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

ENVIRONMENTAL EFFECTS OF MANGANESE AND PROPOSED FRESHWATER GUIDELINES TO PROTECT AQUATIC 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

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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 BC 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 BC. Acute and chronic toxicity tests were conducted on fish, invertebrates and freshwater algae. Acute tests included 48 and 96 hour LC_{50}'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 BC fresh waters. The collective data were evaluated for suitability with respect to the BC 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 BC 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 less than 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 BC 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 water and surface water 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 BC waters. The studies were commissioned by the Water Management Branch of the BC Ministry of Environment, Lands and Parks and were conducted by Environment Canada at their Pacific Environmental Science Center aquatic toxicity laboratory in North Vancouver, BC.

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 BC 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 BC 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 BC Ministry of Environment, Lands and Parks for native BC 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 BC 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 BC 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) for inorganic and organic parameters based on various water uses including drinking water, irrigation, and aquatic life support. BC Environment will adopt CCME guidelines as working values for parameters for which no B.C. guidelines exist.

The BC 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, BC 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 document 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* (Thruston *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 Environment toxicity tests, is expected to provide new information to enhance the data used by EPA to establish the Red Book guidelines on which the BC Environment 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 BC 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.

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. 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 BC Ministry of Environment, Lands and Parks (1998b) are summarized in Table 2.2:

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

	Total Mn Concentration (mg/L)	No. of Samples
Pacific Region	0.01-1.70	155 samples
Cariboo/Omineca/Peace	0.002 - 1.53	10^3 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 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 BC Environment 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 by emssions 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 man-made 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. 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 tricarbonly, 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[1] reports that manganese is an essential trace element for microorganisms, plants and animals and is therefore 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. 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 aquatic 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 BC Environment and are presented in Sections 3 and 4 of this thesis. For ease of presentation, studies that are applicable to species that exist in BC waters have been separated from species not present in BC 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, as follows:

TABLE 2.4: LITERATURE DATA SUMMARY

Organism	Toxicity Test	PH	Temperature (degrees C)	Dissolved Oxygen (mg/L)	Hardness (mg/L $CaCO_3$)	Mn Conc. (mg/L)
D. magna[1]	48 Hour LC_{50}	OECD	OECD	OECD	ASTM Hard Water	4.7-56.1
Rainbow Trout[2]	96 Hour LC_{50}	7.53	14.3	7.73	34.0	4.83
Brown Trout[2]	96 Hour LC_{50}	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 plus or minus 1 12 plus or minus 1 12plus or minus 1	7.8 8.7 9.0	30 150 450	4.67 (IC_{25}) 5.59 (IC_{25}) 8.68 (IC_{25})

Note: 1 - Baird *et. al.*, 1991
2 - Davies and Brinkman, 1994
3 - Stubblefield et. al., 1997

IC_{25} - 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 (Organization for Economic Cooperation and Development), which included an acute immobilization test, was employed and the measured effect was lethality as evidenced by immobility. Hard water, as defined by the American Society for Testing and Materials (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 EC_{50} 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 between the different chemicals. Genotypes that were the most sensitive to one chemical may have been the least sensitive to another chemical and lie near the middle of the response results for another 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 degrees 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 was 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. IC_{25} 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.

IC_{25} 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. The authors recommend the use of IC_{25} 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 IC_{25} 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 concentrations chosen for the test.

An equation to calculate hardness-based IC_{25} values is provided by the study. The equation, which was determined by plotting the IC_{25} values from the study against the natural logarithms of the water hardness values, is shown below:

$$IC_{25(\text{at specified hardness})} = e^{0.2064(\ln \text{hardness}) + 7.7092}$$

The regression analysis used to develop the equation had a 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 IC_{25} 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 that are fairly consistent with the 4.67 mg/L dissolved manganese IC_{25} concentration the authors 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 LC_{50} 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 LC_{50} 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 degrees 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. Measured manganese concentrations and 96 hour acute mortality data are presented in the Table 2.5.

