data for all tests, with the exception of the Microtox® IC50 and the 72 Hour IC50 algal bioassay. These tests did not meet the requirements of primary data because the manganese concentrations in the test solution were only measured at the beginning of the test (this shortcoming for the Microtox® IC50 relates more to the fact that the test was of such short duration, making a second concentration measurement redundant). A note following Table 4.1 indicates that although the tests were static rather than flowthrough, laboratory personnel stated that manganese concentrations were stable during the tests and the data could therefore be considered primary. The data met all requirements for secondary data.

The Stubblefield (1987). study on brown trout at three water hardnesses, the acute and chronic data from the Davies and Brinkman (1994) study on exposed and unexposed rainbow and brown trout in soft water, and the acute data for brown trout in hard water (Davies and Brinkman, 1995) were also classified using the primary and secondary data classification protocol.

TABLE 4.2: FRESHWATER AQUATIC TOXICITY TESTING – DATA CLASSIFICATION

Type of Test	Primary Data Requirements					
	Acceptable Lab Practices Used?	Concentrations Measured at Beginning/End?	Was the Test Flowthrough?	Partial or Full Life Cycle Endpoints?	Were Controls Responses Measured?	Temp., pH, DO and Hardness Reported?
62 Day Chronic Brown Trout[1]	Yes	Yes	Yes	Yes	Yes	Yes
4 Month Chronic Rainbow Trout[2]	Yes	Yes	n.a.	Yes	Yes	Yes
4 Month Chronic Brown Trout[2]	Yes	Yes	n.a.	Yes	Yes	Yes
96 Hour LC50 Rainbow Trout[2]	Yes	Yes	n.a.	Yes	Yes	Yes
96 Hour LC50 Brown Trout[2,3]	Yes	Yes	n.a.	Yes	Yes	Yes

Note: Acceptable lab practices are based on standardized test protocols for fish, invertebrates and plants (see Section IX.3, Appendix C)
Preferred toxicity test endpoints for primary classification for partial or full life cycle tests include effects on embryonic development, hatching or germination success, survival of juvenile stages, growth, reproduction and survival of adults
Preferred toxicity endpoints for secondary classification include those listed above for primary as wells as pathological, behavioural and physiological effects (see Appendices B and C))
1 – Stubblefield et. al. (1997)
2 – Davies and Brinkman (1994)
3 – Davies and Brinkman (1995)
n.a. – not available, the report did not indicate whether it was static or flowthrough

The Davies and Brinkman (1994, 1995) reports did not specify whether the tests were static or flowthrough. Based on the information provided in the materials and methods sections of the referenced studies, the remaining data are considered to meet the B.C. Protocol requirements for primary data (and consequently for secondary data).

4.1.2 Full/Interim Guideline Classification

Literature studies completed by Stubblefield et. al (1997) and Davies and Brinkman (1994, 1995) were considered to be suitable for inclusion in the data set to be used for guideline derivation. This was based on the evaluation of the data from these studies with respect to the requirements for primary and secondary data. This literature data was combined with the new toxicity test data and the B.C. Protocol was applied to determine the extent to which the combined data set met the requirements for full or interim guideline development. Table 2.1 of Appendix B summarizes the minimum requirements for guideline development. A summary of the full and interim guideline requirements and an evaluation of the combined data set is presented in Table 4.3.

TABLE 4.3: BC ENVIRONMENT FRESHWATER CRITERIA DATA REQUIREMENTS				
Organism	Full Requirement	Interim Requirement	BCMELP/Colorado Data	Notes
Acute Criterion				
Fish	3 acute studies on 3 freshwater species resident in B.C., at least 2 cold water species (e.g. trout)	2 acute and/or chronic studies; at least 1 study on a cold water species resident in B.C.	4 acute and 4 chronic studies on cold water species resident in B.C.	Meets full requirements
Invertebrates	2 acute studies on 2 invertebrates from different classes including 1 planktonic species resident in B.C.	2 acute and/or chronic studies on 2 invertebrates from different classes, including 1 planktonic species resident in B.C.	1 chronic and 1 acute study on a planktonic species and 2 other acute studies on 2 invertebrates from different classes	Meets full requirements
Plants	Not required as manganese is not a highly phytotoxic substance	Not required	1 acute study on an algal species resident in B.C.	Not required
Chronic Criterion				
Fish	3 chronic studies on 3 freshwater species resident in B.C., at least 2 cold water species (e.g. trout)	2 acute and/or chronic studies; at least 1 study on a cold water species resident in B.C.	4 acute and 4 chronic studies on cold water species resident in B.C.	Meets full requirements
Invertebrates	2 chronic studies on 2 invertebrates from different classes including 1 planktonic species resident in B.C.	2 acute and/or chronic studies on 2 invertebrates from different classes, including 1 planktonic species resident in B.C.	1 chronic and 1 acute study on a planktonic species and 2 other acute studies on 2 invertebrates from different classes	Meets interim requirements
Plants	1 study on a freshwater vascular plant or algal species resident in B.C.	Not required	1 acute study on an algal species resident in B.C.	Meets full requirements

The requirements for type and number of toxicity tests were met for development of a full acute criterion and an interim chronic criterion.

4.1.3 Summary of Data Sufficiency

The new toxicity test data combined with the Stubblefield (1997) and Davies and Brinkman (1994, 1995) data did not meet the requirements for full guideline derivation for either acute or chronic guideline derivation. For both acute and chronic criteria, this was due to use of static testing procedures rather than flowthrough and the absence of information from the Davies and Brinkman (1994, 1995) acute methodology specifying whether the tests were flowthrough. As noted beneath Table 3.2, the new B.C. toxicity data may meet the primary data requirements and the Davies and Brinkman (1994, 1995) acute studies may have been flowthrough. As this was the only acute data deficiency, there may be sufficient information for full acute criteria derivation. For chronic criteria derivation, only one rather than two chronic studies on invertebrates was available. The available invertebrate data met the requirement for one chronic study on a planktonic species. However, a chronic study on a non-planktonic species was lacking as only LC50 tests were conducted on Chironomid tentans and Hyalella azteca. No additional invertebrate studies on non-planktonic species were identified in the literature. Therefore, the available data are sufficient to derive interim guidelines but fall short of the requirements for full guideline development.

4.2 B.C. ENVIRONMENT TOXICITY TEST RESULTS

As discussed in Section 3.1, nominal water hardness values of 25 mg/L $CaCO_3$, 100 mg/L $CaCO_3$ and 250 mg/L $CaCO_3$ were evaluated as part of the testing program for some of the test/organism combinations. Replicate testing was conducted for several of the bioassays to further check the agreement of the results between replicate tests. Results of the toxicity testing program conducted on B.C. species are presented in the following sections. The data have been separated into acute and chronic results under the categories of fish, invertebrates and plants. Test results are summarized and presented in Appendix D.

4.2.1 Fish

Acute test results generated for fish at each water hardness value under study are presented in Table.4.4:

TABLE 4.4: ACUTE AQUATIC TOXICITY TEST RESULTS – FISH

Type of Test	Experimental Concentration	Actual (Day 0) Concentration	Corrected Concentration	Measured Hardness
Nominal Water Hardness = 25 mg/L $CaCO_3$				
96 Hour LC50 Coho Salmon	Rep. A: 2.4 mg/L Rep. B: 2.4 mg/L Rep. C: 2.2 mg/L	Rep. A: 2.4 mg/L	n.a.	Rep. A: 25.2
96 Hour LC50 Rainbow Trout	Rep. A: 2.2 mg/L Rep. B: 2.1 mg/L Rep. C: 2.0 mg/L	n.a.	n.a.	Rep A: 47.6
Nominal Water Hardness = 100 mg/L $CaCO_3$				
96 Hour LC50 Coho Salmon	Rep. A: 10.3 mg/L Rep. B: 15.8 mg/L Rep. C: 13..5 mg/L	Rep. A: 13.2 mg/L	Rep. A: 13.1 mg/L	n.a.
96 Hour LC50 Rainbow Trout	Rep. A: 21.1 mg/L Rep. B: 19.1 mg/L Rep. C: 22.4 mg/L Pooled: 20.7 mg/L	n.a.	n.a.	n.a.
Nominal Water Hardness = 250 mg/L $CaCO_3$				
96 Hour LC50 Coho Salmon	Rep. A: 17.7 mg/L Rep. B: 19.1 mg/L Rep. C: 20.5 mg/L	Rep. A: 17.4 mg/L	n.a.	Rep. A: 250
96 Hour LC50 Rainbow Trout	Rep. A: 19.1 mg/L Rep. B: 15.8 mg/L Rep. C: 13.5 mg/L	Rep. A: 12.7 mg/L	n.a.	Rep. A: 259

Note: Experimental concentration is based on the unverified concentration calculated by the laboratory technician
Actual concentration is calculated using ICP analyzed manganese concentration on Day 0 for Replicate A
Corrected concentration is the average of the actual toxicity values using Day 0 and final test day ICP manganese concentrations
n.a. - not available

Rainbow trout LC50 concentrations were the lowest at water hardness values of 25 (measured at 47.6) and 250 mg $CaCO_3$/L, while the coho salmon LC50 concentration was the lowest value at a hardness of 100 mg $CaCO_3$/L. The lowest LC50 concentrations were observed at a nominal water hardness of 25 mg $CaCO_3$/L for both species.

Actual and corrected concentrations were not determined for all tests. Manganese concentrations were apparently not determined for some tests on Day 0 (actual) and for most tests on final day (corrected). It appears that the laboratory assumed that differences between actual and true concentrations did not vary sufficiently to warrant analysis. This was supported by the coho salmon data at a water hardness of 100 mg/L $CaCO_3$. However, more variability was noted between experimental and actual concentrations; some concentrations were in good agreement (coho salmon at hardnesses of 25 and 250) while others were not (rainbow trout at 250 hardness).

Chronic test results on fish are provided in Table 4.5

TABLE 4.5: CHRONIC AQUATIC TOXICITY TEST RESULTS – FISH

Type of Test	Experimental Concentration	Actual (Day 0) Concentration	Corrected Concentration	Measured Hardness
Nominal Water Hardness = 25 mg/L $CaCO_3$				
7 Day Early Life Stage EC50 Rainbow Trout	16.6 mg/L	n.a.	14.6 mg/L	25.7
Nominal Water Hardness = 100 mg/L $CaCO_3$				
7 Day Early Life Stage EC50 Rainbow Trout	20.9 mg/L	n.a.	20.0 mg/L	n.a.
Nominal Water Hardness = 250 mg/L $CaCO_3$				
7 Day Early Life Stage EC50 Rainbow Trout	29.5 mg/L	n.a.	22.7	252

Note: Experimental concentration is based on the unverified concentration calculated by the laboratory technician
Corrected concentrations are based on the final test day ICP manganese concentrations
n.a. - not available

EC50 concentrations increased with increasing hardness, but were similar for water hardnesses of 100 and 250 mg/L $CaCO_3$. It is noteworthy that the minimum LC50 concentrations for the acute tests were lower than the chronic values presented in Table 4.5, suggesting a less sensitive life stage used in the chronic study.

At a water hardness of 25 mg/L $CaCO_3$, two initial replicate tests resulted in 37.5%-45.8% non-viable organisms in the control groups, well in excess of the 10% threshold. A third replicate resulted in a corrected concentration of 14.6 mg/L.

4.2.2 Invertebrates

Acute results for toxicity tests conducted on invertebrates are presented in Table 4.6.

TABLE 4.6: ACUTE AQUATIC TOXICITY TEST RESULTS – INVERTEBRATES

Type of Test	Experimental Concentration	Actual (Day 0) Concentration	Corrected Concentration	Measured Hardness
Nominal Water Hardness = 25 mg/L $CaCO_3$				
48 Hour LC50 Daphnia Magna	Rep. A: 1.0 mg/L Rep B: 1.0 mg/L	Rep. A: 0.9 mg/L	Rep. A: 0.8 mg/L	Rep. A: 26.3
96 Hour LC50 Chironomid Tentans	Rep. A: 8.0 mg/L Rep. B: 4.0 mg/L Rep. C: 5.9 mg/L	Rep. A: 5.8 mg/L	Rep. A: 5.8 mg/L	Rep. A: 27.2
96 Hour LC50 Hyalella Azteca	Rep. A: 3.4 mg/L Rep. B: 3.4 mg/L Rep. C: 3.8 mg/L	Rep. A: 3.5 mg/L	Rep. A: 3.6 mg/L	n.a.
Microtox IC50 (5 and 15 Minute) Vibrio Fischeri	5 min = 872.7 mg/L 15 min = 73.1 mg/L	n.a.	n.a.	n.a
Nominal Water Hardness = 100 mg/L $CaCO_3$				
48 Hour LC50 Daphnia Magna	Rep. A: 29.9 mg/L Rep. B: 23.2 mg/L	Rep. A: 30.6 mg/L	Rep. A: 28.7 mg/L	n.a
96 Hour LC50 Chironomid Tentans	Rep. A: 35.5 mg/L Rep. B: 43.5 mg/L Rep. C: 43.5 mg/L	Rep. A: 42.2 mg/L	n.a.	n.a.
96 Hour LC50 Hyalella Azteca	Rep. A: 13.5 mg/L Rep. B: 21.8 mg/L Rep. C: 22.0 mg/L	Rep. A: 21.4 mg/L	Rep. A: 22.2 mg/L	n.a.
Microtox IC50 (5 and 15 Minute) Vibrio Fishceri	5 min = 3808.3 mg/L 15 min = 88.0 mg/L	n.a.	n.a.	n.a.
Nominal Water Hardness = 250 mg/L $CaCO_3$				
48 Hour LC50 Daphnia Magna	Rep. A: 82.2 mg/L Rep. B: 71.0 mg/L	Rep. A: 79.7 mg/L	Rep. A: 76.3 mg/L	267
96 Hour LC50 Chironomid Tentans	Rep. A: 82.3 mg/L Rep. B: 432 mg/L Rep. C: 152.7 mg/L	Rep. A: 101.0 mg/L	Rep. A: 94.3 mg/L	Rep. A: 272
96 Hour LC50 Hyalella Azteca	Rep. A: 31.3 mg/L Rep. B: 29.9 mg/L Rep. C: 33.6 mg/L	Rep. A: 32.7 mg/L	Rep. A: 31.0 mg/L	Rep. A: 269
Microtox IC50 (5 and 15 Minute) Vibrio Fischeri	5 min = 10542.4 mg/L 15 min = 124.3 mg/L	n.a.	n.a.	n.a.

Note: Experimental concentration is based on the unverified concentration calculated by the laboratory technician
Actual concentration calculated using ICP analyzed manganese concentration on Day 0 for Replicate A
Corrected concentration is the average of the actual concentrations using Day 0 and final test day ICP manganese concentrations
IC50 - statistical manganese concentration resulting in a 50% decrease in the exposure endpoint of interest (e.g. light production for Microtox)
n.a. - not available

Daphnia magna was the least tolerant species at a water hardness of 25 mg/L $CaCO_3$, while Hyalella azteca was the least tolerant at hardnesses of 100 and 250 mg/L $CaCO_3$. LC50 concentrations were observed to increase with increasing water hardness.

Actual and true concentrations were determined for the majority of tests. Actual and true concentrations showed good agreement. However, some experimental concentrations varied considerably from the actual and true values (most notably Chironomid tentans).

Chronic toxicity test data on invertebrates are presented in Table 4.7.

TABLE 4.7: CHRONIC AQUATIC TOXICITY TEST RESULTS – INVERTEBRATES				
Type of Test	Experimental Concentration	Actual (Day 0) Concentration	Corrected Concentration	Measured Hardness
Nominal Water Hardness = 25 mg/L $CaCO_3$				
21 Day Chronic Daphnia Magna	Excess Control Deaths due to soft water	n.a.	n.a.	n.a
Nominal Water Hardness = 100 mg/L $CaCO_3$				
21 Day Chronic Daphnia Magna	NOEC = 3.4 mg/L LOEC = 6.8 mg/L IC25 = 5.3 mg/L	NOEC= 3.5 mg/L LOEC = 6.7 mg/L IC25 = 5.3 mg/L	NOEC = 3.6 mg/L LOEC = 6.9 mg/L IC25 = 5.4 mg/L	n.a.
Nominal Water Hardness = 250 mg/L $CaCO_3$				
21 Day Chronic Daphnia Magna	NOEC = 6.8 mg/L LOEC = 13.5 mg/L IC25 = 9.1 mg/L	NOEC = 7.2 mg/L LOEC = 13.6 mg/L IC25 = 9.4 mg/L	NOEC = 7.3 mg/L LOEC = 13.4 mg/L IC25 = 9.4 mg/L	269

Note: Experimental concentration is based on the unverified concentration calculated by the laboratory technician
Actual concentration is calculated using ICP analyzed manganese concentration on Day 0
Corrected concentration is the average of the actual concentrations using Day 0 and final test day ICP manganese concentrations
n.a. - not available

Corrected IC25 concentrations of 5.4 and 9.4 mg Mn/L were observed at water hardnesses of 100 and 250 mg/L $CaCO_3$, respectively. The laboratory noted that excessive control deaths occurred at a water hardness of 25 mg/L $CaCO_3$ and attributed this to the softness of the test water. There was good agreement between the experimental, actual and corrected concentrations determined for the chronic D. magna testing.

4.2.3 Aquatic Plants

Table 4.8 presents the results of the toxicity testing conducted on aquatic plants.

TABLE 4.8: ACUTE AQUATIC TOXICITY TEST RESULTS – PLANTS				
Type of Test	Experimental Concentration	Actual (Day 0) Concentration	Corrected Concentration	Measured Hardness
Nominal Water Hardness = 25 mg/L $CaCO_3$				
72 Hour IC50 Selenastrum Capricomutum	n.a.	n.a.	n.a.	n.a.
Nominal Water Hardness = 100 mg/L $CaCO_3$				
72 Hour IC50 Selenastrum Capricomutum	8.29 mg/L	n.a.	n.a.	n.a.
Nominal Water Hardness = 250 mg/L $CaCO_3$				
72 Hour IC50 Selenastrum Capricomutum	n.a.	n.a.	n.a.	n.a.

Note: Experimental concentration is based on the unverified concentration calculated by the laboratory technician
IC50 - statistical manganese concentration resulting in a 50% decrease in the exposure endpoint of interest (e.g. growth for S. capricomutum
n.a. - not available

Toxicity testing on the freshwater alga Selenastrum capricomutum was limited to a 72 hour IC50 growth inhibition test at a water hardness of 100 mg/L $CaCO_3$. A manganese IC50 concentration of 8.29 mg/L was determined.

4.2.4 Summary of Test Results

The lowest recorded manganese concentrations at which toxic responses occurred for the three water hardnesses under study are summarized in Table 4.9:

TABLE 4.9: MINIMUM ACUTE AND CHRONIC TOXICITY CONCENTRATIONS - MG MN/L					
Water Hardness = 25 mg/L $CaCO_3$		Water Hardness = 100 mg/L $CaCO_3$		Water Hardness = 250 mg/L $CaCO_3$	
Acute	Chronic	Acute	Chronic	Acute	Chronic
0.8 mg/L	14.6 mg/L	13.1 mg/L	6.9 mg/L LOEC 3.6 mg/L NOEC 5.3 mg/L IC25	12.7 mg/L	13.4 mg/L LOEC 7.3 mg/L NOEC 9.1 mg/L IC25
48 hr LC50 Daphnia Magna	7 Day E-test Rainbow Trout	96 hr LC50 Coho Salmon	21 day D. Magna	96 hr LC50 Rainbow Trout	21 day D. Magna

For the species under study, the results indicated that salmonids were the most sensitive species for acute exposure at water hardnesses of 100 mg/L $CaCO_3$ and 250 mg/L $CaCO_3$, while Daphnia magna was most sensitive at a water hardness of 25 mg/L $CaCO_3$. The sensitivity of Daphnia magna may be attributable in part to water hardness as evidenced by the 21 day chronic test results on Daphnia magna at a hardness of 25 mg/L $CaCO_3$. Boron was tested prior to manganese and chronic test results for boron at a water hardness of 25 mg/L $CaCO_3$ indicated control group mortality rates of 0% after Day 2, but 70% after Day 5. The Environment Canada Pacific Environmental Science Center aquatic toxicity laboratory concluded that the control deaths were related to the low water hardness. The test was therefore terminated and chronic Daphnia testing at a water hardness of 25 mg/L $CaCO_3$ was discontinued for boron and for manganese. Environment Canada laboratory personnel reported that high mortality rates in Daphnia have been observed at water hardness values of $\leq$50 mg/L $CaCO_3$ and thus, the observed mortality for the chronic Daphnia magna test was not unexpected (Environment Canada, 1998/1999). This may have also influenced the 48 hour LC50 results for Daphnia magna; the 0.8 mg Mn/L LC50 value may be due in part to water hardness, with the short duration of the test masking any contributory toxic effect of water hardness. The acute Daphnia magna result for a water hardness of 25 mg/L $CaCO_3$ will therefore not be included in the derivation of an acute guideline.

