Evaluation of Potential Standardization Models for Canadian Water Quality Guidelines

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Prepared by:

Zajdlik & Associates Inc.,

G. Craig & Associates, I. Middelraad and D.G. Dixon

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Table of Contents

List of Tables

List of Figures

Table 1: List of Acronyms

Acronym	Definition
BLM	biotic ligand model
CCC	criteria continuous concentration
CMC	criteria maximum concentration
DOC	dissolved organic carbon
DOM	dissolved organic matter
FACR	final acute chronic ratio
FAV	final acute value
H	hardness (as mg $CaCO3/L$)
HMTV	hardness modified trigger value
ME	generic metal
LCx	concentration producing lethality in x% of organisms.
LOEC	lowest observed effect concentration
MTC	maximum tolerable concentration
NOM	natural organic matter
OM	organic matter
pKa	= $-\log_{10}$Ka, where Ka is the equilibrium dissociation constant for a weak acid in water
QSAR	quantitative structural activity relationship
SMAV	species mean acute value
TMF	toxicity modifying factor
TV	Toxicity value
TrV	trigger value
WQG	water quality guidelines

1 Introduction

WQGs have long been used to protect the aquatic environment from the discharge or loss of materials. Prohibiting the discharge or loss of any materials to the aquatic environment certainly prevents potential associated adverse effects but this extreme position places large or impossible restrictions on human activity. In some instances discharge to the environment can result in no adverse effects and no regulation is necessary. However, a lack of regulation has historically led to severe and at times irreversible (in the case of habitat loss) environmental degradation.

The judicious choice of WQG restricts discharges to the environment to the point where ecosystems will remain viable in the long term (at least from the perspective of discharges). Given the historical lack of knowledge regarding interactions between contaminants, factors that modify toxicity, contaminant fate and transport and ecosystem function, safety factors have been applied to WQGs. The safety factors represent the state of knowledge regarding a particular chemical; in the absence of knowledge more stringent safety factors are used. While safety factors embrace the precautionary principle from the perspective of protecting the environment, overly stringent safety factors result in unnecessary costs to dischargers.

The toxicity of all chemicals discharged to the environment is affected by the characteristics of the media in which they reside (US EPA, 1991). Effects of the receiving environment relevant to this project include changes to bioavailability and/or mitigation/exacerbation. The bioavailability of polar organic compounds and some inorganic compounds (ammonia, cyanide, and hydrogen sulphide) is affected by their respective pKa and the pH of the receiving water. The bioavailability of neutral organic compounds, as well as others is affected by adsorption on to inert (carbon or other) particles or other dissolved organics. The effects of the receiving environment on metal toxicity have been intensively studied. Within the last few decades substantial progress has been made in understanding:

- the sites of toxic action for metals;
- the physiological effects of metals;
- the relationships between aquatic chemistry and the bioavailability of metals; and,
- the effects of toxicity modifying factors (TMFs) over wider taxonomic ranges.

Factors that mitigate toxicity to chemicals other than metals have been less well studied and therefore are not often used as modifying factors for WQGs. However, some jurisdictions do acknowledge the effect of TMFs on WQGs. Examples include the combined effects of pH and temperature used to adjust ammonia toxicity WQGs (CCME, 2006, and US EPA, 1998, 2006), and the adjustment of pentachlorophenol toxicity by pH (US EPA, 2006).

The purpose of this project is to determine whether recent knowledge regarding TMFs can be incorporated into new or existing paradigms for WQG development. The goal of incorporating TMFs into WQGs is to develop guidelines that incorporate scientific knowledge and reduce the degree of uncertainty regarding the application of a WQG to a specific receiving environment.

Other approaches to addressing site-specificity such as direct toxicity assessment and water-effect ratios are not discussed herein.

1.1 Selection of Toxicity Modifying Factors (TMFs) to Examine

The TMFs to examine in detail were identified following the three step process below:

1. A general review of the literature published in the last five years to identify modifying factors that have been shown to affect the toxicity of organic compounds, metals, and other inorganic compounds to aquatic organisms.

2. Review the latest guidelines published by regulatory agencies to identify what factors have been incorporated into guidelines, in what way and with what supporting rationale. This review is presented in Table 2 through Table 7**Error! Reference source not found.**, below.

3. Review the published literature to identify advances that include either new modifying factors or revised approaches to using factors already incorporated in guidelines. Attention was directed to both short and long term guidelines if differentiation was made among various studies or agencies.

The purpose of the three step review is to identify what TMFs have been used to modify aquatic guidelines, globally. This information along with more recent information on modification of guidelines for TMFs and new approaches for guideline derivation will be used to define potential approaches for modifying Canadian water quality guidelines.

1.2 Jurisdictional Review of Guideline Modifications for TMFs

Table 2: Jurisdictional Review of Guideline Modifications for TMFs – Australia/New Zealand

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Cadmium	hardness	exponential	HMTV = TV (H/30)^0.89	The TV (toxicity value) is from US EPA 1995 chronic toxicity algorithms at 30 mg/L hardness. The HMTV (hardness modified toxicity value) is calculated using log LC50 and hardness regression slopes determined from the data set for the metal.	fresh surface water (≤2.5‰ salinity)	Markich et al, (2001), US EPA (1995a,b)	Trigger Value (TrV) is taken from probability effect concentration Table 3.4.1. Data used to generate empirical relationships are from US EPA data (US EPA 1995a, b). For further discussion see section 2.1.2.4
Chromium	hardness	exponential	HMTV = TV (H/30)^0.82		fresh surface water (≤2.5‰ salinity)	Markich et al, (2001)	
Copper	hardness	exponential	HMTV = TV(H/30)^0.85		fresh surface water (≤2.5‰ salinity)	Markich et al, (2001)	
Lead	hardness	exponential	HMTV = TV(H/30)^1.27		fresh surface water (≤2.5‰ salinity)	Markich et al, (2001)	
Nickel	hardness	exponential	HMTV = TV(H/30)^0.85		fresh surface water (≤2.5‰ salinity)	Markich et al, (2001)	
Zinc	hardness	exponential	HMTV = TV(H/30)^0.85		fresh surface water (≤2.5‰ salinity)	Markich et al, (2001)	

Table 3: Jurisdictional Review of Guideline Modifications for TMFs – Canada

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Aluminum	pH, DOC, Ca	step	"step" at pH 6.5		Ca<4mg/L; DOC<>2mg/L		
Ammonia (total)	pH, temperature		Table look up	Guideline decreases as pH and temperature increase.		CCME (2001)	Empirical adjustment, based upon predicted amount of unionized ammonia not exceeding 0.019 mg/l.
Cadmium	hardness	log	10^(0.86*log (hardness) - 3.2)	Originating from a LOEL and a 0.1 safety factor, the guideline is adjusted using the slope of a cladoceran toxicity regression of LC50 against hardness normalized at 48.5 mg/L.	none mentioned; freshwater only	Lewis and Porter, (1990)	This relationship was derived using acute toxicity data to cladocerans from various sources as described in Lewis and Porter, (1990). Therefore the application of this relationship assumes that: the relationship between hardness and acute Cd toxicity for cladocerans applies to all freshwater organisms and that the acute relationship holds for chronic exposures.
Copper	hardness	step	none	The US EPA 1985 final acute plus acute/chronic ratio algorithm is multiplied by 0.2 and rounded to the nearest integer; No rationale for the selection of hardness steps is presented. See discussion of error in comment column.	<120, <180,>180 mg $CaCO_3$/L	US EPA (1985b), CCREM (1987)	CCREM (1987) uses three hardness levels without explicitly stating their selection. An implied rationalization is that the three hardness levels represent soft, moderate and hard waters in Canada. The values for hardness >120 and >180 are miscalculated and should be 3 ug/L (not 4ug/L) and 4 ug/L (not 6 µg/L).
Lead	hardness	step	none	The US EPA 1985 chronic toxicity algorithm was used for each hardness step. No rationale for the selection of hardness steps is presented.	<60,<120,<180, >180 mg $CaCO_3$/L	CCREM, (1987) US EPA (1985d)	

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Nickel	hardness	step	none	The US EPA 1980 chronic toxicity algorithm was used for each hardness step. No rationale for the selection of hardness steps is presented.	<60,<120,<180, >180 mg CaCO3/L	CCREM, (1987). US EPA (1986)	

Table 4: Jurisdictional Review of Guideline Modifications for TMFs – European Union

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Copper	hardness	step	none		<10, <50, <100, >300 mg CaCO3/L	Commission of European Communities, (1978)	
Zinc	hardness	step	none		<10, <50, <100, >500 mg CaCO3/L		Different scale for salmonid and cyprinid waters

Table 5: Jurisdictional Review of Guideline Modifications for TMFs – United Kingdom

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Copper Table 2 - Imperative	hardness	step	none	Not stated or referenced	<10, <50,<100, >100 mg/L hardness	Commission of European Communities, (1978)	Table 2: Freshwater Fish Directive: summary of Guideline standards Guideline (G) values - these are quality standards that should be achieved where possible. Values have been set here for other chemical parameters, such as copper, biochemical oxygen demand and suspended solids.
Zinc Table 1 - Imperative	hardness	step	none	Not stated or referenced	<10, <50,<100, >100 mg/L hardness		The Directive identifies two categories of water; those suitable for: • salmonid fish (salmon and trout): These are generally fast flowing stretches of river that have a high oxygen content and a low level of nutrients. • cyprinid fish (coarse fish - carp, tench, barbel, rudd, roach): These are slower flowing waters, that often flow through lowlands. There are two types of standards within each water category: Imperative (I) values - these are standards that must be met if the stretch is to pass the Directive (for the stretch to be 'compliant'). Values have been set for dissolved oxygen, pH, non-ionized ammonia, total ammonium, total residual chlorine, zinc and (for thermal discharges) temperature. Table 1: Freshwater Fish Directive: summary of Imperative standards Guideline (G) values. These are quality standards that should be achieved where possible. Values have been set here for other chemical parameters, such as copper, biochemical oxygen demand and suspended solids.