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 LC_{50} 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 LC_{50} 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 degrees C for a four day period to acclimate. Brown trout fingerlings were similarly placed in aquaria containing 6 degrees 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 LC_{50} 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 methods[19]. 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 LC_{50} concentrations were determined using the Spearman-Karber (Hamilton *et. al.*, 1978) method 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 LC_{50} (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 degrees C	7.73
Unexposed	4.83	4.18 - 5.58	42.0	0.65	34.0	7.53/14.3 degrees 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 LC_{50} 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 LC_{50} 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 the authors stated that the "96 hour LC_{50}s were only slightly different in the exposed and unexposed groups."

The 96 hour LC_{50} results for exposed and unexposed brown trout are presented in Table 2.7.

TABLE 2.7: 96 HOUR LC_{50} ACUTE TOXICITY TEST RESULTS - BROWN TROUT

Group	96 Hour LC_{50} (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 degrees C	7.63
Unexposed	3.77	3.17 - 4.41	138.1	28.54	38.0	7.54/14.4 degrees 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 LC_{50} 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 degrees C	8.08
Unexposed	1.04/0.60	0.79	87.1	7.02	36.8	7.56/15.0	8.17

						degrees C	

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 degrees C	7.07
Unexposed	3.59/2.03	2.70	151.4	39.22	37.5	7.19/15.2 degrees 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 plus or minus 1.8 g) were exposed to 0.1 micrograms Mn/L for 21 days, with ^{54}Mn used as a tracer. Fish were exposed at pH of 7.5 plus or minus 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 and the following results were presented:

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 micrograms/L would represent a very low concentration of total manganese relative to naturally occurring levels observed in BC surface waters.

2.4.2 Other Studies

Wepener, Van Vuren and Du Preez (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 chronic sub-lethal 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, Santhakumar and Nair (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 EC_{50} (reduction in frond growth in 50% of test organisms vs. controls) of 31 mg/L was derived.

Stauber and Florence (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). The author 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 BC resident species of fish, invertebrates and algae. Literature data were also gathered in support of the data collected from toxicity testing on BC species. All relevant data were used to improve the existing freshwater aquatic life guideline for manganese contained in the BC Environment document *Approved And Working Criteria For Water Quality* (BCMELP, 1995).

3.1 B.C. PROTOCOL

The BC 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). This 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 BC Environment procedures (BC 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) presented in Appendix C.

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

1. Review of published and unpublished literature data
2. Determination of data requirements where literature sources do not provide sufficient data for water quality derivation purposes.
3. Use of 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 BC 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 LC_{50} 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 LC_{50} Invertebrate Bioassay 96 Hour LC_{50} Invertebrate Bioassay	*Daphnia Magna* Chironomid Tentans Larvae (3rd instar)	Survival as measured by immobility and lethality
96 Hour LC_{50} 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® IC_{50} - 5 and 15 Minute	*Vibrio fischeri*	Concentrations resulting in 50% decrease in light production after 5 and 15 minutes
72 Hour IC_{50} Freshwater Algal Bioassay	*Selenastrum capricomutum*	50% reduction in growth as measured by cell number/mass

Note: LC_{50} - interpolated concentration at which 50% lethality occurs in test organisms versus control group

IC_{50} - 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 ground water 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 BC PROTOCOL

Classification of toxicity testing data as primary, secondary or unacceptable is required under the BC 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 LC_{50}s and IC_{25}s. 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 require 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 BC 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 BC Environment, included nine separate test/organism combinations. A summary of the degree to which the studies conducted by Environment Canada/BC Environment met the data requirements for primary and secondary data is presented in Table 4.1.

TABLE 4.1: BC 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 LC_{50} Rainbow Trout	Yes	Yes	No	Yes	Yes	Yes