The calculated IC25 manganese concentration of 5.3 mg/L for Daphnia magna is considered to be a more effective measure of toxicity than either the LOEC or the NOEC concentrations. The LOEC and NOEC values are pre-selected manganese concentrations that are based on the concentrations chosen in the experimental design and a comparison of the exposure endpoint (i.e. survival, mobility) for the study organisms versus the control group relative to a preset level of statistical significance (usually $p < 0.05$). By definition, the actual concentration at which an observable effect would occur must fall between the NOEC and the LOEC concentrations for the preset level of statistical significance. The IC25 concentration is based on the experimental data and is an estimate of the concentration at which an adverse effect would be expected in 25% of organisms. Choosing 25% as an acceptable percentage of affected organisms is largely arbitrary and may be based more on societal values than scientific principles. However, the IC25 has become widely

accepted as a reasonable level of protection for aquatic organisms. In the case of the D. magna chronic toxicity test, the IC25 concentration of 5.3 mg/L fell midway between the NOEC (3.6 mg/L) and the LOEC (6.9 mg/L). As the actual LOEC and NOEC must fall somewhere between 3.6 mg/L and 6.9 mg/L, the IC25 value represents a good estimate of the actual NOEC/LOEC concentrations.

4.2.5 Water Hardness and Aquatic Toxicity

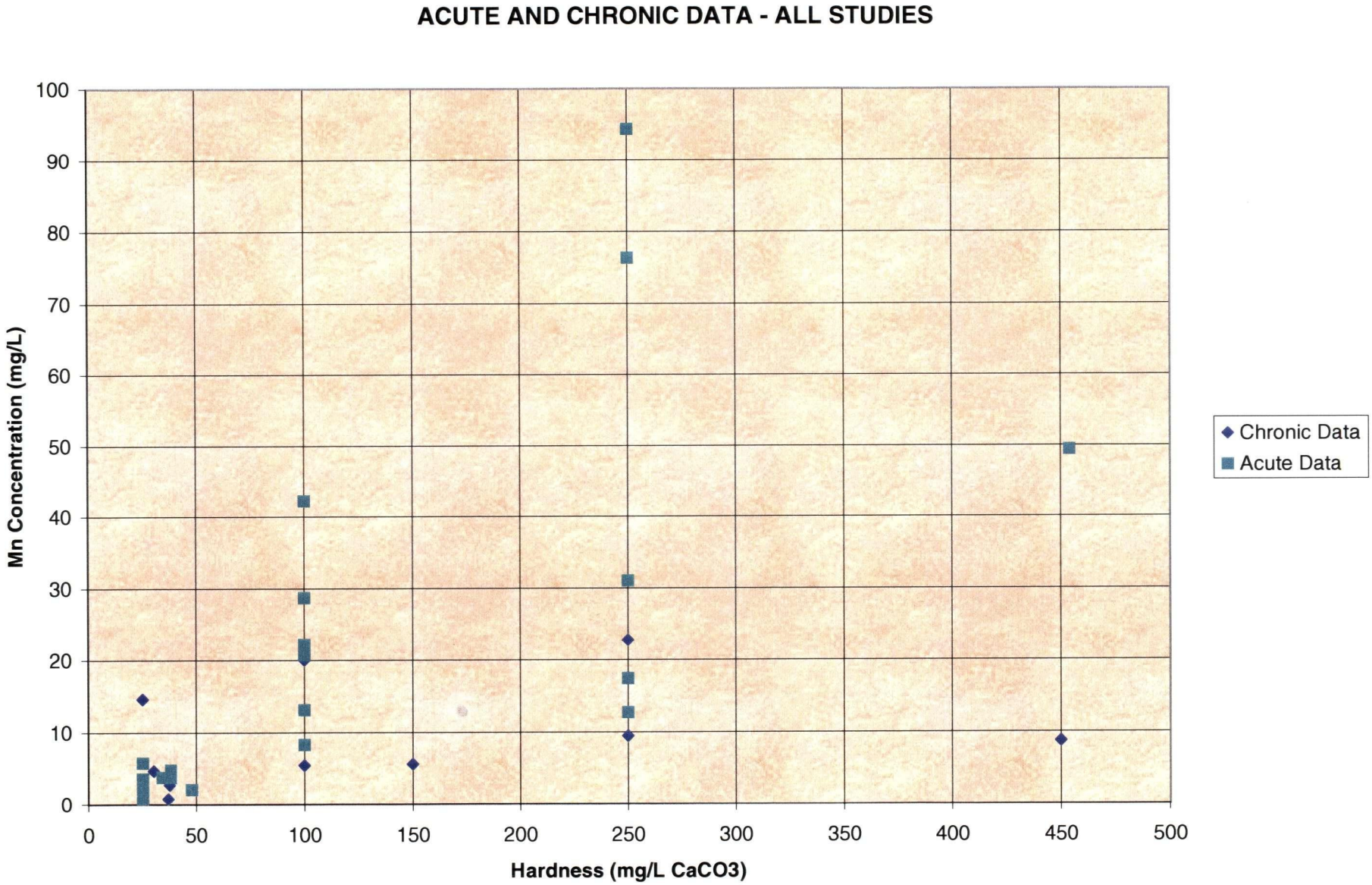
The test results show a trend whereby the manganese concentrations at which toxic responses were observed increase with increasing water hardness. This trend is apparent for most organisms studied, with the exception of rainbow trout, which exhibited higher tolerance prior to the occurrence of a toxic response at a water hardness of 100 versus a water hardness of 250 for the 96 hour LC50 test. Replicate 96 hour LC50 tests confirmed this result. It is not clear why this pattern emerged for rainbow trout. No data were found in the literature to support the conclusion that rainbow trout may be more sensitive to manganese when water hardness is increased from 100 mg/L to 250 mg/L $CaCO_3$. Similarly, there was no information to indicate whether or not the particular rainbow trout used in these experiments were sensitive to higher water hardness.

The hardness relationship was apparent for Daphnia magna for the 21 day chronic test; however, no manganese concentration was determined for a water hardness value of 25 mg/L $CaCO_3$ due to the unacceptably high incidence of experimental control deaths. Thus, it is probable that soft water would not constitute suitable habitat for Daphnia magna irrespective of the presence of manganese.

The 5 and 15 minute Microtox IC50 values were observed to increase with increasing water hardness. These increases were most notable for the 5 minute test, with values increasing from 873 mg/L for a water hardness of 25 mg/L to 10542 mg/L for a water hardness of 250 mg/L $CaCO_3$, an approximate twelve fold increase. The 15 minute IC50 test results increased from 73.1 mg/L to 124.3 mg/L, an increase of about 1.8 times. The results indicate the presence of a hardness dependent relationship; however, the effect of hardness would appear to decrease with increased exposure time for the toxicity endpoint under consideration (light production).

4.3 TOXICTY TEST RESULTS – ALL STUDIES

The toxicity test results (acute and chronic) for the studies commissioned by BCMELP for water hardnesses of 25, 100 and 250 mg/L $CaCO_3$ are presented in graphical form on the following page. The Microtox IC50 values have not been included as the results were the highest recorded among the tests conducted. Data from literature sources that met the B.C. Protocol requirements for primary and/or secondary data are also plotted on this graph.


ACUTE AND CHRONIC DATA - ALL STUDIES
Mn Concentration (mg/L)
Hardness (mg/L CaCO3)
Chronic Data
Acute Data
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400
450
500

The graphical presentation illustrates the general trend of increased manganese concentration with increased water hardness. The exception was the 96 hour acute LC50 test for rainbow trout as discussed in Section 4.2.5. The graph also illustrates the trends in acute data versus chronic data. The levels of manganese at which adverse effects were observed increased with increasing hardness more quickly for the acute tests than for the chronic tests. This pattern is expected as a higher level of exposure without adverse effects would be anticipated for a shorter term (acute) exposure versus a longer term (chronic) exposure.

For purposes of applying the B.C. Protocol to derive water quality criteria, the data have been separated into acute and chronic categories as presented in Sections 4.3.1 and 4.3.2. This will allow determination of separate acute and chronic guidelines for the protection of freshwater aquatic life.

4.3.1 Acute Toxicity Data – All Studies

In order to apply the BCMELP water quality guideline derivation procedures, data collected from all suitable acute studies were combined and are presented in Table 4.10.

TABLE 4.10: ACUTE DATA FROM ALL STUDIES

Water Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)	Toxicity Test
25	2.4*	Coho – Early Life 96 Hour LC50
	3.6	Hyalella Azteca – 96 Hour LC50
	5.8	Chironomid Tentans – 96 Hour LC50
	0.8	Daphnia Magna –48 Hour LC50
34	3.77*	Brown Trout – Early Life 96 Hour LC50
38	4.83	Rainbow Trout – Early Life 96 Hour LC50
	3.8*	Brown Trout – 96 Hour LC50
47.6	2.1*	Rainbow Trout – 96 Hour LC50
100	13.1	Coho – Early Life 96 Hour LC50
	20.7	Rainbow Trout – 96 Hour LC50
	22.2	Hyalella Azteca – 96 Hour LC50
	42.2	Chironomid Tentans – 96 Hour LC50
	28.7	Daphnia Magna –48 Hour LC50
	8.29*	Selenastrum Capricornutum – 72 Hour IC50
250	17.4	Coho – Early Life 96 Hour LC50
	12.7*	Rainbow Trout – 96 Hour LC50
	31.0	Hyalella Azteca – 96 Hour LC50
	94.3	Chironomid Tentans – 96 Hour LC50
	76.3	Daphnia Magna –48 Hour LC50
454	49.9	Brown Trout – 96 Hour LC50

Note: * - denotes value that was used in the regression analysis

Linear regression was performed on the toxicity test data denoted by an asterisk in Table 4.10, values which generally represented the lowest acute manganese concentrations for each of the hardness values. The lowest values were chosen because the objective of establishing freshwater guidelines is to protect sensitive aquatic receptors; the most sensitive test results correspond to the lowest manganese concentrations and guidelines

developed from lower values should result in lower guidelines that will be more protective of sensitive species. At a water hardness of 25 mg/L $CaCO_3$, the coho salmon 96 Hour LC50 value of 2.1 mg/L was used in the regression. As discussed in Section 4.2.4, low water hardness likely contributed to toxic effects observed in the Daphnia magna 48 Hour LC50. Thus, toxicity was unlikely to be due only to concentrations of manganese and the 0.8 mg/L concentration was not included in the regression analysis. The brown trout 96 Hour LC50 at a hardness of 454 mg/L $CaCO_3$ was also omitted from the regression analysis. This decision was based on the following:

1. The absence of other data points at high hardness values, making in unclear whether brown trout was a sensitive species at high water hardnesses as compared to rainbow trout or other organisms for which no test data were available.
2. The observed decrease in slope of the resulting regression line when the 49.9 mg/L value was excluded, thus resulting in more conservative (lower concentration) values on which to base acute guidelines.
3. Water hardness values >300 mg/L $CaCO_3$ are uncommon in British Columbia fresh waters.

All acute values used in the regression analysis were 96 Hour LC50 concentrations with the exception of the 72 Hour IC50 value for S. capricomutum. The 72 Hour test duration was based on Environment Canada's standard procedures for this test (see Appendix A). The resultant equation and the statistical data associated with the regression line are provided in Appendix E and summarized below.

$$Y = 0.0441X + 1.81$$

where X = hardness in mg/L $CaCO_3$ and Y = Mn concentration in mg/L

correlation $r^2 = 0.902$ standard error = 1.46

For a water hardness of zero, the predicted manganese concentration would be 1.81 mg/L. A positive Y-intercept value makes sense because some level of tolerance of manganese would be expected even at very low water hardnesses. Manganese is a naturally occurring substance and it is expected that a threshold level would exist, below which no toxic responses would occur in aquatic organisms exposed to manganese regardless of variations in water hardness or other physical properties.

Table 4.11 summarizes predicted manganese concentrations for various hardness values.

TABLE 4.11: PREDICTED MANGANESE CONCENTRATIONS – ACUTE DATA					
Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)	Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)	Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)
25	2.9	125	7.3	225	11.7
50	4.0	150	8.4	250	12.8
75	5.1	175	9.5	275	13.9
100	6.2	200	10.6	300	15.0

The manganese concentrations predicted by the regression equation ranged from 2.9 mg/L for a hardness of 25 mg/L $CaCO_3$ to 15.0 mg/L for a hardness of 300 mg/L $CaCO_3$. The hardness range of 25 to 300 mg/L covers the likely range of values that occur naturally in B.C. fresh waters.

4.3.2 Chronic Toxicity Data – All Studies

Chronic toxicity test data for the BCMELP tests and data from literature sources screened in Section 4.1 were also combined for application of the BCMELP water quality guideline derivation procedures. The results are presented in Table 4.12:

TABLE 4.12: CHRONIC DATA FROM ALL STUDIES		
Water Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)	Toxicity Test
25	14.6	Rainbow Trout – 7 Day E-Test
30	4.67	Brown Trout – 62 Day IC25
36.8	0.79*	Rainbow Trout – 4 Month Growth/Survival
37.5	2.7*	Brown Trout – 4 Month Growth/Survival
100	20.0 5.4*	Rainbow Trout – 7 Day E-Test Daphnia Magna – 21 Day IC25
150	5.59*	Brown Trout – 62 Day IC25
250	22.4 9.4*	Rainbow Trout – 7 Day E-Test Daphnia Magna – 21 Day IC25
450	8.68*	Brown Trout – 62 Day IC25

Note: * - Denotes values that were used in the regression analysis

Linear regression analysis was performed on the manganese concentrations denoted by an asterisk in Table 4.12. The chosen values were the lowest concentrations at each of the test water hardness values. The lowest values were chosen because the objective of establishing freshwater guidelines is to protect sensitive aquatic receptors; the most sensitive test results correspond to the lowest manganese concentrations and guidelines developed from lower values should result in lower guidelines that will be more protective of sensitive species. The 7 Day E-Test result at a water hardness of 25 and the brown trout 62 Day IC25 result at a water hardness of 30 were not used as the values were considered to be too high (not sufficiently conservative). Other chronic data were available with similar hardnesses (36.8 and 37.5) and, in the case of brown trout, two chronic test results (hardnesses of 30 and 37.5 mg/L $CaCO_3$) were available and the more conservative value (2.7 mg/L for 4 month growth/survival) was considered to be the most appropriate choice.

The resultant equation and the statistical data associated with the regression line are presented in Appendix E and are summarized below.

$$Y = 0.0176X + 2.42$$

where X = hardness in mg/L $CaCO_3$ and Y = Mn concentration in mg/L

correlation $r^2 = 0.702$ Standard Error = 2.03

As with the acute data, a positive Y-intercept value is predicted by the equation. As discussed in Section 4.3.1, this is logical because a threshold concentration of manganese tolerable to most or all aquatic organisms would be expected to exist, below which no toxic responses would be anticipated. The slope of the chronic regression line (0.0176) is flatter than the slope of the acute regression line (0.0441); this also makes sense because a higher level of sensitivity would be expected under chronic exposure conditions.

The higher chronic Y-intercept (2.42 vs. 1.81 mg/L at a water hardness of 0) is a product of the data used to derive the regression lines. With sufficient data, it would be expected that the chronic Y-intercept would be lower than the acute Y-intercept. Although both the acute and chronic equations were based on six data points, the correlation factor (r^2) of 0.902 for the acute equation was notably higher. Substitution of the acute Y-intercept value was therefore given consideration as a conservative measure. This would result in the equation Y = 0.0176X + 1.81 and would predict chronic values that are 0.61 mg/L (2.42 – 1.81) lower than those predicted the chronic regression equation. However, application of a factor of safety (0.1 to 0.5 as outlined in Section 4 of Appendix B) would result in modified chronic manganese concentrations differing by 0.06 to 0.3 mg/L. This was not considered significant given other uncertainties associated with extrapolating toxicity test data (e.g. species differences, variable environmental conditions).

Table 4.13 presents the predicted manganese concentrations for the chronic regression equation.

TABLE 4.13: PREDICTED MANGANESE CONCENTRATIONS – CHRONIC DATA

Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)	Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)	Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)
25	2.9	125	4.6	225	6.4
50	3.3	150	5.1	250	6.8
75	3.7	175	5.5	275	7.3
100	4.2	200	5.9	300	7.7

The predicted manganese concentrations ranged from 2.9 mg/L at a water hardness of 25 mg/L $CaCO_3$ to 7.7 mg/L at a water hardness of 300 mg/L $CaCO_3$. The predicted acute and chronic values were the same at a water hardness of 25 mg/L $CaCO_3$, with lower chronic values for all water hardnesses >25 mg/L.

4.4 DERIVATION OF FRESHWATER GUIDELINES

The document *Derivation Of Water Quality Criteria To Protect Aquatic Life In British Columbia* (BCMELP, 1995), presented in Appendix B,. was referenced to derive proposed manganese guidelines. The predicted acute and chronic manganese concentrations presented in Tables 4.11and 4.13 will be used to derive interim acute and chronic fresh water guidelines for the protection of aquatic life. The acute interim guidelines would apply to short term exposure only while the chronic interim results would be intended for general application.

Section 4.1.1 of the B.C. Derivation document provides guidance on the application of safety factors in the derivation of aquatic life guidelines. The appropriate safety factors are listed as typically falling between 0.1 and 0.5 and are decided on a case by case basis using scientific judgement. A factor of safety of 0.25 was chosen for both the acute and chronic data sets based on the following:

1. The bulk of the toxicity test data was in the hardness range of 25 to 250 mg/L $CaCO_3$, which encompasses the range of hardnesses that would occur in most B.C. surface fresh waters.
2. The overall number of test results (19 acute, 10 chronic) meeting primary and/or secondary data requirements.
3. The variety of organisms for which suitable data were available (sufficient to meet full acute and interim chronic guideline requirements)
4. Use of minimum or near minimum concentrations (i.e. the most sensitive receptors in the toxicity test data set) in the acute and chronic regression line derivations.

The chronic safety factor chosen was the same as the acute safety factor despite the larger data set available for acute effects. The smaller data set was offset by the longer durations of the chronic tests, which typically result in more reliable toxicity values as opposed to acute values which tend to show more variability due to the shorter test durations. Some uncertainty was also associated with the acute data due to the BCMELP 96 Hour LC50 test on rainbow trout, which did not fit the pattern of increasing manganese tolerance with increasing water hardness.

Four of the six chronic values used in the chronic regression equation derivation were the IC25 concentrations for Daphnia magna at water hardnesses of 100 and 250 mg/L $CaCO_3$ and for brown trout at water hardnesses of 100 and 250 mg/L $CaCO_3$. The NOEC (no observed effect concentrations) values reported for these tests and the ratios of the NOEC to IC25 values are presented in Table 4.14.

TABLE 4.14: IC25/NOEC RATIOS FOR DAPHNIA MAGNA AND BROWN TROUT							
Daphnia Magna				Brown Trout			
Hardness ($mg/L\ CaCO_3$)	NOEC	IC25	NOEC/IC25	Hardness ($mg/L\ CaCO_3$)	NOEC	IC25	NOEC/IC25
100	3.6	5.4	0.67	150	4.41	5.59	0.79
250	7.3	9.4	0.78	450	8.68	8.68	1.0

The NOEC values are concentrations at which no adverse impacts were observed for chronic exposure to manganese. The NOEC/IC25 ratios varied between 0.67 and 1.0 for one fish species and one invertebrate species; this suggests that a factor of safety of 0.25 should be sufficiently protective for chronic exposure of aquatic life to manganese. A less conservative factors of safety (e.g. 0.4 or 0.5) was not chosen because the available toxicity data did not meet the requirements for full guideline derivation. There were not sufficient chronic tests on invertebrates and the types and numbers of species in the data set do not encompass all potentially sensitive species that exist in B.C. fresh waters. In addition, the 4 month chronic toxicity test value of 0.79 mg/L for rainbow trout at a hardness of 36.8 mg/L $CaCO_3$ (Davies and Brinkman, 1994) would be exceeded if a safety factor of 0.4 or 0.5 had been chosen. Rainbow trout is an important species in B.C. fresh water and the need to ensure protection of such a species was taken into account.

4.4.1 Acute Guidelines

The acute regression equation concentration data from Table 4.11 and the concentrations resulting from application of a factor of safety of 0.25 are presented in Table 4.15.

TABLE 4.15: MODIFIED MANGANESE CONCENTRATIONS – ACUTE					
Hardness ($mg/L\ CaCO_3$)	Manganese Concentration (mg/L)	Modified Manganese Concentration (mg/L)	Hardness ($mg/L\ CaCO_3$)	Manganese Concentration (mg/L)	Modified Manganese Concentration (mg/L)
25	2.9	0.7	175	9.5	2.4
50	4.0	1.0	200	10.6	2.7
75	5.1	1.3	225	11.7	2.9
100	6.2	1.6	250	12.8	3.2
125	7.3	1.8	275	13.9	3.5
150	8.4	2.1	300	15.0	3.8

Note: Modified Mn Concentration is the predicted Mn concentration multiplied by a factor of safety of 0.25

The modified acute manganese concentrations ranged for 0.7 mg/L to 3.8 mg/L within the range of water hardnesses from 25 to 300 mg/L $CaCO_3$.