Table 6: Jurisdictional Review of Guideline Modifications for TMFs – United States

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Aluminum	pH, hardness	step	none		pH 6.5-6.6 ; hardness ,<10mg/L pH 6.5-9.0	US EPA, (2006)	
Ammonia - Total - CCC 30day	pH, Temp.	exponential	CCC = ((0.0577/(1 + 10^(7.688-pH))) + (2.487/(1 + 10^(pH-7.688)))) x MIN (2.85, 1.45·10^(0.028·(25-T)))		pH 6.5-9; T<25C; Early life stages present	US EPA, (1998, 2006)	Early life stages present
Ammonia - Total - CCC 30day	pH, Temp.	exponential	CCC = ((0.0577/(1 + 10^(7.688-pH))) + (2.487/(1 + 10^(pH-7.688)))) x 1.45·10^(0.028·(25-MAX(T,7)))		pH 6.5-9 ; T<25C; Early life stages NOT present	US EPA, (1998, 2006)	Early life stages NOT present
Ammonia - Total - CMC one hour average	pH, Temp.	exponential	CMC = (0.275/(1 + 10^(7.204-pH))) + (39.0/(1 + 10^(pH-7.204)))		pH 6.5-9 Salmonids present	US EPA, (1998, 2006)	Salmonids present
Ammonia - Total - CMC one hour average	pH, Temp.	exponential	CMC = (0.411/(1 + 10^(7.204-Ph))) + (58.4/(1 + 10^(pH-7.204)))		pH 6.5-9 salmonids NOT present	US EPA, (1998, 2006)	Salmonids NOT present

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Arsenic	none	none	none		none	US EPA, (2006)	This recommended water quality criterion was derived from data for arsenic (III), but is applied here to total arsenic, which might imply that arsenic (III) and arsenic (V) are equally toxic to aquatic life and that their toxicities are additive. In the arsenic criteria document (PDF, 74 pp., 3.2M) (EPA 440/5-84-033, January 1985), Species Mean Acute Values are given for both arsenic (III) and arsenic (V) for five species and the ratios of the SMAVs for each species range from 0.6 to 1.7. Chronic values are available for both arsenic (III) and arsenic (V) for one species; for the fathead minnow, the chronic value for arsenic (V) is 0.29 times the chronic value for arsenic (III). No data are known to be available concerning whether the toxicities of the forms of arsenic to aquatic organisms are additive.
Cadmium	hardness	exponential	CMC=exp(1.0166(ln hardness) - 3.924) Conversion Factor[1] =1.136672-((ln hardness)(0.041838))		none	US EPA, (2006)	

[1] "The term "Conversion Factor" (CF) represents the recommended conversion factor for converting a metal criterion expressed as the total recoverable fraction in the water column to a criterion expressed as the dissolved fraction in the water column." US EPA (2006).

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Cadmium	hardness	exponential	CCC=exp(0.7409(ln hardness) - 4.719) Conversion Factor[1] =1.101672-((ln hardness)(0.041838))		none	US EPA, (2001, 2006)	
Chloride	none	exponential				US EPA, (1998)	
Chlorine	ammonia, Temp.	exponential				US EPA, (1985a)	Temperature mentioned briefly on p.4 of reference document. Ammonia a factor but not discussed.
Chromium III	hardness	exponential	CMC=exp(0.8190(ln hardness) - 3.7256) Conversion Factor =0.316		none	US EPA, (2006)	
Chromium III	hardness	exponential	CCC=exp(0.8190(ln hardness) - 0.6848) Conversion Factor =0.860		none	US EPA, (2006)	
Chromium VI	none				none	US EPA, (2006)	
Copper	hardness		CMC=exp(0.9422(ln hardness) - 1.700) Conversion Factor =0.960		none	US EPA, (2006)	
Copper	hardness		CCC=exp(0.8545(ln hardness) - 1.702) Conversion Factor =0.960		none	US EPA, (2006)	

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Copper	Hardness and combined effects of various water quality variables (e.g., pH and alkalinity)	mechanistic via BLM	CCC = FAV/FACR CMD = FAV/2	FAV adjusted to site-specific conditions using BLM		US EPA, (2003)	A draft version of copper toxicity modification using the BLM has existed since 2003 but has not yet been implemented. It is expected to be released in January, 2007. (L. Cruz, US EPA pers. comm.)
Cyanide	pH, temperature		(ug of free cyanide as CN/L) = (ug of HCN/L)(1 + 10 $^{pH-pK_{HCN}}$) x mol. Wt. CN / mol. Wt. HCN; pKHCN = 1.3440 + 2347.2 / T + 273.6;		pH 6.5-9	US EPA (1995e)	
Lead	hardness	exponential	CMC=exp(1.273(ln hardness) - 1.460) Conversion Factor =1.46203-((ln hardness)(0.145712))		none	US EPA (1985d, 2006)	
Lead	hardness	exponential	CMC=exp(1.273(ln hardness) - 4.705) Conversion Factor =1.46203-((ln hardness)(0.145712))		none	US EPA (1985d, 2006)	

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Mercury	alkalinity, pH, DO, hardness, temperature, chloride, organic complexing agents, sediment					US EPA, (2006) US EPA, (1985c)	This recommended water quality criterion was derived from data for inorganic mercury (II), but is applied here to total mercury. If a substantial portion of the mercury in the water column is methylmercury, this criterion will probably be under protective. In addition, even though inorganic mercury is converted to methylmercury and methylmercury bioaccumulates to a great extent, this criterion does not account for uptake via the food chain because sufficient data were not available when the criterion was derived. Increase in water temperature leads to increase of mercury in tissue residues. Low dissolved oxygen increases respiration rate leading to higher uptake rate.
Nickel	hardness	exponential	CMC=exp(0.8460(ln hardness) + 2.255) Conversion Factor =0.998		none	US EPA, (2006)	
Nickel	hardness	exponential	CCC=exp(0.8460(ln hardness) + 0.0584) Conversion Factor =0.997		none	US EPA, (2006)	
Pentachlorophenol	pH	exponential	CMC = exp(1.005(pH)-4.869);			US EPA, (2006)	

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
Pentachlorophenol	pH	exponential	CCC = exp(1.005(pH)-5.134).			US EPA, (2006)	
Pentachlorophenol - CCC 4 day average once over 3 years	pH	exponential	CCC =e^(1.005(pH)-5.134)		pH 6.5-9	US EPA, (2006)	
Pentachlorophenol - CMC one hour average once over 3 years	pH	exponential	CMC = e^(1.005*(pH)-4.869)		pH 6.5-9	US EPA, (2006)	
Selenium	none	fixed	none		none	US EPA, (2006)	The CMC = 1/[(f1/CMC1) + (f2/CMC2)] where f1 and f2 are the fractions of total selenium that are treated as selenite and selenate, respectively, and CMC1 and CMC2 are 185.9 g/l and 12.82 g/l, respectively. This recommended water quality criterion for selenium is expressed in terms of total recoverable metal in the water column. It is scientifically acceptable to use the conversion factor (0.996- CMC or 0.922- CCC) that was used in the GLI to convert this to a value that is expressed in terms of dissolved metal.
Silver	hardness	exponential	CMC=exp(1.72(ln hardness) - 6.59) Conversion Factor =0.85		none	US EPA, (2006)	EPA is considering using the BLM to adjust for site-specific TMFs for silver following the general paradigm used in EPA (2003). (L. Cruz, US EPA pers.

Guideline / Parameter	TMF	Relationship Type	Equation	Type of Adjustment	Limits	Reference	Comments
							comm.)
Zinc	hardness	exponential	CMC=exp(0.8473(ln hardness) + 0.884) Conversion Factor =0.978		none	US EPA, (2006)	EPA is considering using the BLM to adjust for site-specific TMFs for zinc following the general paradigm used in EPA (2003). (L. Cruz, US EPA pers. comm.)
Zinc	hardness	exponential	CCC=exp(0.8473(ln hardness) + 0.884) Conversion Factor =0.986		none	US EPA, (2006)	

1.3 Other Jurisdictional Reviews

Other jurisdictions were reviewed but do not use adjustments for TMFs. These are summarized in Table 7, below.

Table 7: Jurisdictions that do not Adjust WQGs for TMFs

Jurisdiction	Citation	Comment
Denmark	Samsoe-Petersen and Pedersen, (1995)	In Denmark, the derivation of quality criteria is based primarily on information of toxicity of the substance and consequently their criteria are often called "effects based quality criteria".
Netherlands	Crommentuijn et al, (1997)	Netherlands view the US EPA approach to using modifying factors questionable because few if any criteria are based on modification of chronic data. US EPA criteria presume the modifying factors applied to acute toxicity are transferable to chronic toxicity when there is no substantive evidence for such effects.
OECD	OECD, (1995)	A maximum tolerable concentration (MTC) in water indicates a maximum concentration of a chemical where no unacceptable adverse effects on the ecosystem are expected. ….In the hierarchy of toxicity data, QSARs have a lower status than acute test and chronic test have a higher status than acute tests. A reliable and representative field test had the highest status. As a consequence, an MTC derived in the confirmatory stage will in general be more suitable for setting environment quality objectives, whereas sometimes an MTC based on an initial effects assessment may be used only for setting priorities for further studies.
Sweden	"Metals in Lakes and Watercourses" www.internat.naturvardsverket.se/documents/legal/assess/assedoc/lakedoc/metal2.htm	Swedish EPA ranks surface waters into Classes 1-5 according to concentrations of metals and associates a narrative risk of effect with each class.

1.4 Selection of TMFs and Analytes to Evaluate Standardization Models

Appendix 1: Review of Recent Studies on TMFs and Metal Toxicity presents a wealth of information but is difficult to digest. Therefore the information has been recast in Table 8 below, to merely indicate the presence of studies describing factors affecting toxicity of specific metals to organism groups.

Table 8: Existence of Metal Toxicity Studies using Three Organism Groups for which TMFs have been Described

	Acute (< 7 days)					Chronic (> 14days or > 1 generation)				
	Cu	Ni	Zn	Cd	Ag	Cu	Ni	Zn	Cd	Ag
	Freshwater Organisms									
Fish	*	*	*	*		*		*	*	*
Invertebrates	*	*	*	*	*	*	*	*	*	*
Algae	*			*	*	*			*	
	Marine Organisms									
Fish	*					*				
Invertebrates	*				*	*				*

Both columns in Table 8 above indicate that TMFs for copper toxicity have been investigated for freshwater fish, invertebrates and algae and marine fish and invertebrates for both acute and chronic responses. Since Cu has the most comprehensive acute and chronic exposure data set, it is the best candidate for a case study to assess the practicality of incorporating TMFs into guidelines. Also, Cu and Ag have received the most attention (as of 2002) with respect to modeling by the BLM (Paquin et al, 2003).

Hardness is one of the primary TMFs for Cu in the studies described in Table 13 within Appendix 1: Review of Recent Studies on TMFs and Metal Toxicity. Therefore, Cu and hardness were selected as the contaminant and TMF for assessing the practicality of incorporating TMFs into guidelines, in conjunction with the project authority.

It may be possible to assess whether the simultaneous effects of two or more TMFs may be incorporated into WQGs.

2 Adjustment of Metal Toxicity by TMFs

A systematic review and synthesis of the factors that affect metal toxicity is far beyond the scope of this document. However some general comments regarding TMFs and aquatic toxicity of metals are found below. The interested reader is also directed to the research cited in Appendix 1.

2.1 pH

As pH increases between 6 and 9, metal toxicity usually decreases because as pH increases, the concentration of CO_3^{2-} increases allowing for increased binding with metals such as Cu^{2+} (Meyer, 1999). Metals within this pH range can precipitate as metal hydroxides or salts. This observation does not apply to amphoteric metals such as Al.

