CCME

Canadian Council of Ministers of the Environment

Le Conseil canadien des ministres de l'environnement

SCIENTIFIC CRITERIA DOCUMENT FOR THE DEVELOPMENT OF THE CANADIAN WATER QUALITY GUIDELINES FOR THE PROTECTION OF AQUATIC LIFE

Zinc

PN 1580
ISBN 978-1-77202-043-4 PDF

NOTE TO READERS

The Canadian Council of Ministers of the Environment (CCME) is the primary minister-led intergovernmental forum for collective action on environmental issues of national and international concern.

This document was developed by Environment and Climate Change Canada and reviewed by the Water Management Committee's Guidelines Project Team. It provides the background information and rationale for the development of the Canadian Water Quality Guidelines for zinc.

CCME would like to thank Environment and Climate Change Canada's Science Horizons Youth Internship Program (a Youth Employment Strategy program) and Minnow Environmental for their support of the writing of this document, and Eric Van Genderen, David DeForest, Chris Mebane and Lucinda Tear for their reviews of draft documents.

For additional scientific information regarding these water quality guidelines, please contact:

Environment and Climate Change Canada
Place Vincent Massey
351 St-Joseph Blvd.
Gatineau, QC K1A 0H3
Phone: 800-668-6767 (in Canada only) or 819-997-2800 (National Capital Region)
Email: ec.rqe-eqg.ec@canada.ca

This scientific criteria document is available in English only. Ce document scientifique du soutien n'est disponible qu'en anglais avec un résumé en français.

Reference listing:
Canadian Council of Ministers of the Environment. 2018. Scientific criteria document for the development of the Canadian water quality guidelines for the protection of aquatic life: zinc. Canadian Council of Ministers of the Environment, Winnipeg, MB.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Appendix

LIST OF ABBREVIATIONS

AGNES	absence of gradients and Nernstian equilibrium stripping
BCF	bioconcentration factor
BLM	biotic ligand model
CAS	Chemical Abstract Service
CCC	criteria continuous concentration
CCME	Canadian Council of Ministers of the Environment
CCU	cumulative criterion unit
CDF	cumulative distribution function
CI	confidence interval
CMC	criteria maximum concentration
CWQG	Canadian Water Quality Guideline
dw	dry weight
DOC	dissolved organic carbon
DOM	dissolved organic matter
EC_x	effect concentration: concentration affecting x% of the test organisms
Eh	redox potential
IC_x	inhibitory concentration: concentration causing x% inhibition
ICP-MS	inductively coupled plasma-mass spectrometry
ICP-AES	inductively coupled plasma-atomic emission spectrometry
LC_x	lethal concentration for x% of the test organisms
ln	natural logarithm
LOEC	lowest-observed-effect concentration
LOEL	lowest-observed-effect level
MATC	maximum acceptable toxicant concentration
MLR	multiple linear regression
NEC	no-effect concentration
NOEC	no-observed-effect concentration
PNEC	predicted no-effect concentration
POM	particulate organic matter
SSD	species sensitivity distribution
TLm	median tolerance limit
TOC	total organic carbon
US EPA	United States Environmental Protection Agency
ww	wet weight

EXECUTIVE SUMMARY

Zinc is an essential metal found widely in nature. It is a metal belonging to Group 12 of the periodic table. It can form complexes with various organic ligands and has a variety of salts. It has a density of 7.14 $g \cdot cm^{-3}$, a molecular weight of 65.39 $g \cdot mol^{-1}$ and a vapour pressure of 31 Pa at 450ºC (Lide 2006). Its Chemical Abstract Service (CAS) number is 7440-66-6. While metallic zinc is insoluble in water, several of its salts are soluble, including zinc sulphate, zinc chloride, zinc bromide and zinc nitrate (Budavari 1996; Lide 2006). Zinc is present in the earth's crust, most rocks, certain minerals and some carbonate sediments. Weathering of these sources can release soluble zinc compounds into aquatic environments. Anthropogenic sources of zinc release include urban runoff, mine drainage, and industrial effluents from smelters and refineries.

Zinc generally occurs in association with the metals copper and lead, so mining and milling operations usually recover these substances as co-products. Open-pit mining methods are used to extract zinc from orebodies near the surface, while orebodies at greater depths require underground mining operations (NRCan 2007*b*). Recent primary production of zinc in Canada dropped from 1,026,864 tonnes in 1997 to 580,534 tonnes in 2011 (NRCan 2012*a*). The major industrial use of zinc is to galvanize iron and steel products to render them resistant to corrosion and rust.

Modern analytical methods used to measure zinc in aquatic samples include various types of spectrometry (e.g., inductively coupled plasma spectroscopy, flame atomic absorption spectroscopy and graphite furnace atomic absorption spectrometry), anodic and cathodic stripping voltammetry, X-ray diffraction, and flow-injection analysis (ATSDR 2005). For several methods, detection limits for water are below 1 $\mu g \cdot L^{-1}$.

In water, zinc can be found in both suspended and dissolved forms and in different chemical species. Its speciation is influenced by several abiotic variables, most importantly pH, alkalinity, redox potential and dissolved organic matter content. The most common dissolved zinc species in natural waters under aerobic conditions are zinc monohydroxide ($ZnOH^{+)}$, the aqueous zinc ion (Zn^{2+}) and zinc carbonate ($ZnCO_3$) (Florence 1977; Stumm and Morgan 1981), and most zinc introduced into an aquatic system is partitioned into suspended and bottom sediments (Eisler 1993). Several processes control zinc concentrations and mobility in the water column and thus its bioavailability to aquatic organisms. Of those processes, sorption and precipitation seem to be the most important in limiting zinc bioavailability.

The biological effect (i.e., toxicity) of zinc is strongly related to its speciation. Of all zinc species found in aquatic environments, Zn^{2+} is believed to be the most toxic (Australian and New Zealand Environment and Conservation Council 2000). The toxic mode of action of zinc involves interference with calcium uptake at the gills and, less significantly, a disturbance of sodium and chloride fluxes (Hogstrand *et al.* 1994; Spry and Wood 1985). Zinc is also an essential element needed for a variety of biological functions. If concentrations of zinc in the surrounding environment either exceed or fall below an organism's optimal range, the organism's homeostatic capacity will fail and the effects of zinc toxicity or deficiency may be observed (Muyssen and Janssen 2002*a*).

Water chemistry conditions affect the environmental fate and behaviour of zinc, and can also influence the toxicity of zinc to aquatic organisms. The empirical relationships, discussed herein, were derived for both short-term and long-term studies to convert toxicity data to standardized water chemistry for hardness, pH and dissolved organic carbon (DOC). Accordingly, the Canadian Water Quality Guideline (CWQG) and short-term benchmark for freshwater exposure to zinc are presented as multi-variable equations that are a function of water hardness, pH and DOC and allow users to derive guidelines and benchmarks based on the water chemistry of the site under consideration. The short-term benchmark equation includes variables for hardness and DOC, and the long-term guideline equation includes variables for hardness, DOC and pH, based on data availability and the significance of toxicity modifying factors.

The freshwater short-term benchmark concentration and long-term CWQG for zinc for the protection of aquatic life were developed based on the Canadian Council of Ministers of the Environment (CCME) protocol using the statistical or Type A approach (CCME 2007). CCME did not derive a marine water quality guideline for zinc at this time and hence no marine value is recommended. It is not appropriate to apply the zinc freshwater guideline to marine or estuarine environments.

CWQG for the protection of aquatic life for zinc

	Short-term exposure[a] ($\mu g \cdot L^{-1}$)	Long-term exposure[b] ($\mu g \cdot L^{-1}$)
Freshwater	37[c]	7.0[d]
Marine	Not assessed	Not assessed

[a] The short-term exposure benchmark is meant to estimate severe effects and to protect most species against lethality during intermittent and transient events (e.g., spills, infrequent releases of short-lived and non-persistent substances).

[b] The long-term exposure guideline is meant to protect against all negative effects during indefinite exposures.

[c] The short-term benchmark is for **dissolved** zinc and is calculated using this equation:

Benchmark = exp(0.833[ln(hardness mg·L^{-1})] + 0.240[ln(DOC mg·L^{-1})] + 0.526). The value given in the table is for surface water of 50 mg $CaCO_3 \cdot L^{-1}$ hardness and 0.5 mg·L^{-1} DOC. The benchmark equation is valid between hardness 13.8 and 250.5 mg $CaCO_3 \cdot L^{-1}$ and DOC 0.3 and 17.3 mg·L^{-1}, which is the range of data used to derive the hardness and DOC slopes. Extrapolations should not be made above the upper hardness limit of 250.5 mg $CaCO_3 \cdot L^{-1}$ or above the upper DOC limit of 17.3 mg·L^{-1}. For hardness below 13.8 mg $CaCO_3 \cdot L^{-1}$ or DOC below 0.3 mg·L^{-1}, where users want a more stringent benchmark, they should extrapolate with caution and contact their local authority for advice.

[d] The long-term CWQG is for **dissolved** zinc and is calculated using this equation:

CWQG = exp(0.947[ln(hardness mg·L^{-1})] − 0.815[pH] + 0.398[ln(DOC mg·L^{-1})] + 4.625). The value given in the table is for surface water of 50 mg $CaCO_3 \cdot L^{-1}$ hardness, pH 7.5 and 0.5 mg·L^{-1} DOC. The CWQG equation is valid between hardness 23.4 and 399 mg $CaCO_3 \cdot L^{-1}$, pH 6.5 and 8.13, and DOC 0.3 to 22.9 mg·L^{-1}, which is the range of data used to derive the hardness, pH and DOC slopes. Extrapolations should not be made above the upper hardness limit of 399 mg $CaCO_3 \cdot L^{-1}$, above the upper DOC limit of 22.9 mg·L^{-1} or below the lower pH limit of 6.5. For hardness below 23.4 mg $CaCO_3 \cdot L^{-1}$, DOC below 0.3 mg·L^{-1} or pH above 8.13, where users want a more stringent WQG, they should extrapolate with caution and contact their local authority for advice.

Note: The freshwater benchmark and CWQG equations must be used in order to obtain a site-specific benchmark and CWQG, respectively, based on the DOC, pH and hardness of the water body of interest (see tables below for examples of benchmark and guideline values at various levels of water chemistry).

The short-term benchmark and long-term guideline are for dissolved concentrations of zinc. Where guideline users have only water sample concentrations of total zinc, they should first compare these samples to the dissolved guideline, and where there is an exceedance, re-sample for a dissolved concentration.

Marine guidelines were not derived at this time and hence no marine value is recommended. Note that it is not appropriate to apply this zinc freshwater guideline to marine or estuarine environments.

Example short-term benchmark concentrations (µg·L^{-1}) for dissolved zinc in fresh water at various levels of water hardness and DOC

DOC (mg· L^{-1})	Hardness (mg· L^{-1})							
	15	25	50	75	100	150	200	250.5 (upper limit)
0.5	14	21	37	52	66	93	118	143
2	19	29	52	73	93	130	165	199
5	24	36	65	91	115	162	206	248
10	28	43	77	107	136	191	243	293
17.3 (upper limit)	32	49	87	122	155	218	277	334 (maximum)

Example long-term CWQGs for the protection of aquatic life (µg·L^{-1}) for dissolved zinc in fresh water at various levels of water hardness, pH and DOC

pH 6.5						
DOC (mg·L^{-1})	Hardness (mg $CaCO_3$·L^{-1})					
	25	50	75	100	200	399
0.5	8.2	16	23	30	59	113
2	14	27	40	53	102	195
5	20	39	58	76	146	281
10	27	52	76	100	193	371
22.9	37	72	106	139	268	516 (maximum)
pH 7.0						
DOC (mg·L^{-1})	Hardness (mg $CaCO_3$·L^{-1})					
	25	50	75	100	200	399
0.5	5.4	10	15	20	39	75
2	9.4	18	27	35	68	130
5	14	26	38	50	97	187
10	18	35	51	67	128	247
22.9	25	48	70	93	178	343
pH 7.5						
DOC (mg·L^{-1})	Hardness (mg $CaCO_3$·L^{-1})					
	25	50	75	100	200	399
0.5	3.6	7.0	10	13	26	50
2	6.3	12	18	23	45	87
5	9.0	17	26	34	65	125
10	12	23	34	44	85	164
22.9	17	32	47	62	119	228
pH 8.0						
DOC (mg·L^{-1})	Hardness (mg $CaCO_3$·L^{-1})					
	25	50	75	100	200	399
0.5	2.4	4.6	6.8	8.9	17	33
2	4.2	8.1	12	16	30	58
5	6.0	12	17	22	43	83
10	7.9	15	22	29	57	109
22.9	11	21	31	41	79	152

RÉSUMÉ

Le zinc est un métal essentiel, largement répandu dans la nature. C'est un métal qui appartient au groupe 12 du tableau périodique. Il peut former des complexes avec divers ligands organiques et il existe une grande variété de sels de zinc. Sa masse volumique est de 7,14 g cm^{-3}, sa masse moléculaire est de 65,39 g·mol^{-1} et sa pression de vapeur est de 31 Pa à 450 ºC (Lide 2006). Son numéro dans le Chemical Abstract Service (CAS) est 7440-66-6. Bien que le zinc métallique soit insoluble dans l'eau, plusieurs de ses sels sont solubles, y compris le sulfate de zinc, le chlorure de zinc, le bromure de zinc et le nitrate de zinc (Budavari 1996; Lide 2006). Le zinc est présent dans la croûte terrestre, dans la plupart des roches, dans certains minéraux et dans certains sédiments carbonatés. La météorisation de ces sources peut rejeter des composés de zinc solubles dans les milieux aquatiques. Les sources anthropiques de rejet de zinc comprennent le ruissellement urbain, le drainage minier et les effluents industriels provenant des fonderies et des raffineries.

Le zinc est généralement associé au cuivre et au plomb métalliques, et par conséquent ils sont habituellement récupérés sous forme de coproduits par les opérations d'extraction et de concentration (RNCan 2007b). On utilise des méthodes d'extraction à ciel ouvert pour extraire le zinc des corps minéralisés près de la surface, et on a recours à l'exploitation souterraine pour les corps minéralisés en profondeur (RNCan 2007b). La production primaire de zinc au Canada au cours de la dernière décennie est passée de 1 026 864 tonnes en 1997 à 580 534 tonnes en 2011 (RNCan 2012*a*). La principale utilisation industrielle du zinc est la galvanisation des produits de fer et d'acier, afin de leur procurer une résistance contre la corrosion et la rouille.

Les méthodes analytiques modernes utilisées pour mesurer les concentrations de zinc dans les échantillons aquatiques font appel à divers types de spectrométrie (p. ex., la spectrométrie de masse couplée à un plasma inductif, la spectrométrie d'absorption atomique par flamme, la spectrométrie d'absorption atomique en four de graphite, la voltammétrie par redissolution anodique et cathodique, la diffraction des rayons X et l'analyse par injection de flux (ATSDR 2005). Pour plusieurs de ces méthodes, les seuils de détection dans l'eau sont inférieurs à 1 µg·L^{-1}.

Dans l'eau, le zinc est présent en suspension et sous forme dissoute, et dans différentes espèces chimiques. Sa spéciation dépend de plusieurs variables abiotiques, les plus importantes étant le pH, l'alcalinité, le potentiel d'oxydo-réduction et la teneur en matières organiques dissoutes. Les espèces de zinc dissous les plus courantes dans les eaux naturelles, dans des conditions aérobies, sont le $ZnOH^+$, le Zn^{2+} et le $ZnCO_3$ (Florence 1977; Stumm et Morgan 1981), et la majeure partie du zinc qui se retrouve dans un système aquatique est répartie entre les sédiments en suspension et de fond (Eisler 1993). Plusieurs mécanismes influent sur les concentrations et la mobilité du zinc dans la colonne d'eau, et donc sur sa biodisponibilité pour les organismes aquatiques. Parmi ces mécanismes, la sorption et la précipitation semblent être les plus importants pour ce qui est de limiter la biodisponibilité du zinc.

Les effets biologiques (c.-à-d. la toxicité) du zinc sont étroitement associés à sa spéciation. De toutes les espèces de zinc présentes dans les milieux aquatiques, l'ion de zinc aqueux (Zn^{2+}) est, croit-on, le plus toxique (ANZECC 2000). Le mode d'action toxique du zinc consiste à interférer

avec l'absorption de calcium au niveau des branchies, et de façon moins importante, à perturber les flux de sodium et de chlorure (Hogstrand *et al.* 1994; Spry et Wood 1985). Le zinc est également un élément essentiel requis par diverses fonctions biologiques. Les concentrations de zinc dans le milieu ambiant qui dépassent grandement dans un sens ou dans l'autre la plage optimale d'un organisme peuvent provoquer la défaillance de la capacité homéostatique de cet organisme, et on peut observer les effets de la toxicité du zinc sous forme de carence (Muyssen et Janssen 2002*a)*.

Les conditions de la chimie de l'eau peuvent influer sur la toxicité du zinc pour les organismes aquatiques, en plus de l'effet qu'elles ont sur le devenir et le comportement du zinc dans l'environnement. Des relations empiriques, décrites dans le présent rapport, ont été établies pour les études à court terme et à long terme afin de convertir les données de toxicité en paramètres chimiques de l'eau normalisés, pour la dureté, le pH et le carbone organique dissous (COD). Par conséquent, la recommandation canadienne pour la qualité des eaux (RCQE) et la concentration limite à court terme pour l'exposition au zinc en eau douce sont présentées sous forme d'équations à variables multiples qui sont fonction de la dureté de l'eau, du pH et du COD, et qui permettent aux utilisateurs d'établir des recommandations et des concentrations limites selon la chimie de l'eau du site particulier. L'équation de la concentration limite à court terme comprend des variables pour la dureté et le COD et celle de la recommandation à long terme comprend des variables pour la dureté, le COD et le pH; ces deux équations reposent sur la disponibilité des données et l'importance des facteurs modifiant la toxicité.

La concentration limite à court terme et la RCQE à long terme dans l'eau douce pour protéger la vie aquatique contre l'exposition au zinc ont été établies d'après le protocole du Conseil canadien des ministres de l'Environnement (CCME) selon l'approche statistique ou de type A (CCME 2007). Comme le CCME n'a toujours pas établi de recommandation pour la qualité de l'eau de mer pour le zinc, il n'existe à l'heure actuelle aucune valeur liée à l'eau de mer. Il n'est pas approprié d'appliquer les recommandations pour le zinc en eau douce aux environnements marins ou estuariens.