96 Hour LC_{50} Coho Salmon	Yes	Yes	No	Yes	Yes	Yes
7 Day Early Life Stage Rainbow Trout	Yes	Yes	No	Yes	Yes	Yes
48 Hour LC_{50} Invertebrate Bioassay	Yes	Yes	No	Yes	Yes	Yes
96 Hour LC_{50} Invertebrate Bioassay	Yes	Yes	No	Yes	Yes	Yes
96 Hour LC_{50} Amphipod Bioassay	Yes	Yes	No	Yes	Yes	Yes
21 Day Chronic Invertebrate Bioassay	Yes	Yes	No	Yes	Yes	Yes
Microtox® IC_{50} - 5 and 15 Minute	Yes	No	No	Yes	Yes	Yes
72 Hour IC_{50} 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 BC Environment met the requirements for primary data for all tests, with the exception of the Microtox® IC_{50} and the 72 Hour IC_{50} 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® IC_{50} relates more to the fact that the test was of such short duration, making a second concentration measurement redundant). The first note following Table 4.1 indicates that although the tests were static rather than flowthrough, laboratory personnel stated that stable manganese concentrations

did not change 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 LC_{50} - Rainbow Trout[2]	Yes	Yes	n.a.	Yes	Yes	Yes
96 Hour LC_{50} - 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 (ref. table IX-5, Appendix IX, Canadian Water Quality Guidelines, CCME 1991)
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 BC 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 BC 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	BC Environment/Colorado Data	Notes
Acute Criterion				
Fish	3 acute studies on 3 freshwater species resident in BC, at least 2 cold water species (*e.g.* trout)	2 acute and/or chronic studies; at least 1 study on a coldwater species resident in BC	4 acute and 4 chronic studies on cold water species resident in BC	Meets full requirements
Invertebrates	2 acute studies on 2 invertebrates from different classes including 1 planktonic species resident in BC	2 acute and/or chronic studies on 2 invertebrates from different classes, including 1 planktonic species resident in BC	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 BC	Not required
Chronic Criterion				
Fish	3 chronic studies on 3 freshwater species resident in BC, at least 2 cold water species (*e.g.* trout)	2 acute and/or chronic studies; at least 1 study on a coldwater species resident in BC	4 acute and 4 chronic studies on cold water species resident in BC	Meets full requirements
Invertebrates	2 chronic studies on 2 invertebrates from	2 acute and/or chronic studies on 2	1 chronic and 1 acute study on a planktonic	Meets interim requirements

	different classes including 1 planktonic species resident in BC	invertebrates from different classes, including 1 planktonic species resident in B.C.	species and 2 other acute studies on 2 invertebrates from different classes	
Plants	1 study on a freshwater vascular plant or algal species resident in BC	Not required	1 acute study on an algal species resident in BC	Meets full requirements

The requirements for type and number of toxicity test 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 BC 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 were 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 LC_{50} tests were conducted on *Chironomis 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 BC 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 LC_{50} - 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 LC_{50} - 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 LC_{50} - 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 LC_{50} - 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 LC_{50} - 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 LC_{50} - 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 analysed manganese concentration on Day 0 for Replicate A
Corrected concentrations is the average of the actual toxicity values using Day 0 and final test day ICP manganese concentrations
n.a. - not available

Rainbow trout LC_{50} concentrations were the lowest at water hardness values of 25 (measured at 47.6) and 250 mg $CaCO_3$/L while the coho salmon LC_{50} concentration was the lowest value at a hardness of 100 mg $CaCO_3$/L. The lowest LC_{50} 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 EC_{50} - 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 EC_{50} - 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 EC_{50} - 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

EC_{50} 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 LC_{50} 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 LC_{50} - *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 LC_{50} - *Chironomis 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 LC_{50} - *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 IC_{50} (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 LC_{50} - *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 LC_{50} - *Chironomis 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 LC_{50} - *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 IC_{50} (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 LC_{50} - *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 LC_{50} - *Chironomis 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 LC_{50} - *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 IC_{50} (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 analysed 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
IC_{50} - 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 $CaCO_3$. LC_{50} 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 agreements. However, some experimental concentrations varied considerably from the actual and true values (most notably *Chironomis 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 IC_{25} = 5.3 mg/L	NOEC= 3.5 mg/L LOEC = 6.7 mg/L IC_{25} = 5.3 mg/L	NOEC = 3.6 mg/L LOEC = 6.9 mg/L IC_{25} = 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 IC_{25} = 9.1 mg/L	NOEC = 7.2 mg/L LOEC = 13.6 mg/L IC_{25} = 9.4 mg/L	NOEC = 7.3 mg/L LOEC = 13.4 mg/L IC_{25} = 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 analysed 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 IC_{25} 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 IC_{50} - *Selenastrum capricomutum*	n.a.	n.a.	n.a.	n.a.
Nominal Water Hardness = 100 mg/L $CaCO_3$				
72 Hour IC_{50} - *Selenastrum capricomutum*	8.29 mg/L	n.a.	n.a.	n.a.
Nominal Water Hardness = 250 mg/L $CaCO_3$				
72 Hour IC_{50} - *Selenastrum capricomutum*	n.a.	n.a.	n.a.	n.a.