4.4.2 Chronic Guidelines

The chronic regression equation concentration data from Table 4.13 and the concentrations resulting from application of a factor of safety of 0.25 are presented in Table 4.16.

TABLE 4.16: MODIFIED MANGANESE CONCENTRATIONS – CHRONIC

Hardness (mg/L $CaCO_3$)	Manganese Concentration (mg/L)	Modified Manganese Concentration (mg/L)	Hardness (mg/L $CaCO_3$)	Manganese Concentration (mg/L)	Modified Manganese Concentration (mg/L)
25	2.9	0.7	175	5.5	1.4
50	3.3	0.8	200	5.9	1.5
75	3.7	0.9	225	6.4	1.6
100	4.2	1.0	250	6.8	1.7
125	4.6	1.2	275	7.3	1.8
150	5.1	1.3	300	7.7	1.9

Note: Modified Mn Concentration is the predicted Mn concentration multiplied by a factor of safety of 0.25

The modified chronic manganese concentrations ranged for 0.8 mg/L to 3.9 mg/L within the range of water hardnesses from 25 to 300 mg/L $CaCO_3$. The modified chronic values were lower than the modified acute values for all water hardnesses.

4.4.3 Application of Guidelines

The derived acute and chronic guidelines presented in Sections 4.4.1 and 4.4.2 can be applied to fresh water as maximum acceptable concentrations at the corresponding hardness ranges. For water hardness values of 350 mg/L $CaCO_3$ or greater, the guidelines provided in Tables 5.1 and 5.2 could be applied. The acute guidelines would only apply for exposure durations of 96 hours or less. Exposures of longer duration would be considered chronic and the chronic guidelines would apply.

The guidelines reflect total manganese concentrations in fresh water. Natural variability exists for total manganese concentrations in surface water due to environmental factors such as the range of manganese concentrations that are present in different rock and soil types, the solubility of naturally occurring manganese compounds, the weathering rate of the soil/rock, and the amount of sediment suspended in the water. Section 2.1 of this thesis indicates that total manganese concentrations observed in B.C. surface waters range from <0.001 mg/L to 1.70 mg/L (CCME, 1987; BCMELP, 1998b), with concentrations in excess of 1.0 mg/L rarely observed. Higher concentrations were typically associated with higher seasonal flows. Application of chronic water quality guidelines for manganese should reflect the natural occurrence of peak events and the presence of non-anthropogenic sources of manganese in surface waters.

The modified manganese concentrations may be exceeded by naturally occurring manganese in stream water at water hardnesses below 100 mg/L $CaCO_3$ (acute) and 250 mg/L $CaCO_3$ (chronic). Surface fresh water data (BCMELP, 1998) suggest that higher concentrations occur during periods of higher stream flow (e.g. during spring runoff) and lower concentrations occur downstream of lakes (which act as settling areas for sediment). This natural variability should be taken into account when applying the proposed guidelines because the intent is to protect aquatic life from anthropogenic sources of manganese rather than naturally occurring manganese. Sampling of surface water upstream and downstream of discharge areas can provide a means of comparison. Sampling of groundwater adjacent to surface waters where manganese may be of concern could be undertaken to determine the likelihood that manganese concentrations observed in surface water are a result of human activities. End of pipe points of discharge could also be sampled to evaluate manganese concentrations prior to mixing with surface water, particularly during periods of high sediment loads.

The *Contaminated Sites Regulation* (Province of B.C., 1997) provides standards for substance concentrations in groundwater. For aquatic life water use, the current groundwater standard for manganese is 1 mg/L. A dilution factor of 10 for discharge of groundwater to surface water is assumed (i.e. the surface water value of 0.1 mg/L was modified by a factor of 10 to develop the 1 mg/L standard). The proposed chronic guidelines range from 0.6 mg/L to >1.9 mg/L, depending on hardness. Applying a dilution factor of 10 would result in groundwater values of 6 to >19 mg/L, considerably higher than the current 1 mg/L standard. The proposed guidelines are based on toxicity test results for a number of B.C. species and are considered to have a more solid scientific basis. If a groundwater standard for manganese for protection of aquatic life is retained, the proposed guidelines could be used to develop new groundwater standards. The current groundwater standard of 1 mg/L has frequently been exceeded throughout the province. A range of 6 mg/L to >19 mg/L would be founded on a more scientifically sound basis. In practical terms, it would remove many sites from "contaminated status" based on the proximity of a site to nearby surface water.

5.0 CONCLUSIONS

5.1 REVIEW OF THESIS OBJECTIVES

The objectives of this thesis research were as follows:

1. To review the existing freshwater aquatic life guideline for manganese;
2. To evaluate the practicality of application of the guideline;
3. To review the information available in the literature on manganese; and
4. To use new toxicity test data generated by the B.C. Ministry of Environment, Lands and Parks for native B.C. species in order to improve the existing freshwater aquatic life guideline.

Objectives 1 and 2 revealed that the existing freshwater aquatic life guideline is not toxicologically based and is not based on the protection of aquatic life. In order to fulfill Objective 4, toxicity testing was conducted on British Columbia aquatic species and the data generated were used in conjunction with supplemental data from the literature (Objective 3) to improve the existing guideline. Enhancement/modification of the existing manganese freshwater aquatic life guideline (Objective 4) resulted in a hardness dependent relationship, with manganese concentrations increasing with increased water hardness; the proposed guidelines are presented in Section 5.2.

5.2 PROPOSED ACUTE AND CHRONIC GUIDELINES

The modified acute and chronic concentrations presented in Tables 4.15 and 4.16 are proposed as surface water guidelines for manganese. For water hardnesses falling between increments of 25, it is proposed that the lower value be used as a guideline. The proposed acute and chronic guidelines are presented in Tables 5.1 and 5.2.

TABLE 5.1: PROPOSED INTERIM CHRONIC FRESHWATER AQUATIC LIFE GUIDELINES – MANGANESE (mg/L)

Hardness Range	Proposed Guideline	Hardness Range	Proposed Guideline
0-24	0.6	175-199	1.4
25-49	0.7	200-224	1.5
50-74	0.8	225-249	1.6
75-99	0.9	250-274	1.7
100-124	1.0	275-299	1.8
125-149	1.2	300-324	1.9
150-174	1.3	≥325	Mn = (0.0176H + 2.42) x 0.25

Note: H = hardness in mg/L $CaCO_3$

TABLE 5.2: PROPOSED INTERIM ACUTE (≤96 HOUR) FRESHWATER AQUATIC LIFE GUIDELINES – MANGANESE (mg/L)			
Hardness Range	Proposed Guideline	Hardness Range	Proposed Guideline
0-24	0.6	175-199	2.5
25-49	0.8	200-224	2.8
50-74	1.1	225-249	3.1
75-99	1.4	250-274	3.3
100-124	1.7	275-299	3.6
125-149	1.9	300-324	3.9
150-174	2.2	≥325	Mn = (0.0444H + 2.16) x 0.25

Note: H = hardness in mg/L $CaCO_3$

The manganese concentrations from the chronic data set presented in Table 5.1 are proposed as interim guidelines for protection of freshwater aquatic life to replace the existing manganese guideline of 0.1 mg/L, which applied to all water hardness values. For acute exposure (≤96 hour), manganese concentrations presented in Table 5.2 are proposed as interim guidelines.

A hardness dependent relationship where tolerable manganese concentrations increased with increasing water hardness was well supported by most of the BCMELP toxicity test data and the literature data. The exception was the 96 Hour LC50 acute toxicity test on rainbow trout, where the manganese concentrations were lower at a hardness of 250 mg/L $CaCO_3$ than at a hardness of 100 mg/L $CaCO_3$. Although this does not support the manganese/hardness relationship, the chronic regression equation predicted manganese concentration at a hardness of 250 mg/L was 6.8 mg/L while the rainbow trout LC50 concentration was 12.7 mg/L. The proposed guideline manganese concentration of 1.7 mg/L is well below the 12.7 mg/L value. In addition, if a trend exists for rainbow trout where the manganese concentration at which a toxic response occurs decreases with increasing hardness at values >250 mg/L $CaCO_3$, such hardness values are not commonly found in B.C. fresh waters.

From an aquatic life protection perspective, the modified manganese concentrations proposed are considered to be sufficiently protective of rainbow trout as well as other species. The factor of safety of 0.25 used in the derivation was considered to be suitably conservative given the quality and amount of acute and chronic toxicity tests and the range of species for which data were available. A less conservative factor of safety was not chosen because the data did not meet the requirements for full guideline derivation and uncertainties remain regarding sensitive species present in B.C. fresh waters for which no toxicity data is available. For a hardness range of 25 - 50 mg/L $CaCO_3$, a less conservative safety factor would have resulted in a guideline that exceeded the 4 month chronic toxicity value of 0.79 mg/L determined for rainbow trout (Davies and Brinkman, 1994), a species of importance in B.C. fresh waters.

Application of the guidelines to surface water should also reflect the presence of naturally occurring manganese. Where anthropogenic sources are to be regulated, measurement of manganese concentrations prior to discharge to surface water would help to separate non-anthropogenic manganese that may be at elevated levels due to sediment loads in surface waters. Applying the guidelines to end of the pipe effluent concentrations and to concentrations in groundwater immediately adjacent to a surface water body may alleviate concerns regarding naturally occurring manganese versus anthropogenic manganese. For groundwater, the presence of dissolved rather than total manganese may better reflect the mobile fraction that may discharge to surface water.

The former guideline range of 0.1 to 1 mg/L was modified to a range of 0.6 mg/L at a hardness of zero to 1.9 mg/L at a water hardness of 325 mg/L $CaCO_3$.

5.3 RECOMMENDATIONS FOR FURTHER STUDY

The toxicity testing program commissioned by BCMELP and conducted at Environment Canada's Aquatic Toxicity Laboratory were not comprehensive enough to permit derivation of full guidelines; consequently, interim guidelines were developed. In order to meet the requirements for full guideline development, additional aquatic toxicity testing would be required. Use of flowthrough tests or confirmation of Day 0 and final day manganese concentrations in the test water would be required. For invertebrates, an additional chronic study on a non-planktonic species would be required to meet the BCMELP full guideline requirements.

Additional studies on rainbow trout are also needed to establish whether a manganese/hardness relationship exists for this species or whether manganese tolerance in rainbow trout peaks at an intermediate manganese concentration. As discussed in Section 4, the rainbow trout 96 Hour LC50 results from the BCMELP toxicity testing program were the only data that did not fit the pattern of increasing manganese concentration with increased water hardness. Possible explanations for the decrease in tolerable manganese concentrations between hardnesses of 100 mg/L and 250 mg/L $CaCO_3$ are not clear at this time, but may include test organism or species specific intolerance of higher water hardness. Further studies at additional water hardness values such as 50, 150, 200 and 300 would be needed to identify whether manganese tolerance in rainbow trout peaks at a water hardness of between 25 and 250 mg/L or whether the data in the BCMELP study are somewhat anomalous.

Chronic toxicity testing would also be needed to determine if the observed effect on rainbow trout would occur under chronic exposure. The data from the Stubblefield et. al (1997) 62 day chronic study on brown trout, a species that is physiologically similar to rainbow trout and present in B.C. waters, are in direct contrast to the BCMELP acute rainbow trout data with respect to the manganese/hardness relationship. The chronic toxicity values derived in the brown trout study were also lower than those determined from the rainbow trout tests.

Infilling of these data gaps may allow future enhancement of the proposed guidelines by providing additional data that may further refine the regression equations developed to define the manganese/hardness relationship.

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APPENDIX A

Environment Canada Aquatic Toxicity Test Summaries

From: Buday,Craig [PYR] <Craig.Buday@EC.GC.CA>
To: sreimer@seacor.bc.ca <sreimer@seacor.bc.ca>
Date: Thursday, April 22, 1999 8:34 AM
Subject: Use of Report

Regarding your phone call a few days ago; I authorize the use of the information contained in Scott's report.

Craig Buday
EnvCan PESC EnvTox Lab

CCME/Water Quality Guidelines Study

Prepared For:

Aquatic Toxicology Division
Environment Canada
Pacific & Yukon Region
2645 Dollarton Highway
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V7H 1V2

Prepared By:

Scott Steer
Simon Fraser University
Science Co-op
May 1997
I

Organism	Reference
Rainbow Trout, Coho and Chinook	Watts, Ron and David Moul. Standard Operating Procedure for the 96-hour Acute Lethal Static Bioassay using Salmonids. August 1997.
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E-test, Rainbow Trout	Fennell, Michelle and Joy Bruno. Standard Operating Procedure for the Toxicity Test using Early Life Stage of Rainbow Trout. Feb. 1999.
	Environment Canada, "Biological Test Method: Toxicity Tests using Early Life Stages of Salmonid Fish (Rainbow Trout)". Environ. Prot. Serv., Environment Canada Report EPS 1/RM/28, Second Edition1998.
Daphnia magna	Moul, David. Standard Operating Procedure for the 48-hour Acute Lethality Test using *Daphnia magna* (non-legal and legal). August 1997.
	Environment Canada, "Biological Test Method: Acute Toxicity Test using Daphnia spp.". Environ. Prot. Serv., Environment Canada Report EPS 1/RM/11, July 1990, ammended May 1996.
Hyalella azteca	Environment Canada, "Biological Test Method: Test for Survival and Growth in Sediment using the Freshwater Amphipod *Hyalella azteca*". Environ. Prot. Serv., Environment Canada Report EPS 1/RM/33, Dec 1997.
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Chironomus tentans	Environment Canada, "Biological Test Method: Test for Survival and Growth in Sediment using the Larvae of Freshwater Midges (*Chironomus tentans or Chironomus riparius*).". Environ. Prot. Serv., Environment Canada Report EPS 1/RM/32, Dec. 1997.
	Steer, Scott. Standard Operating Procedure for the Testing of Growth and Survival in Sediment using Larvae of the Freshwater Midge, *Chironomus tentans*. Feb 1998.
Chronic Daphnia	Adams, William J. and Alan D. Forbis. Proposed Standard Guide for Conducting *Daphnia magna* Chronic Toxicity Tests, ASTM Subcommittee E47.01 Aquatic Toxicology, Draft No. 6, 1993
Microtox® Vibrio fischeri	Fennell, Michelle. Standard Operating Procedure for the Liquid-Phase Toxicity Test using Luminescent Bacteria (*Vibrio fischeri* formerly *Photobacterium phosporeum*). August 1997.
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Selenastrum capricornutum	Environment Canada, "Biological Test Method: Toxicity Test using the Freshwater Alga *Selenastrum capricornutum*". Environ. Prot. Serv., Environment Canada Report EPS 1/RM/25, 1992.
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Eohaustorius washingtonianus	Environment Canada, "Biological Test Method: Acute Test for Sediment Toxicity using Marine or Estuarine Amphipods". Environ. Prot. Serv., Environment Canada Report EPS 1/RM/26, 1992.
	Yee, Stewart. Standard Operating Procedure for the Acute Test for Sediment Toxicity using Marine or Estuarine Amphipods. August 1997.
Purple sea urchins	Environment Canada, "Fertilization Assay using Echinoids (Sea Urchins and Sand Dollars)". Method Development and Application Section, Environmental Technology Centre, Ottawa, Ontario. Report EPS 1/RM/27, 1992.

Organism	Source	Toxicity Test	Test Criteria		Water Quality Criteria-control dilution water only				Endpoint	Statistical Method
			Control survival	Other	pH	DO	Temp	Salinity		
Coho	Capilano hatchery	96 hour LC50	invalid if >10% mortality in control		5.5-8.5	>70%	15±1°C	n/a	LC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
Rainbow Trout	Spring Valley, Fraser Valley trout farms	96 hour LC50	invalid if >10% mortality in control	fish loading density < 0.5 g/L	5.5-8.5	>70%	15±1°C	n/a	LC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
Chinook	Chilliwack, Tenderfoot and Big Qualicum hatcheries	96 hour LC50	invalid if >10% mortality in control	marine water	5.5-8.5	>70%	15±1°C	28±2ppt	LC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
E-test, Rainbow Trout	Spring Valley trout farm, Campbell Lake hatchery, or BC hatcheries	7 day EC50 embryo viability test	mean percentage of nonviable controls greater than or equal to 30%	test in darkness	6.5-8.5	60-100%	14±1°C	n/a	EC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
Daphnia magna	CCIW Lab, Burlington, Ont. and EPA Corvallis, Oregon	48 hour LC50	invalid if control mortality >10%, or >10% of controls show overt stressed behavior	neonates < 24 hours old	6.0-8.5	90-100%	20±2°C	n/a	LC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
Hyalella azteca	CCIW Lab, Burlington, Ont. and EPA Corvallis, Oregon	96 hour LC50	invalid if mean control survival <90%	2 to 9 day old test organisms	n/a	90-100%	23±1°C	n/a	LC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
Chironomus tentans	SFU, Burnaby BC and EVS Consultants, North Vancouver, BC	96 hour LC50	invalid if mean control survival <90%	Third instar, 10 to 11 days from egg stage	n/a	90-100%	23±1°C	n/a	LC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
Chronic Daphnia	CCIW Lab, Burlington, Ont. and EPA Corvallis, Oregon	21 day reproduction inhibition	invalid if >30% mortality of first generation in controls, invalid if daphnids that lived 21 days in controls did not produce, on average, at least 60 young	first generation less than 24 hours old at test initiation	6.0-8.5	>40%	20±2°C	n/a	LOEC and NOEC . IC25	t-test, p=0.05 . . Bootstrap Icp
Microtox® *Vibrio fischeri*	Azur Environmental (formerly Microbics)	5 min IC50 15 min IC50	valid numerical estimate of IC50 should be based on concentrations showing light inhibition both greater than and less than the inhibition at the IC50		6.0-8.5	40-100%	15±0.3°C	2% NaCl adjusted for freshwater, marine not adjusted	IC50	Gamma- Microbics computer program

Organism	Source	Toxicity Test	Test Criteria		Water Quality Criteria-control dilution water only				Endpoint	Statistical Method
			Control survival	Other	pH	DO	Temp	Salinity		
Eohaustorius washingtonianus	Biologica Environmental Services, Victoria, BC	96 hour LC50	invalid if >10% overall mortality, or >20% mortality in any rep	n/a	n/a	90-100%	15±2°C	28±2ppt	LC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
Purple sea urchins	Biologica Environmental Services, Victoria, BC	10 min EC50 fertilization inhibition	average success of fertilization in control >= 50% and <100%	n/a	7.5-8.5	40-100%	15±2°C	28-34ppt	EC50	Stephen-Nonlinear Interpolation, Moving Average and Probit
Selenastrum capricornutum	University of Toronto Botany Department	72 hour IC50 growth inhibition	controls increased 16 x in 72h, homogeneity in controls (CV<20%, Hartley test), no trend or gradient in control	4 to 7 day old culture	6.0-8.5	n/a	24±2°C	n/a	IC50	Toxstat 3.2-Bootstrap

TOXICITY TERMS DEFINED:

LC50-median lethal concentration i.e. the concentration estimated to be lethal to 50% of test organisms

IC50-the median inhibition concentration i.e. the concentration estimated to cause a 50% reduction in growth or light production, compared to a control

ICp-the inhibiting concentration for a specified percent effect

EC50-median effective concentration, the concentration of material in water that is estimated to cause a discernible sublethal toxic effect to 50% of test organisms (e.g. fertilization inhibition)

NOEC-the no-observed-effect-concentration, highest conc. of a test material where no significant change in exposed organisms is apparent compared to control

LOEC-lowest-observed-effect concentration, lowest conc. of a test material where a statistically significant effect in exposed organisms was observed compared to control

If more than one test was done on one organism at the same hardness, only the most recent toxicity value was reported i.e. RBT LC50-hard water was done twice

1. INTRODUCTION

Under the direction of the B.C. Ministry of the Environment, Lands and Parks as well as the CCME, the Aquatic Toxicology section of Environment Canada has been requested to conduct a series of bioassays on various elements over the course of the 1996-97 fiscal year. The aim of the project is to acquire valuable toxicity information for the purpose of broadening the Canadian Water Quality Guidelines. The elements that were selected for review have been deemed high priority by the provincial water quality branch based on several criteria:

- The compound is prevalent in industrial discharges and other forms of aquatic pollution.
- The compound's toxicological data record is limited and/or outdated.
- There is a current environmental concern or issue involving the compound.
- The compound is relatively easy to handle and safe to work with.
- It is possible to obtain the chemical at a reasonable expense.

There are many compounds worthy of a toxicological review such as this but due to constraints on time, manpower and labspace, the project was confined to three elements: boron, manganese and barium.

2. PROJECT SYSTEM

2.1 Project Overview

Because no individual test procedure or organism could satisfy the comprehensive approach required to carry out this project effectively, each compound was run through a battery of toxicological tests involving different organisms and test endpoints. Some of the toxicological endpoints examined were lethality, growth inhibiton, light inhibition and reproduction inhibition. Section 3 provides a general outline for each bioassays performed.

Typically, each test was run with a set of five concentrations plus a control. In order to determine the concentration series that would be run, it was necessary to carry out a *range finder* based on previous toxicology data and a "best-guess" estimate. This procedure was usually only necessary before starting work on a new compound as suitable concentration series' for different organisms could be easily extrapolated from the first test.