Recommandations canadiennes pour la qualité des eaux (RCQE) en vue de protéger la vie aquatique – zinc

	Exposition à court terme[a] ($\mu g \cdot L^{-1}$)	**Exposition à long terme[b] ($\mu g \cdot L^{-1}$)**
Eau douce	37[c]	7.0[d]
Eau salée	Non évaluée	Non évaluée

[a] Les recommandations pour les expositions à court terme ont pour objectif d'estimer les effets graves et de protéger la plupart des espèces contre la mortalité lors d'événements intermittents et momentanés (déversements, rejets peu fréquents de substances non persistantes ou de courte durée de vie, etc.).

[b] Les recommandations pour les expositions à long terme ont pour objectif de protéger contre tous les effets nocifs des expositions indéfinies.

[c] La concentration limite à court terme est pour le zinc dissous et est calculée à l'aide de l'équation suivante :
Concentration limite = exp(0,833[ln(dureté mg·L^{-1})] + 0,240[ln(COD mg·L^{-1})] + 0,526). La valeur indiquée dans le tableau est pour une eau de surface ayant une dureté de 50 mg $CaCO_3 \cdot L^{-1}$ et une teneur en carbone organique dissous (COD) de 0,5 mg·L^{-1}. L'équation pour la concentration limite est valide pour une dureté de 13,8 à 250 mg $CaCO_3 \cdot L^{-1}$ et une valeur COD de 0,3 à 17,3 mg·L^{-1}, ce qui correspond à la plage de données utilisée pour calculer les pentes de dureté et de COD. On ne doit pas extrapoler au-delà de la limite de dureté supérieure de 250,5 mg $CaCO_3 \cdot L^{-1}$ ou au-delà de la limite de COD supérieure de 17,3 mg·L^{-1}. Pour une dureté inférieure à 13,8 mg $CaCO_3 \cdot L^{-1}$ ou une valeur COD inférieure à 0,3 mg L^{-1}, si les

utilisateurs veulent une concentration limite plus stricte, ils doivent extrapoler avec prudence et communiquer avec les responsables locaux pour obtenir leur avis.

[d] La RCQE à long terme est pour le zinc dissous et est calculée au moyen de l'équation : **RCQE = exp(0,947[ln(dureté mg·L^{-1})] – 0,815[pH] + 0,398[ln(COD mg·L^{-1})] + 4,625)..** La valeur indiquée dans le tableau est pour une eau de surface ayant une dureté de 50 mg $CaCO_3 \cdot L^{-1}$, un pH de 7,5 et une teneur en carbone organique dissous (COD) de 0,5 mg·L^{-1}. L'équation pour la RCQE est valide pour une dureté de 23,4 à 399 mg $CaCO_3 \cdot L^{-1}$, un pH de 6,5 à 8,13, et une valeur COD de 0,3 à 22,9 mg·L^{-1}**,** ce qui correspond à la plage de données utilisée pour calculer les pentes de dureté, de pH, et de COD. On ne doit pas extrapoler au-delà de la limite de dureté supérieure de 399 mg $CaCO_3 \cdot L^{-1}$, au-delà de la limite de COD supérieure de 22,9 mg·L^{-1} ou en deçà de la limite de pH inférieure de 6,5. Pour une dureté inférieure à 23,4 mg $CaCO_3 \cdot L^{-1}$, une valeur COD inférieure à 0,3 mg L^{-1}, ou un pH supérieur à 8,13, si les utilisateurs veulent une RCQE plus stricte, ils doivent extrapoler avec prudence et communiquer avec les responsables locaux pour obtenir leur avis.

Remarque : Les équations pour la concentration limite et la RCQE en eau douce doivent être utilisées pour obtenir la concentration limite et une RCQE pour un site particulier d'après les valeurs du COD, du pH et de la dureté de l'eau dans le plan d'eau en question (voir les tableaux ci-dessous qui présentent des exemples de concentration limite et de recommandation pour diverses valeurs des paramètres chimiques de l'eau).

La concentration limite à court terme et la recommandation à long terme concernent les concentrations de zinc dissous. Si les concentrations mesurées dans les échantillons d'eau ne sont exprimées que sous forme de zinc total, il est recommandé de comparer d'abord ces échantillons à la recommandation pour le zinc dissous et de ne prélever de nouveaux échantillons pour établir des concentrations de zinc dissous que s'il y a dépassement.

Comme des recommandations pour le milieu marin n'ont pas encore été calculées, il n'existe à l'heure actuelle aucune valeur liée à l'eau de mer. Il n'est pas approprié d'appliquer les recommandations pour le zinc en eau douce aux milieux marins ou estuariens.

Exemples de concentration limite (µg·L^{-1}) pour l'exposition à court terme au zinc dissous dans l'eau douce pour diverses valeurs de dureté de l'eau et de COD

COD (mg· L^{-1})	**Dureté (mg· L^{-1})**							
	15	**25**	**50**	**75**	**100**	**150**	**200**	**250,5 (Limite supérieure)**
0,5	14	21	37	52	66	93	118	143
2	19	29	52	73	93	130	165	199
5	24	36	65	91	115	162	206	248
10	28	43	77	107	136	191	243	293
17,3 (Limite supérieure)	32	49	87	122	155	218	277	334 **(Maximum)**

Exemples de recommandations pour la qualité de l'eau douce (µg·L^{-1}) pour une exposition à long term au zinc dissous pour diverses valeurs de dureté de l'eau, de pH et de COD

pH 6,5						
COD (mg·L^{-1})	**Dureté (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0,5	8,2	16	23	30	59	113
2	14	27	40	53	102	195
5	20	39	58	76	146	281
10	27	52	76	100	193	371
22.9	37	72	106	139	268	516 (**Maximum)**
pH 7,0						
COD (mg·L^{-1})	**Dureté (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0,5	5,4	10	15	20	39	75
2	9,4	18	27	35	68	130
5	14	26	38	50	97	187
10	18	35	51	67	128	247
22.9	25	48	70	93	178	343
pH 7,5						
COD (mg·L^{-1})	**Dureté (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0,5	3,6	7,0	10	13	26	50
2	6,3	12	18	23	45	87
5	9,0	17	26	34	65	125
10	12	23	34	44	85	164
22.9	17	32	47	62	119	228
pH 8,0						
COD (mg·L^{-1})	**Dureté (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0,5	2,4	4,6	6,8	8,9	17	33
2	4,2	8,1	12	16	30	58
5	6,0	12	17	22	43	83
10	7,9	15	22	29	57	109
22.9	11	21	31	41	79	152

1.0 INTRODUCTION

Zinc is an essential and naturally occurring element that may cause toxicity to aquatic organisms if they are exposed to high concentrations. Canada is a large producer and exporter of zinc and zinc products. Zinc also occurs in association with copper and lead. Mining and milling activities of these three metals can redistribute zinc and may cause concentrations in ambient water to exceed background concentrations, which in turn could lead to adverse environmental effects.

The Canadian Water Quality Guidelines (CWQGs) compile and interpret aquatic toxicity data, providing an important tool in the evaluation of ambient water quality. Aquatic life long-term guidelines are derived to indefinitely protect the most sensitive species at all life stages. By comparing environmental concentrations with zinc toxicity data and the guideline value, it is possible to determine the level of zinc below which no adverse impact on the ecosystem is expected.

The 1987 zinc CWQG was an interim guideline. The protocol used to develop this guideline was revised in 2007 (Canadian Council of Ministers of the Environment [CCME] 2007). The goals of the revised protocol, *A Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life*, include (i) accounting for the unique properties of contaminants, which influence their toxicity; and (ii) incorporating a species sensitivity distribution (SSD) method, which uses all available toxicity data (provided these data pass quality control criteria) in a more flexible approach. The actual CWQG is thus an updated zinc guideline that was developed to accommodate the changes in the protocol for deriving guidelines. All the customary components of scientific supporting documents have been included (physical and chemical properties, production and uses, environmental fate and behaviour, environmental concentrations, and toxicity data). In addition, new cornerstones of the protocol, such as bioavailability and toxicity modifying factors, have been given special attention.

2.0 PHYSICAL AND CHEMICAL PROPERTIES OF ZINC

Zinc is an essential metal for life that is naturally present and found widely in nature. Its Chemical Abstract Service (CAS) number is 7440-66-6. It is a heavy metal with a density of 7.14 $g \cdot cm^{-3}$, a molecular weight of 65.39 $g \cdot mol^{-1}$ and a vapour pressure of 31 Pa at 450ºC (Lide 2006).

Zinc belongs to group IIB of the periodic table. It is able to form complexes with a variety of organic ligands and has a variety of salts (World Health Organization [WHO] 2001). Although zinc metal is insoluble in water, several of its salts are freely soluble (Table 2.1). Zinc can occur naturally in five stable isotopes (Table 2.2). The metallic form, Zn(0), can be found only in highly reducing environments (Lindsay 1979). The predominant oxidation state in natural environments is thus the aqueous zinc ion (Zn^{2+}).

Table 2.1 Physical and chemical properties of common zinc compounds

Name	Formula	CAS number	Molecular weight ($g \cdot mol^{-1}$)	Melting point (°C)	Boiling point (°C)[a]	Solubility ($g \cdot 100^{-1}$ g H_2O)
Zinc	Zn	7440-66-6	65.39	419.53	907	insoluble
Zinc acetate dihydrate	$Zn(C_2H_3O_2)_2 \cdot 2H_2O$	5970-45-6	219.527	237[b]	-	30.0 [c]
Zinc bromide	$ZnBr_2$	7699-45-8	225.217	402	≈670	488 [d]
Zinc carbonate	$ZnCO_3$	3486-35-9	125.418	140[b]	-	0.000091 [c]
Zinc chloride	$ZnCl^2$	7646-85-7	136.315	290	732	408[d]
Zinc nitrate hexahydrate	$Zn(NO_3)_2 \cdot 6H_2O$	10196-18-6	297.510	36 [b]	-	120 [d]
Zinc oxide	ZnO	1314-13-2	81.408	1974	-	insoluble
Zinc phosphate	$Zn_3(PO_4)_2$	7779-90-0	386.170	900	-	insoluble
Zinc sulphate	$ZnSO_4$	7733-02-0	161.472	680 [b]	-	57.7 [d]
Zinc sulphate monohydrate	$ZnSO_4 \cdot H_2O$	7446-19-7	179.487	238 [b]	-	57.7[d]
Zinc sulphate heptahydrate	$ZnSO_4 \cdot 7H_2O$	7446-20-0	287.578	100 [b]	-	57.7[d]
Zinc sulphite dihydrate	$ZnSO_3 \cdot 2H_2O$	7488-52-0	181.503	200 [b]	-	0.224 [d]
Zinc ethylene-bis(dithio-carbamate) (Zineb)	$Zn(CS_2NHCH_2)_2$	12122-67-7	-	-	-	practically insoluble
Zinc dimethyldithio-carbamate (Ziram)	$Zn(SCSNCH_3CH_3)_2$	137-30-4	305.83	250	-	practically insoluble

[a] referred to 101.325 KPa
[b] decomposes
[c] at 20°C
[d] at 25°C
Source: Budavari (1996) and Lide (2006).

Table 2.2 Zinc stable isotopes and their relative abundance

Stable isotope	Atomic weight ($g \cdot mol^{-1}$)	Abundance (%)
	63.929	48.268
^{66}Zn	65.926	27.975
^{67}Zn	66.927	4.102
^{68}Zn	67.925	19.024
^{70}Zn	69.925	0.631

Source: Lide (2006).

3.0 ANTHROPOGENIC AND NATURAL SOURCES AND EMISSIONS OF ZINC

Zinc is present as a mineral in the earth's crust, with a content ranging from 10 to 300 $\mu g \cdot g^{-1}$ (Malle 1992). Zinc is also present in most rocks, certain minerals and some carbonate sediments, and weathering of these sources can form and release soluble zinc compounds into bodies of water (Clement Associates 1989). Erosion of soil particles that contain zinc naturally is a process that accounts for a large input of zinc into water.

Anthropogenic sources of zinc released into the Canadian environment include urban runoff, mine drainage and industrial effluents from primary and secondary zinc smelters and zinc refineries (Clement Associates 1989; Newhook *et al.* 2003). Additionally, zinc can enter the natural environment through anthropogenic use and disposal of zinc-containing products.

Table 3.1 shows the on-site releases of zinc and its compounds into air, water and land from Canadian facilities in 2013. The table also provides information about on- and off-site disposal and off-site recycling of zinc.

Table 3.1 Release, disposal and recycling data for zinc from facilities in Canada in 2013

Province	On-site releases (tonnes)				Disposal (tonnes)		Off-site recycling (tonnes)
	Air	Water	Land	Total	On-site	Off-site	
Alberta	11	18.6	29.5	59.1	2,666	1,732	303
British Columbia	228	39.8	20	288	5,249	320	530
Manitoba	17.7	14.0	5.0	36.8	19,374	391	31.9
New Brunswick	11.3	29.9	29.0	70.1	7,109	15.0	48.8
Newfoundland and Labrador	1.2	18.2	0	19.4	2,687	0	0
Northwest Territories	0.001	0.046	0	0.047	605	0	0
Nova Scotia	0.37	0.36	0	0.73	27.2	55.8	141
Nunavut	0	0	0	0	370	0	0
Ontario	70.3	39.8	7.8	118.6	10,947	5,876	24,322
Prince Edward Island	0.076	0.0	0	0.076	0	12.5	0
Québec	103	93.7	249	445	19,093	5,425	3,834
Saskatchewan	33.2	0.40	14	47.6	155	2,628	52.5
Yukon*	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total	**476**	**255**	**354**	**1,085**	**68,282**	**16,455**	**29,263**

*Information from Yukon was not available
Source: NPRI (2014).

4.0 PRODUCTION AND USES OF ZINC

Zinc generally occurs in association with the metals copper and lead. Therefore, mining and milling operations usually recover these substances as co-products. Minor by-products such as gold, silver and cadmium are also recovered. Open-pit mining methods are used extract zinc from orebodies near the surface, while orebodies at greater depths require underground mining operations (NRCan 2007*b*). The mined zinc ores contain a zinc content that is too low to directly reduce and refine; therefore, the ores must first be concentrated. The concentration process involves crushing and grinding the ore, followed by separation or flotation using gravity or magnetic methods (ATSDR 2005).

The major use of zinc is for coating iron and steel products to render them resistant to corrosion and rust. This process, known as galvanizing, accounts for approximately 48% of the global use of zinc (NRCan 2007*b*). Commonly galvanized products include tubes, pipes, wire, and sheet and strip steel. Zinc is not strong, so it is frequently alloyed with other metals, including aluminium, copper, titanium and magnesium. These alloys have various uses, including casting and wrought applications, construction, and use in household electrical components (ATSDR 2005). Additionally, many zinc compounds are used in dentistry, medicinal and household products. Zinc salts act as solubilizing agents in pharmaceuticals. Zinc is used in the rubber industry as a reinforcing agent, a heat conductor and a UV absorber. Zinc oxides are used in paint as pigment and as acid buffers, in cosmetics and drugs for their fungicidal properties, and in dentistry as cement (ATSDR 2005). Medicinal applications of zinc chloride include use as an antiseptic, disinfectant and deodorant. Zinc sulphate is used as a trace component and disease-control agent in fertilizers and animal feed. Zinc acetate is used as a wood preservative, a catalyst and a waterproofing agent (ATSDR 2005).

Canada is one of the largest producers and exporters of zinc. The United States purchases approximately 90% of Canadian exported refined zinc, and other major customers are Japan, Hong Kong, Indonesia and Taiwan (NRCan 2007*b*).

Mine output was highest in 1997, and primary production was highest in 2000, but both declined approximately 40% between 2000 and 2011 (Table 4.1). Although refined production was greatest in 2006, no consistent temporal trend is evident (Table 4.1). Domestic shipments of refined production increased between 1997 and 2007, with the largest amount in 2002 and a gradual decline from then on. Based on available data, New Brunswick, Québec, Ontario and Manitoba are the largest recent producers, although there are mines in British Columbia and Newfoundland and Labrador as well (Table 4.2). Reported use of zinc in Canada, including recycled and primary material, has remained relatively constant from 1999 to 2006, with the lowest amount in 2007 (Tables 4.3 and 4.4).

Table 4.1 Production of zinc (in tonnes) in Canada, 1997–2011

Year	Mine output[1]	Primary production[2]	Refined production	Domestic shipments of refined production
2011	611,577	580,534	662,151	144,220
2010	649,065	609,567	691,221	147,276
2009	699,145	669,879	685,504	138,027
2008	750,502	704,780	764,310	162,622
2007	622,945	587,183	802,103	171,655
2006	637,726	598,297	824,465	179,188
2005	666,664	618,844	724,035	173,203
2004	791,373	734,035	805,438	185,184
2003	788,063	757,307	761,199	181,391
2002	916,220	923,931	793,410	186,900
2001	1,064,744	1,012,048	661,172	173,405
2000	1,002,242	1,051,442	779,892	168,780
1999	1,020,982	963,321	776,927	164,621
1998	1,061,645	991,584	745,131	166,697
1997	1,076,385	1,026,864	703,798	153,981

[1] Metal content in concentrates produced
[2] Recoverable metal in concentrates shipped
Source: NRCan (2008; 2012*a*).

Table 4.2 Production of zinc (in thousands of tonnes) in Canada by province, 2000–2010

Year	Newfoundland and Labrador	Prince Edward Island	Nova Scotia	New Brunswick	Québec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Yukon	Northwest Territories	Nunavut
2010	13.8	0	0	203	201	81.8	74.8	0	0	35.1	0	0	0
2009	18.9	0	0	251	193	103	76.3	0	0	28.1	0	0	0
2008	18.9	0	13.9	263	165	111	98.3	0	0	35.2	0	0	0
2007	16.6	0	4.93	244	103	89.7	106	0	0	30.2	0	0	0
2006	0	0	0	260	94.9	108	105	0.541	0	32.9	0	0	0
2005	0	0	0	244	103	114	105	3.96	0	49.2	0	0	0
2004	0	0	0	245	256	83.5	100	5.17	0	45.0	0	0	0
2003	0	0	0	278	253	71.7	83.5	5.39	0	65.7	0	0	0
2002	0	0	0	257	237	101	96.8	5.17	0	68.0	0	0	160
2001	0	0	0	313	252	77.8	91.8	2.05	0	109	0	0	166
2000	0	0	0	238	215	71.6	99.9	1.10	0	146	0	0	185

Source: NRCan (2012*b*).