Note: Experimental concentration is based on the unverified concentration calculated by the laboratory technician

IC_{50} - 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 IC_{50} growth inhibition test at a water hardness of 100 mg/L $CaCO_3$. A manganese IC_{50} 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	Water Hardness = 100 mg/L	Water Hardness = 250 mg/L

$CaCO_3$		$CaCO_3$		$CaCO_3$	
Acute	Chronic	Acute	Chronic	Acute	Chronic
0.8 mg/L 48 hr LC_{50} Daphnia Magna	14.6 mg/L 7 Day E-test Rainbow Trout	13.1 mg/L 96 hr LC_{50} Coho Salmon	6.9 mg/L LOEC 3.6 mg/L NOEC 5.3 mg/L IC_{25} 21 day *D. magna*	12.7 mg/L 96 hr LC_{50} Rainbow Trout	13.4 mg/L LOEC 7.3 mg/L NOEC 9.1 mg/L IC_{25} 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 less than 50 mg/L $CaCO_3$ and thus, the observed mortality for the chronic *Daphnia magna* test was not unexpected. This may have also influenced the 48 hour LC_{50} results for *Daphnia magna*, the 0.8 mg Mn/L LC_{50} 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 CaCO3 will therefore not be included in the derivation of an acute guideline.

The calculated IC_{25} 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 IC_{25} 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 IC_{25} has become widely accepted as a reasonable level of protection for aquatic organisms. In the case of the *D. magna* chronic toxicity test, the IC_{25} 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 IC_{25} value represents a good estimate of the actual NOEC/LOEC concentrations.

4.2.5 Water Hardness and Aquatic Toxicity

The test results generally 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 LC_{50} test. Replicate 96 hour LC_{50} 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 IC_{50} 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 IC_{50} 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 BC Environment for water hardnesses of 25, 100 and 250 mg/L $CaCO_3$ are presented in graphical form on the following page. The Microtox IC_{50} values have not been included as the results were the highest recorded among the tests conducted. Data from literature sources that met the BC Protocol requirements for primary and/or secondary data are also plotted on this graph.

The graphical presentation illustrates the general trend of increased manganese concentration with increased water hardness. The exception was the 96 hour acute LC_{50} 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 what would be 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 BC 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 BC Environment water quality guideline derivation procedures, data collected from all suitable acute studies were combined and are presented in Table 4.10, as follows:

TABLE 4.10: ACUTE DATA FROM ALL STUDIES

Water Hardness (mg/L $CaCO_3$)	Mn Concentration (mg/L)	Toxicity Test
25	2.4* 3.6 5.8 0.8	Coho - Early Life 96 Hour LC_{50} *Hyalella azteca* - 96 Hour LC_{50} *Chironomis tentans - 96 Hour LC_{50}* *Daphnia magna -48 Hour LC_{50}*
34	3.77*	Brown Trout - Early Life 96 Hour LC_{50}
38	4.83 3.8*	Rainbow Trout - Early Life 96 Hour LC_{50} Brown Trout - 96 Hour LC_{50}
47.6	2.1*	Rainbow Trout - 96 Hour LC_{50}
100	13.1	Coho - Early Life 96 Hour LC_{50}

	20.7 22.2 42.2 28.7 8.29*	Rainbow Trout - 96 Hour LC_{50} *Hyalella azteca* - 96 Hour LC_{50} *Chironomis tentans* - 96 Hour LC_{50} *Daphnia magna* -48 Hour LC_{50} *Selenastrum capricornutum* - 72 Hour IC_{50}
250	17.4 12.7* 31.0 94.3 76.3	Coho - Early Life 96 Hour LC_{50} Rainbow Trout - 96 Hour LC_{50} *Hyalella azteca* - 96 Hour LC_{50} *Chironomis tentans* - 96 Hour LC_{50} *Daphnia magna* -48 Hour LC_{50}
454	49.9	Brown Trout - 96 Hour LC_{50}

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 LC_{50} 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 LC_{50}. 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 LC_{50} 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.