As pH decreases metal toxicity may also decrease due to competitive binding at the gill (Campbell and Stokes, 1985). This effect may be most important at pH < 6 as the H^+ concentration becomes high enough to compete with Ca^{2+} and metals such as Cu^{2+} (Meyer, 1999).

Thus pH as a TMF has a dual mode of action making adjustment of WQGs by this TMF more difficult than for TMFs with a single[2] mode of action.

2.2 Organic Matter

OM mitigates metal toxicity. As early as 1973 (Zitko) showed that dissolved OM affected the toxicity of Cu to Atlantic salmon. In 1974, Pagenkopf et al showed that complexation of Cu by organic matter mitigated toxicity to fish. Playle et al (1993) discusses how DOC affects binding of Cu and Cd to fish gills.

More recently, it has been recognized that the type of natural OM affects the degree of complexation (Benedettti et al, 1995). Ryan et al, (2004) demonstrate that the there are significant differences in Cu toxicity to larval fathead minnows attributable to the source of NOM. Playle et al (2002) assessed the simultaneous effects of hardness, pH and suspended solids on Ni toxicity to fish. Sarathy and Allen (2005) show that OM of human origin also has different Cu-binding affinities than natural OM.

[2] The single mode of toxic action refers to environmentally relevant levels of TMFs. For example, OM acts as a TMF by complexing metals making them less bioavailable.

2.3 Hardness

Toxicity is often inversely proportional to hardness. Zitko (1976) showed that Ca^{2+} and Mg^{2+} competitively bind with cationic metals at the sites of toxic action, thereby reducing metal toxicity. Howarth and Sprague (1978) developed an empirical relationship between hardness and Cu toxicity to rainbow trout. Welsh et al, (2000) and Naddy et al (2002), showed that even the ratio of the two cations usually used to describe water hardness (Ca^{2+} and Mg^{2+}) can affect the ameliorative effects of hardness on metal toxicity.

Some of the generic literature relevant to hardness and Cu and Cd toxicity is reviewed below.

2.4 Incorporating Metal Speciation into Guidelines

Markich et al, (2001) advocate including speciation into Australian guidelines if the acid soluble total metal concentration at a site exceeds the TV. ANZCEC (2000) follows[3] this recommendation by first adjusting the TV for the modifying effect of hardness at that location. Then, if the acid soluble total metal concentration is greater than the hardness-modified TV, the dissolved[4] metal concentration is measured, again comparing against the hardness-modified TV. If the dissolved metal concentration exceeds the hardness-modified TV, speciation models or direct species measurements may be used to further refine the estimated bioavailable fraction.

Of interest here is the adjustment of Cd, Cr Cu, Pb, Ni and Zn guidelines for hardness in freshwaters.

Table 9: Australian / New Zealand Metal Guideline Adjustments for Hardness

Metal	Formula
Cadmium	$HMGV = GV(H/30)^{0.89}$
Chromium(III)	$HMGV = GV(H/30)^{0.82}$
Copper	$HMGV = GV(H/30)^{0.85}$
Lead	$HMGV = GV(H/30)^{1.27}$
Nickel	$HMGV = GV(H/30)^{0.85}$
Zinc	$HMGV = GV(H/30)^{0.85}$

from: Markich et al, (2001)

HMGV- hardness modified guideline value (µg/L)
GV – guideline value (µg/L) at a hardness of 30 mg /L as $CaCO_3$

[3] It is odd that the recommendation date (2001) follows the acceptance date (2000).
[4] Dissolved metal is that which passes through a 0.45 µm (or smaller) filter. This is usually assumed to be the measurable surrogate for bioavailable metal, which really can only be modelled.

H – site-specific hardness (mg /L as $CaCO_3$)

The guideline values are adjusted to a hardness value of 30 although any reasonable value can be chosen. In the case where measured hardness at a site is 30 mg /L as $CaCO_3$, the HMGV = GV.

Markich et al, (2001) rationalize the exponent's range of 0.82 to 1.27 which is centered on 1, using the arguments of Meyer et al (1999). The exponents are derived using US EPA data (US EPA 1995a, b). The exponents represent the pooled slopes from the regression between ln(LC50) and ln(hardness) with hardness as (mg /L as $CaCO_3$) for fish and crustaceans. In the data sets used the hardness varied from 25 - 400 mg/L (as $CaCO_3$). Without the raw data it is unwise to use the equations in Table 9 much outside this range. However Markich et al, (2001) encourage readers to do this, with the (reasonable) justification that not adjusting the guideline value normalized at a hardness of 30 mg/L (as $CaCO_3$) will underprotect soft waters and overprotect hard waters.

2.4.1 Adjustment for Other TMFs

2.5 Adjustments for TMFs are described in Jurisdictional Review of Guideline Modification for TMFs

The TMF adjustments used by various jurisdictions are summarized in Table 2 through Table 6. Aside from hardness, the most prevalent TMFs are pH for some organic compounds, metalloids and metals, and temperature for ammonia, chlorine and cyanide.

Adjustments to WQGs for TMFs are made using step functions, for example pH for Al (CCME, 2006 and US EPA, 2006) or models, for example ammonia toxicity modified by pH and temperature (CCME, 2006).

The contaminant for which the most TMF adjustments are possible is Hg as advocated by US EPA (1985c and 2006). The Hg guideline may be modified by alkalinity, pH, DO, hardness, temperature, chloride, organic complexing agents, and sediment.

ANZECC (2002, section 8.3.5.17) provides the following generic recommendations for TMFs other than hardness:

- Conduct a literature review and if there are additional factors shown to affect toxicity and "if there are any quantitative relationships …
- … examine the original data and adjust each point to the critical parameter at the specific site then recalculate the trigger value using the original method; or

- if the range for the parameter relating to the original data is small, it may be easier to determine the average figure for that parameter and adjust the trigger value to suit the parameter at the site.

- Alternatively, more refined methods of measuring the bioavailable fraction of the toxicant may be employed, where available."

Note that although hardness is the only TMF for most metals in the US, US EPA (2006) states that although not explicitly adjusted for, the hardness adjustment does in fact address alkalinity due to the close relationship between hardness and alkalinity.

2.5.1 Summary

Australia/ New Zealand (ANZECC, 2002) and possibly the United States (US EPA, 2006) currently have the most sophisticated general paradigms in place to modify WQGs for the effects of TMFs. With respect to metals, these are largely[5] restricted to hardness adjustments with recommendations to modify the WQG quantitatively if numerical relationships between toxicity and TMFs exist. If no such relationships exist, the WQG can be modified qualitatively.

Direct adjustment for other metal TMFs is infrequent. The effect of OM is partially addressed through the use of filtered water samples. DOM that is not captured by filtration (at 45 µm) can still significantly mitigate toxicity. The effect of alkalinity is tacitly addressed through the relationship between alkalinity and hardness.

Given that the primary TMF currently being used is hardness, we make recommendations for improving the hardness adjustments for metal WQGs, below.

2.5.2 Recommendations for Hardness Adjustment

If hardness is selected as the only TMF to adjust acute WQGs in Canada we recommend that:

- the coefficients presented in Table 9 should be recalculated using updated data[6]; and

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[5] Some empirical step functions exist to modify metal toxicity as summarized in

1.1 Jurisdictional Review of Guideline Modifications for TMFs

Table 2.

[6] Colleagues across Canada suggested that the metal measurement techniques used prior to the mid 1980's may be inaccurate. The unknown degree of inaccuracy may affect the estimated hardness dependent algorithm coefficients. However given, the range of coefficients around the expected value of 1 (Borgmann, 1983 and Pagenkopf 1983) the degree of measurement error may not be significant in practical terms.

- the coverage of species should be expanded using available data or by generating data. The hardness-dependent algorithms currently being used were largely derived using metal toxicity data for fish and crustaceans. It is assumed that hardness-dependence is the same for all freshwater organisms. The need to generate group-specific hardness-dependent algorithms should be assessed.

A general relationship between hardness and chronic metal toxicity for aquatic organisms has not been defensibly demonstrated (discussed in section 3). An application factor is used to convert the short-term guideline values to long-term guideline values by US EPA (US EPA, 2006). The application factor approach may have to be adopted in Canada as well, despite the short-comings of application factors.

Hardness is almost always described as the sum of Ca and Mg ions. However, Welsh et al, (2000) and Naddy et al, (2002) describe the differential effects of these ions in mitigating toxicity. Therefore adjustment of a WQG using an integrative measurement such as "hardness" may not be the most scientifically valid approach. Pragmatically, hardness as usually defined, does significantly mitigate acute toxicity of metals and therefore adjustment of a WQG by hardness is reasonable, and an improvement over no adjustment. Refining a generic "hardness" adjustment of WQGs by addressing the individual ions responsible for hardness (Ca and Mg) will further increase the scientific validity of a site-specific WQG. Going a step further it may at some point be feasible to adjust WQGs for the suite of analytes which often co-vary with Ca and Mg in natural waters.

One potential concern regarding hardness adjustment brought forward by a reviewer[7] is that calcium carbonate can be used to neutralize acid mine drainage. Adjustment of a WQG based upon the high measured "hardness" could lead to an incorrect adjustment. A TMF adjustment paradigm that accounts for the individual ions might address this problem (if such a model existed). This concern can be addressed by requiring that WQG adjustments for TMFs should be made on the basis of receiving[8] water chemistry which sidesteps this particular problem.

[7] T. Fletcher (MOE).
[8] Care must be taken in characterizing receiving environments where an effluent stream comprises a significant proportion of the receiving environment (either standing volume, proportion of flow, etc.).

2.6 The Biotic Ligand Model

Morel, (1983) developed a general model describing the interaction between metals, metal species, metal complexes and constituents of the exposure water such as hardness and pH with binding to the site of toxic action. Pagenkopf (1983) looked at the effects of hardness, pH and complexation on the binding of specific metals on the gill surface. Morel's free ion activation model and Pagenkopf's gill surface interaction model form the bases for the biotic ligand model.

Briefly, the BLM assumes that toxicity of a metal is associated with a critical metal concentration at the site of toxic action (the biotic ligand) (MacRae et al, 1999 and Meyer, 1999). The LC50 represents the easily measured total or dissolved aqueous metal concentration associated with 50% mortality and varies with water quality. The LC50 is related to a constant per cent occupancy of the metal binding sites on the biotic ligand through the BLM. The BLM adjusts the measured aqueous concentration of a metal to account for speciation and binding to predict whether the "bioavailable" metal fraction will upon equilibrium with the biotic ligand, exceed the critical % occupancy of the binding sites. More sophisticated versions of the BLM allow for toxicity due to ME (generic metal) complexes rather than just the free ion (de Schamphelaere and Janssen, 2002 and Santore et al, 2001).

In order to "use" the biotic ligand model, the density of binding sites within the biotic ligand and the affinity of the metal for the binding site must be estimated (Playle et al, 2003a, b). Paquin et al (2002) provide a relatively recent review of methods for estimating binding site density.

The BLM makes several assumptions:

1) The BLM is a steady-state model and does not account for the time required for metals to reach equilibrium with ligand-Me complexes, other ME complexes and ME species.