Table 4.3 Use of zinc (in tonnes) in Canada, 1999–2007

Year	Reported use (tonnes)
2007	134,966
2006	152,732
2005	149,658
2004	152,175
2003	145,596
2002	149,908
2001	144,590
2000	147,913
1999	143,188

Source: NRCan (2007*a*; 2012*c*).

Table 4.4 Mines, mills and concentrators for zinc in Canada

Company	Type of operation	Name	Province/ Territory	Location	Start-up date	Other products
Breakwater Resources Ltd.	Underground mine, concentrator	Myra Falls operations	British Columbia	Buttle Lake	1966	Copper, gold, silver
Hudbay Minerals Inc.	Underground mine	Trout Lake mine	Manitoba	Snow Lake	1982	Copper, gold, silver
Hudbay Minerals Inc.	Underground mine, concentrator	Chisel North mine	Manitoba	Snow Lake	2001	Copper
Hudbay Minerals Inc.	Underground mine	Callinan mine	Manitoba	Flin Flon	1990	Copper, gold, silver
Hudbay Minerals Inc.	Underground mine	777 mile	Manitoba	Flin Flon	2004	Copper, gold, silver
Hudson Bay Mining and Smelting Co. Ltd. (now Hudbay)	Mill	Snow Lake mill	Manitoba	Snow Lake	1979	---
Hudson Bay Mining and Smelting Co. Ltd. (now Hudbay)	Mill	Flin Flon mill	Manitoba	Flin Flon	1931	Copper
Xstrata Zinc Division (now Glencore)	Underground mine, mill	Brunswick Mining division	New Brunswick	Bathurst	1964	Copper, gold, lead, silver
Xstrata Zinc Division (now Glencore)	Underground mine, concentrator	Kidd mine	Ontario	Timmins	1965	Cadmium, copper, indium, silver
Agnico-Eagle Mines Limited	Underground mine, concentrator	LaRonde division	Québec	LaRonde mine, Cadillac, Twp.	1988	Copper, gold, silver
Campbell Resources Inc.	Underground mine, concentrator	Chibougamau mines, Copper Rand mine	Québec	Southeast of Chibougamau	1959	Copper, gold, silver

Source: NRCan (2007*c*).

5.0 ANALYTICAL METHODS AND DETECTION LIMITS FOR ZINC

In natural waters, sampling and measuring of zinc generally involve either total or dissolved zinc, total extractable, or a form of zinc speciation analysis. Measurement of total zinc does not involve filtration and acid preservation of the sample is frequently done in pre-treatment (WHO 2001). Concerning dissolved zinc, particulate species are separated through filtration (generally 0.45 µm membrane filters) followed by separate analyses for the individual phases. Concerning speciation, the principal concern for water quality criteria is the bioavailable species. A variety of procedures are used, including ultrafiltration, dialysis, ligand exchange and chelating resin separations, as well as measurement methods that differentiate between labile and non-labile zinc, such as anodic and cathodic stripping voltammetry (WHO 2001). Table 5.1 shows various standard analytical methods for analyzing zinc in environmental samples, including the detection limits. Herein, unless otherwise reported, dissolved zinc is defined as filtered through a 0.45 µm filter.

Table 5.1 Analytical methods for zinc in environmental samples[1]

Analytical method	Sample matrix	Detection limit
Inductively coupled plasma-atomic emission spectrometry (ICP-AES)	Air	0.6 $\mu g \cdot L^{-1}$
	Water	1.2 $\mu g \cdot L^{-1}$
	Soil, solid waste, sludge	2 $\mu g \cdot L^{-1}$ (in solution)
Flame atomic absorption spectroscopy	Air	3 $\mu g \cdot sample^{-1}$
	Water	5 $\mu g \cdot L^{-1}$
	Seawater	0.05 $\mu g \cdot L^{-1}$
	Crude oil	0.8 $\mu g \cdot g^{-1}$
	Soil, solid waste, sludge	0.005 $\mu g \cdot L^{-1}$
X-ray diffraction	Air (as zinc oxide)	5 $\mu g \cdot sample^{-1}$
Inductively coupled plasma-mass spectrometry (ICP-MS)	Water	0.14 $\mu g \cdot L^{-1}$
Graphite furnace atomic absorption spectroscopy	Water	0.14 $\mu g \cdot L^{-1}$
Anodic stripping voltammetry	Water	< 1 $\mu g \cdot L^{-1}$
Cathodic stripping voltammetry	Seawater	0.005 $\mu g \cdot L^{-1}$
Flow-injection analysis	Water	3 $\mu g \cdot L^{-1}$

Source: ATSDR (2005).

[1] There is potential for other references to cite lower detection limits.

Additionally, two techniques specifically measure the concentration of the free zinc ion in various environmental media: absence of gradients and Nernstian equilibrium stripping (AGNES), which is an electrochemical technique, and the Donnan membrane technique, which uses a cation exchange membrane (Chito *et al.* 2012). Using the AGNES technique, Galceran *et al.* (2007) reported a detection limit of 0.012 $\mu g \cdot L^{-1}$ for seawater.

6.0 ENVIRONMENTAL CONCENTRATIONS OF ZINC

Zinc occurs naturally in the environment. Some areas contain naturally elevated concentrations of zinc in underlying rock, which can be released and transported to other environmental media. In other areas, anthropogenic activity may cause elevated concentrations of zinc that exceed the natural background levels. In such situations, statistical methods and comparisons with pristine environments can distinguish anthropogenic contributions of zinc from natural background levels. Natural background levels of zinc are site specific and lead to locally adapted ecological communities that may respond differently to anthropogenic releases of zinc compared to non-adapted communities. Therefore, these background levels cannot be incorporated into a guideline value applicable across the country. When the recommended Canadian guideline value falls below the natural background concentration, it may be necessary to derive a site-specific guideline.

The following sections include all readily available information for natural background levels and concentrations of zinc at impacted sites in Canadian surface waters, soil and sediments, and aquatic biota. Data gaps exist for natural background levels of zinc in Canadian environments, and further studies are recommended in this area. All study sites identified as impacted sites in the data source are reported as such in the following text.

6.1 Concentrations of Zinc in Surface Water

Various statistical methods for estimating natural background levels have been investigated. Currently the most commonly accepted method is to use the 95th percentile as an approximation of the upper limit of the normal range (Stantec Consulting Ltd 2008; Tri-Star Environmental Consulting 2006, available from Environment and Climate Change Canada, Gatineau QC, mailto:ec.rqe-eqg.ec@canada.ca). Reported estimates of natural background concentrations in the current section have been calculated using this method, and data were acquired from the Metals in the Environment database (Stantec Consulting Ltd 2008; Tri-Star Environmental Consulting 2006, available from Environment and Climate Change Canada, Gatineau QC, mailto:ec.rqe-eqg.ec@canada.ca).

6.1.1 Northwest Territories

The Great Bear River, Northwest Territories, is a site essentially undisturbed by anthropogenic activity. Its estimated natural background concentration of zinc is 5.32 $\mu g \cdot L^{-1}$ (Tri-Star Environmental Consulting 2006, available from Environment and Climate Change Canada, Gatineau QC, mailto:ec.rqe-eqg.ec@canada.ca). No further information was available for this Canadian region.

6.1.2 British Columbia

Concentrations of zinc in surface water in British Columbia were available for both relatively undisturbed and potentially disturbed sites. A background site in the Tsolum River had total zinc

concentrations ranging from below detection (10 µg·L^{-1}) to 20 µg·L^{-1} (Deniseger and Pommen 1995). The Kicking Horse River and Beaver River are two mountain watersheds that represent essentially pristine conditions. The Kicking Horse River's natural background zinc estimate was 9.0 µg·L^{-1}, and the Beaver River's was 7.0 µg·L^{-1}. The regional background concentration was 8.755 µg Zn·L^{-1} (Tri-Star Environmental Consulting 2006, available from Environment and Climate Change Canada, Gatineau QC, mailto:ec.rqe-eqg.ec@canada.ca). In the Similkameen River, total zinc concentrations ranged up to 5 µg·L^{-1} in uncontaminated areas (Swain 1990). In the Oyster River watershed, Nagpal (1990) reported total zinc concentrations below detection (10 µg·L^{-1}) in most samples. Only seven (of 185) samples exceeded the detection limit, and of these, the three highest concentrations were 290, 180 and 40 µg·L^{-1} and were attributed to analytical anomaly or potential contamination from an abandoned mine (Nagpal 1990). In the Sumas River (an agricultural watershed), zinc concentrations were consistently at or below detection limits of 10 µg·L^{-1} in 1993–1994 and 2003–2004 (Smith *et al.* 2007).

Data were available for sites in British Columbia potentially impacted from mining. Cahill Creek is a potentially impacted site (from mine development in the mid 1980s) and had estimated natural background levels of 16 µg·L^{-1} total zinc and 10 µg·L^{-1}dissolved zinc (Tri-Star Environmental Consulting 2006, available from Environment and Climate Change Canada, Gatineau QC, mailto:ec.rqe-eqg.ec@canada.ca). Monitoring of zinc concentrations in Buttle Lake (subject to contamination from heavy metals of a copper-lead-zinc mine) found concentrations reaching 2,310 µg·L^{-1} downstream of the mine prior to the treatment of the water in 1980, while post-treatment concentrations decreased to 500 µg Zn·L^{-1} in 1984 (Deniseger *et al.* 1990). More recent data from the British Columbia Ministry of the Environment reported concentrations of total zinc in Buttle Lake below 50 µg·L^{-1} between 1990 and 1996 (BC MOE 2004).

6.1.3 Québec

The St. Lawrence River receives urban effluents between Lake Ontario and the mouth of the river near Québec City, and these effluents contribute to the total metal flux. The mouth of the St. Lawrence River in Québec had mean total, dissolved and particulate zinc concentrations of 10.4, 8.6 and 3.4 µg·L^{-1}, respectively, sampled between 1974 and 1976 (Yeats and Bewers 1982). Sampled in 1987, zinc concentrations for the St. Lawrence River stretching between the outflow of Lake Ontario to Québec City ranged from 0.434 to 0.939 µg·L^{-1} for dissolved zinc and 0.228 to 0.437 µg·L^{-1} for particulate zinc (Lum *et al.* 1991). In 2000–2001, dissolved zinc concentrations near the mouth of the river were 0.812 µg·L^{-1} and particulate concentrations were 0.234 µg·L^{-1} (Gobeil *et al.* 2005). These concentrations represent water composition typical of the St. Lawrence River with respect to zinc and other trace metals, elements and nutrients.

At the outlet of the Montréal wastewater treatment plant, the concentrations of zinc as dissolved particulate matter were 0.232 (± 0.00758) µg·L^{-1} and concentrations of suspended particulate matter were 0.480 (± 0.136) µg·L^{-1} (Gobeil *et al.* 2005). In 2009, median concentrations of dissolved zinc in the St. Lawrence from Montréal to Île d'Orléans were under the detection limit of 0.7 µg·L^{-1} at all sites (S. Hébert, Ministry of Sustainable Development, Environment and Parks [MDDEP], personal communication, 2010). Between 1995 and 1997, dissolved zinc concentrations in the Upper St. Lawrence River were 0.30 µg·L^{-1}, and at Cornwall in the north

shore tributary in Ottawa, dissolved zinc concentrations were 0.589 $\mu g \cdot L^{-1}$. Concentrations of dissolved zinc in the south shore tributaries were 0.589 $\mu g \cdot L^{-1}$ in Richelieu, 0.903 $\mu g \cdot L^{-1}$ in St. Francois, 1.282 $\mu g \cdot L^{-1}$ in Yamaska and 0.543 $\mu g \cdot L^{-1}$ in Nicolet (Rondeau *et al.* 2005). In 2009, all median concentrations for those tributaries were under the detection limit of 0.7 $\mu g \cdot L^{-1}$ and concentrations of dissolved zinc in four north shore tributaries were 0.5 $\mu g \cdot L^{-1}$ in L'Assomption, 1.3 $\mu g \cdot L^{-1}$ in St. Maurice, 2.8 $\mu g \cdot L^{-1}$ in Batiscan and 1.9 $\mu g \cdot L^{-1}$ in Jacques-Cartier (S. Hébert, MDDEP, personal communication, 2010).

6.1.4 Ontario

In the Great Lakes, including Ontario, Erie and Superior, there is a large input of trace metals from anthropogenic activity. However, Nriagu *et al.* (1996) reported that average dissolved concentrations remain quite low (0.087–0.277 $\mu g\ Zn \cdot L^{-1}$, detection limit not reported) due to rapid scavenging by seston and rapid turnover in the water column. In general, higher trace metal concentrations are found near shores, urban centres and polluted river mouths (Nriagu *et al.* 1996). Dissolved zinc concentrations specifically in Lake Ontario have been reported between less than 0.0026 and 0.331 $\mu g\ Zn \cdot L^{-1}$ (mean 0.160 $\mu g\ Zn \cdot L^{-1}$, detection limit = 0.0026 $\mu g \cdot L^{-1}$). Atmospheric deposition is responsible for most zinc flux in surface waters, followed by industrial and municipal discharge (Coale and Flegal 1989; Nriagu *et al.* 1996). Dissolved concentrations in Lake Erie range from 0.0256 to 0.377 $\mu g\ Zn \cdot L^{-1}$ (mean 0.087 $\mu g\ Zn \cdot L^{-1}$), with atmospheric contribution accounting for half the total zinc input and most of the remainder caused by industrial and municipal discharge (Coale and Flegal 1989; Nriagu *et al.* 1996). In Lake Superior, dissolved concentrations ranged from 0.144 to 0.867 $\mu g\ Zn \cdot L^{-1}$ with a mean of 0.277 $\mu g\ Zn \cdot L^{-1}$ (Nriagu *et al.* 1996).

Water quality monitoring data for Ontario streams were available from the Ontario Provincial (Stream) Water Quality Monitoring Network (PWQMN) for 2014. Data were collected from 298 stations throughout the year for total, unfiltered zinc. Mean, median, minimum and maximum zinc concentrations were 15, 12, 0.03 and 537 $\mu g\ Zn \cdot L^{-1}$, respectively (PWQMN 2016).

6.1.5 Alberta, Saskatchewan and Manitoba

The Regional Aquatics Monitoring Program (RAMP) database provided water quality monitoring data for the Athabasca oil sands region of Alberta for the year 2014. Data were collected from 40 sites throughout the year for both dissolved and total zinc concentrations. For dissolved zinc, mean, median, minimum and maximum concentrations were 1.6, 1.0, 0.13 and 41 $\mu g\ Zn \cdot L^{-1}$, respectively (RAMP 2016). For total zinc, mean, median, minimum and maximum concentrations were 3.4, 1.6, 0.3, and 47 $\mu g\ Zn \cdot L^{-1}$, respectively (RAMP 2016).

Most surface water data for the Prairie provinces included information for sites impacted by mines. Ross Lake, near Flin Flon, Manitoba, is a shallow lake adjacent to the Hudson Bay Mining and Smelting Company. This lake had average total zinc concentrations in 1996 between 222 and 838 $\mu g\ Zn \cdot L^{-1}$ (Evans 2000). Data from mine-impacted sites in the Rocky Mountains area of Alberta can be compared to reference streams unaffected by anthropogenic activity. Median zinc concentrations at the Gregg River and Luscar Creek, Alberta, which are impacted by coal mines, ranged from 3.86 $\mu g\ Zn \cdot L^{-1}$ in 2001 to 4.78 $\mu g\ Zn \cdot L^{-1}$ in 2003. Reference sites

unaffected by mine activity, including McLeod River, Whitehorse Creek, Wildhay River, South Berland and Berland rivers, South Sulphur River, and several sites on the Gregg River upstream of mines, had median zinc concentrations ranging from 0.55 µg $Zn \cdot L^{-1}$ in 2002 to 0.13 µg $Zn \cdot L^{-1}$ in 2003 (Wayland and Crosley 2006).

6.1.6 Nova Scotia

The Mersey River in Nova Scotia (potentially impacted due to acidic deposition) has an estimated natural background zinc concentration of 7.135 $\mu g \cdot L^{-1}$ (Tri-Star Environmental Consulting 2006, available from Environment and Climate Change Canada, Gatineau QC, mailto:ec.rqe-eqg.ec@canada.ca). Background concentrations of zinc reported for stream water sampled throughout Nova Scotia range from a minimum of less than 5.0 µg $Zn \cdot L^{-1}$ to a maximum of 11 µg $Zn \cdot L^{-1}$ (Reimann and De Caritat 1998). Surface water monitoring data from Nova Scotia Environment were available for a variety of brooks, lakes and ponds. Thirty-six sites sampled in 1984 had zinc concentrations ranging from 6.1 to 29.4 $\mu g \cdot L^{-1}$, with a median of 7.9 $\mu g \cdot L^{-1}$, while 44 sites sampled in 1996 had a concentration range of 10 to 150 µg $Zn \cdot L^{-1}$ with a median of 40 µg $Zn \cdot L^{-1}$. Forty-five sites sampled between 1998 and 2000 had a zinc concentration range of 10 to 100 $\mu g \cdot L^{-1}$ with a median of 20 $\mu g \cdot L^{-1}$, while 58 sites sampled between 2002 and 2005 had zinc concentrations ranging from 2 to 241 $\mu g \cdot L^{-1}$ with a mean of 6.5 $\mu g \cdot L^{-1}$ (Nova Scotia Environment 2008). As different sites were sampled each year, the varying concentration ranges do not reflect temporal patterns of changing zinc concentrations.

6.2 Concentrations of Zinc in Soil and Sediments

Most Canadian data for zinc concentrations in soil and sediment samples are available for sites impacted by anthropogenic activity and will therefore not be representative of all Canadian environments. Further studies on natural background concentrations and concentrations of zinc in areas with minimal anthropogenic impact are recommended.