The concentrations denoted by an asterisk in Table 4.10 were used in the regression analysis. All acute values used were 96 Hour LC_{50} concentrations with the exception of the 72 Hour IC_{50} 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 B.C. Environment tests and data from literature sources screened in Section 4.3 were also combined for application of the B.C. Environment 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 IC_{25}
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 IC_{25}
150	***5.59***	Brown Trout - 62 Day IC_{25}
250	22.4 ***9.4***	Rainbow Trout - 7 Day E-Test *Daphnia magna* - 21 Day IC_{25}
450	***8.68***	Brown Trout - 62 Day IC_{25}

Note: Bolded and italicized values 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 IC_{25} 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 $CaCO_3$, but were lower 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). 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 acute and chronic interim guidelines for the protection of fresh water 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 BC 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 duration of the tests. Some uncertainty was also associated with the acute data due to the BC Environment 96 Hour LC_{50} 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 IC_{25} 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 IC_{25} values are presented in Table 4.14:

TABLE 4.14: IC_{25}/NOEC RATIOS FOR DAPHNIA MAGNA AND BROWN TROUT

Daphnia magna				Brown Trout			
Hardness	NOEC	IC_{25}	NOEC/IC25	Hardness	NOEC	IC25	NOEC/IC25

(mg/L $CaCO_3$)				(mg/L $CaCO_3$)			
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/IC_{25} 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 BC 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 BC 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 $CaCO_3$)	**Mn Concentration** (mg/L)	**Modified Mn Concentration** (mg/L)	**Hardness** (mg/L $CaCO_3$)	**Mn Concentration** (mg/L)	**Modified Mn 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 multiplies 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 $CaCO_3$)	**Mn Concentration** (mg/L)	**Modified Mn Concentration** (mg/L)	**Hardness** (mg/L $CaCO_3$)	**Mn Concentration** (mg/L)	**Modified Mn 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 acute and chronic guidelines derived 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 equations 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 document indicates that total manganese concentrations observed in BC surface waters range from <0.001 mg/L to 1.70 mg/L (CCME, 1987; BCMELP 1998), 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, if used as guidelines, 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 ground water adjacent to surface waters where manganese may be of concern could also 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 BC, 1997) provides standards for substance concentrations in ground water. For aquatic life water use, the current ground water standard for manganese is 1 mg/L. A dilution factor of 10 for discharge of ground water 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 ground water 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 BC species and are considered to have a more solid scientific basis. If a ground water standard for manganese for protection of aquatic life is retained, the proposed guidelines could be used to develop new ground water standards. The current ground water 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 BC Ministry of Environment, Lands and Parks for native BC 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 (less than 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 modified manganese concentrations from the chronic data set (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 (less than 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 BC Environment toxicity test data and the literature data. The exception was the 96 Hour LC_{50} 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 LC_{50} 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 BC 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 BC 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 BC 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 ground water immediately adjacent to a surface water body may alleviate concerns regarding naturally occurring manganese versus anthropogenic manganese. For ground water, 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 LC_{50} 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 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 BC 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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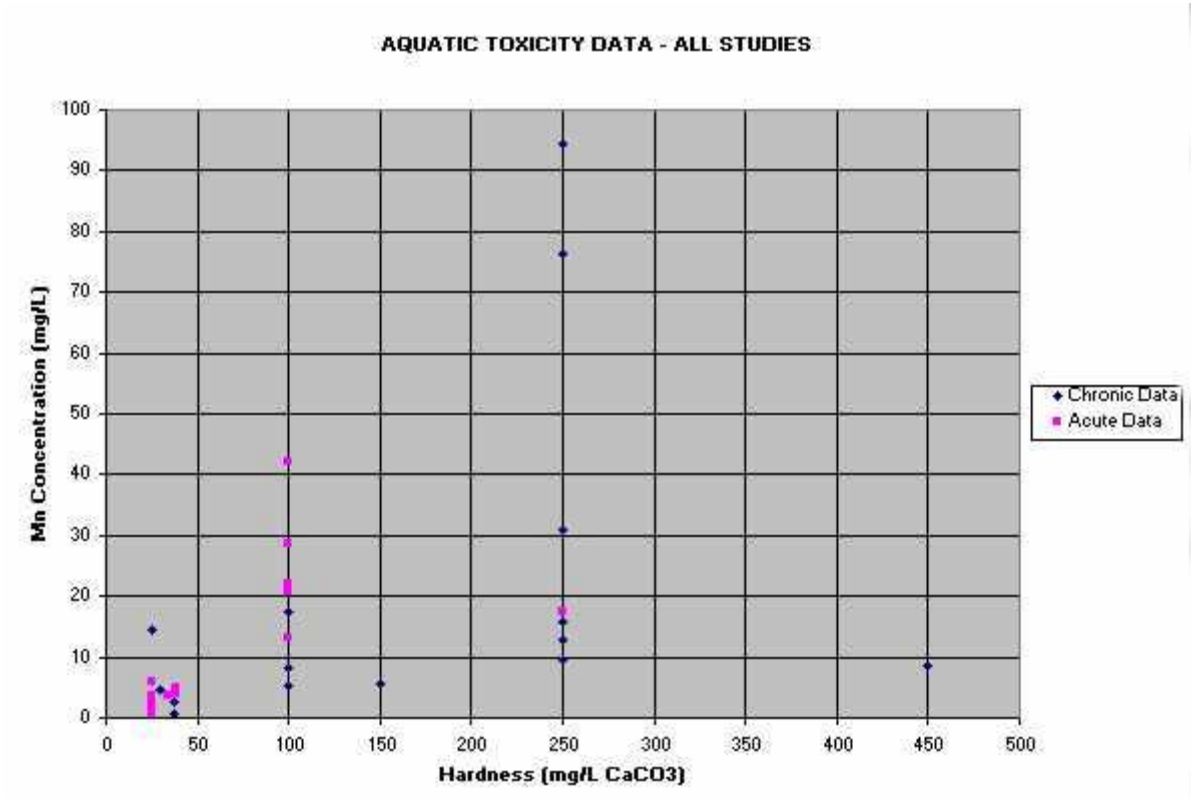
Data Charts and Tables
Aquatic Toxicity Data - All Studies