At the start (Day 0) and end of each bioassay, a subsample from each prepared concentration was taken and analyzed to determine the *actual* test agent concentrations and to ensure there was no cross-contamination between solutions. On Day 0 of each test, an additional subsample of control/dilution water was taken to verify water hardness. Most results were generated using Environment Canada's statistical package for calculating LC50's, based on Stephan (1977).

2.2 Test Apparatus

Table 1 summarizes the various materials and equipment used to perform each bioassay.

Bioassay	Materials Used
Fish	salmonid underyearlings, aquaria, appropriate glassware and labware, scale, nets, thermometer, pH meter, dissolved oxygen meter
Chironomid	chironomid larvae, small paint brush, pipette, 250 mL beakers, silica sand, appropriate glassware and labware, scale, pH meter, D.O meter
Hyalella	2-9 day-old hyalella, pipette, 250 mL beakers, gauze, appropriate glassware and labware, scale, pH meter, D.O. meter
Daphnia/Chronic Daphnia	daphnia neonates, pipettes, beakers, appropriate glassware and labware, scale, pH meter, D.O. meter
Microtox	bacteria, Microtox Model 500 Analyzer (photometer), vials, disposable glass cuvettes, pipettors and pipettes, appropriate glassware and labware
Marine Amphipod	E. Washingtonius amphipods, 1 liter jars, scale, appropriate glassware and labware, pH meter, D.O. meter
Algae Growth Inhibition	Coulter Counter, microscope, centrifuge, photometer, filter apparatus, burner, gas source, microplates, pipettes, test tubes, petri dishes, aluminum foil, weigh plates, appropriate glass/labware.
Echinoderm Sperm Inhibition	sea urchins, Hemacytometer, slides, depression well slide, syringe, counting chamber, compound microscope, appropriate glass/labware.
E-Test	Rainbow Trout eggs, sperm, plastic testing vessels, large plastic stock vessels, air line tubing, fluid pump, weigh boats, pH meter, D.O meter

Table 1. Bioassay Materials

2.3 Test Organisms

The test organisms employed for the purposes of this project represented several taxonomic groups and habitat types. The following table describes each test organism in terms of taxonomic classification, habitat and some basic life history.

Organism	Taxonomy	Habitat and Habit
Rainbow Trout	Fish	Freshwater (lakes, streams and rivers) Feed on aquatic invertebrates
Coho	Fish	Freshwater and Marine
Chinook	Fish	Freshwater and Marine
Chironomus tentans	Insect	Larvae found within the sediment and water column of lakes and rivers. Important food source for fish.
Hyalella azteca	Crustacean	Epibenthic and sediment burrower of lakes, ponds and slow flowing streams. Important food source for fish.
Daphnia magna	Crustacean	Freshwater zooplankton (lakes and ponds). Often the dominant herbivore in lakes.
Photobacterium phosphoreum	Bacteria	Found in marine habitats. Produces blue-green light by enzymatic reactions.
Selenastrum capricornutum	Algae	Most freshwater habitat. Important food source for many species of zooplankton.
Purple Sea Urchin	Echinoderm	Marine. Found on rocky substrates. Feed on algae and are important food source of many marine animals.
E. Washingtonianus	Crustacean	Marine or estuarine habitats. Feed on detritus Important food source for fish and other vertebrates.

Table 2. Test Organisms

2.4 Water Types

Several water types were used in this project. The property of water hardness refers to the concentration of dissolved calcium carbonate ($CaCO_3$) a sample of water has. The water hardness at a given location is highly dependent upon the climatic conditions in the area as well as its geological makeup and, therefore, this property of water is highly variable throughout British Columbia. Compounds and chemicals behave differently at different water hardnesses

and, as a result, their toxicities to biota also vary. To account for this, each bioassay was run in three distinct water hardnesses; soft, medium and hard water.

"Soft water" refers to water with $CaCO_3$ concentrations in the range of 10-50 mg/L. Initially, Capilano River water was selected as the soft water of choice for the project, but its use was abandoned when it was found to be too soft for many test organisms to survive. The river water was replaced by reconstituted deionized water which had a more tolerable hardness value of 25 mg/L $CaCO_3$.

Pacific Environmental Science Centre well water has a hardness of approximately 100 mg/L $CaCO_3$ which fell nicely within the acceptable range of "moderately hard water" which is 80-100.

Water is deemed "hard" if it has a hardness value in excess of 160. For the purposes of the toxicity project, hard water (hardness ~250) was prepared by reconstituting well water.

3. TEST PROCEDURES

3.1 96-Hour Acute Lethal Fish Bioassay

Underyearling salmonid fish are placed in multiple concentrations of the compound being tested and monitored over a period of 96 hours. 35 litre rectangular fish tanks are used as test vessels. Coho and Rainbow Trout were tested with soft, medium and hard freshwater. Chinook salmon were tested in seawater. The bioassay is conducted in environmental chambers set at 15 ± 1 °C and an approximate light intensity of 480 lux. At the conclusion of the test, Environment Canada's statistical package is used to calculate the LC50.

3.2 96-HOUR ACUTE LETHAL CHIRONOMID BIOASSAY

Test worthy chironomids (third instar larvae) are placed in multiple concentrations of the metal being tested and monitored over a period of 96 hours. 250 mL beakers are used as test vessels in which a small quantity of silica sand is placed to act as a substrate for the organisms. The bioassay is conducted at 23 ± 1 °C and an approximate light intensity of 480 lux. The 96 hour LC50 value is computed by comparing the mortalities in the various test concentrations at the end of the test using the Stephan program.

3.3 96-HOUR ACUTE LETHAL HYALELLA BIOASSAY

The acute hyalella bioassay is essentially identical to the acute chironomid test. The only major difference between the two tests is that cheese cloth or gauze is used as a substrate in the hyalella test as opposed to the silica sand used in the chironomid bioassay.

3.4 48-HOUR ACUTE LETHAL DAPHNIA BIOASSAY

Daphnia magna neonates are placed in replicated, multiple, concentrations of the metal being tested and monitored over a period of 48 hours (Report EPS 1/RM/11, July 1990). 250 mL beakers are used as test vessels. The bioassay is conducted at 20 ± 1°C and an approximate light intensity of 480 lux. The 48 hour LC50 is computed by comparing the mortalities in the various test concentrations at the end of the test.

3.5 21-DAY CHRONIC DAPHNIA BIOASSAY

Due to the labor-intensive nature of this bioassay, three test concentrations and a control are run over a 21 day period. The acute lethal NOEC (i.e./ the concentration where there was No Observed Effect in the acute test) is set as the highest concentration in the chronic test. The test is started with daphnia no more than 24 hours of age. Three times per week, the number of neonates produced at each concentration is counted and total reproduction is calculated at the conclusion of the test. It is assumed that differences in reproductive output is due to the effect of the compound being tested. Chronic NOEC and chronic LOEC are calculated via statistical analysis of reproduction data.

3.6 MICROTOX TEST

Living bioluminescent bacteria are exposed to a test sample, and the toxic effect of the sample on the organisms is measured. The Microtox test system measures the light output of rehydrated lyophilized bacteria after exposure to a specified dilution series of a sample, and compares it to the light output of a control blank (i.e./bacterial cell suspension in diluent only). It is assumed that the difference in light production is due to the effect of the compound being tested. The degree of light loss (degree of metabolic inhibition in the bacteria) indicates the degree of toxicity of the sample. A dose-response curve is determined, on which the IC50 is located. The IC50 is the inhibiting concentration of a sample causing a 50 % decrease in the bacteria light output under defined conditions of exposure time and test temperature.

3.7 96-Hour Marine or Estuarine Amphipod Bioassay

This bioassay may be performed on any of several marine amphipod species depending on the purpose and conditions of the test. For the purposes of this project, the test species of choice was *Eohaustorius washingtonianus*. Test vessels used are 1 litre mason jars. The test is conducted at 15 ± 1 °C and in complete darkness, which is preferred by the amphipods 20 amphipods are placed in each vessel in the 5 concentration series, which are capped with lids and placed in black bags to eliminate light. At the conclusion of the test the Stephan computer program is used to determine the LC50.

3.8 Growth Inhibition Test Using Freshwater Algae

Selenastrum capricornutum is used as the freshwater alga in this bioassay. A small volume of test solution from each test concentration is inoculated with a known number of algal cells and incubated with nutrients for 72 hours. At the conclusion of the test, growth of the algae in the test concentrations are compared with algae growth in a control. Using Environment Canada's statistical package for calculating an LC50, the IC50 of the test is calculated.

3.9 Echinoderm Sperm Inhibition Bioassay

Sperm cells are exposed to solutions of toxic agent for 10-15 minutes prior to addition of eggs to the solution for fertilization. Reduced fertilization success, as indicated by the presence or absence of the fertilization membrane, is used as an indicator of toxic effects on the sperm viability and /or the fertilization response. At the conclusion of the test, an IC50 value is generated using the Stephan method.

3.10 Early Life Stage Toxicity Test Using Salmonid Fish

The early life stage examined in the 7 day E-Test is the freshly-fertilized rainbow trout (*Oncorhynchus mykiss)* egg exposed through the first seven days of embryonic development. Fertilized rainbow trout eggs are exposed to various concentrations of a sample and allowed to develop over a 7 day exposure period at 15 ± 1°C. At the end of the test the embryos are immersed in a fixative to preserve and clear the eggs. Each embryo and or unfertilized egg is scored as viable or non-viable. Viable eggs contain a white embryonic streak while unfertilized eggs, embryos showing significant retardation of development, deformed embryos and Siamese twins are scored as non-viable. For each incubation unit, the percent non-viable embryos is calculated and the EC50 for each test is determined using the Stephan method.

4.0 Results

Tables 3, 4 and 5 present toxicity results obtained from bioassays involving boron, manganese and barium respectively. All results have been presented in terms of milligrams of toxic agent per litre of dilution water. Selenastrum results are not included in this data set as the algae growth inhibition bioassay was a late addition to the program and testing was not complete when this report was being prepared. For a few of the tests performed, chemical verification of test concentrations was not done and as such, actual or corrected data could not be reported. As well, for most bioassays performed with sea water, chemistry samples were not analyzed due to the high salt sensitivity of the chemistry lab equipment.

4.1 Boron

Water Hardness	Bioassay	Result (mg/L) Experimental	Actual(Corrected)
Soft	Coho	348.2	357.4
	Rainbow Trout	409.4	438.7
	Daphnia	20.1	21.3
	Hyalella	26.9	28.9
	Chironomid	114.6	157.3
	E-test	574	598
	Chronic Daphnia	Water too soft. Test not done.	
	Microtox	5 min.- 224.4	
		15 min.- 187.8	
Moderate	Coho	302.5	304.1
	Rainbow Trout	379.2	379.6
	Daphnia	56.2	52.4
	Hyalella	288.6	291.3
	Chironomid	122.4	118.0
	E-Test	808	821
	Chronic Daphnia	LOEC- 25	LOEC- 25.4
		NOEC- 12.5	NOEC- 13.1
	Microtox	5 Min.- 301.7	No chem taken
		15 Min.- 272.8	
Hard	Coho	457.5	477.1
	Rainbow Trout	312	334
	Daphnia	131.2	139.2
	Hyalella	322.3	333.6
	Chironomid	139.1	137.7
	E-test	No result at time of report	
	Chronic Daphnia	LOEC- 25	LOEC- 26.4
		NOEC- 12.5	NOEC- 12.4
	Microtox	5 Min.- 371.3	
		15 min.- 280.8	
Sea	Coho	111.3	122.6
	Marine Amphipod	847.7	
	Echinoderm Fert.	503.3	
	Microtox	5 min.- 270.7	
		15 min.- 198.2	

Table 3. Boron test results.

4.2 Manganese

Water Hardness	Bioassay	Result (mg/L) Experimental	Actual(Corrected)
Soft	Coho	2.3	2.4
	Rainbow Trout	2.1	
	Daphnia	1.0	0.9
	Hyalella	3.5	3.5
	Chironomid	5.5	5.8
	E-test	10.8	11.5
	Chronic Daphnia	Water too soft. Test not done.	
	Microtox	5 min.- 872.7	
		15 min.- 73.1	
Moderate	Coho	12.7	13.2
	Rainbow Trout	20.7	
	Daphnia	29.4	30.6
	Hyalella	20.6	21.4
	Chironomid	40.6	42.2
	E-Test	20.9	20.0
	Chronic Daphnia	LOEC- 6.8	LOEC- 6.7
		NOEC- 3.5	NOEC- 3.5
	Microtox	5 Min.- 3808.3	
		15 Min.- 88.0	
Hard	Coho	18.9	17.4
	Rainbow Trout	16.1	12.7
	Daphnia	75.4	79.7
	Hyalella	30.8	32.7
	Chironomid	108	101
	E-test	29.5	22.7
	Chronic Daphnia	No result at report time	
	Microtox	5 Min.- 10542.4	
		15 min.- 124.3	
Sea	Chinook	194.1	214.4
	Marine Amphipod	178.0	193.9
	Echinoderm Fert.	442.2	
	Microtox	5 min.- 7550.0	
		15 min.- 759.6	

Table 4. Manganese test results.

4.3 Barium

Water Hardness	Bioassay	Result (mg/L) Experimental	Actual(Corrected)
Soft	Coho	48.2	
	Rainbow Trout	73.6	65.0
	Daphnia	56.2	50.1
	Hyalella	61.2	57.9
	Chironomid	578.4	
	E-test	No result at report time	
	Chronic Daphnia	Water too soft. Test not done.	
	Microtox	5 min.- 7749.7	
		15 min.- 3673.7	
Moderate	Coho	153.4	157.2
	Rainbow Trout	262.3	274.3
	Daphnia	77.1	68.2
	Hyalella	42.4	
	Chironomid	933.4	975.9
	E-Test	878.3	907.2
	Chronic Daphnia	LOEC- 6.8	LOEC- 6.7
		NOEC- 3.4	NOEC- 3.5
	Microtox	5 Min.- 7036.2	
		15 Min.- 3574.0	
Hard	Coho	578.0	799.0
	Rainbow Trout	635.8	645.9
	Daphnia	342.7	343.8
	Hyalella	294.5	272.7
	Chironomid	765.1	776.7
	E-test	No result at report time	
	Chronic Daphnia	No result at report time	
	Microtox	5 Min.- 9569.2	
		15 min.- 5713.7	
Sea	Coho	2798.1	
	Marine Amphipod	2397	
	Echinoderm Fert.	42.8	
	Microtox	5 min.- 16000.2	
		15 min.- 7020.9	

Table 5. Barium test results.

5. Discussion

Upon inspection of the results, it is difficult to identify patterns among test organisms or the toxic agents they have been exposed to. However, several generalizations can be made concerning species sensitivities and the toxicology of compounds in different types of water. First, it is clear that daphnia and hyalella are the most sensitive species when exposed to any of the three toxic agents tested. These two crustacean species depend heavily on a high level of water quality and have a low tolerance for changes in their aquatic environment. Daphnia, in particular, are highly stressed in soft water and as such exhibit acute sensitivity to toxins under these adverse conditions. In general, the trout and salmon species exhibited lower sensitivities to boron and barium than the other species but proved to be more sensitive to manganese in many cases. It is likely that fish have a greater capacity to process and detoxify pollutants than some of the other species which allows them to be more tolerant to most toxins.

The toxicity of a compound to a test organism varies greatly depending on the hardness of the water in which it is dissolved. Generally, the toxicity of the agents tested increased with a decrease in water hardness. Often, the LC50 of a toxic agent was 1 and sometimes 2 orders of magnitude lower in soft water than in hard water. This phenomenon is due to the concentration of toxic agent that is *bioavailable* to the organism that it is exposed to. When a toxic agent is able to bond to dissolved solids, the amount that is bioavailable to the organism declines and the effective toxicity of the agent decreases. In soft water, there is only a small amount of dissolved solids that the toxic agent can bind to and thus more of the toxin is available to take effect on the test organism. In hard water, the reverse is true. Much of the toxin is bound-up by dissolved solids which essentially decreases the amount that the organism is exposed to.

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Glossary

Acute Toxicity - a discernible adverse effect (lethal or sublethal) induced in the test organism within a short period of exposure to a test material, usually ≤ 4 days for fish.

Control - treatment in an investigation or study that duplicates all the conditions and factors that might affect the results of the investigation, except the specific condition that is being studied. The control is used to determine the absence of measurable toxicity due to basic test conditions (e.g., quality of the control/dilution water, health or handling of test organisms).

Control/dilution water - the water used for diluting the test material, or for the control test, or both.

Deionized Water - water that has been passed through resin columns to remove ions from solution and thereby purify it.

EC_{50} - the median *effective* concentration (i.e., the concentration of material that is estimated to cause a predetermined effect in 50 % of the test organisms).

Endpoint - the variables (ie.,time, reaction of the organisms, etc.) that indicate the termination of a test, and also means the measurement(s) or value(s) derived, that characterize the results of the test (LC50, LT50, etc.).

IC_{50} - the median concentration of material in water that is estimated to be *inhibitory* (i.e., to growth, reproduction etc.) to 50 % of the test organisms after a fixed period of exposure.

LC_{50} - the median concentration of material in water that is estimated to be *lethal* to 50 % of the test organisms. The LC_{50} and its 95 % confidence limits are usually derived by statistical analysis of mortalities in several test concentrations, after a fixed period of exposure.

LT_{50} - the time (period of exposure) estimated to cause 50 % mortality in a group of fish held in a particular test solution.

LOEC - the lowest-observed-effect-concentration. This represents the lowest concentration of a test material to which organisms are exposed and for which a statistically significant effect was observed relative to the control.

NOEC - the no-observed-effect concentration. This represents the highest concentration of a test material to which organisms are exposed and in which no significant effect was observed relative to the control.

Neonate - a newly-born or newly-hatched individual (first-instar daphnid, ≤ 24-h old).

APPENDIX B

B.C. Guideline Derivation Protocol

MINISTRY OF ENVIRONMENT, LANDS AND PARKS
PROVINCE OF BRITISH COLUMBIA

DERIVATION OF WATER QUALITY CRITERIA TO PROTECT AQUATIC LIFE IN BRITISH COLUMBIA

WATER QUALITY BRANCH
ENVIRONMENTAL PROTECTION DEPARTMENT

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SUMMARY

This document has been prepared to outline clearly the procedure used to derive water quality criteria in BC. This procedure will help to identify data gaps, encourage research, and provide a sound basis for defensible criteria. It will also provide a consistent format for the derivation of criteria, and serve as a checklist to ensure the appropriate information has been considered.

The following is a brief overview of the procedure used to derive water quality criteria to protect aquatic life in BC.

Substances of concern at the provincial level are identified and ranked for criteria development after consultation within the Ministry of Environment, Lands and Parks. Substances are then selected for criteria development after consultation with federal and other provincial jurisdictions to avoid duplication of efforts.

For each substance selected, a literature search is conducted to obtain information on the following:

- physical and chemical properties;
- environmental concentrations with special emphasis on BC levels;
- environmental fate and behaviour;
- bioaccumulation potential;
- acute toxicity to aquatic biota;
- chronic toxicity to aquatic biota;
- mode of toxic action; and
- information from other jurisdictions.

To proceed with criteria derivation, certain minimum toxicity and environmental fate data requirements should be met. In cases where there is insufficient information to set criteria, interim criteria can be derived providing that a less stringent data set is available.

Key toxicity studies found in the literature search are evaluated to ensure that acceptable laboratory practices were used in the design and execution of the experiments. Each key study is judged on its scientific acceptability.

When available, the lowest reliable LC50 or EC50 from an acute toxicity test and the lowest-observed-effect level (LOEL) from a reliable chronic exposure study, on sensitive native BC species, are selected. These values are then multiplied by an appropriate safety factor to derive an acute and a chronic criterion. For certain substances, only a single criterion is set which is based on the LOEL from a chronic exposure study or on bioaccumulation. Other factors taken into account include no-observed-effect levels (NOEL), and ambient background concentrations for naturally occurring substances. Alternatively, the most sensitive LC50 or EC50 from an acute exposure study is multiplied by an acute/chronic ratio or appropriate application factor to determine an interim criterion concentration.

TABLE OF CONTENTS

1. INTRODUCTION

BC Environment is developing province-wide ambient water quality criteria for substances or physical attributes that are important in both fresh and marine surface waters of British Columbia. This work has the following goals:

- to provide a basis for the evaluation of data on water, sediment and biota for water quality assessments;
- to provide a basis for the establishment of site-specific ambient water quality objectives;
- to identify areas with degraded conditions;
- to provide a basis for establishing wastewater discharge limits; and
- to provide part of the information needed to establish waste discharge fees.

The definition adopted for *criterion* is:

"A maximum and/or minimum value for a physical, chemical or biological characteristic of water, sediment or biota, applicable province-wide, which should not be exceeded to prevent specified detrimental effects from occurring to a water use, including aquatic life, under specified environmental conditions".