6.2.1 New Brunswick and Nova Scotia

Data from the Atlantic provinces were available for various regions of New Brunswick and Nova Scotia. Zinc concentrations in the metal-contaminated sediments of Dalhousie Harbour and Belledune Harbour, New Brunswick, were 519.5 and 6,285.5 $\mu g \cdot g^{-1}$, respectively, compared to 41.5 $\mu g \cdot g^{-1}$ at an uncontaminated control site of Grand Desert Beach, Nova Scotia (Samant *et al.* 1990). Chaleur Bay, located between northern New Brunswick and the south shore of Gaspésie in Québec, receives metals from a variety of sources and had zinc concentrations in sediments ranging from 22 to 3,200 $\mu g \cdot g^{-1}$, with a mean of 100 $\mu g \cdot g^{-1}$ (Parsons and Cranston 2006). Sample sites along the New Brunswick coast of the Bay of Fundy were investigated for metal accumulation in surface salt marsh sediments, and sites were chosen in undisturbed areas and away from point sources of pollution. The mean zinc concentration for all sites was 66 $\mu g \cdot g^{-1}$, with a range of 33 to 100 $\mu g \cdot g^{-1}$ (Hung and Chmura 2007). At each study site, the concentration of zinc appeared to be within its natural range (Hung and Chmura 2007).

6.2.2 Québec

The St. Lawrence River is exposed to a multitude of anthropogenic stresses, including 70% of the Québec population and 75% of Québec industries which are located on its shores (Desrosiers *et al.* 2008). In the fall of 2004 and 2005, concentrations of zinc in sediment samples of three fluvial lakes of the St. Lawrence River and in the Montréal Harbour area were investigated. The concentration ranges of total recoverable zinc were 49–330 $\mu g \cdot g^{-1}$ dry weight (dw) in Lake Saint-François, 31–312 $\mu g \cdot g^{-1}$ dw in Lake Saint-Louis, 62–210 $\mu g \cdot g^{-1}$ dw in Lake Saint Pierre and 61–550 $\mu g \cdot g^{-1}$ dw in Montréal Harbour. The concentration ranges of reactive zinc for each site were 44–310 $\mu g \cdot g^{-1}$ dw in Lake Saint-François, 25–280 $\mu g \cdot g^{-1}$ dw in Lake Saint-Louis, 56–170 $\mu g \cdot g^{-1}$ dw in Lake Saint Pierre and 38–390 $\mu g \cdot g^{-1}$ dw in Montréal Harbour (Desrosiers *et al.* 2008). Natural background levels of zinc in the fluvial section of the St. Lawrence River were determined by taking core samples and adopting the 90th percentile value of the data. In pre-industrial sediment samples (representing concentrations before 1920), zinc concentrations were 86 $\mu g \cdot g^{-1}$, while in postglacial clay samples (representing concentrations 8,000 years ago) zinc concentrations were 150 $\mu g \cdot g^{-1}$ (Environment Canada and Ministère du Développement durable 2007). Concentrations were higher in postglacial clays due to differences in mineralogy and the sources of material making up the sediment matrix (Environment Canada and Ministère du Développement durable 2007).

6.2.3 Ontario

A literature review compared data on background and surface concentrations of zinc in sediments of different areas of the Great Lakes (Table 6.1) (Mudroch *et al.* 1988). Surface measurements refer to samples taken from a sediment depth of 1–5 cm and represent conditions current to the time of study, while background measurements refer to the pre-industrial sediments that were determined through pollen analysis and radiometric dating.

Table 6.1. Reported ranges of surface and background zinc concentrations in the sediments of the Great Lakes (µg·g-1) (number of surveyed reports)

	Ontario	Erie	St. Clair	Huron	Superior
Depositional basins:					
-surface	87–3,507 (3)	18–536 (6)	8–107 (2)	8.2–233 (4)	143–195 (1)
-background	83–163 (2)	8–128 (3)	-	60–88 (2)	53–137.1 (1)
Non-depositional zones:					
-surface	6–1,120 (9)	16–351 (1)	-	-	165–202 (1)
-background	100 (1)	-	-	-	105–117 (1)
Embayments:					
-surface	14–1,225 (1)	-	-	6–230 (6)	36–150 (2)
-background	-	-	-	78–116 (3)	-
Harbours:					
-surface	5–2,010 (6)	12–650 (3)	9–132 (1)	-	-
-background	210 (1)	40–500 (2)	-	-	-
River mouth:					
-surface	24.5–500 (2)	15.7–220.8 (1)	31.2–330.3 (1)	5.7–257.2 (1)	3.0–85.5 (1)

Source: Murdoch et al. (1988).

The Niagara River, which flows northerly from Lake Erie to Lake Ontario, carries fine-grained material in suspension to Lake Ontario, where it is deposited. A study investigating metal

concentrations in the sediment of the Niagara River, at sites exposed to various pollution sources including industrial and municipal discharges and outfall from chemical plants and sewers, found total zinc concentrations ranging from 172 to 1,072 $\mu g \cdot g^{-1}$ dw (Mudroch and Duncan 1986). Other impacted sites investigated in Ontario included areas downwind of the Copper Cliff, Falconbridge and Coniston smelters in the Sudbury smelting area. Mean total zinc concentrations in topsoil samples were 63 (32–146) $\mu g \cdot g^{-1}$ at Copper Cliff, 50 (13–144) $\mu g \cdot g^{-1}$ at Falconbridge and 54 (43–67) $\mu g \cdot g^{-1}$ at Coniston (Adamo *et al.* 2002). Another study evaluated zinc concentrations in surficial sediments of 10 metal-contaminated lakes in the Sudbury region and found concentrations to be greatest in lakes closest to local smelters with a rapid decrease as distance from the smelter increased (Table 6.2) (Bradley and Morris 1986).

Table 6.2 Concentrations of zinc in surficial sediments from various lakes near Sudbury, Ontario (means ± standard error)

Lake	Distance from Sudbury smelters (km)	Concentration of zinc in surficial sediments ($\mu g \cdot g^{-1}$ dw)
Nepawhin	10	448 ± 20
Whitewater	10	340 ± 5
Minnow	10	545 ± 35
Nelson	30	270 ± 35
Ashigami	35	160 ± 15
Vermilion	35	220 ± 1
Fairbank	35	220 ± 20
Kukagami	40	155 ± 10
Tyson	40	235 ± 10
Skeleton	180	130 ± 50

Source: Bradley and Morris (1986).

Sediment samples from 15 lakes in the Sudbury and Muskoka regions of Ontario, which were unclassified as to the level of anthropogenic impact, had zinc concentrations ranging from 37.5 to 105.8 $\mu g \cdot g^{-1}$ dw (see Table 6.3) (Reimer and Duthie 1993).

Table 6.3 Concentrations of zinc ($\mu g \cdot g^{-1}$ dw) in sediment samples from 15 lakes in central Ontario

Lake	Sediment zinc concentration
Clearwater	37.5
Crosson	72.5
Dickie	85.2
Fawn	76.5
Gullfeather	64.5
Hannah	86.8
Harp	90.8
Heney	105.8
Leech	85.8
Leonard	87.9
Lohi	53.9
McKay	102.3
Moot	90.7
Plastic	86.0
Ril	79.0

Source: Table adapted from Reimer and Duthie (1993).

Agricultural areas can receive metal contamination from acidic deposition, commercial fertilizers, pesticides and sewage sludge application (Stone and Droppo 1996). The concentrations of zinc in riverbed sediments of two agricultural catchments in southwestern Ontario were evaluated. For Big Creek, total zinc concentrations in size-fractionated bed sediments ranged from 26.3 to 230.5 $\mu g \cdot g^{-1}$ in summer and from 35.4 to 209.2 $\mu g \cdot g^{-1}$ in spring. For Big Otter Creek, total zinc concentrations in size-fractionated bed sediments ranged from 28.8 to 113.3 $\mu g \cdot g^{-1}$ in summer and from 56.1 to 325.6 $\mu g \cdot g^{-1}$ in spring. With decreasing grain size, there was an increase in concentration and potential bioavailability of zinc in the size-fractionated sediments (Stone and Droppo 1996). Ontario has measured background concentrations of various inorganics in soil, including zinc, and these measurements are referred to as Ontario typical range measurements. The Ontario typical range measurement for zinc is 160 mg/kg (based on 98th percentile) (OMOE 2011). This measure is for rural parklands, where parkland is any area that is not residential, commercial or industrial, transportation right of ways, agricultural, or golf courses. Parkland would therefore include parks, cemeteries, schools, forest or woodlots, and most large undeveloped areas.

6.2.4 Manitoba

The average content of zinc in sediment samples from Ross Lake in Flin Flon, Manitoba, which is adjacent to the Hudson Bay Mining and Smelting Company smelter, were 27,428 $\mu g \cdot g^{-1}$ using an aqua-regia extraction, with a standard deviation of 10,563 $\mu g \cdot g^{-1}$ (Evans 2000). In addition, sequential extractions on sediment samples were performed to extract four fractions: soluble and exchangeable metals (fraction 1), organically bound metals (fraction 2), specifically adsorbed metals (fraction 3) and oxide-bound metals (fraction 4). The average content of zinc in the sediments for fractions 1, 2, 3 and 4 were 1.2, 266, 192 and 64.7 $\mu g \cdot g^{-1}$ respectively (Evans 2000).

Henderson *et al.* (1998) investigated the soil concentrations of zinc in humus and till at 23 sites near a copper-zinc smelter in Flin Flon, Manitoba. Concentrations of zinc at the background sites were 172 and 24 $\mu g \cdot g^{-1}$ in the humus, and 70 and 20 $\mu g \cdot g^{-1}$ in the till (Henderson *et al.* 1998). At sites extending 82 km from the smelter, the range of zinc concentrations were 134–7,908 $\mu g \cdot g^{-1}$ in the humus and 96–426 $\mu g \cdot g^{-1}$ in the till. At sites extending 40 km from the smelter, the range of zinc concentrations were 74–7,428 $\mu g \cdot g^{-1}$ in the humus and 52–756 $\mu g \cdot g^{-1}$ in the till. Samples collected at a distance of 5 km from the smelter were enriched to a maximum of 94 times the background value for the region. At a 10 km distance the zinc concentration was approximately 40 times the regional background value, at 20 km it was 16 times, at 40 km it was 5 times, and at 80 km was 1.6 times (Henderson *et al.* 1998).

6.2.5 British Columbia

One study measured the local natural background level of zinc in surface soil for the Trail area in British Columbia as 168 $\mu g \cdot g^{-1}$ using the 95th percentile (Goodarzi *et al.* 2002). Another study reported the mean, median and upper limit of the natural background range as 94.1, 88 and 152 $\mu g \cdot g^{-1}$, respectively, using the median ± 2 median absolute deviations (Sanei *et al.* 2007). Goodarzi *et al.* (2002) found that bulk concentration of surface soil sampled in the area around

the Trail smelter had zinc concentrations ranging from 85 to 1,632 $\mu g \cdot g^{-1}$ with a mean of 345.2 $\mu g \cdot g^{-1}$ (Goodarzi *et al.* 2002).

The Sumas River is a watershed impacted by intensive agricultural activity. Between 1993 and 1994, minimum and maximum zinc concentrations were 26.6 and 164 $\mu g \cdot g^{-1}$. In 2003 and 2004 concentrations were higher, reaching 190 $\mu g \cdot g^{-1}$, compared to 34 $\mu g \cdot g^{-1}$ at a reference site outside the vicinity of agricultural activity (Smith *et al.* 2007). Vancouver Harbour, impacted by industrial and municipal discharges, marinas, and shipping activities, had zinc concentrations in sediment reaching 410 $\mu g \cdot g^{-1}$ dw compared to 45 $\mu g \cdot g^{-1}$ dw at an unimpacted reference site (Bolten *et al.* 2003).

Additional information on regional background levels of zinc in soil in British Columbia was available from the British Columbia Contaminated Sites Regulation, Protocol 4 (BC MOE 2010). Background concentration estimates ranged from 85 to 200 $\mu g \cdot g^{-1}$: 85 $\mu g \cdot g^{-1}$ at Cariboo; 90 $\mu g \cdot g^{-1}$ in the Greater Vancouver area; 100 $\mu g \cdot g^{-1}$ at Vancouver Island, Lower Mainland, Thompson, Nicola and Okanagan; 150 $\mu g \cdot g^{-1}$ in Skeena and Omineca Peace; and 200 $\mu g \cdot g^{-1}$ at Kootenay (BC MOE 2010).

6.3 Concentrations of Zinc in Aquatic Biota

6.3.1 Concentrations of Zinc in Fish Species Found in Canadian Waters

In 1973, zinc concentrations in fish tissues from Lake Huron at Baie du Doré and Toronto Harbour were analyzed using atomic absorption spectrophotometry. The species studied included alewife, brown bullhead, carp, freshwater drum, gizzard shad, golden shiner, lake whitefish, largemouth bass, longnose sucker, pumpkinseed, rainbow smelt, rock bass, white bass, white sucker and yellow perch. Metal concentrations among the various species were similar, and the authors presented the study results as pooled data for all species (Brown and Chow 1977). Table 6.4 shows these results.

Table 6.4 Concentrations of zinc ($\mu g \cdot g^{-1}$) in tissues of fish of Baie du Doré and Toronto Harbour sampled in 1973

Tissue	Baie du Doré		Toronto Harbour	
	Mean	Range	Mean	Range
Muscle	4.69	2.85–9.20	36.02	16.08–81.98
Liver	15.10	7.58–27.06	89.04	36.62–239.56
Kidney	26.09	6.51–54.46	59.41	44.99–277.78

Source: Table adapted from Brown and Chow (1977).

A monitoring study evaluated the concentrations of zinc in various fish species from 10 metal-contaminated lakes in the Sudbury region of northeastern Ontario. The study investigated the levels of zinc in muscle, liver and kidney of various fish species from the various lakes and found no evidence that the mean concentration differed among species or among lakes (Bradley and Morris 1986). Table 6.5 summarizes the data.

Table 6.5 Range of concentrations (mean ± standard error) (µg·g-1 dw) of zinc in fish muscle, liver and kidney tissue from various lakes near Sudbury, Ontario

Tissue	Yellow perch	Walleye	Smallmouth Bass	Northern pike	White sucker	Lake whitefish	Lake trout
Muscle	23.2 ± 0.5–43.3 ± 1.4	15.7 ± 0.5–33.6 ± 0.8	17.8 ± 0.8–31.0 ± 0.8	16.8 ± 0.5–20.9 ± 1.3	18.6 ± 0.5–25.2 ± 0.8	12.2 ± 0.6–16.4 ± 1.4	11.8 ± 1.6–12.9 ± 0.8
Liver	78.1-171	92.5 ± 5.7–111 ± 7	83.0 ± 7.7–88.6 ± 0.7	98.5 ± 9.1–153 ± 13	106 ± 6–165 ± 20	159 ± 13–175 ± 17	116 ± 11–153± 13
Kidney	N/A	N/A	N/A	N/A	N/A	N/A	104 ± 9–132 ± 11

Source: Bradley and Morris (1986).

Deniseger *et al.* (1990) measured zinc concentrations in fish tissues from Buttle Lake on Vancouver Island, British Columbia, between 1967 and 1986. The ranges of zinc concentrations found in the muscle tissues of *Oncorhynchus mykiss* (rainbow trout), *Oncorhynchus clarkii* (cutthroat trout) and *Salvelinus malma* (Dolly Varden char) were approximately 18–38, 18–28 and 38–40 $\mu g \cdot g^{-1}$, respectively, with control values of approximately 18, 18 and 20 $\mu g \cdot g^{-1}$, respectively. The ranges of zinc concentrations found in the liver tissues of *O. mykiss*, *O. clarkii* and *S. malma* were approximately 110–175, 110 and 175–280 $\mu g \cdot g^{-1}$, respectively, with control values of approximately 110, 110 and 125 $\mu g \cdot g^{-1}$, respectively (Deniseger *et al.* 1990).

In 1999, a study analyzed zinc concentrations in the muscle tissue of the fish species English sole (*Pleuronectes vetulus*) from five samples sites in Vancouver Harbour. Zinc concentrations were determined using flame atomic absorption, and detection limits were 0.02 $\mu g \cdot g^{-1}$ dw. Concentrations of zinc in fish tissues ranged from approximately 16 to 24 $\mu g \cdot g^{-1}$ dw (Bolten *et al.* 2003).

6.3.2 Concentrations of Zinc in Plant Species Found in Canadian Waters

Reimer and Duthie (1993) examined the concentrations of zinc in the tissue of four aquatic macrophytes from lakes in the Sudbury and Muskoka regions of Ontario (Table 6.6.). Neutron activation analysis was used to determine the concentrations of zinc in *Eriocaulon septangulare* (pipewort), *Nuphar variegatum* (yellow water lily), *Nymphaea odorata* (white water lily) and *Pontederia cordata* (pickerelweed) (Reimer and Duthie 1993).

Table 6.6 Concentrations of zinc (µg·g-1 dw) in aquatic macrophytes sampled from lakes in central Ontario

Lake	*Eriocaulon septangulare*	*Nuphar variegatum*		*Nymphaea odorata*		*Pontederia cordata*	
	Whole plant	Shoot	Root	Shoot	Root	Shoot	Root
Clearwater	15.9	-	-	-	-	-	-
Crosson	42.7	7.3	15.7	15.2	14.7	16.7	38.3
Dickie	85.7	11.2	19.2	12.2	16.4	18.8	56.4
Fawn	52.6	10.7	14.7	18.7	28.5	19.2	30.9
Gullfeather	49.1	10.2	17.3	19.0	23.4	16.3	40.3
Hannah	59.0	8.1	15.8	15.3	20.7	1.2	27.0
Harp	53.6	9.1	17.8	13.1	20.6	18.4	78.9
Heney	77.8	16.5	21.2	14.5	13.2	25.8	64.5
Leech	63.2	6.3	16.4	13.2	24.7	20.4	68.2
Leonard	62.3	14.6	15.8	14.4	14.6	19.5	39.2
Lohi	22.7	9.3	8.8	-	-	-	-
McKay	66.1	10.0	18.1	16.0	23.3	29.4	69.7

Lake	*Eriocaulon septangulare*	*Nuphar variegatum*		*Nymphaea odorata*		*Pontederia cordata*	
Moot	47.4	14.1	21.3	18.7	22.4	19.1	37.1
Plastic	59.4	9.2	14.0	13.3	10.5	26.3	70.5
Ril	50.9	12.1	15.9	11.4	7.3	18.8	42.6

Source: Table adapted from Reimer and Duthie (1993).