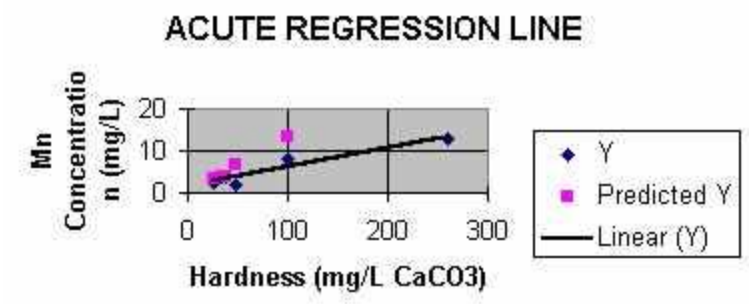
Hardness	Mn Concentration	Hardness	Mn Concentration
mg/L $CaCO_3$	mg/L	mg/L $CaCO_3$	mg/L
450	8.68	250	17.4
250	15.8	100	13.1
250	12.7	100	20.7
250	31	100	22.2
250	94.3	100	42.2
250	76.3	100	28.7
250	9.4	38	3.8
150	5.59	38	4.83
100	17.5	34	3.77

100	8.29	25	2.1
100	5.4	25	2.4
37.5	2.7	25	3.6
36.8	0.79	25	5.8
30	4.67	25	0.8
25	14.6		