2) Toxicity is associated with a certain proportion of binding sites on the gill being bound to ME. The implication of this is that toxicity (by this measure) is independent[9] of water quality. However, Paquin et al, (2002) state that Ca in addition to mitigating toxicity by competitive inhibition of ME binding, also serves to stabilize the gill. In this instance toxicity is not independent of water quality.

3) The nature of DOM-metal complexes is not well understood due to the vast number of types of DOM. At low metal concentrations where binding sites are unsaturated, simple BLMs relying upon on a single DOM-ME binding coefficient

[9] This statement, although made by various authors should be qualified. Some qualifications are that exposure temperature and traditional water quality parameters such as pH, are not affecting the organism.

(such as that used by McGeer et al, 2000) may provide unreliable predictions. This problem can be side-stepped if DOM fractions are identified and corrections to bioavailability (due to DOM) are made using models such as WHAM (Tipping, 1994).

One of the significant advantages of the BLM is that metal-water quality TMF interactions are species invariant (since metal – ligand binding coefficients are constants). The coefficients required to run these steady-state models already exist in software. See for example CHESS[10] – chemical equilibria in soils and solutions (Santore and Driscoll, 1995) and WHAM[11] - Windermere humic aqueous model (Tipping, 1994)).

2.6.1 The BLM and Chronic Exposures

The BLM has been developed in the context of acute toxicity. The authors do not believe it is currently possible to defensibly extend BLM-derived guidelines that account for TMFs, to chronic exposures for the following reasons:

- Niyogi and Wood (2003) have found that gill-binding constants for Cu, Cd and Zn vary with exposure history in the rainbow trout. Therefore any guidelines derived for these metals must consider the exposure history of any toxicity tests used.

- Hollis et al (2000), working specifically on chronic exposure of rainbow trout to Cd, concluded that "the present gill modelling approach (i.e. acute gill surface binding model or BLM) does work for soft and hard water exposures but there are complications when applying the model to fish chronically exposed to cadmium."

- de Schamphelaere and Janssen (2004a) state "it was demonstrated that the acute copper biotic ligand model (BLM) for D. *magna* could not serve as a reliable basis for predicting chronic copper toxicity".

- Janssen et al (2003) state: "…it should be recognized that most initial BLM efforts have concentrated on predicting short-term metal toxicity to fish. Continued research towards the development of similar models (1) for use with invertebrates and algae and (2) to predict long-term toxicity effects, is required to further explore the possibilities and limitations of incorporating these tools in environmental management schemes.

- In an experiment to assess dietary versus aqueous exposure of rainbow trout to Cd, Szebedinszky et al (2001) maintained the same gill-load of Cd using two different exposure routes; aqueous or dietary. The authors found that the critical

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[10] CHESS (Chemical Equilibrium of Species and Surfaces) is an aquatic speciation model that can be used to predict the degree of complexation of M^{n+} with inorganic ligands at equilibrium.
[11] WHAM is a model that combines an inorganic speciation model with the "Humic Ion-Binding model" to predict the degree of complexation of M^{n+} with organic ligands under the assumption of equilibrium.

BLM parameters (gill binding affinity and binding capacity) varied significantly with exposure route.

- Finally, Paquin et al, (2002) state that the sub-models implicit in the BLM (WHAM and CHESS) need further work to reliably predict metal speciation at chronic levels.

The authors suggest that the modification of WQGs by TMFs should at least be stratified using empirical relationships derived for each of chronic and acute exposures.

2.6.2 Validation of the BLM in the Receiving Environment

2.6.2.1 Acute Cu Toxicity to Daphnids

De Schamphelaere et al, 2002 assessed the predictive ability of the BLM to predict the 48h EC50 for Cu, to D. *magna*. The BLM was adjusted to account for the observed toxicity of $CuCO_3$ at pH >8. Using the adjusted BLM, the predicted 48h EC50 for Cu was within a factor of two for 25 artificial waters with varying levels of pH, Ca, Mg and Na and 19 natural European waters.

Villavicencio et al (2005) validated three versions (De Schamphelaere and Janssen. 2002, De Schamphelaere et al, 2002 and Hydroqual V2, 2002) of the acute BLM for Cu exposure to daphnids for natural and artificial Chilean waters. They found that the three models generally predicted toxicity to within a two-fold range around observed toxicity.

They also compared BLM derived WQG with derivations based upon observed toxicity to a native daphnid and the US EPA hardness-adjusted value (US EPA 1985b, 1996, 2006) for Cu. They found good agreement between the BLM derived WQG and WQGs based upon observed toxicity. The US EPA hardness-adjusted values were similar in low DOC water but were conservative in high DOC water.

This latter experiment points out the utility of adjusting by multiple TMFs when such effects are quantifiable. Doubtless other validation experiments will confirm this observation.

2.6.2.2 Acute Cd Toxicity to Daphnids

Brooks et al (2004) performed experiments in mesocosms to assess the efficacy of WQGs for effluent-dominated streams. They state that the BLM used supports the establishment of site-specific WQGs for acute exposure to Cd but not for chronic exposure.

2.6.2.3 Chronic Cu Toxicity to Daphnids

De Schamphelaere and Janssen (2004a) developed a chronic Cu BLM for D. *magna* as the acute Cu BLM did not reliably predict Cu toxicity. Differences in the two models are differences in binding coefficients, the insignificance of Ca, Mg or combined effects on toxicity[12], increased bioavailability of Cu-hydroxide, Cu-carbonate complexes, an increase in proton competition, etc. Seventy nine per cent of chronic Cu BLM predicted 21-day EC50s were within a factor of two, from natural observed values.

2.6.2.4 Zn Toxicity to Algae, Crustaceans and Fish

De Schamphelaere et al (2005b) assessed the utility of the BLM to describe Zn toxicity to D. *magna*, *O. mykiss* and *Pseudokirchneriella subcapitata*. They used 8 natural waters spiked with Zn to estimate the EC10 for growth of P. *subcapitata* following 72-hour exposure, reproduction of D. *magna* following 21-day exposure, immobility of D. *magna* following 48-h exposure, and growth and mortality of *O. mykiss* following 30-day exposure. (Only mortality was used for *O. mykiss* since it was much more sensitive than growth). They found the models predicted toxicity within a factor of two of observed toxicity; however chronic toxicity to D. *magna* was under-predicted by a factor of 3-4 in water with pH = 8. They developed ranges of pH and Ca over which they state the models are valid.

2.6.3 Using the BLM to Generate WQGs

The BLM combines powerful geochemical speciation models with recent developments in physiology and toxicology to predict toxicity as a function of water chemistry and exposure to a single metal. Because of its mechanistic basis, Meyer et al (1999) suggest that the BLM may be an important regulatory tool. US EPA (2003) concurs stating that the BLM addresses criticisms that US metal criteria incorporate only hardness and not other TMFs.

[12] Interestingly this parallels the findings of Lewis and Porter (1990) with respect to the lack of effects of hardness on chronic toxicity to cladocerans.

The BLM does address the effects of multiple TMFs on metal toxicity in a mechanistic manner. Its implementation in generating WQGs may be premature at the current time for the following reasons:

1) The application of the BLM requires accurate estimation of gill binding constants. Niyogi and Wood (2003) go so far as to say that estimation of these constants is "critical to the successful application of the BLM approach in fish". The critical gill binding constants have been shown to vary with exposure history in the rainbow trout at least (Niyogi and Wood, 2003) and also with hardness acclimation and prior dietary composition Franklin[13] et al, (2005). WQGs generated using the BLM will be subject to the criticism that the sensitivity of the BLM toxicity predictions (through their effects on gill binding constants) to factors such as dietary and acclimation history has not been assessed.

2) The application of the BLM to taxa other than fish is relatively rare. De Schamphelaere et al, (2005a) found that surficially bound Cu and internal Cu were better predictors of growth rate in long-term (48 and 72h) exposures to two algal species than free Cu^{2+}. They state that this "observation is the first step toward considering the use of the cell surface as the algal biotic ligand for Cu in a similar way as fish gills fulfill this role in the biotic ligand model for predicting metal toxicity to fish species."

3) The generic BLM may not be applicable to non-fish species. Borgmann et al (2005) found that a two-binding site model is required to describe the effects of pH on *H. azteca* using the BLM. They speculate that the same two-binding site model (with different coefficients) may be applicable to daphnids and fish but further work is required to evaluate cationic effects at varying pHs. Also, Hassler et al, (2004) found that competitive interactions with Ca and Pb uptake were not predicted by the BLM for *Chlorella kesslerii*. This observation may not be germane as the SSDs for acute WQGs at least, will not include algae CCME (2006).

   If (aside from species/taxa-specific binding coefficients) different functional forms of the BLM are required to deal with the effects of TMFs such as pH, should the BLM be considered as a generic TMF-adjustment model?

4) The BLM does not address the effects of temperature. Hassler et al, (2004) found that a change of 17° C resulted in a 2-5-fold decrease in the permeability of the membranes of *Chlorella kesslerii* to Pb and Zn. Temperature is an acknowledged TMF for ammonia (CCME, 2000, US EPA, 1998, 2006), cyanide (US EPA, 1995e), chlorine (US EPA, 1985a) and mercury (US EPA, 2006, 1985c). We are not aware of any studies on the effect of temperature on BLM predictions for other taxa.

[13] One startling observation regarding this statement is that the dietary exposure was more than 15,000 times greater than the aqueous exposure. It is possible that this discrepancy in exposure reasons affects their conclusion.

5) The physiological portion of the BLM is based upon the mechanisms of acute metal toxicity. Researchers have begun to extend the BLM to chronic exposures but the authors do not believe it is currently possible to defensibly derive chronic WQGs using BLM-adjusted toxicity test results. Reasons for this statement are discussed in section 2.6.1 and summarized below.

   a) Critical gill-binding constants for Cu, Cd and Zn vary with exposure history in the rainbow trout at least. Therefore any guidelines derived for these metals must consider the exposure history of any toxicity test results used.

   b) Authors have shown that the BLM does not apply to fish chronically exposed to Cd (Hollis et al, 2000) nor daphnids chronically exposed to Cu (de Schamphelaere and Janssen, 2004a). This may be due to extension of the geochemical sub-models to metal levels lower than those with which they were calibrated (Paquin et al, 2002) or the requirement for more sophisticated BLMs (De Schamphelaere and Janssen, 2004a and Borgmann et al, 2005)

6) Validation experiments in the natural receiving environment are limited. De Schamphelaere et al (2005b) validate the BLM for mortality (an acute response) in rainbow trout over a restricted range of pH (5-8) and Ca (5-160 mg/l).

7) There is currently insufficient information to apply the BLM to the myriad species within an SSD (Janssen et al, 2003). However US EPA (2003) has attempted to generate a WQG for Cu using the BLM approach but this document has remained in a draft form for the last three years[14].

Finally, some authors have explicitly stated that the BLM requires further research work prior to being used for chronic exposures.