Pugh *et al.* (2002) examined plants near the Anvil Range lead/zinc mine in Faro, Yukon, for accumulation of zinc in foliage. ICP-AES was used to measure zinc concentrations. Five sites were sampled; sites F1 through F4 were of increasing distance from the mine mill site, and site SC was a control site. For *Ledum groenlandicum* (Labrador tea), zinc concentrations from plants at sites SC, F1, F2, F3 and F4 were approximately 25, 80, 20, 20 and 15 $\mu g \cdot g^{-1}$, respectively. For willow (*Salix* sp.), zinc concentrations from plants at sites SC, F1, F2, F3 and F4 were approximately 100, 375, 190, 150 and 45 $\mu g \cdot g^{-1}$, respectively. For *Vaccinium uliginosum* (bog blueberry), zinc concentrations for sites SC, F1, F2, F3 and F4 were approximately 40, 230, 100, 25 and 55 $\mu g \cdot g^{-1}$, respectively (Pugh *et al.* 2002).

6.3.3 Concentrations of Zinc in Aquatic Mammal Species Found in Canadian Waters

Wagemann *et al.* (1996) assessed zinc concentrations in tissues of whales and seals from the Canadian Arctic. Mean zinc concentrations collected from beluga whales (*Delphinapterus leucas*) in the Western Arctic from 1993 to 1994 were 65.8 $\mu g \cdot g^{-1}$ wet weight (ww) in muktuk (skin and blubber), 25.7 $\mu g \cdot g^{-1}$ ww in muscle, 27.9 $\mu g \cdot g^{-1}$ ww in liver and 26.4 $\mu g \cdot g^{-1}$ ww in kidney tissues. Mean zinc concentrations for *D. leucas* of the Eastern Arctic sampled between 1984 and 1994 for muktuk, muscle, liver and kidney tissues were 86.6, 24.4, 28.8 and 29.4 $\mu g \cdot g^{-1}$ (ww), respectively. Mean zinc concentrations in muktuk, muscle, liver and kidney tissues of the narwhal (*Monodon monoceros*) were 64.8, 24.9, 40.3 and 34.0 $\mu g \cdot g^{-1}$ (ww), respectively (Wagemann *et al.* 1996). For the ringed seal (*Phoca hispida*) sampled in the Western Arctic between 1987 and 1993, mean zinc concentrations in muscle, liver and kidney tissues were 27.1, 41.5 and 38.7 $\mu g \cdot g^{-1}$ (ww), respectively. *P. hispida* sampled in the Eastern Arctic from 1989 to 1993 had mean zinc concentrations of 23.2, 47.9 and 50.4 $\mu g \cdot g^{-1}$ (ww) in muscle, liver and kidney tissues, respectively. *P. hispida* sampled in 1994 in Eureka had mean zinc concentrations of 22.0, 44.6 and 40.8 $\mu g \cdot g^{-1}$ (ww) in muscle, liver and kidney tissues, respectively (Wagemann *et al.* 1996).

6.3.4 Concentrations of Zinc in Invertebrate Species Found in Canadian Waters

Total zinc concentrations in Dreissenid mussels sampled at the outflow of Lake Ontario were assessed using flame atomic absorption spectrophotometry. The mean total zinc concentrations for zebra mussels (*Dreissena polymorpha*), for dry weight of soft tissue, were 171 $\mu g \cdot g^{-1}$ in 1992, 153 $\mu g \cdot g^{-1}$ in 1993, 177 $\mu g \cdot g^{-1}$ in 1994 and 53.4 $\mu g \cdot g^{-1}$ in 1995. Mean total zinc concentrations, for dry weight of soft tissue, for *Dreissena bugensis* (quagga mussel) were 64.3 $\mu g \cdot g^{-1}$ in 1993, 62.1 $\mu g \cdot g^{-1}$ in 1994 and 69.9 $\mu g \cdot g^{-1}$ in 1995 (Johns and Timmerman 1998).

Mytilus edulis (blue mussels) were sampled at nine stations in the Halifax inlet in 1988, which had been receiving untreated industrial and urban wastes for over 200 years. Pools of reproductively active mussels had mean tissue zinc concentrations ranging from 92.1 to 201.7

$\mu g \cdot g^{-1}$ dw. For non–reproductively active mussels, the mean zinc concentrations in tissue ranged from 108.3 to 552.0 $\mu g \cdot g^{-1}$ dw (Ward 1990).

Trace elements, including zinc, were assessed in aquatic insects from reference streams and coal mine-affected streams of the Rocky Mountains in Alberta between 2001 and 2003 (Table 6.7). Three insect taxa were assessed, including the mayfly family Heptageniidae, the caddisfly family Hydropsychidae and the stonefly *Megarcys*. Zinc concentrations were analyzed using ICP-MS.

Table 6.7 Concentrations of zinc ($\mu g \cdot g^{-1}$ dw) in macroinvertebrate taxa from reference and coal mine-impacted sites from the Rocky Mountains in Alberta, 2001–2003. Values are medians, with 25th and 75th percentiles in parentheses

Insect	Reference site			Mine-impacted site		
	2001	2002	2003	2001	2002	2003
Heptageniidae	-	182 (148–204)	189 (179–211)	-	609 (244–714)	725 (670–789)
Hydropsychidae	151 (146–169)	154 (144–170)	184 (178–192)	223 (195–320)	221 (179–292)	293 (268–299)
Megarcys	225 (195–279)	216 (187–258)	250 (235–292)	263 (259–271)	268 (226–290)	319 (298–358)

Source: Table adapted from Wayland and Crosley (2006).

7.0 ENVIRONMENTAL FATE AND BEHAVIOUR OF ZINC

7.1 Speciation of Zinc in the Aquatic Environment

In water, zinc is found in both suspended and dissolved forms and in different chemical species. Several abiotic variables influence the speciation of zinc, the most important of which are pH, alkalinity, redox potential (Eh) and dissolved organic matter (DOM) content. The most common dissolved zinc species in natural waters under aerobic conditions are $ZnOH^+$, Zn^{2+} and $ZnCO_3$ (Florence 1977; Stumm and Morgan 1981).

The predominance of certain zinc forms depends on the abiotic variables cited above. Models based on stability constants for several zinc species can compute zinc speciation in water. For example, Hem (1972) derived a model that gives the predominant solid and dissolved zinc species in water in relation to pH and Eh (redox potential) (Figure 7.1). As speculated by this model, hydroxide-zinc complexes are expected to be the predominant forms at high pH. Zn^{2+} would predominate in acidic and low-alkalinity water, while at a neutral pH $ZnCO_3$ is presumed to be the main zinc species in the system. Under anoxic conditions with low Eh, such as sediments, and in the presence of sulphide ions, zinc is most commonly found as zinc sulphide (ZnS) (Hem 1972; Spear 1981; Turner *et al.* 1981; WHO 2001; EU 2006).

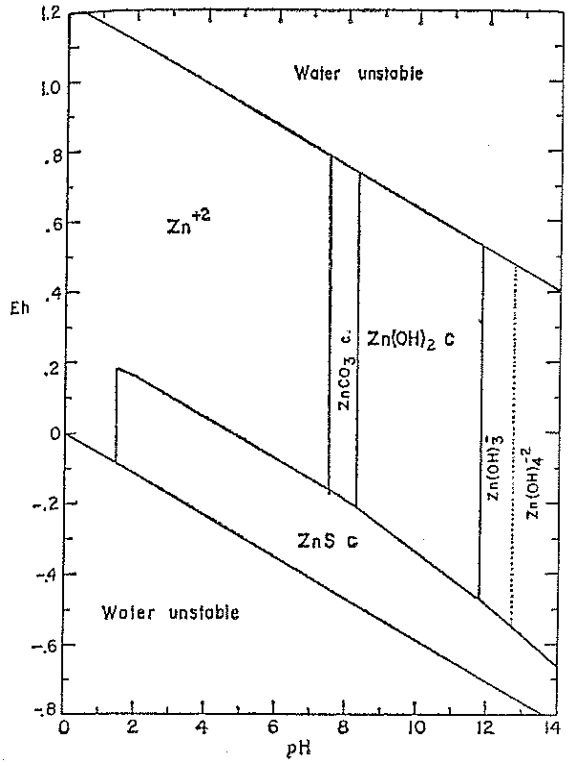


Figure 7.1 Predominant solid and dissolved zinc species in an aqueous system at 25°C in relation to pH and Eh

Source: Figure is from Hem (1972).

7.2 Partitioning of Zinc within the Aquatic Ecosystem

Zinc enters the aquatic environment via atmospheric wet and dry depositions, as dissolved or particulate zinc from terrestrial runoff or even directly from urban and industrial effluents (Clement Associates 1989; WHO 2001). In aquatic environments, zinc can occur in both suspended and dissolved forms, but most zinc introduced into an aquatic system is partitioned into suspended and bottom sediments (Eisler 1993). Consequently, concentrations of dissolved zinc are usually low compared to total zinc concentrations (which includes dissolved and particulate zinc) and zinc concentrations found in sediments.

Several processes control zinc concentrations and mobility in the water column and thus control bioavailability of zinc to aquatic organisms. The following sections briefly summarize the role of sorption, precipitation or co-precipitation, and desorption and dissolution processes on zinc bioavailability for aquatic organisms.

7.2.1 Sorption

The term *sorption* includes different processes occurring at the solid-liquid interface. Adsorption, complexation and absorption are examples of sorption processes controlling zinc partitioning. Adsorption is a process that occurs between a solute molecule and the surface of a solid. It

involves binding forces of different strength, such as low van der Waals forces and strong covalent binding (Stumm and Morgan 1981). The adsorption of zinc on particles in the aquatic system plays an important role in zinc's toxic behaviour because it removes the aqueous zinc from solution. In general, zinc adsorption is not anticipated in acidic water (Spear 1981). In the aquatic environment, several macromolecules, colloids and particulate matter may interact with metals. Zinc can be adsorbed on the surface of colloidal and particulate organic matter (POM) such as cell walls of phytoplankton, which can reduce bioavailability (Spear 1981). Zinc also binds to inorganic material such as clay, silicon, sulphides or manganese and iron hydroxides (Hem 1972; Spear 1981; Stumm and Morgan 1981). Zinc also forms complexes with organic particles such as humic acids. Those substances contain hydrophilic functional groups that contain oxygen, nitrogen and sulphur groups that form strong complexes with metals (Stumm and Morgan 1981). Absorption, or intracellular uptake, of zinc into phytoplankton is also an important process controlling zinc concentrations in surface waters (Sigg *et al.* 2000; Xue and Sigg 1994). Because zinc is an essential nutrient, its uptake by microorganisms leads to an important depletion of zinc concentrations from the photic zone in lakes and oceans (Morel and Hering 1993).

7.2.2 Precipitation or Co-precipitation

Precipitation of zinc controls its mobility and concentration in reducing environments such as sediments. Zinc soluble concentrations vary greatly with pH (Hem 1972). For example, in a freshwater system with a water hardness of approximately 100 $mg \cdot L^{-1}$ as calcium carbonate ($CaCO_{3)}$, the predicted saturated zinc concentration varies from 10.0 $mg \cdot L^{-1}$ for a pH of 6 and 0.01 $mg \cdot L^{-1}$ for a pH of 9 (Hem 1972; Spear 1981). Precipitation of soluble zinc is important only when zinc concentrations are high and in systems under reducing conditions and elevated pH (Cleven *et al.* 1993). Zinc can precipitate as zinc sulphides (ZnS), hydroxides ($Zn(OH)_2$) or carbonates ($ZnCO_3$). Precipitated zinc can thus be found in sediments (United States Environmental Protection Agency [US EPA] 1987).

Zinc can also co-precipitate with all adsorbing agents of high molecular weight, such as those mentioned above (Spear 1981). Zinc bound to the solid phase is scavenged from the water column and buried into sediments. It is thus removed from the water column (Sigg *et al.* 2000). For example, zinc co-precipitates with oxidized iron and manganese hydroxides, which are assumed to be important precipitating agents of metals (Callahan *et al.* 1979). Zinc adsorbed on organic particles also precipitates. In dimictic and eutrophic lakes, the maximum sedimentation rate of zinc together with biological material appears to be during summer stratification (Xue and Sigg 1994).

7.2.3 Desorption and Dissolution

Desorption and dissolution are important processes that control zinc concentrations in solution and in sediments. Zinc organic complexes have low stability compared to other metals (Spear 1981). Other cations, such as calcium, have higher affinity for organic ligands and can thus compete with and replace zinc on particle adsorption sites. This cation competition results in desorption of zinc from particle surfaces. As a result, important displacement and desorption of zinc occurs in estuarine waters with increasing salinity (Callahan *et al.* 1979).

The oxidation state of sediments also influences the solubility of zinc in sediments and overlying water. In chemically reducing conditions, some zinc complexes such as iron and manganese hydroxides are dissociated, leading to the release of dissolved zinc in porewater and its subsequent diffusion through the water column (Spear 1981). Zinc may also dissociate from organic complexes in low Eh environments (Spear 1981).

7.3 Speciation, Bioavailability and Toxicity of Zinc

Besides those physical processes acting on zinc partitioning, all other water quality factors influencing zinc speciation can affect its toxicity (see Section 9.2) because the biological effect of zinc and thus its toxicity is strongly related to its speciation. To cause toxicity, zinc has to interact with biological ligands, such as those found on the surface of gills. Of all zinc species found in aquatic environments, most bioavailability and possible toxicity depend upon the free Zn^{2+} concentration (ANZECC 2000). Less-soluble forms of zinc such as zinc hydroxide ($Zn(OH)_2$) and zinc carbonate $ZnCO_3$, other common forms found in the environment, are considered to be non-toxic (Cairns *et al.* 1971; Spear 1981). Variation in environmental conditions influencing zinc speciation can change zinc bioavailability and toxicity (see Section 9 for detailed discussion). When zinc is above saturation concentration, a modification of environmental conditions, such as a reduction of Eh enhancing zinc precipitation, could also reduce zinc bioavailability and toxicity (Spear 1981).

8.0 EXPOSURE AND UPTAKE PATHWAYS OF ZINC FOR AQUATIC ORGANISMS

8.1 Zinc Exposure and Route of Uptake

The derivation of water quality guidelines focusses on studies in which the exposure route was water, rather than dietary or sediment exposure. Most aquatic zinc toxicity tests attempt to isolate water as the main route of exposure. In long-term studies, where animals must be fed, most studies manage food based on approximate consumption from previous feeding, and excess food is removed. In the environment, zinc may partition into sediment, and accordingly, direct or incidental sediment uptake/ingestion may be a route of exposure. Sediment ingestion is likely an issue with benthic deposit feeders. The zinc CWQG for the protection of aquatic life accounts for aqueous exposure. Sediment exposure of zinc is captured in the CCME sediment quality guidelines for the protection of aquatic life.

8.2 Zinc Bioavailability and the Biotic Ligand Model

The biotic ligand model (BLM) describes the relationship between metal accumulation at a specific site (the biotic ligand) of an aquatic organism and toxicity. For fish, the biotic ligand is considered to be the sodium or calcium channel proteins on the surface of the gill, which regulate blood ionic balance (Di Toro *et al.* 2001). The BLM is an adaptation of both the gill surface

interaction model (GSIM) and the free ion activity model (FIAM) (Di Toro *et al.* 2001). The BLM is built on the assumption that other constituents present in the surrounding water influence the degree of metal binding to the biotic ligand (Paquin *et al.* 2002). For example, dissolved organic carbon (DOC) forms complexes with metals, thus reducing its free ion activity, and ions such as calcium and sodium compete with metals for binding sites on the biotic ligand. If enough metal accumulates at the biotic ligand, toxicity will occur, and this toxicity can be predicted using the model (Paquin *et al.* 2002). Figure 8-1 shows a conceptual diagram of the BLM for zinc (Santore *et al.* 2002).

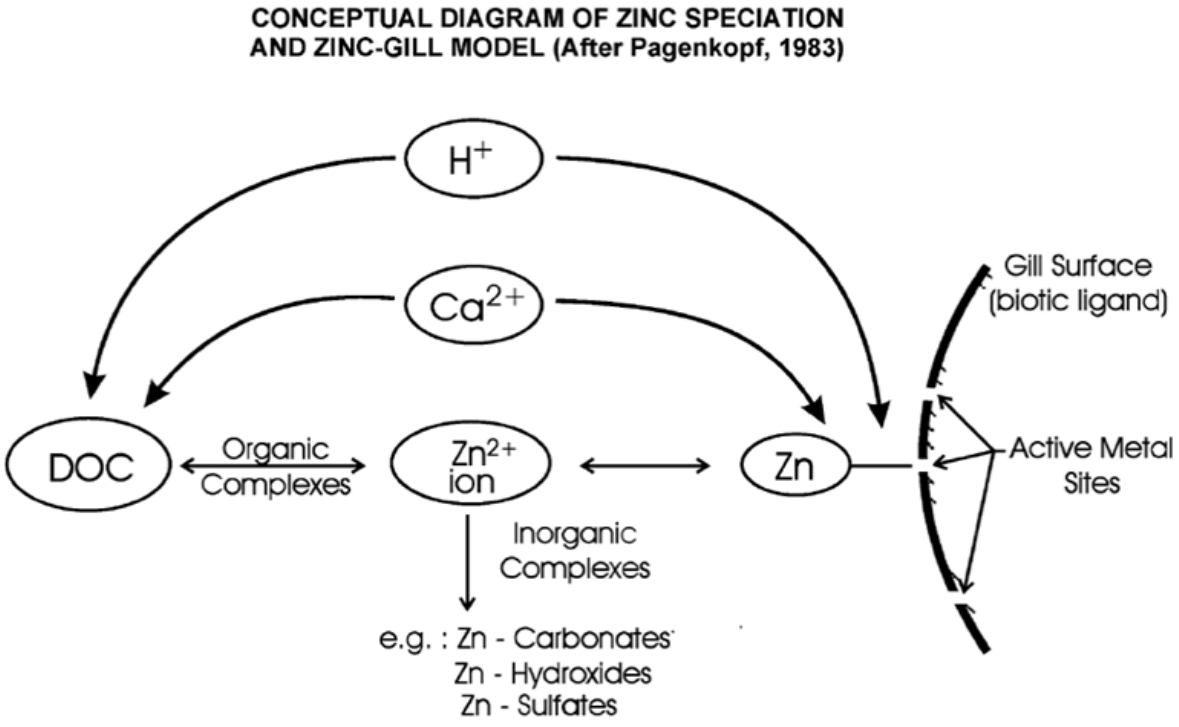


Figure 8.1 A conceptual diagram of the BLM for zinc

Source: Figure is from Santore *et al*. (2002) and is used with permission.