Acute Regression Line/Acute Regression Line - All Studies

SUMMARY OUTPUT



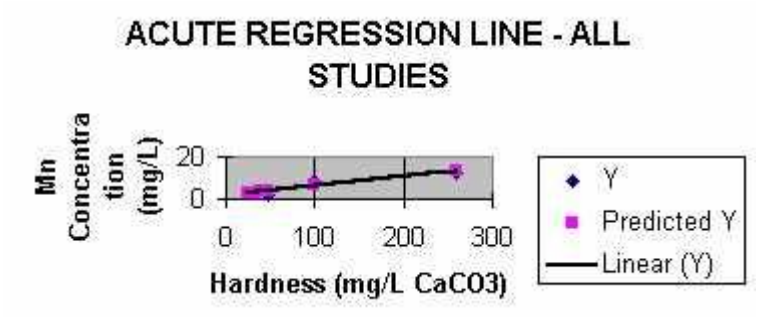
Regression Statistics	
Multiple R	0.969215243
R Square	0.939378188
Adjusted R Square	0.919170917
Standard Error	1.231648238
Observations	5

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	70.51900786	70.51900	46.48713	0.006453893
Residual	3	4.550872143	1.516957		
Total	4	75.06988			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.162165242	0.801499292	2.697650	0.07393	-0.38856561	4.71289609	-0.38856561	4.712896095
X Variable 1	0.04440531	0.006512811	6.818147	0.00653	0.023678618	0.06513200	0.023678618	0.065132003

RESIDUAL OUTPUT		
Observation	*Predicted Y*	*Residuals*
1	3.272298005	-1.172298005
2	3.671945799	0.098054201
3	3.849567041	-0.049567041
4	6.602696291	1.687303709
5	13.26349286	-0.563492864

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	36.89291	0.0037119
Residual	4	8.478378209	2.119594		

Total	5	86.6764			

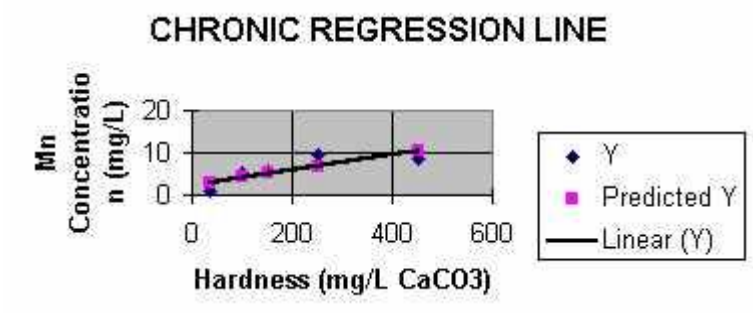
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.808610518	0.851245248	2.124664	0.100811	-0.554830078	4.17205111	-0.554830078	4.172051114
X Variable 1	0.044064161	0.007254609	6.073953	0.003711	0.023922095	0.06420622	0.023922095	0.064206226

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

Hardness	Mn Concentration
(mg/L $CaCO_3$)	(mg/L)
25	2.4
34	3.77
38	3.8
48	2.1

100	8.29
259	12.7

Manganese vs. Hardness Chronic Regression - 6 Point



Regression Statistics	
Multiple R	0.837590538
R Square	0.701557909
Adjusted R Square	0.626947386
Standard Error	2.031840132
Observations	6

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	38.81883605	38.81883	9.402935	0.037423327
Residual	4	16.51349729	4.128374		
Total	5	55.33233333			

	Coefficie	*Standard*	*t Stat*	*P-*	*Lower*	*Upper*	*Lower*	*Upper*

	nts	*Error*		*value*	*95%*	*95%*	*95.0%*	*95.0%*
Intercept	2.422968012	1.283577768	1.887667	0.132105	-1.14082258	5.986758605	-1.14082258	5.986758605
X Variable 1	0.017594642	0.005737844	3.066420	0.037423	0.001663801	0.033525483	0.001663801	0.033525483

RESIDUAL OUTPUT		
Observation	*Predicted Y*	*Residuals*
1	3.070450843	-2.280450843
2	3.082767092	-0.382767092
3	4.182432225	1.217567775
4	5.062164331	0.527835669
5	6.821628543	2.578371457
6	10.34055697	-1.660556967

Hardness	Mn Concentration	
(mg/L $CaCO_3$)	(mg/L)	
36.8	0.79	
37.5	2.7	
100	5.4	
150	5.59	
250	9.4	
450	8.68	