- Janssen et al (2003) state: "…it should be recognized that most initial BLM efforts have concentrated on predicting short-term metal toxicity to fish. Continued research towards the development of similar models (1) for use with invertebrates and algae and (2) to predict long-term toxicity effects, is required to further explore the possibilities and limitations of incorporating these tools in environmental management schemes.

- US EPA (2003) states: "While the BLM is currently considered appropriate for use to derive an updated freshwater CMC, further development is required before it will be suitable for use to evaluate a saltwater CMC or a CCC or chronic value."

[14] The draft EPA Cu fresh and saltwater criteria document was released in 2003. This draft used the BLM to adjust the acute freshwater Cu value and for "combined effects of various water quality variables (e.g., pH and alkalinity)". Review comments were solicited for one year and then extended by one year. EPA took one year to respond to comments. Release of the document is expected in January of 2007. All comments on timelines were provided by L. Cruz, US EPA pers. comm. Nov. 28, 2006)

One reviewer (P. Welsh, MOE) suggested that the BLM could be used to adjust acute toxicity test results for TMFs. Using the rule-of-thumb from validation studies that the BLM works well if it predicts observed toxicity within a factor of 2 a safety factor of 0.5 could be used to ensure that the BLM does not under-predict toxicity. This suggestion only applies to those species/exposures that have been modeled with the BLM.

3 Thoughts on Extrapolating the Effect of Hardness based on Acute Results to Chronic Results

This section explores how TMFs might be utilized in general and specifically, to standardize WQGs. Hardness and Cu and possibly Cd were selected as TMFs and test substances, respectively by the contractor and project authority. The following section expands upon the relationships between hardness and Cu (or Cd) mentioned in Table 2 through Table 6 and following a literature review.

3.1 Empirical Results for Hardness Adjustments

3.1.1 Lewis and Porter on Cd Toxicity

Lewis and Porter (1990) investigated the potential relationship between hardness and Cd toxicity for a variety of data sets partitioned by source of data, acute versus chronic exposure and organism type. Their results are summarized below.

The “significance” of the result denotes statistical significance using the reported p-values for the Pearson product moment correlation coefficient at a 5% level of significance. Lewis and Porter (1999) seem to use the word “acute” to describe mortality based on the association between the word “acute” and LCx. The word “chronic” is not defined but occasionally endpoints are reported as “various”, reproductive effects or LOECs. The time associated with “chronic” studies is not reported.

Table 10: Summary of Relationships between Cd Toxicity Test Endpoints and Hardness - Lewis and Porter (1990)

Organism	Exposure Type	Data Set	Pearson rho	P-value	Relationship?
salmonid	acute	48h LC50	0.830	0.0819	n
		96h LC50	0.7573	0.0003	y
		combined			y?
	chronic	data set from P. Outridge	-0.0728	0.760	n
		data set from WHO, MOE, EPA and P. Outridge	NA	NA	n
		data set from J. Sprague	0.8411	0.0001	y[15]
non-salmonids	acute	24h LC50	0.950	0.0001	y
		48h LC50	0.628	0.0122	y
		96h LC50	0.243	0.288	n
		combined	0.449	0.0015	y
	chronic	data set from P. Outridge	0.429	0.126	n
		data set from WHO, MOE, EPA and P. Outridge	NA	NA	n[16]
invertebrates excluding insects and *Tubifex tubifex*	acute	24h LC50	0.544	0.0196	y
		48h LC50	0.382	0.0281	y
		96h LC50	0.755	0.0000	y
all invertebrate data – Figure 6 seems to represent a subset of data used to generate Figure 7. Conclusions are the same across each set of figures.		24h LC50	0.540	0.0114	y
		48h LC50	0.174	0.170	n
		72h LC50	-0.552	0.156	n[17]
		96h LC50	0.534	0.000	y[18]
invertebrates excluding insects	chronic	P. Outridge data excluding insects (various endpoints)	0.261	0.267	n
		all data from WHO, MOE and P. Outridge (various endpoints)	-0.134	0.345	n
		as above plus EPA data (various endpoints)	0.146	0.252	n
insects	acute	24h LC50	0.461	0.0626	n[19]
		48h LC50	-0.3992	0.126	n
cladocera	acute	24h LC50	0.871	0.0238	y
		48h LC50 data from P. Outridge	0.815	0.0001	y
		48h LC50 data from WHO, MOE and P. Outridge	0.514	0.0007	y
		48h LC50 data from WHO, MOE, EPA and P.	NA	NA	y?

[15] Lewis and Porter argue that relationship is spurious
[16] fitted equation seems incorrect as it does not describe the data well (B. Zajdlik)
[17] inverse relationship between mortality and hardness that is not statistically significant
[18] significance likely an artifact of sample size given very poor predictability (B. Zajdlik)
[19] only marginally “insignificant”

Organism	Exposure Type	Data Set	Pearson rho	P-value	Relationship?
		Outridge			
		96h LC50 data from WHO, MOE, EPA and P. Outridge	-0.432	0.334	n
	chronic	data from WHO, MOE and P. Outridge (various endpoints)	-0.0094	0.956	n
		data from WHO, MOE, EPA and P. Outridge (various endpoints)	0.170	0.301	n
		chronic impairment to reproduction, data from WHO, MOE, EPA and P. Outridge	0.363	0.247	n

The results in Table 10 suggest that the strength of the relationship between hardness and toxicity decreases as the duration of exposure increases for fish.

With respect to invertebrates (excluding insects), the number of significant relationships between hardness and toxicity test endpoints is markedly higher for "acute" exposures relative to "chronic" exposures. Investigations of cladocera separately, lead to the same conclusions.

In summary, Lewis and Porter (1990) were unable to establish a relationship between chronic Cd toxicity and hardness for cladocerans but were able to establish such a relationship for acute toxicity. This observation is generally correct for salmonids and invertebrates (excluding insects) and is always correct for non-salmonid fishes within this compilation of data.

3.1.2 US EPA on Cd Toxicity

US EPA (2001) reviewed the effect of hardness on Cd chronic toxicity. Data in (their) table 2a were screened to create a data set such that the effect of hardness on toxicity test endpoints could be assessed. This screening produced data for three species; 4 observations for D. *magna*, 2 for fathead minnow and 2 for brown trout. The data for D. *magna* were so divergent that one observation was removed leaving a D. *magna* data set generated by a single author. The very small data sets (two observations to fit a regression model for each of two species) were re-fit by the author[20].

The final model adopted by US EPA removed one aberrant D. *magna* observation because the D. *magna* data set was otherwise "too divergent". The resulting D. *magna* data set used was generated by a single author. The fitted model possibly suffers from

[20] In fitting the data we noted that the slope expressed by US EPA used two different units for hardness (mg/l) and Cu (µg/l). Users should be aware of this odd discrepancy.

some unduly influential observations (which is not surprising given the very small size of the data set).

The interpretation should be used cautiously due to the small data set representing limited species (two fish species with two observations each and one invertebrate species with three observations generated by a single author and no plants or amphibians) and the deletion of one observation that did not fit the expected trend.

3.1.3 US EPA on Cu Toxicity

The derivation of a hardness adjustment for chronic Cu toxicity in the US goes back to the 1985 Cu ambient water quality criterion document (US EPA, 1985b). In that document, US EPA (1985b) states: "The available information concerning the effect of hardness on the chronic toxicity of Cu is inconclusive." However, the authors go on to choose (not estimate) parameters for hardness adjustment in the chronic case, for pragmatic reasons. US EPA (1985b, pg. 13) states "The combination of a chronic intercept of -1.465 and a chronic slope of 0.8545 provides the lowest chronic slope that will keep the Final Chronic Value below the Criterion Maximum Concentration down to a hardness of 1 mg/L".

The water quality criteria for Cu were revisited in 2003 (US EPA, 2003). This documents states (US EPA, 2003, pg. 7) that while the BLM may be used to derive an acute Cu criterion in freshwater it is not currently considered suitable for establishing a freshwater chronic criterion. The authors investigate the relationship between chronic Cu toxicity and hardness and conclude that the relationship is equivocal (section 5.3.2). In the end, US EPA uses acute-chronic Cu toxicity ratios to adjust the acute value to obtain a chronic Cu WQG.

3.1.4 Rainbow Trout Growth and Cu Toxicity

Hansen et al, (2002a) compared studies on the combined effects of Cu and hardness on rainbow trout growth with studies on the combined effects of Cu and hardness on rainbow trout survival. They concluded that the effect of hardness was greater on growth than survival based upon the size of the slope parameter when regressing growth (expressed as an IC20) or survival (expressed as an LC50) on water hardness. The coefficients are respectively 1.423 and 0.831. Unfortunately the standard errors of these parameters are not presented and therefore a test of the equality of these two slopes cannot be conducted without obtaining and refitting the raw data.

Are the coefficients substantively different? Possibly. In any case it is clear that hardness does mitigate the effects of Cu on growth in rainbow trout based upon the three studies reviewed.

3.1.5 The BLM and Chronic Cu Toxicity to Daphnids

De Schamphelaere and Janssen (2004a) developed a chronic Cu BLM for D. *magna* and found that neither Ca, Mg separately or together affected toxicity. In a parallel study investigating the joint effects of DOC concentration (ranged from 1.6-18.4 mg/l) and pH (ranged from 5.3 – 8.7) had a significant effect on copper toxicity but hardness (ranged from 25-500 mg/l) did not (De Schamphelaere and Janssen, 2004c).

3.2 Conclusions

CCME intends to provide both short and long-term WQGs. “Short-term exposure guidelines are meant to estimate severe effects and to protect most species against lethality during in transient events (e.g., spill events to aquatic receiving environments, or infrequent releases of short-lived / non-persistent substances. Long-term exposure guidelines are meant to protect against all negative effects during indefinite exposures.” CCME (2006).

It is the opinion[21] of B. Zajdlik and G. Craig that the effect of hardness in modifying the Cu and Cd toxicity has not been sufficiently demonstrated for long term exposures[22]. This opinion is also that of Crommentuijn et al (1997), who view the US EPA (1985) approach to using hardness as a modifying factor for Cu questionable because the hardness-toxicity relationship is established on the basis of acute toxicity test results using relatively high metal concentrations.

They also state that:

a. most experiments on hardness as a modifying factor of toxicity dilute naturally hard waters using distilled or de-ionized water. The effect of the dilution reduces hardness but also simultaneously affects alkalinity, conductivity and pH[23]. They conclude these effects (which affect bioavailability and uptake) confound the conclusion that hardness is responsible for the observed modifications in acute toxicity.

b. the relationship between hardness and chronic toxicity is not strong and is sometimes the opposite of the expected relationship.

Because of these reservations, no modification of guidelines based upon hardness is made in the Netherlands.