The criteria are province-wide in application, but use-specific, and are being developed for the following water uses:

- Drinking, public water supply and food processing[1]
- Aquatic life (and their consumers) and wildlife
- Agriculture (livestock watering and irrigation)
- Recreation and aesthetics[2]
- Industrial (water supplies)

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1 The criteria apply to the ambient raw water source before it is diverted or treated for domestic use. The Ministry of Health regulates the quality of water for domestic use after it is treated and delivered by a water purveyor.

2 Criteria relating to public health at bathing beaches are the same as those used by the Ministry of Health which regulates their use.

The criteria are set after considering the scientific literature, criteria from other jurisdictions, and conditions in British Columbia. The scientific literature gives information on the effects of toxicants on various life forms. This information is rarely conclusive because it is usually based on laboratory tests on a limited number of species which only approximates field conditions. To compensate for this uncertainty, criteria have built-in safety factors. We use safety factors which are conservative, but the ambient background conditions in the province are also considered for those substances that occur naturally.

This document describes how criteria are derived to protect aquatic life, and applies to toxic chemical substances more than the physical properties of water (e.g., temperature, pH, suspended solids). Derivation of water quality criteria to protect other water uses will be described under separate cover.

Neither criteria, nor objectives[3] which are derived from them, have any legal standing. They are intended as a tool to provide policy direction to those making decisions affecting water quality provided that they do not allow legislated effluent standards to be exceeded. The objectives can be used to establish the allowable limits in waste discharges. These limits are set out in waste management permits, plans, or operating certificates which do have legal standing. The objectives are not usually incorporated as conditions of the permit.

1.1 Background

This document is required to:

- maintain consistency in the derivation of water quality criteria;
- lay out the procedure in clear terms;

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3 The ambient water quality objectives for specific waterbodies are based on the province-wide criteria as well as on present and future uses, waste discharges, hydrology/limnology/oceanography, and existing background water quality. The process for establishing water quality objectives is more fully outlined in "Principles for Preparing Water Quality Objectives in British Columbia", copies of which are available from the Water Quality Branch.

- serve as a checklist to ensure that all aspects are considered; and
- identify data gaps, to encourage research and to provide a better basis upon which to set more defensible criteria.

The approach used by the Province to derive water quality criteria is similar in many respects to that used by the Canadian Council of Ministers of the Environment (CCME) to derive national water quality guidelines (which are analogous to BC criteria). The latter is outlined in "Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life; April, 1991 (CCME, 1991). This similarity is due, in part, to input by BC. Environment scientists as members of the CCME review team. The CCME document was chosen as a template upon which to build and refine these Provincial procedures.

Certain fundamental aspects of criteria derivation in BC differ from the CCME approach. One change worth noting is that, for some water quality variables, both acute and chronic criteria are recommended by the Province to address acute and chronic toxicity independently, whereas the CCME specifies only a single concentration to address all conditions. In developing the BC approach, a review of all existing approaches (including the U.S. EPA Water Quality Criteria, and the Ontario and CCME Water Quality Guidelines) was performed. In addition, we recognized that scientific judgement is an important and vital part of the process for deriving practical and useable water quality criteria. Accordingly, this document is designed to serve as a guide for those involved in deriving water quality criteria; it is not intended as a protocol to be followed rigidly in every respect.

Water quality criteria to protect aquatic life have been prepared for many substances of concern and criteria for other substances continue to be prepared including other priority substances judged to be most urgently needed for water quality assessments and objectives in BC. Until criteria for certain substances are approved by the Ministry Executive, the Water Quality Branch is using what is termed 'working' criteria for water quality, many of which have been recommended by the CCME – formerly known as the Canadian Council of Resource and Environment Ministers (CCREM). This is consistent with Ministry policy that the CCME Guidelines are to be used in developing water quality criteria and objectives and in assessing water quality, but recognizes that site-specific factors may necessitate modification of the CCME Guidelines (Pommen, 1991).

1.2 Guiding Principles for the Development of Water Quality Criteria for Aquatic Life

There are several over-riding principles used in developing water quality criteria in BC. These are:

- In deriving water quality criteria to protect aquatic life in BC, all components of the aquatic ecosystem (e.g., algae, macrophytes, invertebrates, amphibians, fish) are considered if the data are available. Where data are available but limited, interim criteria are deemed preferable to no criteria.
- The approach to the development of criteria for aquatic life follows that of the CCME (1991), which reflects the philosophy that all forms of aquatic life and all aquatic stages of their life cycle are to be protected during indefinite exposure. It should be noted however, that this approach may not protect individuals weakened to some degree through age, illness, or injury. Whether this goal can be realized is a separate issue and does not influence the criteria derivation procedure.
- For some substances both an acute and a chronic criterion are recommended as provincial water quality criteria, provided sufficient toxicological data are available. Both conditions should be met to protect aquatic life. For other substances which may not be acutely toxic due to their low water solubilities (e.g., PCBs and Dioxins), but may be of concern due to their accumulation in aquatic life, the criterion is a single value which should not be exceeded. This value is based on a long-term, no-effect level.
- Unless otherwise specified, a criterion refers to the total concentration of a substance in an unfiltered sample. Total concentrations will apply unless it can be demonstrated that the relationship between other measures of the substance and their toxicity is firmly established, and analytical techniques have been developed that unequivocally identify the toxic fraction of a substance in a consistent manner using routine field-verified measurements.

2. Data Requirements For Criteria Derivation

To set water quality criteria, certain basic data should be available. Where insufficient data are available to set criteria, interim criteria may be set. The interim criteria may be upgraded to full criteria status when the data gap is filled. While minimum data requirements have been recommended for both criteria and interim criteria, it is important to emphasize that these are intended as a guide, not as a strict requirement.

Flexibility and the use of scientific judgement as well as innovative new approaches are recognized as necessary and important components of the derivation process. For example, consideration must be given to the nature of the substance such as its mode of toxic action, its bioaccumulation potential, or if it exhibits delayed toxicity. Exemptions from the minimum data requirements may be considered on a case-by-case basis provided they are documented and scientifically justified. The final decision of whether criteria or interim criteria are recommended is based, in part, on the confidence the authors have in the criteria. If interim criteria are set, then it is the responsibility of the authors to justify their position and to recommend the information needed to elevate interim criteria to full criteria status.

2.1 Minimum Aquatic Toxicity Data Requirements for Freshwater Criteria

The goal of freshwater aquatic criteria is the protection and maintenance of all forms of aquatic life and all aquatic life stages in the freshwater environment. Therefore, it is essential that data for fish, invertebrates, and plants be included in the criteria derivation process. For this purpose, minimum data requirements have been recommended (Table 2.1). In the derivation process (see Section 3), criteria or interim criteria may be derived from studies involving species not required in the minimum data set (e.g., amphibians, protozoa, bacteria), when reasonable justification exists.

Table 2.1 Minimum Data Requirements for Freshwater Criteria

Fish

- To set a chronic criterion, at least three chronic studies on three or more freshwater species resident in BC, including at least two cold-water species (e.g.,trout).

- To set an acute criterion, at least three acute studies on three or more freshwater species resident in BC, including at least two cold-water species.

Invertebrates

- To set a chronic criterion, at least two chronic (partial or full life-cycle) studies on two or more invertebrate species from different classes, one of which includes a planktonic species resident in BC (e.g., daphnid).

- To set an acute criterion, at least two acute studies on two or more invertebrate species from different classes, one of which includes a planktonic species resident in BC.

Plants

- at least one study on a freshwater vascular plant or freshwater algal species resident in BC.

- for highly phytotoxic substances, three acute and/or chronic studies on non-target freshwater plant or algal species.

The reduced requirements for plant toxicity studies were deemed necessary because fewer studies on plants have been conducted (Swanson and Peterson 1988). The minimum data requirements for plants could be increased in the future if data availability improves.

In cases where the minimum data requirements for criteria derivation are not met, interim water quality criteria may be developed provided the minimum data set requirements shown in Table 2.2 are met.

Table 2.2 Minimum Data Requirements for Interim Freshwater Criteria

Fish
• at least two acute and/or chronic studies on two or more fish species, one of which includes a coldwater species (e.g., trout) resident in BC.
Invertebrates
• at least two acute and/or chronic studies on two or more invertebrate species from different classes, one of which includes a planktonic species resident in BC (e.g., daphnid).

If a toxicity study indicates that a plant species is the most sensitive species in the data set, then this study shall be used in the interim criteria derivation process. However, in the absence of data on plants, interim criteria can be derived provided that this data gap is noted. The information that is required to elevate interim criteria to full criteria status needs to be clearly identified to stimulate research that will generate the necessary data.

2.2 Minimum Aquatic Toxicity Data Requirements for Marine Criteria

Recognizing that toxicants may react differently in marine water than in fresh water, and that different species are involved, the data requirements are different to reflect the need for separate criteria for the marine situation. This need for separate marine criteria has been demonstrated by the U.S. EPA and supported by the CCME.

For most substances, however, there are fewer data available for marine species, particularly phytoplankton and macroalgae, than are available for the fresh water environment (Hansen 1989). Since the goal of marine aquatic criteria is the protection and maintenance of all forms of aquatic life and aquatic life stages in the marine environment, it is recommended that data for marine fish, invertebrates, and plants be included in the criteria derivation process. As with the requirements for fresh water aquatic life criteria, minimum data requirements have been recommended (Table 2.3). In this data set, marine species include those species found in estuarine, coastal, and open-ocean habitats, any of which may be used to derive a criterion or interim criterion.

Table 2.3 Minimum Data Requirements for Marine Criteria

Fish

- To set a chronic criterion, at least three studies on three or more temperate marine fish species, including at least two chronic (partial or full lifecycle) studies.

- To set an acute criterion, at least three acute studies on two or more temperate marine fish species.

Invertebrates

- To set a chronic criterion, at least two chronic (partial or full lifecycle) studies on two or more temperate marine invertebrate species from different classes.

- To set an acute criterion, at least two acute studies, on two or more temperate marine invertebrate species from different classes.

Plants

- at least one study on a temperate marine vascular plant or marine algal species.

In cases where the minimum data requirements are not met, interim water quality criteria can be derived providing the minimum data requirements shown in Table 2.4 are met.

Table 2.4 Minimum Data Requirements for Interim Marine Criteria

Fish

- at least two acute and/or chronic studies on two or more marine fish species, one of which is a temperate species.

Invertebrates

at least two acute and/or chronic studies on two or more marine species from different classes, one of which is a temperate species.

If a toxicity study indicates that a plant species is the most sensitive species in the data set, then this study shall be used in the interim criteria derivation process. However, in the absence of data on plants, interim criteria can be derived provided that this data gap is clearly identified. As with freshwater aquatic life criteria, the information that is required

to elevate interim criteria to criteria status needs to be clearly identified to stimulate research that will generate the necessary data.

2.3 Minimum Environmental Fate and Behaviour Data Requirements

In addition to the minimum toxicity data requirements outlined above, studies that have investigated the major environmental fate processes and persistence of the substance in water, soil, sediment, air, and biota are required. Potential fate processes include volatilization, hydrolysis, oxidation, photolysis, aerobic and anaerobic biodegradation, long-range transport, soil and sediment sorption/desorption, bioconcentration and bioaccumulation. It is not necessary to have information on each potential fate process. Rather, the intent is to be able to identify the major environmental pathways and fate of a substance in the aquatic environment. Specifically, the following should be determined:

- the mobility of the substance and the compartments of the aquatic environment in which it is most likely to be distributed;
- the kinds of chemical and biological reactions that occur during transport and after deposition;
- the eventual chemical form(s);
- the persistence of the substance in water, sediment, and biota;
- physical and chemical properties; and
- ambient background concentrations for those substances that occur naturally (criteria for some substances are based solely on background concentrations when they occur naturally and fluctuate widely, e.g., turbidity and suspended solids).

Where possible, the persistence of a substance should be expressed in terms of its half-life. Where significant environmental fate information is lacking, interim criteria are set. In these cases, the information required to elevate the interim criterion to full criterion status needs to be clearly identified to stimulate the necessary research.

2.4 Additional Information

The following are not required elements of the minimum data set, but should be included when available because they are useful in assessing the potential hazard of a substance:

- production and uses;
- organoleptic effects (taste, odour, fish flesh tainting);
- sources to the aquatic environment;
- methods of analysis and current detection limits;
- concentrations in the aquatic environment;
- mode of toxic action;
- toxicity of the metabolites and breakdown products;
- sensitivity of birds and wildlife consuming aquatic organisms; and
- criteria, guidelines, objectives, and standards from other jurisdictions.

3. Evaluation Of Toxicity Data

Since standard protocols for toxicity testing may become outdated or are not always available or followed, a great deal of variability exists in the quality of published data. To ensure a consistent scientific evaluation for each substance, the data included in the minimum data set should meet certain standards. These include information on test conditions/design (e.g., flow-through, renewal, static), test concentrations, temperature, hardness, pH, adjuvants (i.e., synergistic effects), experimental design (controls, number of replicates), and a description of the statistics used in evaluating the data.

A variety of standardized test protocols have been developed for fish, invertebrates and plants. When appropriate, these should be consulted during the evaluation process (for example, see BC Ministry of Environment 1982; EPS 1980; ASTM 1980; OECD 1981; Rand and Petrocelli 1985; U.S. EPA 1985a, 1985b, 1985c; Sergy 1987; Swanson and Peterson 1988). Information useful for interpreting toxicity data is also available (Buikema *et al.* 1982; Rand and Petrocelli 1985, ch. 1-11) and should be consulted when necessary. When consulting test protocols, it is important to be aware of the following limitations:

- protocols consider only a few well-studied species and biological processes;
- our knowledge of extrapolation from one species to another (i.e., comparative ecotoxicology) is very limited;
- there is limited knowledge of the effects of metabolites and other environmentally transformed products of the parent chemicals;
- protocols do not take into account cumulative effects of chemicals or compensatory responses of organisms (such as acclimation or reduced density-dependent mortality amongst juveniles); and
- the predictability of laboratory exposures and effects on aquatic ecosystems has not been adequately tested (Sheenan *et al.* 1984; Arthur 1988; Petersen and Petersen 1988).

Therefore, it is essential that the evaluation of toxicity data not follow a rigidly fixed format. Once evaluated, key data are classified as primary, secondary, or unacceptable as described in Table 3.1.

All data included in the minimum data set should be primary for criteria derivation to proceed. For interim criteria derivation, primary or secondary data may be used. In either

case, a weight-of-evidence approach always should be an underlying principle of criteria derivation. Unacceptable data cannot be used in either derivation procedure.

Table 3.1 Classification of Toxicity Data

Primary Data

- Toxicity tests must employ currently acceptable laboratory practices of exposure and environmental controls (see, for example, citations in text). Other types of tests using more novel approaches will be evaluated on a case-by-case basis.
- As a minimum requirement, substance concentrations must be measured at the beginning and end of the exposure period. Calculated concentrations or measurements taken in stock solutions are unacceptable.
- Generally, unrenewed static tests are unacceptable unless it can be shown that substance concentrations did not change during the test and that adequate environmental conditions for the test species were maintained.
- Preferred endpoints from a partial or full lifecycle test include a determination of effects on embryonic development, hatching, germination success, survival of juvenile stages, growth, photosynthesis, reproduction, and survival of adults.
- Endpoints should be demonstrated to be ecologically relevant toxic endpoints. These generally include reproduction, growth, development and survival of young and adults.
- Response and survival of controls must be measured and should be appropriate for the life stage of the test species used.
- Measurements of abiotic variables such as temperature, pH, dissolved oxygen, and water hardness should be reported so that any factors that may affect toxicity can be included in the derivation process.

Secondary Data

- Toxicity tests may employ a wider array of methods (e.g., measuring toxicity while test species is exposed to additional stresses such as low temperatures, lack of food, or high salinity).
- Static tests are acceptable.
- Preferred test endpoints include those listed for primary data as well as pathological, behavioural, enzymatic, and physiological effects.
- Calculated substance concentrations are acceptable.
- All relevant environmental variables should be measured and reported. The survival of controls must be measured and reported.
- Data that meet all the conditions of primary data but are obvious outliers when compared to the results of at least two other tests performed under the same or similar conditions on the same or closely related organisms. In other words, weight-of-evidence principle may be applied to discard outliers.

Unacceptable Data

- Toxicity data that do not meet the conditions of primary or secondary data.

4. CRITERIA DERIVATION

There are four levels or categories of water quality criteria to protect aquatic life in BC. These are:

- acute and chronic criteria derived from acute and chronic studies, respectively;
- a single criterion derived from chronic studies;
- a single criterion derived from bioconcentration studies; and
- an interim criterion derived from acute and/or chronic studies.

The choice of which level to apply depends on a number of factors such as the quantity and quality of toxicity data, and the nature of the substance.

Criteria derived from chronic studies are preferably based on the lowest-observed-effect level (LOEL), using a non-lethal endpoint for the most sensitive life stage, of the most sensitive aquatic species investigated. However, when these types of data are unavailable, interim criteria can be derived from acute studies by converting short-term median lethal or median effective concentrations (LC50, EC50) to long-term no-effect concentrations using acute/chronic ratios or safety factors. Species not required in the minimum data set (e.g., amphibians) may be used in either derivation procedure provided that the life stage under investigation is completely aquatic. In addition, bioconcentration data may be used to derive criteria to protect the organisms, or consumers of the organisms, from harmful effects. Each study chosen for the criteria derivation procedure must have demonstrated a clear dose/response relationship and the LOEL must be statistically significant (95% confidence level).

4.1 Derivation of Acute and Chronic Criteria from Acute and Chronic Studies

4.1.1 Qualifications and Setting Criteria

To qualify for this category, the nature of the substance must first be considered. For example, if persistence, bioconcentration, bioaccumulation or delayed mortality is a concern then the substance would not qualify for this dual level approach. If the substance

meets this first set of conditions then the toxicity data are summarized in a tabular and/or graphical format and separated into acute and chronic data. The decision of whether data are acute or chronic depends primarily upon the exposure period. Acute toxicity data generally refer to the results of short-term tests with toxicity endpoints that occur within 96 hours of exposure (e.g., ≤96-h LC50). Chronic toxicity data generally refer to tests with lethal or sublethal endpoints that exceed 96 hours of exposure duration (>96-h LC50 or EC50). However, the normal longevity of the animal tested also must be considered in this decision. For example, 96 hours is a relatively short time in the life cycle of most fish, whereas it may constitute most or all of the life cycle of some invertebrates or lower life forms. Again, scientific judgment is appropriate here.

Another condition that must be met to qualify for this dual approach is that sufficient acute and chronic data must be available to set both an acute and chronic criterion (see Table 2.1 for freshwater and Table 2.3 for marine water). This decision is not always possible at this stage, especially if the toxicity of a substance is affected by some environmental factor such as water hardness or pH. However, there is usually some indication of such a relationship in the scientific literature. To test this relationship, the toxicity data are plotted against the modifying environmental factor. The acute and chronic data are identified by different symbols. This graphical presentation summarizes the toxicity data and serves several useful purposes in the process of criteria derivation and evaluation, as well as during their application. These are:

- to provide an indication of whether a relationship exists between the substance toxicity and any modifying environmental factor;

- to determine if there is a distinction in magnitude between acute and chronic data so that both an acute and chronic criterion can be set [4];

- to serve as an initial screening tool for identifying the key acute and chronic toxicity data; and

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[4] If there is no distinction, then there is no justification for setting both an acute and chronic criterion. Therefore, the derivation process must default to category (2) outlined in Section 4.2.

- to provide a visual representation of the relationships among the toxicity data, the criteria once set, and criteria from other jurisdictions.

Once the key acute and chronic data have been identified (i.e., the relevant lower concentrations that induce acute and chronic toxicity in the most sensitive species tested), they are evaluated in terms of their scientific soundness and rated as primary, secondary, or unacceptable (see Table 3.1). Appropriate safety factors (typically between 0.1 and 0.5) are then applied to the primary key acute and chronic data to derive acute and chronic criteria. If NOELs for sensitive life stages of sensitive species fall within this safety range for chronic data (i.e., between the LOEL and the calculated safe value), then the NOEL may be adopted as the chronic criterion. It should be noted that the magnitude of the safety factor may vary from substance to substance depending upon the quality and quantity of toxicity data (the toxicity of some substances is well-defined so that the safety factor need not be as large as for other substances less well understood). The actual size of the safety factor is decided on a case-by-case basis and involves the use of scientific judgement to maintain some flexibility in the derivation process.

Ambient background concentrations for substances that occur naturally may also play a role in the size of a safety factor. Criteria set far below levels that occur naturally in BC waters, and in which aquatic life thrive, would be impractical and unusable for assessing the environmental impact of anthropogenically generated substances.

When there is a relationship between the toxicity of the substance and some modifying environmental factor, then the criterion may be specified in terms of a regression equation and shown on a graph.

4.1.2 Averaging Periods

The use of acute and chronic criteria for certain substances is an improvement over the use of a single criterion. A single criterion maximum, based on chronic toxicity studies, can often be over-restrictive for many situations and the consequences of exceeding the criterion for short periods are uncertain. In contrast, the dual criteria approach is more refined, reflecting more closely the thresholds of acute and chronic toxicity. This approach allows concentrations of a substance to fluctuate above and below the chronic criterion provided that the acute criterion is never exceeded, and the chronic criterion is met over the specified averaging period. The goal is to provide a balance between acceptable levels of

protection to counter acute and chronic toxicity without being too stringent, and the practical application of the criteria in terms of monitoring requirements.