A central premise of the BLM is that the water chemistry of the system is at equilibrium, and that therefore thermodynamic and conditional binding constants can be used to calculate the metal concentrations in the system, including metal bound to the biotic ligand (Paquin *et al.* 2002). The conceptual model has three components: (i) the chemistry of the solution in bulk water, allowing estimation of the free metal ion of interest; (ii) the binding of the metal of concern to the biotic ligand; and (iii) the relationship between the binding of the metal to the biotic ligand and the toxic response (Paquin *et al.* 2002). Although the free metal ion is considered the principal metal species of concern, the BLM does account for toxicity of other species as well (e.g., MOH^n, MCl^n) (Paquin *et al.* 2002). The BLM associates the short-term binding of metals with the short-term toxicity of metals, using the LA_{50} (lethal accumulation) to predict the LC_{50} (Niyogi and Wood 2004). Chronic zinc BLMs that are not based on short-term binding of zinc are also available.

Acute and chronic versions of the zinc BLM have been developed, validated and calibrated for several aquatic species. Acute versions have been developed for *O. mykiss*, *Pimephales promelas* (fathead minnow) (Santore *et al.* 2002), *Daphnia magna* (Heijerick *et al.* 2002*b*) and *Daphnia pulex* (Clifford and McGeer 2009). Chronic BLM versions that are not based on short-term binding of zinc are available for *O. mykiss* (De Schamphelaere and Janssen 2004) and *Daphnia magna* (Heijerick *et al.* 2005). Chronic BLMs have also been calibrated for the rotifer *Brachionus calyciflorus* and the snail *Lymnaea stagnalis* (De Schamphelaere and Janssen 2010). Additionally, BLM versions have been developed for the green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) (De Schamphelaere *et al.* 2005; Heijerick *et al.* 2002*a*) and *Chlorella* (Wilde *et al.* 2006). A unified zinc BLM has also been developed by averaging BLM binding constants for zinc and competing cations from existing zinc BLMs to predict acute and chronic zinc toxicity (DeForest and Van Genderen 2012).

Using the BLM to make site-specific predictions of toxicity involves several steps, including calculating speciation based on water chemistry, calculating free metal activity at the toxicity threshold and calculating the dissolved metal concentration at the toxicity threshold. The full BLM is relatively complex and has intensive data input requirements for ions and chemical parameters of the site-specific water. In order to incorporate a BLM approach into CWQG development, a specialized software package that has simplified data input requirements, is user-friendly to operators without a background in chemical modelling and is customized in alignment with CCME principles and protocol would be required. Currently, some simplified user-friendly programs are available, but they have not been tailored to CCME specifications in terms of data selection, data requirements and protection goals. A multiple linear regression (MLR) approach was used to address toxicity modifying factors of zinc, as it accounts for the most important toxicity modifying factors, is transparent and user-friendly, considers the most up-to-date toxicity data, and meets CCME guiding principles with respect to protection. The main water quality variables affecting zinc toxicity to algae, invertebrates and fish are pH, Ca and DOC (Schamphelaere and Janssen 2004; Brinkman and Woodling 2005; De Schamphelaere *et al*. 2005; Heijerick *et al.* 2005; Wilde *et al.* 2005; Van Sprang *et al.* 2009; De Schamphelaere and Janssen 2010). The MLR approach considered and incorporated these important variables.

8.3 Bioaccumulation of Zinc by Aquatic Organisms

Bioaccumulation, as summarized by Arnot and Gobas (2006) is

> a process in which a chemical substance is absorbed in an organism by all routes of exposure as occurs in the natural environment, i.e., dietary and ambient environment sources. Bioaccumulation is the net result of competing processes of chemical uptake into the organism at the respiratory surface and from the diet and chemical elimination from the organism… (p. 260)

Biomagnification, as summarized by Gobas and Morrsion (2000), is “a process in which the chemical concentration in an organism achieves a level that exceeds that in the organism’s diet, due to dietary absorption” (p. 193).

The study of bioaccumulation of naturally occurring metals can be complicated by several factors, including physicochemical differences between organic and inorganic substances, accumulation of essential and nonessential elements from natural background, homeostatic control of accumulation, and internal detoxification and storage (McGeer *et al.* 2003).

McGeer *et al.* (2003) analyzed bioconcentration data for zinc across eight species groups: algae, insects, mollusks, arthropods, salmonids, centrarchids, killifish and other fish. The mean bioconcentration factor (BCF) was 1,900, when aqueous zinc exposures were between 10 and 110 $\mu g \cdot L^{-1}$ (where chronic toxicity might be expected to occur). The authors found internal zinc content to be well regulated; only slight increases in whole-body zinc concentrations were observed when exposure concentrations were dramatically increased. Due to the lack of increased whole-body concentrations at higher exposure levels, the zinc BCF data demonstrated an inverse correlation with aquatic exposure concentration (McGeer *et al.* 2003). Data analysis of field studies by DeForest *et al.* (2007) also found that BCFs (and bioaccumulation factors) for zinc were inversely related to exposure concentration from both dietary and waterborne exposure.

The accumulation of zinc in tissues of aquatic invertebrates, fish, plants and algae has been widely studied in laboratory experiments. De Schamphelaere *et al.* (2004*a*) assessed internal zinc burdens in the cladoceran *Daphnia magna* after dietary exposure. The food source, green algae, was exposed to zinc concentrations of 0, 20, 30 and 60 $\mu g \cdot L^{-1}$ for 64 hours and had resulting mean internal zinc concentrations of 130, 200, 320 and 490 $\mu g \cdot g^{-1}$, respectively. *D. magna* exposed for 21 days to the algae from the control, 20, 30 and 60 $\mu g \cdot L^{-1}$ treatments (8×10^6 cells per day from day 0 to day 6, 12×10^6 cells per day from day 7 to day 8, and 16×10^6 cells per day from day 9 to day 20) had body burdens of 62.3, 50.9, 55.6, and 84.0 $\mu g\ Zn \cdot g^{-1}$, respectively. The authors suggest the higher body burden of the control group compared to the 20 and 30 $\mu g \cdot L^{-1}$ exposure groups could be due to a regulation mechanism initiated upon exposure to dietary zinc (De Schamphelaere *et al.* 2004*a*).

Table 8.1 presents the internal zinc body concentrations for *D. magna* following aqueous zinc exposure (Muyssen *et al.* 2006).

Table 8.1 Mean internal zinc body concentrations ($\mu g\ Zn \cdot g^{-1}$) for *Daphnia magna* after chronic exposure to various concentrations of water-borne zinc ($\mu g \cdot L^{-1}$)

Duration	Control	80	115	170	250	340
2 d	147	137	181	180	221	200
7 d	155	177	191	229	255	281
14 d	106	105	137	152	-*	-*
21 d	96	103	131	173	-*	-*

*At day 7 all organisms alive at 250 and 340 $\mu g \cdot L^{-1}$ were used for measuring physiological endpoints, so no 14-d or 21-d data are available for these concentrations.
Source: Data adapted from Muyssen et al. (2006).

For fish, the *Cirrhinus mrigala* (mrigal carp) was assessed for zinc accumulation in various tissues in a 96-h static exposure to a range of zinc concentrations (Table 8.2). The heart, kidney, liver and skin of the carp accumulated the greatest amount of zinc, followed by the bones and operculum, followed by the muscles, gills and alimentary canal (Gupta and Sharma 1994).

Table 8.2 Accumulation (ng·100^{-1} mg tissue) of zinc in fingerlings of *Cirrhinus mrigala* during a 96-h bioassay

Zinc concentration in water (mg •L^{-1})	Skin	Gills	Operculum	Alimentary canal	Liver	Heart	Kidney	Muscles	Bones
18.0	3.193	0.231	0.661	0.233	3.13	17.864	6.38	0.632	0.93
10.0	0.870	0.319	0.872	0.426	5.73	15.075	4.81	0.619	0.516
8.7	1.87	0.603	0.697	0.433	5.92	13.183	5.06	0.491	0.441
7.5	0.178	0.235	0.690	0.501	6.12	12.90	3.95	0.22	0.318
0.0 (Control)	0.033	0.076	0.0116	0.061	0.033	0.045	0.98	0.043	0.032

Source: Table adapted from Gupta and Sharma (1994).

Table 8.3 shows the whole-body content of zinc in *O. mykiss* exposed to 250 µg·L^{-1} zinc for 13 days, and the BCF was calculated as 142 (McGeer *et al.* 2000).

Table 8.3 Whole-body zinc content (µg·g^{-1} ww) of *Oncorhynchus mykiss* exposed to 250 µg·L^{-1} zinc or control conditions (mean ± standard error of the mean)

Exposure day	Unexposed control	Zinc exposed
0	27.5 +/- 0.6	-
1	26.4 +/- 1.9	37.4 +/- 2.9*
2.5	30.1 +/- 0.8	31.6 +/- 2.8
3.5	26.4 +/- 1.4	35.5 +/- 1.6*
5.5	28.4 +/- 1.3	33.0 +/- 1.3*
10	25.3 +/- 1.3	32.5 +/- 0.8*

***** Indicates a significant difference from control value on that day.
Source: Data adapted from McGeer *et al.* (2000).

The green algae *Cladophora glomerata* was exposed to zinc concentrations of 150, 400, 750, 1,000, 1,750, 3,500 and 4,000 µg·L^{-1} for a period of up to three hours. Concentration factors were calculated for zinc and found to range from 1.9 to 5.2 × 10^3 (McHardy and George 1990). The vascular plant common duckweed (*Lemna minor*) was exposed to zinc concentrations covering 0–100% effect on growth. The internal concentration of zinc was measured using atomic absorption spectroscopy, and the BCF, calculated as the ratio of internal to external EC_{50}, was 102 (Drost *et al.* 2007).

Although zinc is accumulated in tissues of aquatic plants and animals, biomagnification of zinc in the food web is not a significant process, as BCFs have been observed to decrease with increasing trophic level (Cleven *et al.* 1993). McGeer *et al.* (2003) also state that although there is little evidence of zinc biomagnification in the aquatic food web, it can accumulate to high levels in aquatic organisms, which could be mistaken as trophic transfer.

8.4 Essentiality of Zinc and Deficiency Toxicity

Although zinc can cause toxic effects when present at high concentrations, it is also an essential element needed for a variety of biological functions. Every biologically essential element in each

species has a particular optimal concentration range, which can be determined by the natural concentration range of the element in the species' natural habitat and the species' homeostatic capacity (Muyssen and Janssen 2002*a*). Concentrations in the surrounding environment that greatly exceed or fall below this range cause the homeostatic capacity of the organism to fail, and the effects of toxicity or deficiency can be observed. Aquatic organisms use active regulation or storage/detoxification, or both to regulate their internal concentrations of essential metals (Muyssen and Janssen 2002*a*).

Zinc deficiency can occur as a result of insufficient dietary intake, decreased absorption or use, increased requirements, increased loss, or genetic disease. Zinc deficiency has many symptoms, including hindered growth, hypogonadism in males, neurosensory impairments and cell-mediated immunological malfunction (El Hendy *et al.* 2001). Zinc is an essential element for the proper functioning of over 200 enzymes, including those involved in DNA and protein synthesis, mitosis, and cell division (El Hendy *et al.* 2001; Muyssen and Janssen 2002*a*). Zinc is also a component of numerous transcription factors and proteins that play regulatory roles in the cell cycle. It is an important element for many species in their reproductive cycle. Additionally, zinc is an essential element in a variety of biochemical processes, including the control of cell proliferation and cell degeneration (El Hendy *et al.* 2001).

Several laboratory studies experimentally withheld zinc from exposure systems to investigate the effects of zinc deficiency. Caffrey and Keating (1997) conducted a zinc deprivation experiment with the daphnid *Daphnia pulex* over 23 generations. Withholding zinc from both the liquid medium and solid food resulted in irregular shortening of the lifespan, decreased fecundity and decreased cuticle integrity. *D. pulex* survived for more than 20 consecutive generations before the reproduction line terminated entirely in generation 23 (Caffrey and Keating 1997). The cladoceran *D. magna* exhibited some suboptimal physiology in the control group compared to those organisms exposed to waterborne zinc at a concentration of 80 $\mu g \cdot L^{-1}$ (Muyssen *et al.* 2006). The mean dry weights of control organisms were 19.7 µg after two days of exposure and 99.8 µg after seven days of exposure. Organisms exposed to 80 µg $Zn \cdot L^{-1}$ after two and seven days had mean dry weights of 137 µg after two days and 177 µg after seven days (Muyssen *et al.* 2006). Knauer *et al.* (1997) found growth of the algae *Scenedesmus subspicatus* was optimal at a pZn between 12 and 5.5, while the growth rate declined by 90% at free zinc concentrations less than 3.16×10^{-14} M. Inhibition of growth also occurred at zinc values above the optimal range (Knauer *et al.* 1997).

During the evaluation of studies for guideline derivation, the concept of zinc deficiency was considered in studies conducted at low zinc concentrations by examining dose-response to determine, if possible or applicable, whether effects of deficiency or toxicity occurred. Aquatic environments in Canada are not likely to have zinc concentrations low enough to cause deficiency. Moreover, organisms from environments with naturally low zinc concentrations are expected to have adapted to such conditions.

O. mykiss are able to take up zinc from either water or food relatively independently. Zinc uptake over 16 weeks in *O. mykiss* fingerlings was studied through simultaneous exposure to a range of dietary and waterborne zinc. Diets ranged in zinc concentrations from deficient to excessive. Waterborne zinc ranged from background to 0.53 mg $Zn \cdot L^{-1}$. Fish on zinc-deficient diets at

ambient levels of zinc in Lake Ontario water showed depressed plasma zinc concentration and stopped growing by week 12. Fish on a zinc-deficient diet were able to compensate by taking zinc up from the water across the gills. Even in those fish on zinc-adequate diets, uptake from the water was as high as 57% of the total zinc intake. There were no signs of toxicity at even the highest waterborne and dietary zinc concentrations (Spry *et al.* 1988).

9.0 TOXICITY OF ZINC TO AQUATIC ORGANISMS

9.1 Toxicity Mechanisms and Effects

As mentioned earlier, zinc is an essential element. Because of this, aquatic organisms have developed efficient mechanisms to accumulate zinc from water. Above a certain concentration of zinc, which varies among species and populations, zinc can cause toxic effects (US EPA 1987). Zinc is known to produce adverse effects on reproduction, biochemical and physiochemical reactions, and behaviour of aquatic organisms (WHO 2001). Zinc exerts its toxic effects in aquatic organisms by several mechanisms.

In fish, zinc interferes with gill uptake of calcium (Hogstrand *et al.* 1994; Spry and Wood 1985). Because calcium is also an essential element, this reduction of calcium uptake causes hypocalcemia, or calcium deficiency (Spry and Wood 1985). Zinc also disrupts calcium homeostasis in invertebrates such as *D. magna* (Muyssen *et al.* 2006). This is due to competition between zinc and calcium for the same uptake sites on the apical membrane of the gill epithelium (Hogstrand *et al.* 1994, 1998). Zinc also disturbs, to a lesser extent, sodium and chloride fluxes, resulting in a net branchial ion loss caused by an increase in gill permeability attributed to alteration of ATPase activities (Spry and Wood 1985).

At higher zinc concentrations, lethal toxicity of zinc to aquatic organisms is due to the irreversible destruction of the gill epithelium. This limits oxygen diffusion, causing subsequent tissue hypoxia, osmoregulatory failure, acidosis and low oxygen tensions in the arterial blood (Skidmore 1970; Hiltibran 1971; Skidmore and Tovell 1972).

9.2 Toxicity Modifying Factors

Water chemistry data have an important role in the development and application of the CWQGs. Because water chemistry (e.g., water hardness, alkalinity, pH, DOC) can modify the toxicity of many metals, consideration of these variables must be taken into account in the development of the water quality guideline, and subsequently must be taken into account when applying the guideline. The CCME 2007 protocol does not specify data requirements for quantifying the influence of toxicity modifying factors. For the purposes of this CWQG, all relevant literature was examined for trends in the effect of a given toxicity modifying factor over a range of species.

9.2.1 Hardness

Hardness is most often defined as the sum of calcium and magnesium cations in solution. These elements enter the aquatic environment mainly via the weathering of rocks. Calcium and magnesium salts in water are mostly combined with bicarbonates and carbonates, which govern temporary hardness. Calcium and magnesium salts can also combine with sulphates, chlorides, and other anions and mineral acids, which in that case regulate permanent hardness (Wetzel 2001). Hardness is expressed frequently as calcium carbonate ($CaCO_3$) equivalent ($mg \cdot L^{-1}$).

Hardness modifies zinc toxicity to aquatic organisms. Several hypothetical biological and chemical mechanisms have been proposed to explain this influence. The majority of the research regarding the influence of hardness on zinc toxicity (both long-term and short-term) has demonstrated an antagonistic effect (i.e., as hardness increases zinc toxicity decreases). Several biological mechanisms have been proposed to explain this relationship. This decrease in toxicity is generally attributed to ion competition for binding sites at biological tissues. Calcium and magnesium, which are positively charged like zinc, can be involved in a competitive inhibition mechanism with zinc at membrane-binding sites, resulting in reduced zinc uptake (Zitko *et al.* 1973; Bradley and Sprague 1985; Heijerick *et al.* 2002*b*). Calcium usually has a stronger protective effect on zinc uptake and toxicity than magnesium (Rai *et al.* 1981; Alsop *et al.* 1999; De Schamphelaere and Janssen 2004). Calcium and magnesium ions have been found to bind to fish gill surfaces with the same strength (log conditional equilibrium binding constant of 4.0) (Macdonald *et al.* 2002). This same strength of binding was evident in stability constants derived for *D. magna*, where calcium and magnesium bound to ligands with a log K value of 3.0 (Heijerick *et al.* 2002*b*).