[21] A considerable amount of effort was expended to come to this conclusion which overturned our prior “understanding” that the effect of at least one TMF, hardness was well documented, understood and generally applicable.
[22] Paquin et al, (2002) state that Ca can stabilize the gill structure.
[23] US EPA (1985b) also states “In most natural waters, alkalinity and pH increase with water hardness and the relative influence of these parameters on toxicity is not easily determined.”

Based upon the discussion in section 3.1.1 through 3.1.5 we recommend that:

1. the effect of hardness as a TMF for chronic toxicity tests be investigated in detail. The preliminary review conducted here cannot be viewed as the final word on the effect of hardness on chronic aquatic toxicity of Cd or Cu.

   Consideration should be given to the differential effects of Ca and Mg (see for example Welsh et al, 2000) and the effect of other analytes that may co-vary with Ca and Mg in the receiving waters of interest. The investigation should address the joint action of other TMFs such as humic acid, and should include a range of species including fish, invertebrates (more than one taxon) and possibly plants.

   It may be reasonable to assemble a database of hardness and chronic Cu/Cd toxicity results (particularly Lewis and Porter, 1990) reassess earlier study results using more sophisticated statistical tools.

2. the investigation should attempt to at least discretize the effect of "hardness" on chronic toxicity to create a WQG adjustment based on "high" and "low" hardness;

3. at the present time, hardness as a TMF should only be applied to short-term exposures.

4 TMF Adjustment Paradigms

4.1.1 Effect of TMF Adjustment Paradigms on WQGs

Gravenmier et al (2005) assessed the sensitivity of threespine sticklebacks, (*Gasterosteus aculeatus*) to Cu, relative to other acute aquatic animal toxicity test results. Prior to assessing the relative sensitivities all toxicity test endpoints were standardized. Two paradigms were used to standardize toxicity test endpoints.

The first standardization paradigm is the US EPA (1985) standardization for hardness only. The second standardization paradigm uses the BLM as described by US EPA (2003). In both cases missing observations were assigned values as described in Gravenmier et al (2005).

54 toxicity test results were standardized following US EPA (1985). Due to the increased data requirements only 39 observations could be defensibly standardized using the BLM. The author's goal was to compare the sensitivity of threespine sticklebacks to Cu, relative to other acute aquatic animal toxicity test results and not to compare the effects of TMF adjustment paradigms. The relative sensitivities for hardness adjustment and BLM-adjustment are 39.6 and 15.8%, respectively.

US. EPA (2003) also compared the US EPA (1985) hardness adjustment toxicity predictions with those produced by the BLM. Given that the two datasets are different, the dataset used for hardness adjustment had a restricted range of DOC, SMAVs (species mean acute values) were used (reducing within species variability) and many missing observations for the BLM-adjusted data set at least were censored and therefore infilled, the two methods produced virtually identical residual mean square errors (unexplained variability) when regressing predicted versus observed toxicity test values. The authors conclude that the BLM model is a slightly better predictor than the hardness model, but this conclusion must be interpreted in terms of the number of parameters used to achieve this improvement and the percent of total variability explained. A more defensible conclusion is that two adjustment methods explained observed results equally well[24], given the caveats mentioned above.

This leaves CCME with the following outstanding questions:

- Does the theoretical improvement in prediction of toxicity test results using the BLM relative to the simpler hardness adjustment translate into observable differences in predicted toxicity in general?

[24] This observation may change as researches begin to generate datasets that contain the variables necessary to fully utilize the BLM.

- What degree of change in predicted toxicity models will cause a significant change in an SSD derived WQG?

The answers to these two questions can be used to address the following question:

- Is the expense of making BLM adjustments (both in terms of deriving WQGs and to the end user in the form of water quality characterization) relative to hardness adjustments of toxicity test results prior to estimating a WQG warranted?

4.2 Pragmatic TMF Adjustment Paradigms for SSDs

Adjustment of toxicity test values for TMFs allows for the derivation of site-specific WQGs. There are a variety of opportunities within the WQG estimation paradigm to make these adjustments. The following schematic discusses the advantages / disadvantages, and assumption of adjustments at various points within the WQG derivation paradigm[25]. These approaches are described as 'pragmatic" as there is no real link between estimation of the SSD parameters and the adjustment of toxicity test results.

Our original intent when beginning this project was to allow the parameters of the SSD to vary as functions of TMFs. The general theory to do this was developed but not implemented due to the unavailability of complete data sets. It is still possible to implement this method for hardness and acute toxicity. This was not done as pragmatic adjustments for hardness already exist but primarily because regulatory agencies are moving away from adjustment of WQGs by one TMF alone.

[25] Note that the advantages / disadvantages do not include those of the SSD approach relative to other approaches and assumptions do not include the general SSD assumptions.

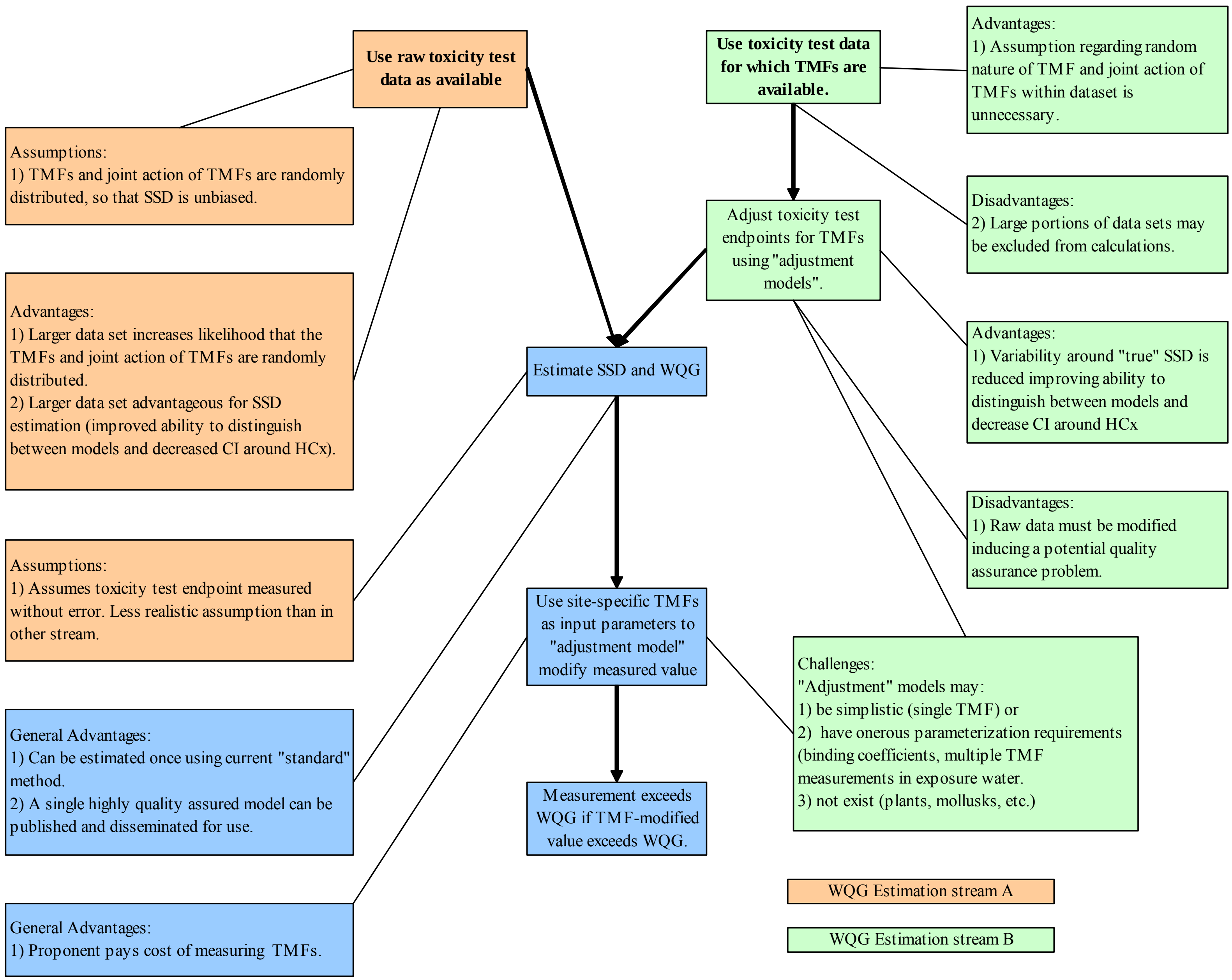

Figure 1: General TMF Adjustment Paradigms

4.2.1.1 WQG Estimation "Stream A" Pros, Cons and Assumptions

WQG Estimation stream A is currently used by ANZECC (2002) and OECD (1995). This approach is pragmatic, using all available data (that meets respective quality assurance requirements) to estimate the parameters of the SSD. The key assumption with respect to TMFs (not including those required to validly use an SSD which include proportional representation of sensitive species, species representative of the ecosystem(s) to which the estimated WQG will be applied, etc.) using this approach is that the distribution of the TMFs is independent of the sensitivity of the organisms tested.

If the distribution of the TMFs varies systematically with sensitivity then the estimated WQG will be biased. To see why this is so consider one potential dependence (cynically attributable to the requirement to publish "positive" results):

Researchers looking for sensitive toxicity test species test species under water quality conditions likely to enhance the sensitivity of the species in question. These test results become part of a toxicity test database that is used to generate an SSD. The lower tail of the SSD is biased downward. The net effect of this bias may be to: 1) bias the parameter estimates and 2) inflate the residual variance term. The effect of the first bias is likely to reduce the estimated HC_5. The result of inflating the residual variance term will be to increase the width of the lower confidence limit, thus biasing the WQG downward.

Table 11: Summary of Advantages and Disadvantages of TMF Adjustment Paradigm "A".

Advantages	Larger data set can increase the likelihood that the joint action of TMFs and TMFs are randomly distributed. This reduces the potential for a biased SSD.
	A larger SSD dataset improves the ability to distinguish between models in the critical lower tail. Larger data sets (reflecting increased knowledge) reduce the size of the confidence interval around the HC_x and consequently allow a "data-supported" increase in a WQG. This is a desirable attribute for any WQG estimation paradigm; reduced uncertainty allows a WQG to creep up to some asymptote reflecting the "correct" WQG (at least as far as the underlying theory is correct).
	In this approach the SSD, HC_x and associated WQG need only be estimated once (or as new data are produced). This allows for a central body to produce a single highly quality-assured model. This advantage is shared by adjustment paradigm "B".
	The cost associated with making site-specific modifications to create a site-specific value is borne by the proponent. This advantage is shared by adjustment paradigm "B".
Assumption / Disadvantage	Assumes that TMFs and the joint action of TMFs are randomly distributed. This reduces the potential for a biased SSD.
	Stream A assumes that the toxicity test endpoint is measured without error. This assumption is incorrect. Toxicity test databases should be compiled with this information required. The effect of ignoring this information is that WQGs will be higher than they should be. Pragmatically, the effect of not acknowledging variability in toxicity test endpoints when estimating WQGs is not likely large.