The averaging period for the chronic criterion may differ depending upon the substance under investigation and is somewhat arbitrary (e.g., five to 30 days have been used for BC water quality objectives). These times were chosen as reasonable and practical durations to address chronic effects and to fit into monitoring timetables for provincial agencies. Five samples are considered the minimum needed to calculate the average; however, in some cases where the concentrations fluctuate widely in nature, more than five samples may be necessary. On the other hand, if concentrations are uniform and rarely exceed the chronic criterion, then less frequent monitoring may be justified. In this case, failure of any individual sample to meet the chronic criterion would serve as an alert signal to increase the monitoring.

For some substances, such as residual chlorine, the BC criteria are time-related whereby the averaging periods for ambient monitoring are based on the toxicity exposure-duration data (Singleton 1989). The minimum duration of the averaging period for residual chlorine is set at the threshold of chronic toxicity. For freshwater this threshold is four days, but for marine waters it is only two hours.

4.2 Derivation of a Single Criterion from Chronic Studies

This category is employed for those substances that defaulted from category 1 (Section 4.1) for such reasons as:

- insufficient acute and chronic data;
- an overlap of acute and chronic toxicities such that the distinction between them is not clear;
- the substance is persistent and has bioconcentration/bioaccumulation potential;
- the substance does not exhibit acute toxicity under normal environmental conditions; or

- the substance has exhibited delayed toxicity after acute exposure. Nevertheless, the minimum data requirements (Table 2.1 for freshwater and Table 2.3 for marine water) should be met.

The derivation process for this category is basically the same as that for the chronic criterion in the foregoing category (Section 4.1). This single criterion typically is based on the LOEL using a non-lethal endpoint and multiplied by an appropriate safety factor (usually between 0.1 and 0.5). A NOEL may be used if it falls within this range provided it is based on the most sensitive life-stage of a sensitive species native to BC waters. This approach is used to derive a preliminary water quality criterion regardless of whether bioconcentration is a concern.

When bioconcentration of a particular substance is a concern, then an additional assessment must be made. If the bioconcentration assessment results in a safe limit lower than the preliminary water quality criterion, then the preliminary criterion can be adjusted accordingly. This derivation process is described more fully in Section 4.3.

4.3 Derivation of Water Quality Criteria from Bioconcentration Data

When a substance bioconcentrates from the water into the tissues of an organism, a separate assessment of bioconcentration in the criteria derivation process is required. To derive a water quality criterion from bioconcentration data, some basic information is necessary:

- reliable laboratory determination of body burdens in aquatic organisms exposed to known concentrations of the substance in water at equilibrium (i.e., a bioconcentration factor, BCF). For those contaminants that accumulate in fatty tissues, the BCF should be lipid-normalized. The BCF test concentrations should be much less than known toxic concentrations.

- information on the harmful effects of body burden levels on the exposed aquatic organism or upon their consumers.

To derive a water quality criterion based on the above information, the lowest tissue residue level that induces a harmful effect in the exposed organism or its consumers should

be determined. This value is then divided by the highest reliable BCF to derive a LOEL for the water. To derive a water quality criterion (WQC) to protect the organism from accumulating harmful body burdens, the LOEL is multiplied by an appropriate safety factor (typically 0.1 to 0.5 - see Section 4.1.1.1) as follows:

$$WQC = \frac{\text{Lowest Harmful Tissue Residue Level x Safety Factor}}{BCF}$$

If a no-harmful-effect-tissue-residue is available, then this value may be used to replace the lowest harmful tissue residue level provided the effects studied involve sensitive chronic endpoints of sensitive species. If this no-effect value is used, then a safety factor may not be required.

To determine the final water quality criterion, the value calculated here is compared to the preliminary water quality criterion determined in Section 4.2. The final water quality criterion should be the most scientifically defensible of the two values. However, given the variability of BCFs for many substances (e.g., laboratory-derived BCFs ranging over three orders of magnitude have been measured for some substances), and the subjective nature of application factors, a statistical value, such as the geometric mean, may be considered as the final criterion if there is a wide range between the values derived by each method. The rationale for this alternative statistical approach is that if the values derived by the two methods are similar, then there is a high level of confidence in the criterion. However, when the range between the two values is wide for a particular substance, there is less confidence that either of the values are accurate. Hence, the assumption was made that the safe level (criterion) probably lies somewhere between the two values.

4.4 Interim Criteria Derivation from Acute or Chronic Studies

The procedure for the derivation of interim water quality criteria is similar to that used to derive criteria, except that the minimum data requirements are not as rigorous (see Table 2.2 for freshwater and Table 2.3 for marine water). In addition, secondary data (Table 3.1) are acceptable for the derivation of interim criteria, but unacceptable data should not be used. Chronic data are preferred over acute data as a basis to derive an interim water quality criterion.

When available, acute/chronic ratios (ACR) can be used to convert the median lethal results of a short-term study to an estimated long-term no-effect concentration for the most sensitive species for which chronic results are unavailable(Kenaga 1982). An ACR is calculated by dividing an LC50 or EC50 by the no-observed-effect level (NOEL) from a chronic exposure test for the same species (i.e., LC50/NOEL). It is important to note that an ACR should only be used from studies that were designed for this purpose to avoid complications arising from different test conditions or different test populations. Further, the use of an ACR needs to be carefully rationalized since the available evidence indicates that for a given substance, ACRs may vary between species with different sensitivities, and across major taxonomic groupings (Mount 1977; Stephan 1985). The interim criterion is derived by dividing the most sensitive LC50 or EC50 by the most appropriate ACR.

In the event that acute/chronic ratios are not available, the alternate method of choice to derive an interim criterion value from an acute study is to multiply the LC50 or EC50 value by a universal application factor. At present, ACRs are not available for all substances and, to meet this situation, universal application factors have been widely used (U.S. EPA 1972). The application factor (AF) for non-persistent substances (half-life in water <8 weeks) is 0.05; for persistent substances, the AF is 0.01. These application factors are now endorsed by the majority of Canadian jurisdictions involved in developing water quality criteria, guidelines, or objectives (e.g., CCME, International Joint Commission, Ontario, Manitoba, and Saskatchewan). However, it must be emphasized that, although the universal application factors have been empirically tested and supported (e.g., Kenaga 1982), several studies (Mount 1977; Buikema *et al.* 1982; Mayer *et al.* 1986) have suggested that these factors may be inappropriate for several substances (e.g., diazinon, zinc). Therefore, universal application factors for deriving an interim criterion should be used only in the absence of chronic data and in the absence of ACRs for acute data.

The information that is required to elevate interim criteria to full criteria status needs to be clearly identified to stimulate research that will generate the necessary data.

5. ADMINISTRATIVE PROCEDURE

The following steps must be followed to establish criteria as Ministry policy:

- carry out an internal review of the first draft of the Technical Report containing all relevant information pertaining to the substance of concern, the recommended criteria and their application, and the Overview report to ensure quality and accuracy of all material,
- carry out a review of the second draft of the Technical Report and the Overview report by Water Quality Branch, Federal Agencies (e.g., Environment Canada, Fisheries and Oceans), scientific experts, and other government and non-government stakeholders,
- carry out a review of the penultimate draft of the Overview report by appropriate Program Directors such as Water Management, Environmental Protection, Fisheries Management, and others,
- submit the Report for approval and sign off by the Executive Director of the Environmental Protection Department (delegated from Deputy Minister), and
- obtain a library catalogue number (CIP) from the legislative library (Catalogue Section) by sending copies of title page and table of contents.

The review time should be limited to about one month for each of the first and second drafts.

Copies of the report are made available through mailing lists, the internet, libraries, and requests to the Water Quality Branch of the Ministry of Environment, Lands and Parks in Victoria.

On occasion, the proposed Water Quality Criteria may need to be incorporated into site remediation plans or an Operational Certificate prior to formal approval through the above process. The proposed Water Quality Criteria should still be put through the formal review and approval process so that it can be treated as policy for future use within the Ministry.

The criteria are subject to review and revision as new knowledge becomes available, or as circumstances dictate.

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APPENDIX C

CCME Protocol

APPENDIX IX

A PROTOCOL FOR THE DERIVATION OF WATER QUALITY GUIDELINES FOR THE PROTECTION OF AQUATIC LIFE (APRIL 1991)

IX.1 INTRODUCTION

Change is an important characteristic of aquatic ecosystems. Species composition, various rate processes, degree of complexity, and many other community characteristics change over time. Changes in aquatic ecosystem structure and function may result from storms, floods, changes in rainfall patterns, sedimentation, and a variety of other natural causes. In addition, changes may result from societal stresses such as toxic chemical inputs and nutrient enrichment. An ecosystem may recover from both types of change, however, the recovery process will rarely produce a system identical to the original when a societal stress is involved (Cairns 1980). The guidelines for freshwater aquatic life in chapter 3 were developed as one of a series of management tools to ensure that societal stresses, particularly the introduction of toxic chemicals, do not lead to the degradation of Canadian fresh waters.

IX.1.1 Background

Chapter 3, Freshwater Aquatic Life, includes water quality guidelines for approximately 65 water quality variables and continues to be updated and expanded with the addition of guidelines for industrial solvents, in-use pesticides, and other variables of concern to freshwater aquatic life (also see preceding appendices). However, since the publication of the Canadian Water Quality Guidelines in 1987, several concerns have been raised regarding the protocol used to develop guidelines for the protection of freshwater aquatic life. The protocol contained in chapter 3 was considered to be incomplete regarding the identification and selection of key studies and the mechanism of guideline derivation. Further, several jurisdictions have since reassessed their protocols for guideline development, while other jurisdictions have requested a similar protocol for the marine environment. In response to these issues, the Canadian Council of Ministers of the Environment (CCME) Task Force on Water Quality Guidelines undertook a review of the protocol used in chapter 3 of the Canadian Water Quality Guidelines. The revised protocol for the derivation of water quality guidelines for the protection and maintenance of freshwater aquatic life is presented in this update. A protocol for the derivation of marine aquatic life guidelines is also presented. **All guidelines previously approved by CCREM (now known as CCME), however, continue to apply until a future review is deemed necessary.**

IX.I.2 Guiding Principles for the Development of Water Quality Guidelines for Aquatic Life

The following is an update of the chapter 3 guiding principles for the development of freshwater aquatic life guidelines as originally adopted by the CCREM Task Force on Water Quality Guidelines. Provincial jurisdictions, however, may aim for greater or lesser levels of protection depending upon circumstances within each jurisdiction.

(a) In deriving Canadian water quality guidelines for aquatic life, all components of the aquatic ecosystem (e.g., algae, macrophytes, invertebrates, fish) are considered if the data are available. Where data are available but limited, interim guidelines are deemed preferable to no guidelines.

(b) The approach to the development of guidelines for aquatic life follows that of the International Joint Commission Water Quality Board (IJC 1975) and the Ontario Ministry of the Environment (OMOE 1979, In press). This approach states that guidelines "are set at such values as to protect all forms of aquatic life and all aspects of the aquatic life cycles." The goal is to protect all life stages during an indefinite exposure to water. Whether this goal can be realized is a water management issue and does not affect the guideline derivation procedure.

(c) For most water quality variables, a single maximum value, which is not to be exceeded, is recommended as a Canadian water quality guideline. This maximum value is based on a long-term no-effect concentration.

(d) Unless otherwise specified, a guideline value refers to the total concentration in an unfiltered sample. Total concentrations will apply unless it can be demonstrated that (i) the relationship between variable fractions and their toxicity is firmly established and (ii) analytical techniques have been developed that unequivocally identify the toxic fraction of a variable in a consistent manner using routine field-verified measurements.

IX.1.3 The Guideline Derivation Protocol

The following is a brief overview of the guideline derivation protocol, which is outlined in detail in sections IX.2, IX.3, and IX.4 (see Fig. IX-1).

Selection of Variables

Variables of concern at the national level are given priority for guideline development. For example, the Canadian Environmental Protection Act includes a Priority Substances List (Canada Gazette 1989) for which water quality guidelines are required. Variables are also selected for guideline development after consultation with federal and provincial jurisdictions.

Literature Search

For each variable selected, a literature search is conducted to obtain information on the following: *(a)* physical and chem-

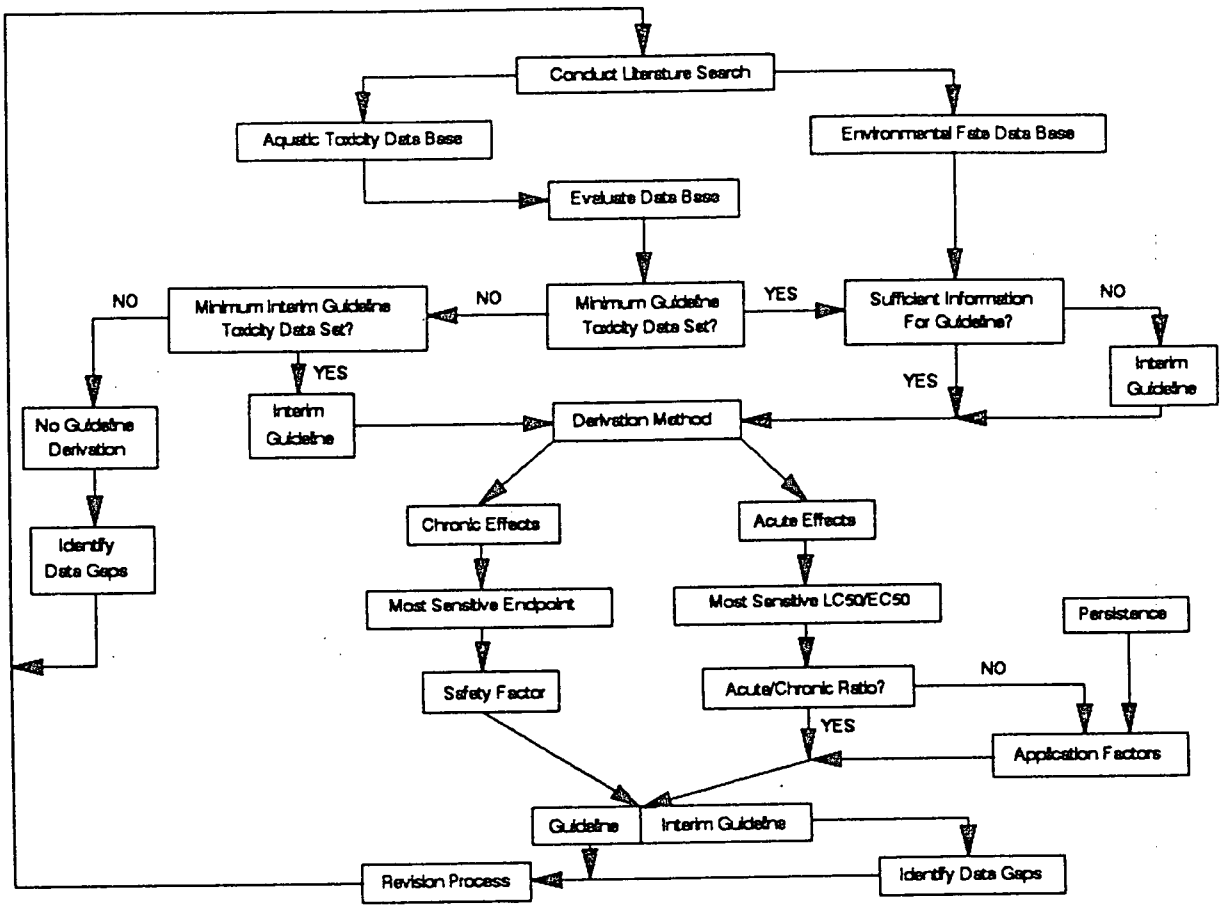

Figure IX-1. The protocol for deriving Canadian water quality guidelines.

ical properties, *(b)* environmental concentrations, *(c)* environmental fate and behaviour, *(d)* bioaccumulation potential, *(e)* acute toxicity to aquatic biota, *(f)* chronic toxicity to aquatic biota, *(g)* genotoxicity, and *(h)* information from other jurisdictions.

Data Set Requirements

In order to proceed with the guideline derivation process, certain minimum toxicological and environmental fate data set requirements must be met (see section IX.2). In cases where there is insufficient information, an interim guideline can be derived providing that a less stringent minimum data set is available.

Evaluation of Toxicological Data

Each toxicological study found in the literature search is evaluated to ensure that acceptable laboratory practices were used in the design and execution of the experiment (see section IX.3). Each study is then classified as primary, secondary, or unacceptable.

Guideline Derivation

When available, the most sensitive lowest-observable-effects level (LOEL) from a chronic exposure study on a native Canadian species is multiplied by a safety factor of 0.1 to arrive at the final guideline concentration (see section IX.4). Alternatively, the most sensitive LC_{50} or EC_{50} from an acute exposure study is multiplied by an acute/chronic ratio or appropriate application factor to determine the final guideline concentration. The derivation protocol is the same for guidelines and interim guidelines.

IX.1.4 The Use of Water Quality Guidelines and Objectives in Water Quality Management

Canadian water quality guidelines for aquatic life are developed to provide basic scientific information about the effects of water quality variables on water uses. This information is used to assess water quality issues and to establish water quality objectives for specific sites (Fig. IX-2).

The need to develop water quality objectives often arises when an industry announces a new project that could affect water quality in a basin. Objectives may also be required to address an existing problem or to provide preventative watershed protection. Those charged with developing objectives (for example, Environment Canada, Indian Affairs and Northern Development, provincial and territorial governments, and water management agencies such as the Prairie Provinces Water Board) must decide what uses are to be protected, obtain the necessary information, formulate the objectives, and present them for approval to the appropriate jurisdiction (Fig. IX-2).

Developing site-specific objectives to protect aquatic life is a complex process, especially when it concerns objectives for toxic substances. At a given site, there are many species, each of which can respond differently to the often large number of toxic substances produced by human activities. To develop a site-specific objective requires an extensive knowledge of the chemical, physical, and biological properties of the water body and, as well, the social and economic characteristics of the local area. Once this information has been acquired, objectives are derived using the same protocol as outlined in section IX.1.3 for guidelines, except that only species and environmental conditions relevant to the site are considered. Social and economic factors are then evaluated to determine if the objectives can realistically be attained. In general, when setting effluent regulations to meet objectives, social and economic factors are factored in by giving longer deadlines to smooth out the transition period. Periodic assessments then fine tune the objectives and pollution control program to ensure that the desired water quality is maintained.

As a minimum, water quality objectives should protect the existing and potential uses of a water body. Where water bodies are considered to be of exceptional value, or where they support valuable biological resources, it is the policy of the CCME that **degradation of the existing water quality should always be avoided**. Similarly, modifications of guidelines to site-specific objectives should not be made on the basis of aquatic ecosystem characteristics that have arisen as a direct result of previous human activities.

IX.1.5 Guideline Derivation Protocols for Other Water Uses

Canadian Water Quality Guidelines includes guidelines that will protect and maintain other water uses (raw water sources for drinking water, recreation and aesthetics, irrigation, livestock watering, and industrial water supplies) not discussed in this appendix. The protocols used to derive guidelines for these water uses are found in the appropriate chapters of the Guidelines. The long-term goal is to prepare revised guideline protocols for each of the major water uses in Canada. Each revised protocol will be made available to interested parties after review and approval by the CCME Task Force on Water Quality Guidelines.

IX.2 DATA REQUIREMENTS FOR GUIDELINE DERIVATION

IX.2.1 Minimum Aquatic Toxicological Data Set Requirements for Freshwater Guidelines

The intended goal of freshwater aquatic guidelines is the protection and maintenance of all forms of aquatic life and all aquatic life stages in the freshwater environment. Therefore, it is essential that data from fish, invertebrates, and plants be included in the guideline derivation process. For this purpose, minimum data set requirements have been set (Table IX-1). In the derivation protocol (see section IX.3), guidelines or interim guidelines may be derived from studies involving species not required in the minimum data set (e.g., amphibians, protozoa, bacteria), provided that the minimum data set requirements are met.