Calcium may exert a more protective effect because the molar concentration of calcium is typically twice that of magnesium in surface waters (Everall *et al.* 1989*b*). Zinc and calcium compete not only for the same non-specific binding sites, but also for binding sites on transport channels. High calcium concentrations in water may cause closure of the apical membrane of chloride cells on fish gills. Because chloride cells appear to be the site of entry of both calcium and zinc, this closure would decrease membrane permeability and uptake of these cations (Spry and Wood 1985; Everall *et al.* 1989*a*; Hogstrand and Wood 1995; Hogstrand *et al.* 1996; Alsop and Wood 1999). Moreover, these high concentrations of calcium in water could also contribute to increased calcium uptake by gills, thus limiting the calcium deficiency of the organisms, one of the effects following zinc exposure (Spry and Wood 1985; Alsop and Wood 1999; Meyer *et al.* 2007). Hard waters have greater ionic strength because of a larger quantity of charged ions in solution. These ions cause electrostatic inhibition of other ions, such as zinc, to approach binding sites on the organism, thereby resulting in lower activity of zinc ions in harder waters (US EPA 1987). Paulauskis and Winner (1988) have also proposed that the decrease in zinc toxicity in hard water could be the result of a difference in the physiological state of the organism. They observed that the addition of calcium and magnesium increased survivorship and brood size in *D. magna*. A portion of hardness effect could thus be independent of its interaction with zinc.

Barron and Albeke (2000) have also observed that without any acclimation in hard water, *O. mykiss* exposed to elevated calcium concentrations during short-term zinc exposure experienced reduced zinc accumulation in whole body and gills. They concluded that even though biological

mechanisms could be responsible for low zinc accumulation in hard water, the dominant mechanism of calcium control of zinc uptake in short-term exposure was mediated by a chemical process. It has been proposed that the free hydrated zinc ion activity could be lowered in hard water. Even though an increase in hardness does not directly affect zinc speciation, increase in hardness is usually followed by an alkalinity and pH increase in natural water. Moreover, in many studies examined, toxicity in hard water was compared to toxicity in soft water obtained from dilution of hard water with distilled water. This softening process results in a simultaneous reduction of pH and alkalinity if not controlled, which is usually the case. The co-variation of pH and alkalinity with hardness thus causes changes in zinc speciation, as mentioned in Section 7.3. The formation of zinc carbonates and solubility reduction in alkaline conditions result in a decrease in the toxic zinc free-ion form (Alsop *et al.* 1999).

Hardness is the most widely studied toxicity modifying factor for zinc. From the large body of research, studies on algal, invertebrate or fish species consistently report a decrease in toxicity with increasing hardness. A regression can be plotted of the natural logarithm (ln) water hardness as the independent variable and ln toxicity as the dependent variable. As can be seen in Figure 9-1 (short-term toxicity) and Figure 9-2 (long-term toxicity), endpoint concentrations increase (representing decreased zinc toxicity) with increasing water hardness in studies ranked as acceptable (primary or secondary) according to the CCME 2007 protocol. Data included in Figures 9-1 and 9-2 are for species where at least two endpoints were available over a range of hardness (following US EPA [2001] guidance that the range of hardness is at least 100 $mg \cdot L^{-1}$ as $CaCO_3$ and the highest hardness is at least three times the lowest). Data for individual species were included from one or multiple studies where hardness was varied and other water and exposure parameters were held constant. Including data for a single species from multiple studies, where possible, allows for a more robust evaluation of the relationship. A slope of the hardness-toxicity regression can be calculated for each individual species. Short-term hardness-toxicity slopes ranged from 0.24 to 2.0 (Figure 9-1), and long-term hardness-toxicity slopes ranged from 0.62 to 1.5 (Figure 9-2). Note these regressions with single, independent variables are data exploration exercises and are not the final models used to correct toxicity responses and become a water quality guideline. Multivariate models (Section 9.3), where effects of other toxicity modifying factors are taken into account, are relevant for guideline derivation.

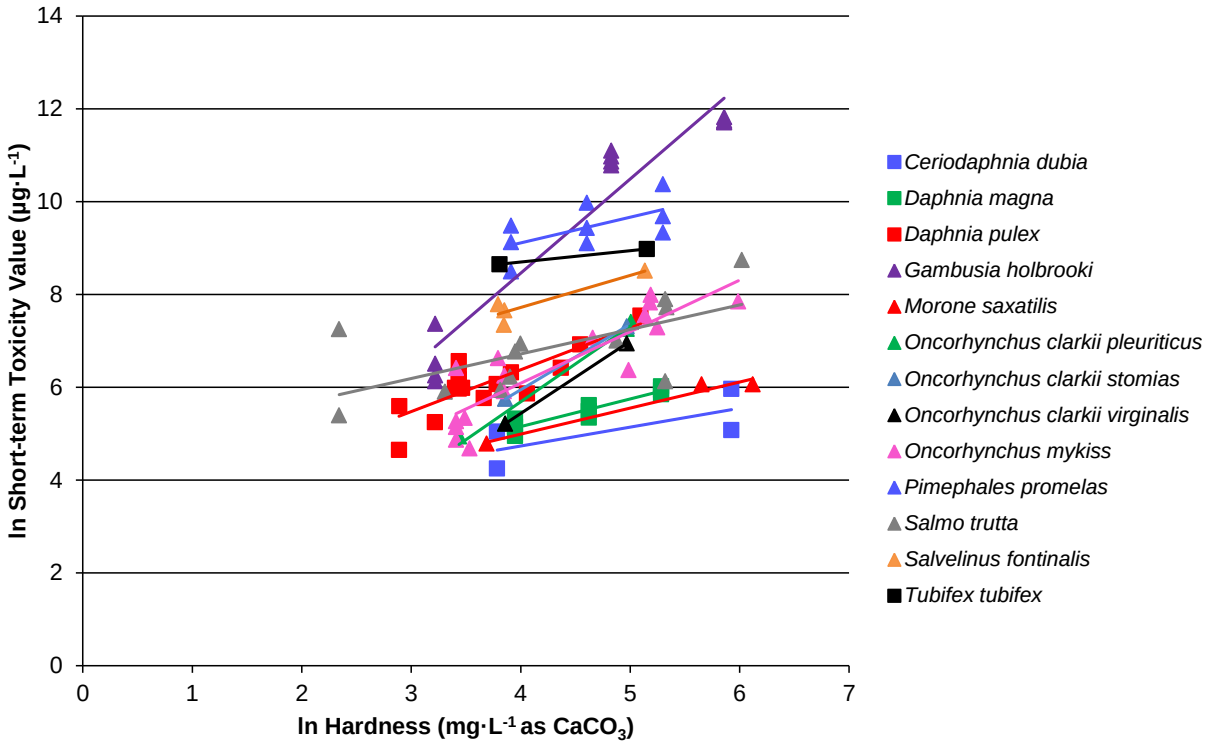


Figure 9.1 Hardness-toxicity regressions for short-term data on a natural logarithmic scale

▲ = endpoints for fish and ■ = endpoints for invertebrates
Endpoints plotted include LC_{50}, EC_{50}, and median tolerance limit (TLm) endpoints.

Data references by species are as follows: *Ceriodaphnia dubia* (Hyne *et al.* 2005); *Daphnia magna* (Paulauskis and Winner 1988); *Daphnia pulex* (Clifford and McGeer 2009); *Gambusia holbrooki* (Pourkhabbaz *et al.* 2011); *Morone saxatilis* (Palawski *et al.* 1985); *Oncorhynchus clarkii pleuriticus* (Brinkman and Hansen 2004; Brinkman and Johnston 2012); *Oncorhynchus clarkii stomias* (Brinkman and Johnston 2012); *Oncorhynchus clarkii virginalis* (Brinkman and Johnston 2012); *Oncorhynchus mykiss* (Holcombe and Andrew 1978; De Schamphelaere and Janssen 2004; Todd *et al.* 2009); *Pimephales promelas* (Mount 1966); *Salmo trutta* (Everall *et al.* 1989*b*; Davies and Brinkman 1999; Davies *et al.* 2002; Davies *et al.* 2003); *Salvelinus fontinalis* (Holcombe and Andrew 1978); *Tubifex* (Rathore and Khangarot 2003).

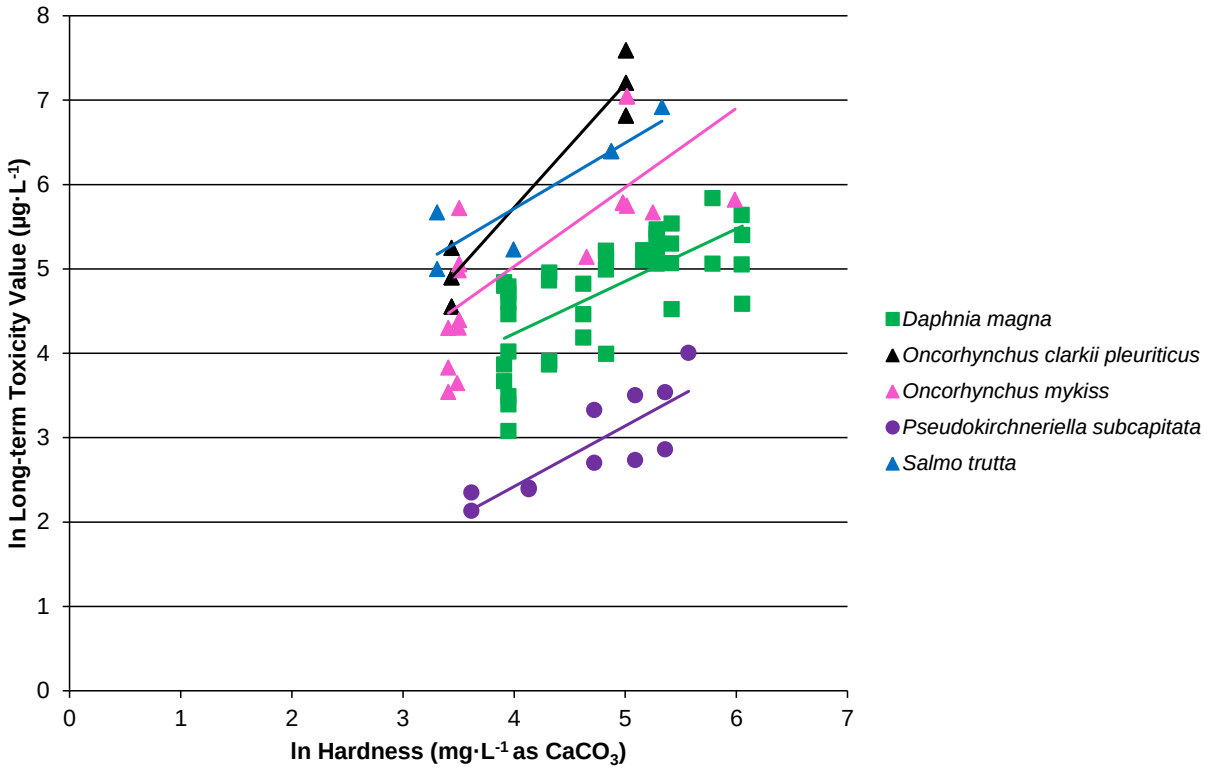


Figure 9.2 Hardness-toxicity regressions for long-term data on a natural logarithmic scale

▲ = endpoints for fish, ■ = endpoints for invertebrates, and ● = endpoints for algae

Data references by species are as follows: *Daphnia magna* (EC_{50} and no-observed-effect concentration [NOEC] endpoints: Heijerick *et al.* [2005]; IC_{10} and maximum acceptable toxicant concentration (MATC) endpoints: Paulauskis and Winner [1988]); *Oncorhynchus clarkii pleuriticus* (NOEC, LOEC and MATC endpoints: Brinkman and Hansen [2004]); *Oncorhynchus mykiss* (MATC endpoints: Brinkman and Hansen [2004]; LC_{10} endpoints: De Schamphelaere and Janssen [2004]); *Pseudokirchneriella subcapitata* (EC_{50} endpoints: Heijerick *et al.* [2002*b*]); *Salmo trutta* (MATC endpoints: Davies and Brinkman [1999]; MATC endpoints: Davies *et al.* [2003]).

Two studies from the literature found that the protective effect of calcium was reduced above a certain level of hardness. Rai *et al.* (1981) observed that increases in calcium concentrations decreased zinc toxicity for the green microalgae *Chlorella vulgaris*, albeit above 20 mg calcium·L^{-1} the detoxifying effect was reduced. However, the study does not report if the other water quality variables were maintained as constant. The observed change in toxicity with increasing hardness could be attributable to a change in pH. In a second study, *D. magna* had higher long-term EC_{10} values at intermediate hardness values of 200–250 mg·L^{-1} as $CaCO_3$, but at very high hardness values there was an adverse effect on daphnids (Heijerick *et al.* 2003). Heijerick *et al.* (2005) later confirmed this positive correlation between hardness and toxicity to very high hardness was attributed to intolerance by the organisms to high hardness level.

Due to the large body of evidence and available data demonstrating the importance of hardness as a toxicity modifying factor for zinc, hardness was considered for inclusion in development of the short-term benchmark and CWQG.

9.2.2 pH

pH (hydrogen and/or hydroxide ions) is often considered a toxicant in itself for aquatic organisms. Low and high pH outside the optimal range for an organism could cause sublethal stress and, when combined with high concentrations of zinc, the toxicity of those two factors could be additive (Everall *et al.* 1989*b*).

Furthermore, pH can also influence zinc toxicity by affecting its speciation and solubility. In natural waters containing organic matter and other types of ligands, increasing concentrations of metals such as zinc are usually observed with a decrease in pH. This is because metal complexes with both organic and inorganic ligands are likely to dissociate as pH decreases, resulting in increased free ion zinc concentrations in water (Campbell and Stokes 1985; Playle 1998). Zinc bioavailability thus increases at acidic pH. At high pH, particulate complexes predominate, which can lead to zinc precipitation. When taking into account pH influence on zinc speciation and thus availability to aquatic organisms, zinc toxicity is likely to decrease with increasing pH.

However, pH also affects zinc interaction at the gills and cell interface in different ways. First, protons could compete with other cations such as zinc for the same binding sites at the cell surface. Secondly, a modification of the water pH could also alter the affinity between zinc and the membrane-binding sites by causing conformational changes in the metal-binding sites. Finally, a reduction of pH could eventually lead to the depolarization of the negatively charged membrane, which would also result in a decrease in affinity between zinc and the cell membrane (Campbell and Stokes 1985). Consequently, the ameliorating effect of low pH on zinc toxicity could be attributed to reduced zinc binding to the cell membrane and thus a decrease in its uptake by organisms.

Because pH may affect zinc toxicity by influencing zinc speciation and biological sensitivity in antagonistic ways, a wide range of effects could be expected depending on the toxicity test and analytical methods. The type of water (e.g., natural water containing ligands versus artificial water) used in the experiments can influence what kind of effect pH will have on zinc toxicity. Moreover, the way zinc concentration is expressed (total versus Zn^{2+}) can influence the effect of pH on zinc toxicity. If zinc concentrations are expressed as Zn^{2+}, only the effect of pH on the interaction of zinc and biotic ligand is measurable. If results are expressed in total concentrations, both speciation and biological processes will be measured.

Many studies that have assessed the effect of pH on zinc toxicity used artificial or filtered water containing no ligands that could control zinc speciation. Therefore, these studies could not assess the effect of pH on zinc speciation because the lack of ligands mostly implies surface sorption reactions with organic and inorganic particles. If no ligands are present, pH should affect zinc toxicity by increasing organisms' sensitivity at high pH due to decrease of zinc binding to competing ligands. Several studies have thus shown an increasing toxicity with rising pH, including Everall *et al.* (1989*b*) for *S. trutta*, Mount (1966) for *P. promelas*, Schubauer-Berigan *et al.* (1993) for *P. promelas* and *Hyalella azteca*, Cusimano *et al.* (1986) for *O. mykiss*, De Schamphelaere and Janssen (2004) for *O. mykiss* and *P. subcapitata*, Wilde *et al.* (2006) for *Chlorella* sp., Heijerick *et al.* (2002*b*) for *P. subcapitatagreen*, and Hyne *et al.* (2005) for *C. dubia*.

Although theory predicts increased toxicity with pH in waters containing no ligands, some studies reported an inverse relationship, unclear patterns or no effect of pH on zinc toxicity. Short-term studies on *D. magna* and *D. pulex* found reduced zinc toxicity with increasing pH (Heijerick *et al.* 2002*b*; De Schamphelaere *et al.* 2004*b*; Clifford and McGeer 2009). Starodub *et al.* (1987*b*) also reported decreased zinc toxicity with increasing pH for the green algae *Scenedesmus quadricauda*. A pH increase of four units produced an increase in the lowest-observed-effect concentration (LOEC) value by a magnitude of five. No clear linear relationship between variation of pH and zinc toxicity was observed when long-term data with *D. magna* were compiled (Heijerick *et al.* 2003, 2005). Spry and Wood (1984) observed that pH had no significant effect on zinc toxicity to *O. mykiss*.

Few studies have assessed the influence of pH in natural water. In these studies, pH could have an antagonistic effect. As mentioned earlier, pH could affect both zinc speciation and biological sensitivity to zinc. All short-term studies done on fish (*O. mykiss* and *S. fontinalis*) reported or predicted that an increase in pH would also result in an increase in toxicity (Cusimano *et al.* 1986; Holcombe and Andrew 1978). However, inconsistent results were reported for invertebrates. Belanger and Cherry (1990) observed that a pH increase of 3.0 units resulted in a mean 1.7 fold increase in toxicity to *C. dubia* in different natural waters. Heijerick *et al.* (2003) observed no general pattern between pH variation and zinc toxicity to *D. magna* in artificial water of different DOM concentration and hardness.