4.2.1.2 WQG Estimation "Stream B" Pros, Cons and Assumptions

WQG Estimation stream B was attempted by US EPA (2003) using the BLM as the "adjustment" model. However this effort was blocked at the beginning of stream B since data sets containing all the parameters required by the BLM version used were not available. US EPA (2003) used various methods to acquire these missing values as described in their Appendix D (Estimation of Water Chemistry Parameters for Acute Copper Toxicity Tests).

Table 12: Summary of Advantages and Disadvantages of TMF Adjustment Paradigm "B".

Advantages	Does not assume that TMFs and the joint action of TMFs are randomly distributed. This reduces the potential for a biased SSD.
	By standardizing toxicity test endpoints prior to estimating the SSD one source of variability is removed (in as much as the adjustment is "correct"). This will result in a more precise estimate of the HC_5 and an associated higher WQG reflecting the increased precision.
	In this approach the SSD, HC_x and associated WQG need only be estimated once (or as new data are produced). This allows for a central body to produce a single highly quality assured model. This advantage is shared by adjustment paradigm "A".
	The cost associated with making site-specific modifications to create a site-specific value is borne by the proponent. This advantage is shared by adjustment paradigm "A".
Assumption / Disadvantage	The unavailability of the parameters necessary to make adjustments to a toxicity test endpoint may require infilling or omitting such data. Any guidelines based upon adjusted data using infilled TMFs will be subject to criticism.
	The raw data must be modified introducing a potential quality assurance problem.
	A very real problem is that suitable models may not exist for adjusting each observation in the SSD. This is particularly true for plants and mollusks.

4.2.2 Dealing with Missing Data

The problem of missing data applies to those TMFs required to adjust a toxicity test endpoint to account for the bioavailability of the test substance under the exposure conditions.

The statistical assessment of missing data begins with a partially complete data set. The partial information is used to impute the missing information. In order to assess the effects of missing TMFs on BLM adjustments prior to estimating WQGs (stream 2 in Figure 1 we compiled an acute Cu data set as described in (Appendix 2: Compilation of Cu-TMF Database). After screening the data, the number of records complete with hardness, alkalinity, some sort of organic carbon measurement (with units so that conversions can be made), and a measured pH is 59. These records still require DIC, Cl, Na, K and some assumption regarding % humic acid composition before they may be used to adjust Cu toxicity using the BLM.

US EPA (2003) also attempted to compile a Cu database that included those TMFs required for the BLM. We contacted US EPA to obtain the raw data used in the draft document. Unfortunately this dataset did not contain any complete[26] datasets despite the considerable efforts expended.

[26] The conclusion that all observations are missing some data, is based upon reading the footnotes associated with each entry in appendix F of US EPA (2003). A re-read of the document implies that some of the datasets may have received a quality ranking of "1" indicating a complete dataset (from the perspective of BLM adjustment). A query has been placed with R. Santore of Hydroqual Inc. requesting clarification.

5 Summary

Section 1 describes which TMFs are used by various jurisdictions and how each TMF is used. The majority of TMFs used pertain to metals and the most common TMF used to adjust WQGs is hardness. Hardness is used to modify toxicity using exponential functions in Australia, New Zealand and the United States. Canada, the United Kingdom and the European Union use step functions.

Hardness along with Cu and Cd were selected to assess the effect of TMF modification on the derivation of WQGs. One unexpected outcome of reviewing the literature is that in our opinion, the validity of using hardness as a TMF for adjusting chronic WQGs is doubtful. Despite this, some jurisdictions do use hardness to modify chronic WGS while at the same time acknowledging doubts about doing so. Authors representing other jurisdictions state that the state of knowledge is insufficient to modify chronic metal toxicity WQGs using hardness.

No jurisdiction currently uses more than one TMF at a time to adjust WQGs for metals using models. Only Al WQGs (Canada and United States) and Hg (United States) acknowledge more than one TMF for metal toxicity, simultaneously. WQGs for these two metals do not use models to adjust WQGs by their respective TMFs.
The BLM is being explored for incorporating multiple TMFs for acute Cu toxicity in the United States (US EPA, 2003). It is expected that the new paradigm for creating site-specific WQGs will be promulgated in January of 2007 (L. Cruz, US EPA pers. comm.). At the present time the BLM TMF adjustment paradigm for Cu will not apply to chronic Cu toxicity.

Currently no models that consider multiple TMFs are used to adjust WQGs although the BLM will be used to adjust acute Cu WQGs imminently. This finding was surprising to the authors and discouraging in the context of this project: to statistically evaluate potential standardization models for Canadian WQGs.

Considerable effort was expended to the questions posed using the BLM, but these efforts were forestalled by the lack of data sets with a complete set of TMFs over a sufficiently broad taxonomic base to generate a WQG. The next section provides recommendations on how to proceed with the evaluation of potential standardization models for Canadian WQGs.

6 Recommendations

Despite the fact that most of the contract deliverables have been fulfilled by this document, one task could not be completed: assessing the effect of missing data on the derived WQG (or the effect of standardizing only portions of a dataset). During conversations with U. Schneider the idea was raised that a demonstration of the insensitivity of a WQG to TMF adjustment would address the concerns underlying the uncompleted task namely; are the guidelines underprotective if the known effects of TMFs are not acknowledged? The following recommendations address this important concern.

Estimate WQGs following the SSD approach (CCME, 2006) using data from US (EPA, 2003) and / or Gravenmier et al (2005). This will allow an empirical assessment of how a WQG is affected by adjusting for one TMF (hardness) relative to multiple TMFs (those used by the BLM) although it still does not address the effect of missing[27] observations.

One limitation of this comparison is that different data sets are used. This limitation can be side-stepped by using the BLM dataset only, but standardizing only by hardness. The WQG derived from this standardization could be compared directly to the WQG derived from the BLM-adjusted data set.

Missing data may be dealt with as follows:

1) Use typical water matrices to infill the missing observations. For example, due to the relationship between pH, hardness and alkalinity, high pH values are not generally associated with soft water. Therefore if for example a pH measurement was missing from a toxicity test dataset and the water was ‘soft” a low pH measurement might be reasonably assumed. This approach was used by US EPA, (2003) when updating water quality criteria for Cu and by Gravenmier et al, (2005) when comparing sensitivity of fish taxa to Cu.

   This option will artificially reduce the variability of the adjusted dataset and by extension artificially increase the LCL of the HC_5 making the estimated WQG underprotective at the expected level.

2) Use historical water characteristics of the exposure water to infill missing observations.

   This approach is practical when a toxicity test is conducted within an established testing facility. Such facilities are required to monitor at least dilution water characteristics. Given the desire to cooperate, laboratory personnel should be able

[27] However see footnote 26.

to provide the missing measurements obtained within the approximate (if not the exact) time frame of the toxicity test result in question.

In our opinion, this option will likely produce very little bias in the missing observation and ultimately little bias in the estimated WQG.

If the toxicity test result in question was generated by a research facility, such detailed records are not required and either the investigator or the data in question may not be available. In this case, the dilution water used or likely to have been used may be measured, making the assumption that the water quality has remained unchanged since the toxicity test was conducted. In a broad geographical sense this approach is sensible since the underlying water characteristics within a municipal water supply will be less variable than the same characteristic over a larger geographic area.

In our opinion, given other sources of variability, this option will produce moderate bias in the missing observation and ultimately a slight downward (conservative) bias in the estimated WQG.

3) Use the worst-case scenario to infill missing observations.

   This option will bias all adjusted toxicity test values downward and have unknown effects on the variability around the SSD. The effects on the variability around the SSD are a function of: 1) what observations are missing data, 2) how many observations are missing data and 3) the position of the observation so affected relative to the true SSD. The effect of this bias is not generalizable.

4) Use the distribution of TMFs in the receiving environment via a Monte Carlo simulation to estimate the "most probable TMF adjusted WQG".

5) Stratify the distribution of TMFs in the receiving environment on a regional basis via a Monte Carlo simulation to estimate the "regionally most probable TMF adjusted WQG". The region may be defined by geopolitical boundaries or by more relevant boundaries such as watershed, geophysiography, etc.

6) Empirically assess the effects of a TMF on WQGs estimated using SSD approach as follows:
   a. Estimate the range in sensitivities of several species for a TMF such as hardness. The species should range from sensitive to insensitive.
   b. Construct an SSD and add a third axis representing a TMF to visually assess the effect of the TMF.
   c. Compare the within-species range to the among-species range visually and/or numerically. A small within-species range relative to the among-species range empirically rationalizes the use of an SSD unstandardized for TMFs.

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Appendix 1: Review of Recent Studies on TMFs and Metal Toxicity

The following table indicates whether a TMF/chemical/organism combination has been studied. One or more studies are indicated by a "*".

Table 13: Studies on TMFs and Metal Toxicity

Chemical	Organism	Acute / Chronic	Hardness	Ca^+	Mg^+	Na^+	K^+	H^+	DOC / DOM	Other	Citation	Comments
Cd	freshwater bivalve, *Hyridella depressa*	acute								Cd2+ only; not affected by hardness, H+, DOC	Markich et al 2003	Measured 48 hr valve movement (duration of valve opening) pH, hardness and DOC were NOT dependant variables regarding Cu effect concentrations.
Cd	FW algae	acute							*		Vigneault and Campbell 2005	Uptake of Cd affected by organic acids through complexation with the free metal ion
Cd	trout	chronic								Ca in diet	Franklin et al 2005	Dietary Ca+ supplement reduced Cd tissue accumulation.
Cd	trout	chronic	*							Cd pre-exposure	Hollis et al, 2000	30 day exposure; tissue accumulation measured
Cd	trout	chronic								dietary vs. water exposure	Szebedinszky et al, 2001	Dietary tissue accumulation more effective via food than water.
Cd	trout / perch	acute	*	*							Niyogi et al, 2004	Gill accumulations between the two species at LC50 exposures were much higher for perch than trout. Metal and hardness pre-exposure also affected Cd gill accumulation

Chemical	Organism	Acute / Chronic	Hardness	Ca^+	Mg^+	Na^+	K^+	H^+	DOC / DOM	Other	Citation	Comments
Cd	various fish & invertbrates	acute	*	*				*			Meyer, 1999	Synthesis of EPA Criteria data for divalent metals leading to the conclusion the amount of transition metal bound to the fish gill at the LC50 concentration is constant.
Cu	D. *magna*	chronic		*	*	*		*	*		de Schamphelaere and Janssen, 2004a	21day EC50s for reproduction; bioaccumulation; different dissolved organic materials have different effects on copper toxicity
Cu	D. magna, D. *pulex*, D. *obtusa*	acute	*						*		Villavicencio et al, 2005, de Schamphelaere et al, 2004, Santore et al, 2001	Citation 1) 48 hr LC50s Citation 2) The binding coefficients between Cu and DOM from 13 locations varied by a factor of 6. 48h EC50s for Cu toxicity to D. *magna* were within a factor of 2 for 90% of predictions. By accounting for the difference in DOM-binding affinities, 90% of predictions were within a factor of 1.3 of observed EC50s. Citation 3) general BLM
Cu	fathead minnow	acute		*				*	*		Welsh et al, (1996)	