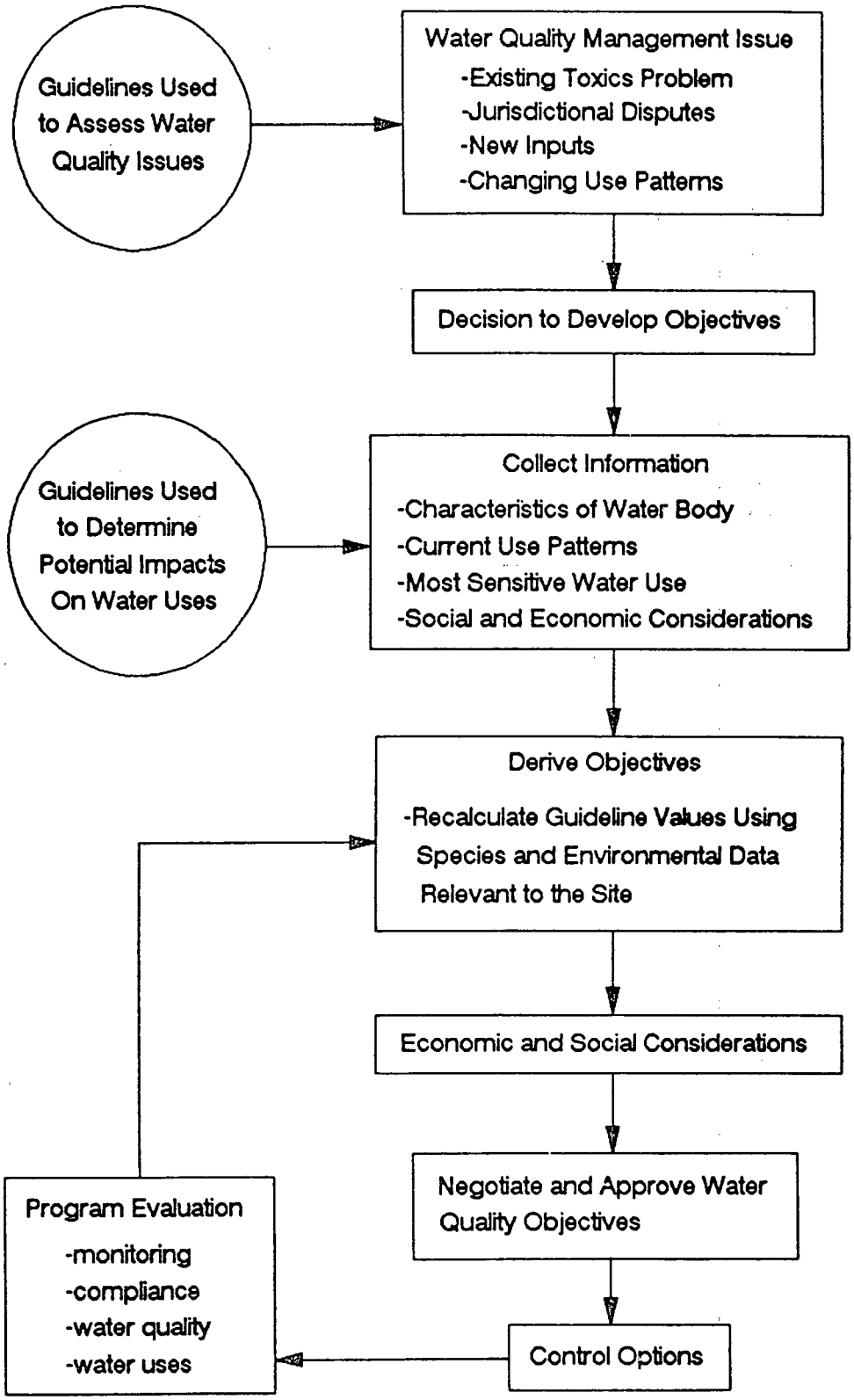

Figure IX-2. The role of water quality guidelines and objectives in water quality management.

Table IX-1. Minimum Data Set Requirements for Freshwater Guidelines

Fish
• at least three studies on three or more freshwater species resident in North America, including at least one cold-water species (e.g., trout) and one warmwater species (e.g., fathead minnow)
• of the above studies, at least two must be chronic (partial or full lifecycle) studies
Invertebrates
• at least two chronic (partial or full lifecycle) studies on two or more invertebrate species from different classes, one of which includes a planktonic species resident in North America (e.g., daphnid)
Plants
• at least one study on a freshwater vascular plant or freshwater algal species resident in North America
• for highly phytotoxic variables, four acute and/or chronic studies on nontarget freshwater plant or algal species

It is important to emphasize that the guideline derivation process for freshwater aquatic life need not always follow a fixed approach. Consideration must also be given to the nature of the variable. For example, the requirement for two chronic studies for fish may be waived when acceptable acute/chronic ratios from fish species exist to convert the results of acute studies, or if the toxicity of the variable has been shown not to increase during chronic exposures. Other scientifically justified exemptions may also be considered on a case-by-case basis.

The reduced requirements for plant toxicity studies were deemed necessary because fewer studies on plants have been conducted (Swanson and Peterson 1988). The minimum data set requirements for plants could be increased in the future if data availability improves.

In cases where the minimum data set requirements for guideline derivation are not met, interim water quality guidelines may be developed provided the minimum data set requirements shown in Table IX-2 are met.

Table IX-2. Minimum Data Set Requirements for Interim Freshwater Guidelines

Fish
• at least two acute and/or chronic studies on two or more fish species, one of which includes a coldwater species (e.g., trout) resident in North America
Invertebrates
• at least two acute and/or chronic studies on two or more invertebrate species from different classes, one of which includes a planktonic species resident in North America (e.g., daphnid)

If a toxicity study indicates that a plant species is the most sensitive species in the data set, then this study shall be used in the interim guideline derivation process. However, in the absence of data on plants, interim guidelines can be derived provided that this data gap is noted. The information that is required to elevate an interim guideline to guideline status needs to be clearly identified in order to stimulate research that will generate the necessary data.

IX.2.2 Minimum Aquatic Toxicological Data Set Requirements for Marine Guidelines

U.S. EPA criterion continuous concentrations (the U.S. equivalent of Canadian water quality guidelines) were calculated separately for fresh and marine waters. When compared, 35% of the freshwater criterion continuous concentrations differed from the marine water criterion continuous concentrations by a factor of greater than five (Hansen 1989). Given this information, Canadian water quality guidelines should be developed separately for freshwater and marine environments. For most variables, however, there is less toxicological information available for marine species, particularly phytoplankton and macroalgae, than is available for the freshwater environment (Hansen 1989). Since the goal of marine aquatic guidelines is the protection and maintenance of all forms of aquatic life and aquatic life stages in the marine environment, it is essential that data from marine fish, invertebrates, and plants be included in the guideline derivation process. As with the requirements for freshwater aquatic life guidelines, minimum data set requirements have been set (Table IX-3). In this appendix, marine species include those species found in estuarine, coastal, and open ocean habitats, any of which may be used to derive a guideline or interim guideline.

Table IX-3. Minimum Data Set Requirements for Marine Guidelines

Fish
• at least three studies on three or more temperate marine fish species, including at least two chronic (partial or full lifecycle) studies
Invertebrates
• at least two chronic (partial or full lifecycle) studies on two or more temperate marine invertebrate species from different classes
Plants
• at least one study on a temperate marine vascular plant or marine algal species

In cases where the minimum data set requirements are not met, interim water quality guidelines can be derived providing the minimum data set requirements shown in Table IX-4 are met.

Table IX-4. Minimum Data Set Requirements for Interim Marine Guidelines

Fish
• at least two acute and/or chronic studies on two or more marine fish species, one of which is a temperate species
Invertebrates
• at least two acute and/or chronic studies on two or more marine species from different classes, one of which is a temperate species

If a toxicity study indicates that a plant species is the most sensitive species in the data set, then this study shall be used in the interim guideline derivation process. However, in the absence of data on plants, interim guidelines can be derived provided that this data gap is clearly identified. As with freshwater aquatic life guidelines, the information required to elevate an interim guideline to a guideline needs to be clearly identified in order to stimulate research that will generate the necessary data.

IX.2.3 Minimum Environmental Fate and Behaviour Data Set Requirements

In addition to the minimum toxicological data set requirements indicated above, studies that have investigated the major environmental fate processes and persistence of the variable in water, soil and sediment, air, and biota are required. Potential fate processes include volatilization, hydrolysis, oxidation, photolysis, aerobic and anaerobic biodegradation, long-range transport, soil and sediment sorption/desorption, and bioaccumulation. However, it is not required to have information on each potential fate process. Rather, the intent is to be able to identify the major environmental pathways and fate of a variable in the aquatic environment. Specifically, the following should be determined: *(a)* the mobility of the variable and the compartments of the aquatic environment in which it is most likely to be distributed, *(b)* the kinds of chemical and biological reactions that take place during transport and after deposition, *(c)* the eventual chemical form, and *(d)* the persistence of the variable in water, sediment, and biota. Where possible, the persistence of a variable should be expressed in terms of its half-life. Where significant environmental fate information is lacking, interim guidelines are set. In these cases, the information required to elevate the interim guideline to a guideline needs to be clearly identified in order to stimulate the necessary research.

IX.2.4 Additional Information

The following are not required elements of the minimum data set, but because they are useful in assessing the potential hazard of a variable, they should be included when available: *(a)* production and uses; *(b)* physical and chemical properties; *(c)* organoleptic effects (taste, odour, fish flesh tainting); *(d)* sources to the aquatic environment; *(e)* methods of analysis and current detection limits; *(f)* concentrations in the aquatic environment; *(g)* mutagenicity, carcinogenicity, and teratogenicity; *(h)* sensitivity of birds and wildlife consuming aquatic organisms; *(i)* guidelines, objectives, and standards of other jurisdictions.

IX.3 EVALUATION OF TOXICOLOGICAL DATA

Since standard protocols for toxicity testing may become outdated or are not always available or followed, a great deal of variability exists in the quality of published toxicity data. To ensure a consistent scientific evaluation for each variable, the data included in the minimum data set should meet certain criteria. These include information on test conditions/design (e.g., flow-through, renewal, static), test concentrations, temperature, hardness, pH, adjuvants, experimental design (controls, number of replicates), and a description of the statistics used in evaluating the data. A variety of standardized test protocols have been developed for fish, invertebrates and plants. When appropriate, these should be consulted during the evaluation process (for example, see EPS 1980; ASTM 1980; OECD 1981; Rand and Petrocelli 1985; U.S. EPA 1985a, 1985b, 1985c; Sergy 1987; Swanson and Peterson 1988). Information useful for interpreting toxicity data is also available (Buikema *et al.* 1982; Rand and Petrocelli 1985, ch. 1–11) and should be consulted when necessary. When consulting test protocols, it is important to be aware of the following limitations: *(a)* protocols consider only a few well-studied species and biological processes; *(b)* our knowledge of extrapolation from one species to another (i.e., comparative ecotoxicology) is very limited; *(c)* there is limited knowledge of the effects of metabolites and other environmentally transformed products of the parent chemicals; *(d)* protocols do not take into account cumulative effects of chemicals or compensatory responses of organisms (such as acclimation or reduced density-dependent mortality amongst juveniles); and *(e)* the predictability of laboratory exposures and effects to aquatic ecosystems has not been adequately tested (Sheehan *et al.* 1984; Arthur 1988; Petersen and Petersen 1988). Therefore, it is essential that the evaluation of toxicological data not follow a rigidly fixed format. Once evaluated, the data are classified as primary, secondary, or unacceptable as described in Table IX-5.

All data included in the minimum data set must be primary in order for guideline derivation to proceed. For interim guideline derivation, primary or secondary data may be used. Unacceptable data cannot be used in either derivation procedure.

IX.4 GUIDELINE DERIVATION

Guidelines or interim guidelines are preferably derived from the lowest-observable-effects level (LOEL) from a chronic study using a nonlethal endpoint for the most sensitive life stage of the most sensitive aquatic species investigated. However, when this type of data is unavailable, guidelines can be derived from acute studies by converting short-term median lethal or median effective concentrations (LC_{50}, EC_{50}) to long-term no-effect concentrations. Species not required in the minimum data set (e.g., amphibians) may be used in either derivation procedure provided that the life stage under investigation was completely aquatic. Each study chosen for the guideline derivation procedure must have demonstrated a clear dose/response relationship and, where applicable, the LOEL must be statistically significant.

IX.4.1 Guideline Derivation from a Chronic Study

The most sensitive LOEL is multiplied by a safety factor of 0.1 to arrive at the guideline value. This safety factor has been chosen to account for differences in sensitivity to a chemical variable due to differences in species, laboratory versus field conditions, and test endpoints (Kimerle 1986; Mayer *et al.* 1986; Mayer and Ellersieck 1988).

Table IX-5. Classification of Toxicity Data

Primary Data

- Toxicity tests must employ currently acceptable laboratory practices of exposure and environmental controls (see, for example, citations in text). Other types of tests using more novel approaches will be evaluated on a case-by-case basis.
- As a minimum requirement, variable concentrations must be measured at the beginning and end of the exposure period. Calculated concentrations or measurements taken in stock solutions are unacceptable.
- Generally, static tests are unacceptable unless it can be shown that variable concentrations did not change during the test and that adequate environmental conditions for the test species were maintained.
- Preferred endpoints from a partial or full lifecycle test include a determination of effects on embryonic development, hatching, or germination success, survival of juvenile stages, growth, reproduction, and survival of adults.
- Responses and survival of controls **must** be measured and should be appropriate for the life stage of the test species used.
- Measurements of abiotic variables such as temperature, pH, dissolved oxygen, and water hardness should be reported so that any factors that may affect toxicity can be included in the evaluation process.

Secondary Data

- Toxicity tests may employ a wider array of methodologies (e.g., measuring toxicity while test species is exposed to additional stresses such as low temperatures, lack of food, or high salinity).
- Static tests are acceptable.
- Preferred test endpoints include those listed for primary data as well as pathological, behavioural, and physiological effects.
- Calculated variable concentrations are acceptable.
- All relevant environmental variables should be measured and reported. The survival of controls **must** be measured and reported.

Unacceptable Data

- Toxicity data that do not meet the criteria of primary or secondary data are not acceptable.

IX.4.2 Guideline Derivation from an Acute Study

When available, acute/chronic ratios (ACR) can be used to convert the median lethal results of a short-term study to an estimated long-term no-effect concentration (Kenaga 1982). An ACR is calculated by dividing an LC_{50} or EC_{50} by the no-observed-effects level (NOEL) from a chronic exposure test for the same species (i.e., LC_{50}/NOEL). It is important to note that an ACR should only be used from studies that were designed for this purpose in order to avoid complications arising from different test conditions or different test populations. Further, the use of an ACR needs to be carefully rationalized since the available evidence indicates that for a given chemical variable, ACRs may vary between species with different sensitivities and across major taxonomic groupings (Mount 1977; Stephan 1985). The guideline value is derived by dividing the most sensitive LC_{50} or EC_{50} by the most appropriate ACR.

In the event that acute/chronic ratios are not available, the alternate method of choice to derive a guideline value from an acute study is to multiply the LC_{50} or EC_{50} value by a universal application factor. At present, ACRs are not available for all variables and, to meet this situation, universal application factors have been widely used (U.S. EPA 1972). The application factor (AF) for nonpersistent variables (t in water < 8 weeks) is 0.05; for persistent variables, the AF is 0.01. These application factors are now endorsed by the majority of Canadian jurisdictions involved in developing water quality criteria, guidelines or objectives (e.g., International Joint Commission, Ontario, Manitoba, Saskatchewan, British Columbia). However, it must be emphasized that, although the above universal application factors have been empirically tested and supported (e.g., Kenaga 1982), several studies (Mount 1977; Buikema *et al.* 1982; Mayer *et al.* 1986) have suggested that these factors may be inappropriate for several variables (e.g., diazinon, zinc). Therefore, the use of universal application factors for deriving a guideline or interim guideline should be used only in the absence of chronic data and in the absence of ACRs for acute data.

IX.5 PROCEDURES FOR THE PREPARATION, REVIEW, AND PUBLICATION OF CANADIAN WATER QUALITY GUIDELINES

Both a technical report, containing all relevant information pertaining to the selected water quality variable, and a CCME guideline report, containing the recommended guideline value and the rationale for selecting the value, are prepared. The technical report and the CCME guideline report are circulated to scientific experts and to the CCME Task Force on Water Quality Guidelines for review.

Once reviewed, the appropriate revisions are incorporated and both reports are returned to the CCME Task Force on Water Quality Guidelines for final approval.

Upon approval by the CCME Task Force, the reports are submitted to the CCME Water Advisory Committee and to the Canadian Council of Ministers of the Environment. The technical report is published in the Environment Canada, Inland Waters Directorate, Scientific Series and is made available through mailing lists and library loans. The CCME guideline report is published by CCME as an appendix to Canadian Water Quality Guidelines and is made available to those requesting guideline updates and library loans.

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APPENDIX D

New B.C. Toxicity Testing Data

Manganese Toxicity Data

WATER HARDNESS	TEST ORGANISM	RESULT EXPERIMENTAL	ACTUAL	TRUE
SOFT WATER (~25 mg/L CaCO3)	COHO	REP A: **2.4 mg/L** REP B: **2.4 mg/L** REP C: **2.2 mg/L**	**2.4 mg/L**	
	LC50	POOLED: **2.3 mg/L**		
	RAINBOW TROUT	REP A: **2.2 mg/L** REP B: **2.1 mg/L** REP C: **2.0 mg/L**		
	LC50	POOLED: **2.1 mg/L**		
	DAPHNIA MAGNA	REP A: **1.0 mg/L** REP B: **1.0 mg/L**	**0.9 mg/L**	**0.8 mg/L**
	LC50	POOLED: **1.0 mg/L**		
	HYALELLA AZTECA	REP A: **3.4 mg/L** REP B: **3.4 mg/L** REP C: **3.8 mg/L**	**3.5 mg/L**	**3.6 mg/L**
	LC50	POOLED: **3.5 mg/L**		
	CHIRONOMID TENTANS	REP A: **8.0 mg/L** REP B: **4.0 mg/L** REP C: **5.9 mg/L**	**5.8 mg/L**	**5.8 mg/L**
	LC50	POOLED: **5.5 mg/L**		
	21-DAY CHRONIC DAPHNIA	* Water too soft Control deaths		
	MICROTOX	5 min. IC50: **872.7 mg/L** 15 min. IC50: **73.1 mg/L**		

Manganese Toxicity Data

WATER HARDNESS	TEST ORGANISM	RESULT EXPERIMENTAL*	ACTUAL**	TRUE***
WELL WATER (~100mg/L CaCO3)	COHO	REP A: **10.3 mg/L** REP B: **15.8 mg/L** REP C: **13.5 mg/L**	13.2 mg/L	13.1 mg/L
	LC50	POOLED: **12.7 mg/L**		
	RAINBOW TROUT	REP A: **21.1 mg/L** REP B: **19.1 mg/L** REP C: **22.4 mg/L**		
	LC50	POOLED: **20.7 mg/L**		
	DAPHNIA MAGNA	REP A: **29.9 mg/L** REP B: **23.2 mg/L**	30.6 mg/L	28.7 mg/L
	LC50	POOLED: **29.4 mg/L**		
	HYALELLA AZTECA	REP A: **13.5 mg/L** REP B: **21.8 mg/L** REP C: **22.0 mg/L**	21.4 mg/L	22.2 mg/L
	LC50	POOLED: **20.6 mg/L**		
	CHIRONOMID TENTANS	REP A: **35.5 mg/L** REP B: **43.5 mg/L** REP C: **43.5 mg/L**	42.2 mg/L	
	LC50	POOLED: **40.6 mg/L**		
	21-DAY CHRONIC DAPHNIA	Chronic LOEC: **6.8 mg/L** Chronic NOEC: **3.4 mg/L**	6.7 mg/L 3.5 mg/L	6.9 mg/L 3.6 mg/L
	MICROTOX	5 min. IC50: **3808.3 mg/L** 15 min. IC50: **88.0 mg/L**		
	SELENASTRUM	IC50: **8.29 mg/L**		

Manganese Toxicity Data

WATER HARDNESS	TEST ORGANISM	RESULT EXPERIMENTAL	ACTUAL	TRUE
HARD WATER (~250 mg/L CaCO3)	COHO LC50	REP A: **17.7 mg/L** REP B: **19.1 mg/L** REP C: **20.5 mg/L** POOLED: **18.9 mg/L**	**17.4 mg/L**	
	RAINBOW TROUT LC50	REP A: **19.1 mg/L** REP B: **15.8 mg/L** REP C: **13.5 mg/L** POOLED: **16.1 mg/L**	**12.7 mg/L**	
	DAPHNIA MAGNA LC50	REP A: **82.2 mg/L** REP B: **71.0 mg/L** POOLED: **75.4 mg/L**	**79.7 mg/L**	**76.3 mg/L**
	HYALELLA AZTECA LC50	REP A: **31.3 mg/L** REP B: **29.9 mg/L** REP C: **33.6 mg/L** POOLED: **30.8 mg/L**	**32.7 mg/L**	**31.0 mg/L**
	CHIRONOMID TENTANS LC50	REP A: **82.3 mg/L** REP B: **432 mg/L** REP C: **152.7 mg/L** POOLED: **108 mg/L**	**101.0 mg/L**	**94.3 mg/L**
	21-DAY CHRONIC DAPHNIA	Chronic LOEC: **50 mg/L** Chronic NOEC: **25 mg/L**		
	MICROTOX	5 min. IC50: **10542.4 mg/L** 15 min. IC50: **124.3 mg/L**		

Manganese Toxicity Data

WATER HARDNESS	TEST ORGANISM	RESULT EXPERIMENTAL	ACTUAL	TRUE
SEAWATER	CHINOOK	REP A: **188.9 mg/L** REP B: **197.8 mg/L** REP C: **184.4 mg/L**	**214.4 mg/L**	**216.6 mg/L**
	LC50	POOLED: **194.1 mg/L**		
	E.WASH	REP A: **180.4 mg/L** REP B: **162.7 mg/L** REP C: **190.9 mg/L**	**193.9 mg/L**	
	LC50	POOLED: **178.0 mg/L**		
	MICROTOX	5 min. IC50: **7550.0 mg/L** 15 min. IC50: **759.6 mg/L**		
	PURPLE SEA URCHINS	EC50: **442.2 mg/L**		

* Experimental toxicity values are based on unverified metal concentrations calculated and prepared for a given bioassay by the toxicity technician.