Chemical	Organism	Acute / Chronic	Hardness	Ca^+	Mg^+	Na^+	K^+	H^+	DOC / DOM	Other	Citation	Comments
Cu	fathead minnow	acute	*	*				*			Meyer et al, 1999	The concentration of a transition metal bound to fish gills ([Mgill]) is predicted to be a constant predictor of mortality. DOC was not an included variable but considered an important competitor for metal binding sites in estimating acute toxicity.
Cu	fathead minnow	acute							*		Ryan et al, (2004), Santore et al, 2001	Citation 2) general BLM
Cu	fathead minnow	acute - chronic	*			*		*	*	TSS	Erickson et al, 1996	24 and 96hr LC50s plus 7day larval growth tests; K+ addition increased toxicity; Alkalinity had not observable effect; clay and humic acid addition reduced toxicity
Cu	freshwater bivalve, *Hyridella depressa*,	acute	*					*	*		Markich et al 2003	Measured 48 hr valve movement (duration of valve opening) pH, hardness and DOC were dependant variables regarding Cu effect concentrations.
Cu	freshwater algae	chronic						*	*		de Schamphelaere et al, 2005	Toxicity predicted based on internal and external bound Cu rather than Cu+ in the medium.
Cu	***Hyalella azteca***	acute		*	*	*	*	*			Borgmann et al, 2005	Acute 7-day LC50 for survival.
Cu	killifish (*Fundulus heteroclitus*)	chronic?								salinity	Blanchard and Grosell, 2005	Copper accumulation in whole body and liver.
Cu	larval fathead minnows (*Pimephales*	acute	*					*	*		Sciera et al, 2004	96hr LC50

Chemical	Organism	Acute / Chronic	Hardness	Ca^{+}	Mg^{+}	Na^{+}	K^{+}	H^{+}	DOC / DOM	Other	Citation	Comments
	promelas)											
Cu	*Mytilus*	chronic							*		Arnold, 2005	48-hr embryo-larval shell development & mortality; the toxicity of copper to the genus on which the U.S. EPA saltwater criteria is based is affected by DOC in a predictable manner (EC50 = 11.53DOC^0.54).
Cu	rainbow trout (*O. mykiss*)	chronic							* Humic acid		McGeer et al, 2002	30d exposure of trout to copper in the presence of humic acid indicates importance of organics to accumulation and effects.
Cu	rainbow trout (*O. mykiss*) bull trout (S. *confluentus*)	acute	*						*	Temp.	Hansen et al, 2002b,c	
Cu	threespine stickleback, *Gasterosteus aculeatus*	acute	*	*	*	*	*	*	*	SO4, humic acid, alkalinity	Gravenmier, et al, 2005	96hr LC50; LC50 Normalized = LC50 Actual/ [(H actual / H normalized)^0:9422].
Cu	Various fish & invertebrates	acute	*	*				*			Meyer, 1999	Synthesis of EPA Criteria data for divalent metals leading to the conclusion the amount of transition metal bound to the fish gill at the LC50 concentration is constant.
Pb	Various fish & invertebrates	acute	*	*				*			Meyer, 1999	Synthesis of EPA Criteria data for divalent metals leading to the conclusion the amount of transition metal bound to the fish gill at the LC50 concentration is constant.

Chemical	Organism	Acute / Chronic	Hardness	Ca^+	Mg^+	Na^+	K^+	H^+	DOC / DOM	Other	Citation	Comments
Ni	C. *dubia*	chronic	*								Keithly et al, 2004	48hr LC50s; 7 day IC50 reproduction; acute lethality was hardness dependant, chronic toxicity was not.
Ni	fathead minnow	acute	*	*				*			Meyer et al, 1999	The concentration of a transition metal bound to fish gills ([Mgill]) is predicted to be a constant predictor of mortality. DOC was not an included variable but considered an important competitor for metal binding sites in estimating acute toxicity.
Ni	fathead minnow	acute	*							suspended solids - clay	Pyle et al, 2002	96hr LC50s; H+ had little effect on toxicity below pH7; pH 8.5 reduced toxicity 4 fold. Increased hardness and clay addition decreased toxicity.
Ni	*Hyalella azteca*	chronic	*								Keithly et al, 2004	96hr LC50; 14-day mortality and growth; chronic survival was hardness dependant, growth was not. Nickel body burden increased with hardness.
Ni	Various fish & invertbrates	acute	*	*				*			Meyer, 1999	Synthesis of EPA Criteria data for divalent metals leading to the conclusion the amount of transition metal bound to the fish gill at the LC50 concentration is constant.
Ag	D. *magna*	chronic				*					Bianchini, and Wood, 2002	Physiological effects to chronic exposure suggest Na+ is key to toxicity for D. magna
Ag	FW algae	acute								Cl-	Lee et al, 2004	Silver uptake related to free Ag+. BLM is applicable for Ag in the presence of Cl. When Cl is present, Ag does not enter cells via passive

Chemical	Organism	Acute / Chronic	Hardness	Ca^+	Mg^+	Na^+	K^+	H^+	DOC / DOM	Other	Citation	Comments
												diffusion or anion transport.
Ag	mysid, *Americamysis bahia*	chronic								salinity	Ward and Kramer, 2002	Three tests were conducted: 1) 28 day survival & reproduction; 2) 7 day survival & growth with < 24 hour old mysids and 3) as above with 7 day old mysids. All results were within a factor of two. Measured ionic Ag was well predicted by total Ag, (not clear from abstract, what complexing agents, etc. were used in calculations). Free ionic Ag varied with total Ag.
Ag	rainbow trout (*Oncorhynchus mykiss*)	chronic							* humic acid		Brauner and Wood, 2002.	51 day exposure of trout embryo to silver in the presence of humic acid indicate protective effects of DOC to chronic silver exposure appear to be less than that observed during acute exposure. BLM for chronic Ag exposure not yet ready for general application.
Ag	rainbow trout (*O. mykiss*) - dispersed gill cells	acute				*		*	*	Cl-	Zhou et al, 2005.	In vitro BLM may provide a way to cost-effectively evaluate site-specific waters.
Zn	D. m*agna*	acute		*	*	*					Heijerick et al, 2002	48h LC50S; K^+, H^+ and Na^+ did not alter acute toxicity significantly; validation tests were conducted with humic acid containing media - 88% were within 1.3x of expected.

Chemical	Organism	Acute / Chronic	Hardness	Ca^+	Mg^+	Na^+	K^+	H^+	DOC / DOM	Other	Citation	Comments
Zn	*D. magna*	chronic		*	*	*		*			Heijerick et al, 2005	Chronic 21-day EC50 for Daphnia reproduction is the estimated endpoint.
Zn	D. magna	chronic	*					*	*		Heijerick et al, 2003	21 day reproduction
Zn	rainbow trout (*O. mykiss*)	chronic		*	*	*		*			de Schamphelaere and Janssen, 2004b	30 day exposure ; survival more sensitive than growth; Given the relative importance of Ca competition (factor 12 variability) as compared to the effects of Mg, Na, and pH (factor 2-3 variability), the Ca effect should be evaluated first. Na has been shown to be more important than Ca and Mg in reducing chronic toxicity of copper, a metal interfering with Na homeostasis (8).
Zn	rainbow trout (*O. mykiss*) bull trout (S. *confluentus*)	acute	*						*	Temp.	Hansen et al, 2002c	
Zn	Various fish & invertbrates	acute	*	*				*			Meyer, 1999	Synthesis of EPA Criteria data for divalent metals leading to the conclusion the amount of transition metal bound to the fish gill at the LC50 concentration is constant.

Appendix 2: Compilation of Cu-TMF Database

Cu toxicity datasets were downloaded from the US EPA ACQUIRE database (June, 2006). Download criteria include all forms of Cu, freshwater exposure

The following steps were taken to refine the data downloaded from the ACQUIRE database in consultation with the project authority:

- Omit all but short-term data. The CCME definitions (CCME, 2006) are ≤ 96h for fish, amphibians and aquatic invertebrates. Data for aquatic plants will be considered on a case-by-case basis for guideline derivation but exposure periods should be ≤ 96h. For the purposes of this document, any plant results with exposure periods ≤ 96h were included in the database. Finally CCME states that algal toxicity tests are generally considered inappropriate for inclusion in the derivation of a short-term guideline.

- Other restrictions based on test duration entries:

  - When test duration was not recorded, the record was kept if the title contained and case-insensitive version of the word "acute" (U. Schneider, pers. comm.).

  - Records with the following entries for test duration were omitted:

    - dd - undefined field code, 10 observations
    - brd - broods, not in units of time, 8 observations
    - frt - days to fertilization, no test duration recorded, 1 observation
    - ge - generations, not in units of time, 43 observations
    - ht - until hatch, no times recorded, 7 observations
    - any test duration followed by a slash (the slash denotes an associated comment) unless time <= 96h (U. Schneider, pers. comm.).

- Restrictions based on test duration unit entries:

  - Retain records where duration units are not recorded but title contains word acute.

- Records with the following entries in the field "common name" were removed:

  - Algae, algal mat
  - Algae
  - Blue-green algae
  - Blue-green algae phylum
  - Green alga

    - Green algae
    - Green algae division
    - Green algae order

- Records with the following entries in the field "endpoint" were retained:

    - LC50 or LC50*
    - EC50 or EC50* if field Effect Measurement = "IMBL" (immobility)
    - IC50 or IC50* if field Effect Measurement = "IMBL" or "MORT", (immobility or mortality.)

- Studies where endpoint was not recorded, were deleted.

- Studies with a delayed response were retained if the exposure time meets criterion (U. Schneider, pers. comm.).

The final database contains 3372 records. Now in order to adjust each endpoint using the BLM, measurements of pH, dissolved organic carbon (DOC) (in mg/L), percent humic acid, temperature; major cations (Ca+, Mg+, Na+, and K+); major anions (SO4-, Cl-); dissolved inorganic carbon (DIC) and sulfide are required.

Comments on Data Requirements for BLM Adjustment

- Dissolved inorganic carbon measurements are not found within the Acquire database but these measurements could be obtained from alkalinity and pH (both of which are available in database).

- Sulfide is not generally present in freshwater except under unusual circumstances (hot springs and sewage treatment plants).

- Cl, Na, K are not available within database. (Simulation options are to select a median value) or run over a range of possible values or a combination of "worst-case" values. The total number of simulations could become very large if many levels for each of these variables is chosen.

- Only 20 records have units referring to humic acid. A range or worst case regarding % humic acid composition could be used in a simulation study.

The number of records complete with hardness, alkalinity, some sort of organic carbon measurement (with units so that conversions can be made), and a measured pH is 59. These records still require DIC, Cl, Na, K and some assumption regarding % humic acid composition as described above.