** Actual toxicity values are calculated using precise metal concentrations obtained via ICP analysis of experimental concentrations conducted by the inorganic chemistry lab. Actual toxicity results are based on initial (Day 0) replica A concentrations only.

*** True toxicity is the average of 'Actual Toxicities' calculated from initial (Day 0) and final (Day 2 or 4) chemistry data.

Note: For most bioassays involving seawater, ICP analyses were not done and final toxicity values have been represented as experimental.

CHRONIC DAPHNIA RESULTS

CHEMICAL	WATER TYPE	NOMINAL (mg/L)			ACTUAL (mg/L)			TRUE (mg/L)		
		LOEC	NOEC	IC25	LOEC	NOEC	IC25	LOEC	NOEC	IC25
Manganese (as Mn)	Well	6.8	3.4	5.3	6.7	3.5	5.3	6.9	3.6	5.4
	Hard	13.5	6.8	9.1	13.6	7.2	9.4	13.4	7.3	9.4

NOMINAL = unverified concentrations calculated and prepared by the toxicology technician
ACTUAL = precise concentrations by the inorganic chemistry lab using Day 0 data
TRUE = precise concentrations by the inorganic chemistry lab using the average of Day 0 & Day 21 data

LOEC = Lowest Observable Effect Concentration
NOEC = No Observable Effect Concentration
IC25 = statistical concentration of Mn to cause a 25% inhibition of reproduction

E-test timeline (FY '96'97)

CCME/Water Quality Guidelines Study (Les Swain Project) - Record of 7-day E-tests performed

Test Start	Test Chemical	Water Type	EC50 (95% confidence range) in mg/L Nominal	EC50 (95% confidence range) in mg/L Corrected	Ref. Tox. Test	Ref. Tox. Result EC50 (mg/L)	*Test details to consider for billing*
12-Jun-96	Boron	Well	n/a: no eggs	n/a: no eggs	$ZnSO_4$ in Well	n/a: no eggs	Day 0 Chemistry only, set-up & then no eggs available
14-Jun-96	Boron	Well	didn't work	didn't work	$ZnSO_4$ in Well	didn't work	Full Chemistry but test unsuccessful, gamete quality ??
11-Jul-96	Boron	Well	didn't work	didn't work	$ZnSO_4$ in Well	didn't work	Full Chemistry but test unsuccessful, gamete quality ??
04-Sep-96	Boron	Well	808 (500-1000)	821 (515-1011)	$ZnSO_4$ in Well	23.7 (20.7-28.4)	Full Chemistry, E-test successful
18-Sep-96	Boron	Hard	<1000	<1005	$ZnSO_4$ in Well	19.8 (17.0-22.9)	Full Chemistry, Soft successful, repeat Hard
		Soft	574 (250-1000)	598 (259-1045)			
30-Oct-96	Manganese	Well	20.9 (18.3-23.2)	20.0 (17.1-22.6)	$ZnSO_4$ in Well	17.5 (14.9-21.5)	Full Chemistry, E-test successful
27-Nov-96	Manganese	Hard	29.5 (23.4-38.2)	22.7 (18.9-27.8)	$ZnSO_4$ in Well	15.8 (14.4-17.6)	Full Chemistry, Hard successful, repeat Soft
		Soft	>6.75	>6.47			
05-Mar-97	Barium	Well	878.3 (734.6-1053.2)	907.2 (743.3-1109.8)	$ZnSO_4$ in Well	6.6 (4.6-8.8)	Full Chemistry, E-test fine, 48.6% nonviab. at highest [ ]
12-Mar-97	Barium	Hard	<899? died off very early		$ZnSO_4$ in Well	14.1 (10.0-18.0)	Full Chemistry, repeat Hard (63.3% nonviab.hard control)
		Soft	<225?	<224?			Full Chemistry, repeat soft
	Manganese	Soft	10.8 (7.7-14.1)	11.5 (8.3-14.9)			Full Chemistry, but...
			questionable control results, repeat FY'97'98				45.8% nonviable in well controls
19-Mar-97	Barium	Well	two higher [ ] for 05-Mar-97 run		$ZnSO_4$ in Well	11.5 (9.3-14.7)	Full Chemistry, E-test successful but well controls?
		Hard	>899?	>1630?			Full Chemistry, fine hard controls (but well controls?)
		Soft	310.8 (295.8-326.6)	294.6 (280.2-309.8)			Full Chemistry, fine soft controls (but well controls?)
	Boron	Hard	see note	see note			Chem revealed B levels 5x too low, repeat
	Manganese	Soft	10.2 (6.8-67.5)	10.6 (7.1-67.0)			Full Chemistry, close to 12-Mar results, but...
			questionable control results, repeat FY'97'98				37.5-43.3% nonviable in well controls

E-test timeline (FY '97'98)

CCME/Water Quality Guidelines Study (Les Swain Project) - Record of 7-day E-tests performed

Test Start	Test Chemical	Water Type	EC50 (95% confidence range) in mg/L Nominal	EC50 (95% confidence range) in mg/L Corrected	Ref. Tox. Test	Ref. Tox. Result EC50 (mg/L)	*Test details to consider for billing*
09-Sep-97	Sulphate	Well	n/a: low fertilization	n/a: low fertilization	$ZnSO_4$ in Well	n/a: low fertilization	Full Chemistry but test unsuccessful, gamete quality ?
16-Sep-97	Sulphate	Well	2005 (1250-2500)	1925 (1200-2400)	$ZnSO_4$ in Well	33.0 (18.0-100)	Day 0 chemistry only
23-Sep-97	Sulphate	Hard	n/a: low fertilization	n/a: low fertilization	$ZnSO_4$ in Well	n/a: low fertilization	Full Chemistry but tests unsuccessful, gamete quality ?
		Soft	n/a: low fertilization	n/a: low fertilization			
21-Oct-97	Iron	Well	>21.0	>19.6	$ZnSO_4$ in Well	40.3 (32-100)	Day 0 chemistry only
28-Oct-97	Boron	Hard	988.0 (939.2-1040.6)	969.0 (934.2-1006.0)	$ZnSO_4$ in Well	28.8 (24.3-35.0)	Full Chemistry and all successful
	Barium	Hard	1351.1 (1326.0-1377.8)	1199.4 (1173.4-1227.1)			
		Soft	626.7 (606.0-648.4)	626.3 (605.1-648.6)			
	Manganese	Soft	16.6 (6.75-67.5)	14.6 (6.14-56.0)			
04-Nov-97	Sulphate	Hard	3351 (3066-3645)	3774 (3483-4071)	$ZnSO_4$ in Well	30.8 (28.2-33.8)	Full Chemistry and all successful
		Soft	1105 (1000-2500)	1193 (1080-2700)			
	Iron	Hard	>54.0	>50.7			
		Soft	>7.0	>6.7			
18-Nov-97	Aluminum	Well	19.5 (17.7-21.3)	17.4 (15.8-19.1)	$ZnSO_4$ in Well	18.6 (16.5-20.9)	Full Chemistry and test successful
17-Feb-98	Xylene*	Well	94.6 (83.6-109.2)	32.6 (28.6-37.9)	$ZnSO_4$ in Well	16.1 (14.3-18.2)	Full Chemistry and test successful
			140.7 (134.8-147.2)	54.8 (53.9-55.7)			
03-Mar-98	Xylene*	Hard	126.4 (107.5-154.3)	41.2 (34.7-50.9)	$ZnSO_4$ in Well	17.5 (15.5-19.9)	Full Chemistry and both tests successful
			170.2 (60-180)	56.4 (18.8-59.8)			
		Soft	106.6 (100.0-113.6)	34.4 (32.2-36.8)			
			174.1 (151.0-210.7)	54.9 (47.8-65.9)			

* = Xylene EC50s have been calculated two different ways, since the interpretation of some of the embryos was not straight-forward. While the "questionable" embryos were unlike the control embryos, it was not obvious whether to count them as viable or not viable. The upper value includes the questionables as nonviable embryos.

HARDNESS

Organism	Test Water	Nominal Total Hardness (mg/L)	Actual Total Hardness (mg/L)	Comments
Coho	Hard	250	250	
	Well	100	n/a	
	Soft	25	25.2	
Rainbow Trout	Hard	250	259	most recent value
	Well	100	n/a	
	Soft	25	47.6	
Chinook	Marine	n/a	n/a	
E-test, Rainbow Trout	Hard	250	252	
	Well	100	n/a	
	Soft	25	25.7	most recent value
Daphnia magna	Hard	250	267	
	Well	100	n/a	
	Soft	25	26.3	
Hyalella azteca	Hard	250	269	
	Well	100	n/a	
	Soft	25	n/a	
Chironomus tentans	Hard	250	272	
	Well	100	n/a	
	Soft	25	27.2	
Chronic Daphnia	Hard	250	269	
	Well	100	n/a	
	Soft	25	n/a	
Microtox® *Vibrio fischeri*	Hard	250	n/a	
	Well	100	n/a	
	Soft	25	n/a	
Eohaustorius washingtonianus	Marine	n/a	n/a	
Purple sea urchins	Marine	n/a	n/a	

Note: water hardness is measured on Day 0

APPENDIX E

Regression Analysis

B.C. ENVIRONMENT TOXICITY TESTS	Manganese Concentration				
Acute					
Hardness	25	48	100	250	(mg/L CaCO3)
Coho - Early Life 96 Hour LC50	2.4		13.1	17.4	(mg/L)
Rainbow Trout - 96 Hour LC50		2.1	20.7	12.7	(mg/L)
Hyalella Azteca - LC50	3.6		22.2	31	(mg/L)
Chironomid Tentans - LC50	5.8		42.2	94.3	(mg/L)
Daphnia Magna - LC 50	0.8		28.7	76.3	(mg/L)
Selanastrum IC50			8.29		(mg/L)
Chronic					
Rainbow Trout - 7 Day E-test	14.6	20	22.7		(mg/L)
Daphnia Magna - 21 Day Chronic LOEL		6.9	13.4		(mg/L)
Daphnia Magna - 21 Day Chronic NOEL		3.6	7.3		(mg/L)
Daphnia Magna - 21 Day Chronic IC25		5.4	9.4		(mg/L)

OTHER TOXICITY TEST DATA

	Manganese Concentration					
Acute						
Hardness	34	38	454			(mg/L CaCO3)
Rainbow Trout - Early Life 96 Hour LC50	4.83					(mg/L)
Brown Trout - Early Life 96 Hour LC50		3.77				(mg/L)
Brown Trout - 96 Hour LC50		3.8	50			(mg/L)
Chronic						
Hardness	30	36.8	37.5	150	450	(mg/L CaCO3)
Rainbow Trout - 4 Month Chronic		0.79				(mg/L)
Brown Trout - 4 Month Chronic			2.7			(mg/L)
Brown Trout - 62 Day Chronic LOEL	7.38			8.81	16.21	(mg/L)
Brown Trout - 62 Day Chronic NOEL	3.94			4.41	8.68	(mg/L)
Brown Trout - 62 Day Chronic IC25	4.67			5.59	8.68	(mg/L)

MANGANESE ACUTE REGRESSION - SIX POINT

Regression Statistics	
Multiple R	0.94983343
R Square	0.902183545
Adjusted R Square	0.877729431
Standard Error	1.45588274
Observations	6

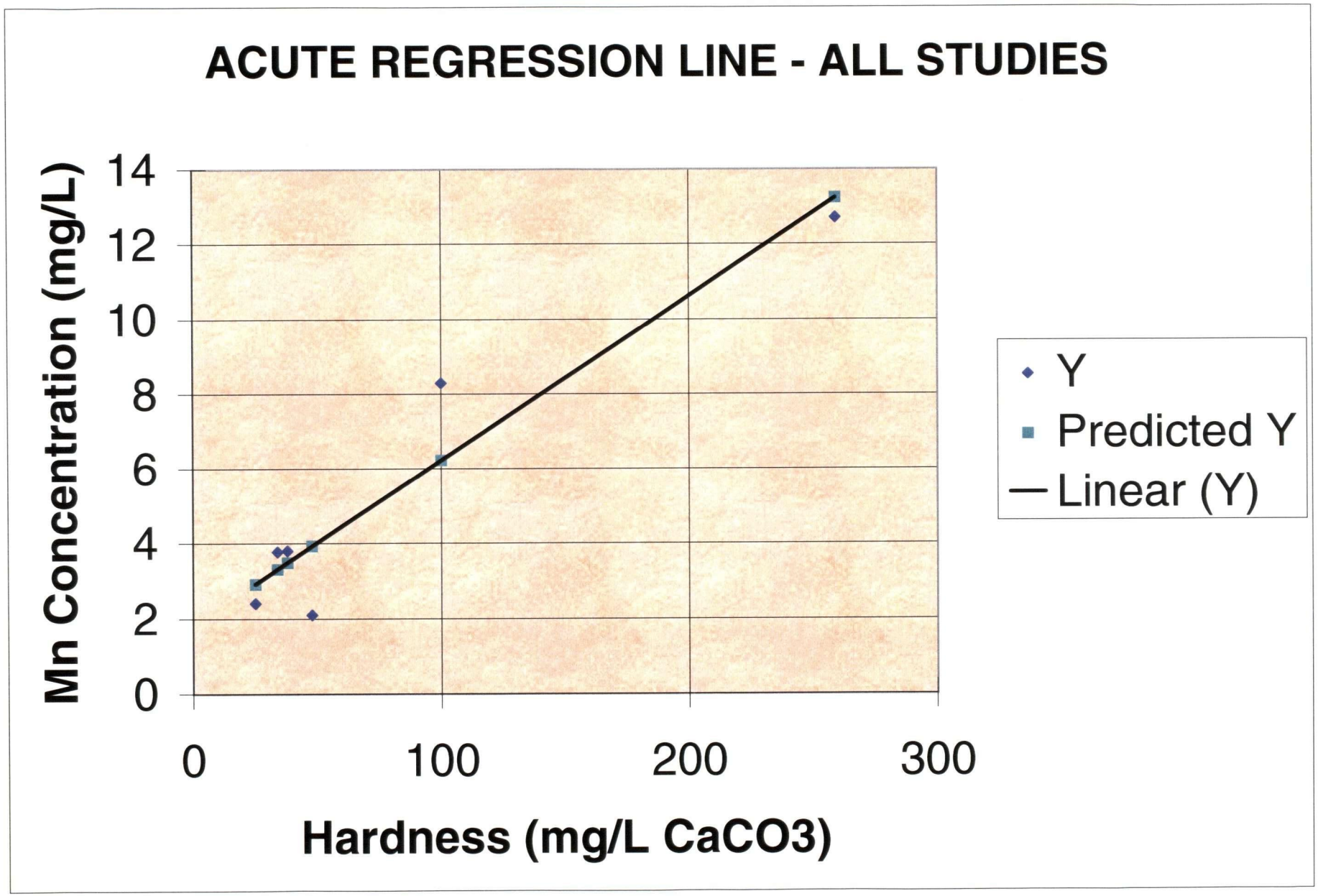
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	78.19802179	78.19802	36.89292	0.0037119
Residual	4	8.478378209	2.119595		
Total	5	86.6764			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.808610518	0.851245248	2.124664	0.100811	-0.554830078	4.1720511	-0.5548301	4.17205111
X Variable 1	0.044064161	0.007254609	6.073954	0.003712	0.023922095	0.0642062	0.02392209	0.06420623

RESIDUAL OUTPUT

Observation	*Predicted Y*	*Residuals*
1	2.91021453	-0.51021453
2	3.306791975	0.463208025
3	3.483048617	0.316951383
4	3.923690222	-1.823690222
5	6.215026568	2.074973432
6	13.22122809	-0.521228088


ACUTE REGRESSION LINE - ALL STUDIES
Mn Concentration (mg/L)
14
12
10
8
6
4
2
0
0
100
200
300
Hardness (mg/L CaCO3)
Y
Predicted Y
Linear (Y)

MANGANESE VS. HARDNESS CHRONIC REGRESSION - 6 POINT

Regression Statistics	
Multiple R	0.83759054
R Square	0.70155791
Adjusted R Square	0.62694739
Standard Error	2.03184013
Observations	6

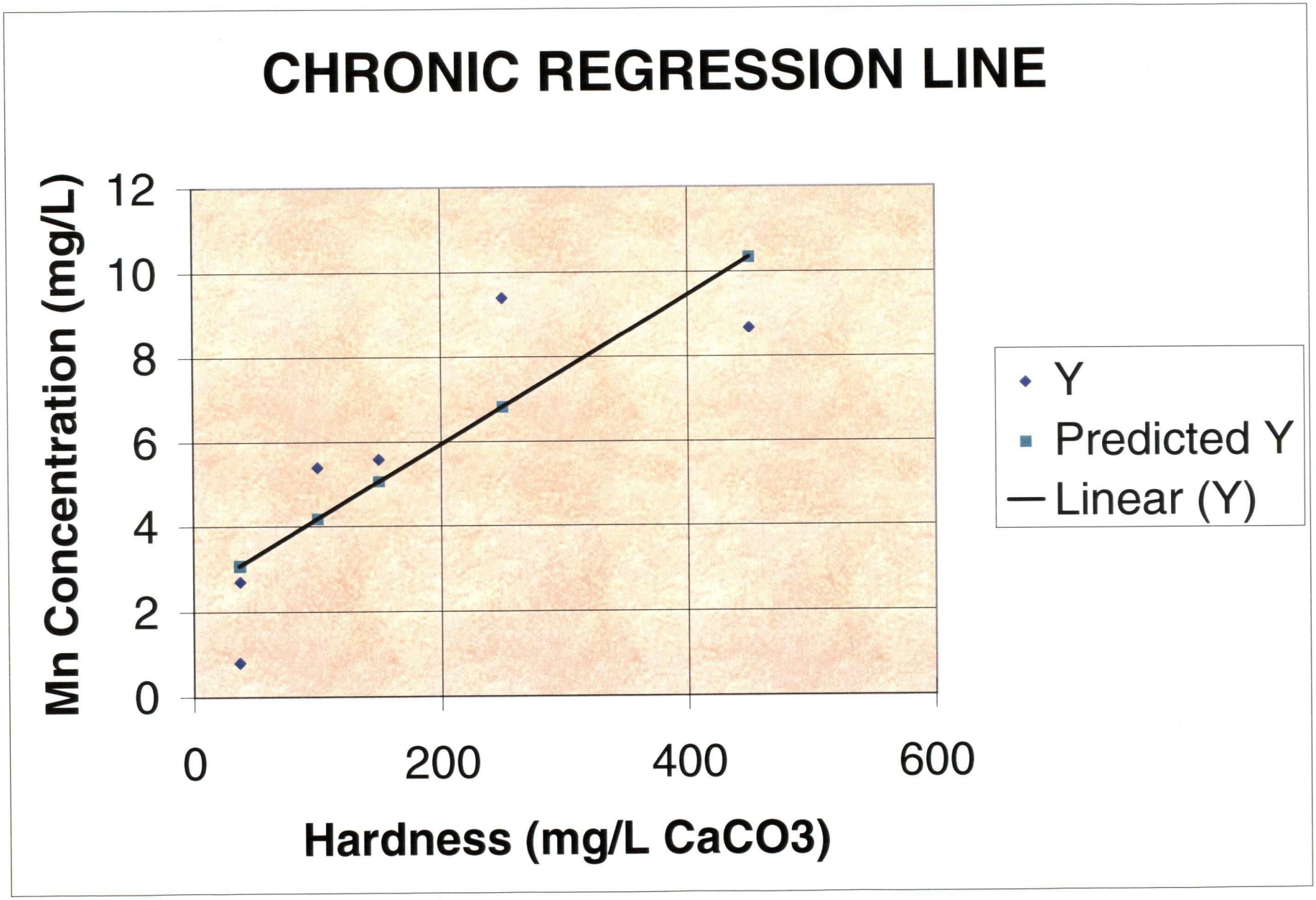
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	38.81883605	38.81884	9.402935	0.037423327
Residual	4	16.51349729	4.128374		
Total	5	55.33233333			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.42296801	1.283577768	1.887667	0.132106	-1.14082258	5.9867586	-1.14082258	5.9867586
X Variable 1	0.01759464	0.005737844	3.066421	0.037423	0.001663801	0.03352548	0.001663801	0.03352548

RESIDUAL OUTPUT

Observation	*Predicted Y*	*Residuals*
1	3.07045084	-2.280450843
2	3.08276709	-0.382767092
3	4.18243222	1.217567775
4	5.06216433	0.527835669
5	6.82162854	2.578371457
6	10.340557	-1.660556967


CHRONIC REGRESSION LINE
Mn Concentration (mg/L)
12
10
8
6
4
2
0
0
200
400
600
Hardness (mg/L CaCO3)
Y
Predicted Y
Linear (Y)