Data from acceptable (primary or secondary) studies where a range of pH was tested were plotted in a regression, with pH as the independent variable and ln toxicity as the dependent variable (Figure 9-3 for short-term toxicity and Figure 9-4 for long-term toxicity). Data for individual species were included from one or multiple studies where, within that study, at least two endpoints were available over a range of pH (spanning 1.5 units), while other water and exposure parameters were held constant. Including data for a single species from multiple studies, where possible, allows for a more robust evaluation of the relationship. A slope of the pH-toxicity regression can be calculated for each individual species. Figures 9-3 and 9-4 demonstrate some variability between species in terms of magnitude and direction of slopes, but most fish and algal species demonstrated increased toxicity with increasing pH. Note these regressions with single, independent variables are data exploration exercises and are not the final models used to correct toxicity responses and become a water quality guideline. Multivariate models (Section 9.3), where effects of other toxicity modifying factors are taken into account, are relevant for guideline derivation.

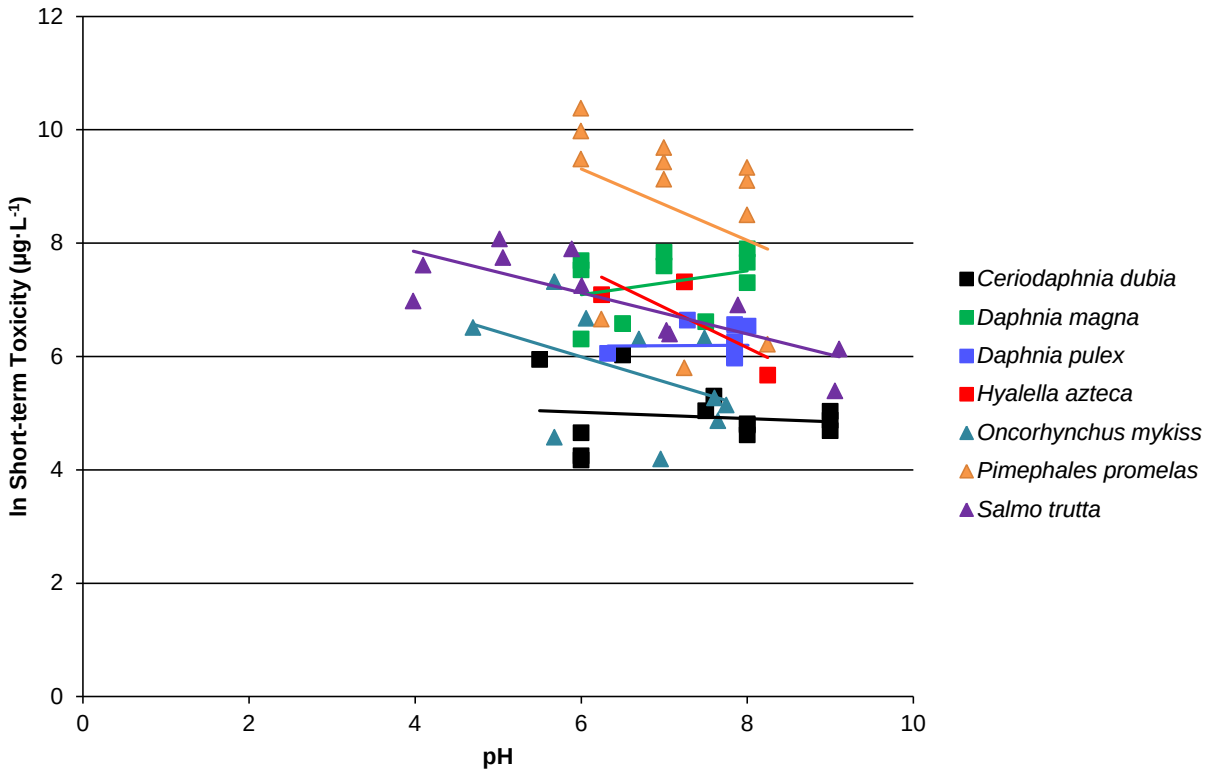


Figure 9.3 pH-toxicity regressions for short-term data on natural logarithmic scale

▲ = endpoints for fish and ■ = endpoints for invertebrates.
Endpoints plotted include LC_{50}, EC_{50} and TL_m endpoints.

Data references by species are as follows: *Ceriodaphnia dubia* (Belanger and Cherry 1990; Hyne *et al*. 2005); *Daphnia magna* (De Schamphelaere *et al.* 2004*b*, Heijerick *et al.* 2002*b*); *Daphnia pulex* (Clifford and McGeer 2009); *Hyalella azteca* (Schubauer-Berigan *et al*. 1993); *Oncorhynchus mykiss* (De Spry and Wood 1984; Cusimano *et al.* 1986; Schamphelaere and Janssen 2004); *Pimephales promelas* (Mount 1966; Schubauer-Berigan *et al*. 1993); and *S. trutta* (Everall *et al*. 1989*b*).

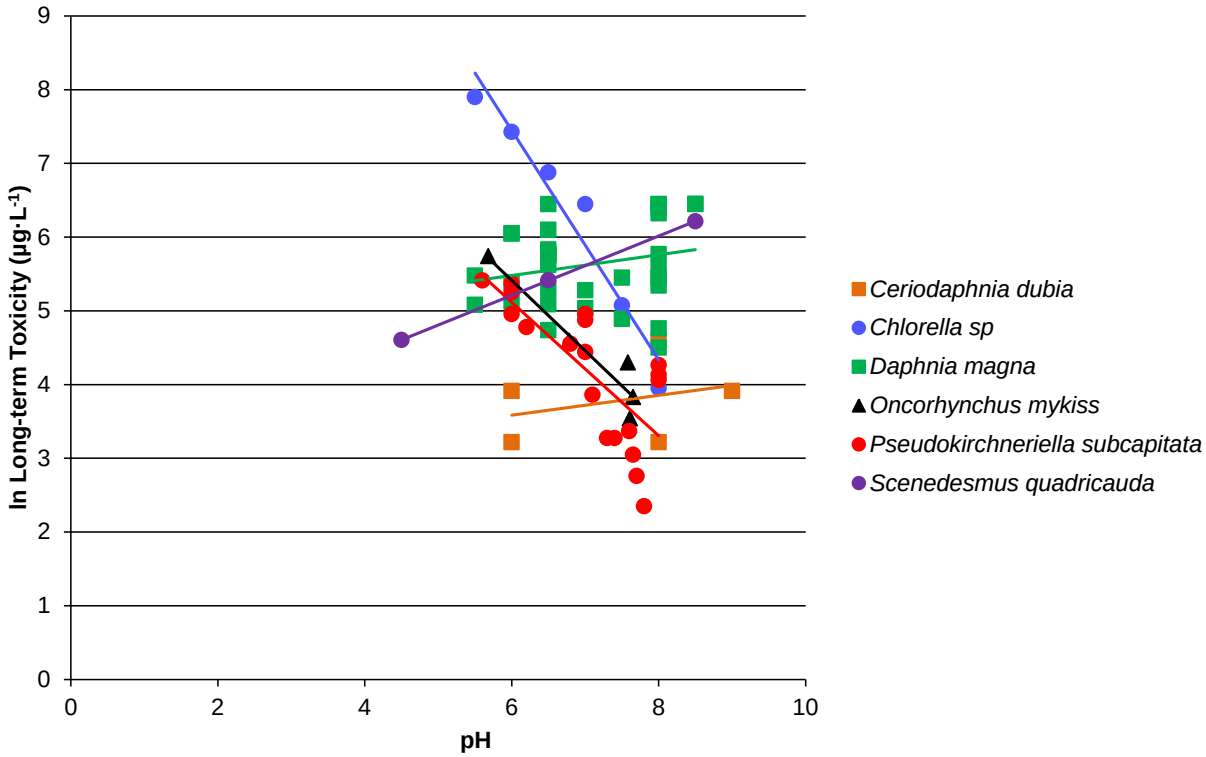


Figure 9.4 pH-toxicity regressions for long-term data on a natural logarithmic scale

▲ = endpoints for fish, ■ = endpoints for invertebrates and ● = endpoints for algae.

Data references by species are as follows: *Ceriodaphnia dubia* (LOEC endpoints: Belanger and Cherry 1990); *Chlorella* sp. (IC_{50} endpoints: Wilde *et al.* 2006); *Daphnia magna* (EC_{10}, EC_{50} and NOEC endpoints: Heijerick *et al.* 2003, 2005); *Oncorhynchus mykiss* (LC_{10} endpoints: De Schamphelaere and Janssen 2004); *Pseudokirchneriella subcapitata* (EC_{50} endpoints: De Schamphelaere *et al.* 2004; Heijerick *et al.* 2002*a*); and *S. quadricauda* (LOEC endpoints: Starodub *et al.* 1987*b*).

Due to the large amount of data assessing the influence of pH on zinc toxicity and considering the increased sensitivity of algal species to zinc as pH increases, this variable was considered in guideline development.

9.2.3 Alkalinity

Alkalinity is defined as the capacity of water to buffer or neutralize acid. In many surface waters, alkalinity is primarily due to carbonate concentrations (Wetzel 2001). In the environment, one main source of both hardness and alkalinity is dissolved limestone ($CaCO_3$), which creates conditions in which hardness and alkalinity can co-vary. An alkalinity increase is also frequently associated with an increase in pH. However, conceptually, hardness, pH and alkalinity alter toxicity through different mechanisms. Alkalinity affects zinc toxicity by reducing free ion concentrations in water. In alkaline waters containing high carbonate concentrations, zinc forms complexes with bicarbonate and carbonate molecules, which affect metal bioavailability (De Schamphelaere and Janssen 2002; Hyne *et al.* 2005). Most of the studies reviewed through this process have not tested the influence of alkalinity and hardness on zinc toxicity separately. Very few studies have assessed the influence of alkalinity alone, which would require maintaining constant water hardness.

In a short-term study with *O. mykiss*, Bradley and Sprague (1985) observed that an increase in alkalinity from 8.4 to 24 $mg \cdot L^{-1}$ at pH 7 did not influence zinc toxicity for either hard or soft water. They concluded that carbonate alkalinity was not an important factor affecting acute toxicity at or below pH 7. In a more recent study, Barron and Albeke (2000) confirmed these results by demonstrating that a seven-fold increase in bicarbonate concentration had no effect on zinc uptake by *O. mykiss*. In another study, Holcombe and Andrew (1978) assessed alkalinity influence by comparing zinc toxicity at two different hardness values, once in allowing alkalinity to co-vary with hardness and in a second experiment by maintaining alkalinity constant at 42–43 $mg \cdot L^{-1}$ as $CaCO_3$. By applying a stepwise multiple regression on their results, they concluded that carbonate alkalinity and hardness were equally important factors governing the toxicity of zinc to *O. mykiss* and *S. fontinalis*.

Although in theory alkalinity could influence zinc toxicity, there are insufficient data to demonstrate this influence without the confounding effect of hardness. Therefore, no adjustment for alkalinity was made in the development of the guidelines.

9.2.4 Dissolved Organic Matter

Organic matter plays an important role in aquatic systems. Organic matter is a mixture of dead plants, microorganisms and animals at different stages of decomposition (Wetzel 2001). Organic matter found in a water system can be from terrestrial sources (allochthonous) or directly from the system, mainly from phytoplankton biomass (autochthonous). Organic matter is a general term that refers to compounds with a large range of sizes and properties. Larger constituents are classified as particulate organic matter (POM). Smaller constituents are classified as dissolved organic matter (DOM). DOM is usually separated from POM by filtration through a 0.45 µm pore filter. DOM constitutes about 85–90% of organic matter in lakes (Wetzel 2001). DOM is classified in two categories. The first is the non-humic substances, which include carbohydrates, proteins, amino acids and other low molecular weight substances. Their concentrations in water are usually low. The second category is humic substances, which represent 70–80% of organic matter in water and are very heterogeneous. Humic substances in soil and sediment can be separated into humic acids, fulvic acids and humin. These three fractions differ in their molecular weight and functional group (Wetzel 2001). Aquatic toxicity studies often report measurement of organic matter in the test water as either DOC or total organic carbon (TOC). TOC consists of DOC and POM.

Because of its carboxylic functional groups, DOM is an important complexing agent for zinc and other metals in the aquatic system. Zinc, and other metals, can bind to fish gills and cause disruptions to ionoregulatory and respiratory functions of the gills (Playle 1998). Competing cations such as calcium (as discussed above) and complexing ligands such as DOM prevent metals from binding to the gills. Generally, DOM reduces zinc bioavailability by forming complexes with large and insoluble organic ligands that are not transported through membranes (Meyer *et al.* 2007). The exception to the general case is where zinc binds strongly to small organic ligands that are lipophilic and membrane soluble. In these cases, DOM would increase zinc uptake by increasing the transfer of metal across biological membrane (Playle 1998).

In a study on the green algae *P. subcapitata* by Errécalde *et al.* (1998), zinc toxicity was enhanced by the addition of 10^{-4} mol·L^{-1} of citrate, a low molecular weight metabolite. Errécalde *et al.* explained these results by demonstrating that the algal membrane recognized citrate and allowed it to permeate. Zinc complexed with citrate could thus enter the cell by accidental transport through algae membrane, resulting in an increased bioavailability and toxicity to *P. subcapitata.*

Dissimilar results were reported in a study done on the marine photobacterium *Vibrio fischeri.* Kungolos *et al.* (2006) observed that adding 10 and 20 mg·L^{-1} of humic acid to the test solution had no significant effect on zinc toxicity. They estimated, from speciation modelling, that at 20 mg·L^{-1} of humic acid, 80% of zinc was still in free ion species because of the low complexation capacity of zinc and humic acid. Kashian *et al.* (2004) obtained comparable results in a study on the influence of organic matter on zinc toxicity and bioaccumulation in an aquatic community. They reported that adding 4.7 mg·L^{-1} of organic matter measured as TOC in test water from a 10-day microcosm experiment did not significantly affect the abundance of macroinvertebrates; number of Ephemeroptera, Plecoptera and Trichoptera taxa; periphyton biomass; or chlorophyll *a* content. They also observed no difference in zinc levels in caddisflies between untreated and TOC-treated artificial streams. However, periphyton accumulated more zinc in streams treated with TOC and zinc compared to zinc alone (Kashian *et al.* 2004). In a short-term toxicity study done on juvenile Atlantic salmon (*Salmo salar*), Zitko *et al.* (1973) and Carson and Carson (1972) observed no effect of 5–10 mg·L^{-1} of humic acid on zinc toxicity. They also attributed this lack of influence to low stability of humic acid-zinc complexes.

On the other hand, most studies done on the influence of DOM on zinc toxicity reported a decrease in zinc toxicity with increasing humic substance content. Hongve *et al.* (1980) observed that in combination with zinc, an increase of only 1.6 mg·L^{-1} of DOM led to partial detoxification of zinc on a phytoplankton community composed mainly of diatoms. Hyne *et al.* (2005) tested the influence of natural DOM in the form of fulvic acid on the acute toxicity of zinc to *C. dubia*. They observed that DOC concentrations between 0.5 and 5mg·L^{-1} had no effect on zinc toxicity. However, the DOC concentration of 10 mg·L^{-1} resulted in a small (1.3-fold) reduction in the toxicity of zinc to *C. dubia*. Clifford and McGeer (2009) reported reduced acute toxicity of zinc to *D. pulex* with increasing DOC when hardness and pH were held constant. In another short-term study on invertebrates, Oikari *et al.* (1992) found that zinc was less toxic to *D. magna* in natural humic water from Lake Louhilampi (Finland) that contained around 20 mg C·L^{-1} compared to a standardized humus-free water. Paulauskis and Winner (1988) obtained similar results in a short-term study, finding a positive and linear relationship between humic acid concentrations and LC_{50} values on *D. magna*. They also tested the influence of humic acid at concentrations up to 1.5 mg·L^{-1} at different water hardness values. They found that reduction of *D. magna* sensitivity due to increased humic acid content was independent of water hardness in short-term exposure. In long-term exposure, humic acid had a stronger protective effect against the toxic effect of zinc on reproduction in soft water than in hard water (Paulauskis and Winner 1988).

In two other long-term studies on *D. magna,* an increase of DOM concentration decreased zinc toxicity (Heijerick *et al.* 2003; Winner and Gauss 1986). Winner and Gauss (1986) observed that the chronic toxicity of zinc on daphnid mortality was significantly (p-value < *0.05*) reduced by

1.5 mg $\cdot L^{-1}$ of humic acid over a 50-d exposure to 125 µg $Zn \cdot L^{-1}$ in soft water. However, this decrease of zinc toxicity was not accompanied by a significant (p-value < *0.05*) reduction of the accumulation of zinc by the organisms. Secondly, in a similar study, Heijerick *et al.* (2003) reported that the addition of 2 to 40 $mg \cdot L^{-1}$ of artificial humic acid reduced zinc toxicity to *D. magna* over a 21-d period. They observed that an increase in DOM reduced net reproductive rate of daphnids, and that this relationship was not affected by water hardness. The relationship was dependent on the pH of the medium, as an increase in pH from 6.5 to 8 led to an increase of EC_{50} value. Finally, a 96-h toxicity study on larval *P. promelas* done with natural DOM from different surface waters in the United States gave similar results: DOM decreased zinc toxicity to *P. promelas* (Bringolf *et al.* 2006). However, as in Hyne *et al.* (2005), a threshold concentration around 11 mg $DOC \cdot L^{-1}$ was needed to decrease zinc toxicity.

Data from acceptable (primary or secondary) studies where a range of DOC was tested were plotted in a regression with ln DOC as the independent variable and ln toxicity as the dependent variable (Figure 9-5 for short-term toxicity and Figure 9-6 for long-term toxicity). Data for individual species were included from one or multiple studies where, within that study, at least two endpoints were available over a range of DOC of 5 $mg \cdot L^{-1}$ (with the highest DOC being three times the lowest DOC), while other water and exposure parameters were held constant. A slope of the DOC-toxicity regression can be calculated for each individual species. The invertebrate species for which this type of univariate data was available demonstrated shallow, positive slopes. Note these regressions with single, independent variables are data exploration exercises and are not the final models used to correct toxicity responses and become a water quality guideline. Multi-variable models (Section 9.3), where effects of other toxicity modifying factors are taken into account, are relevant for guideline derivation.

Due to the availability of data, and the known complexing behaviour of DOC with zinc and other metals that can reduce metal bioavailability, DOC was considered as a variable in guideline development.

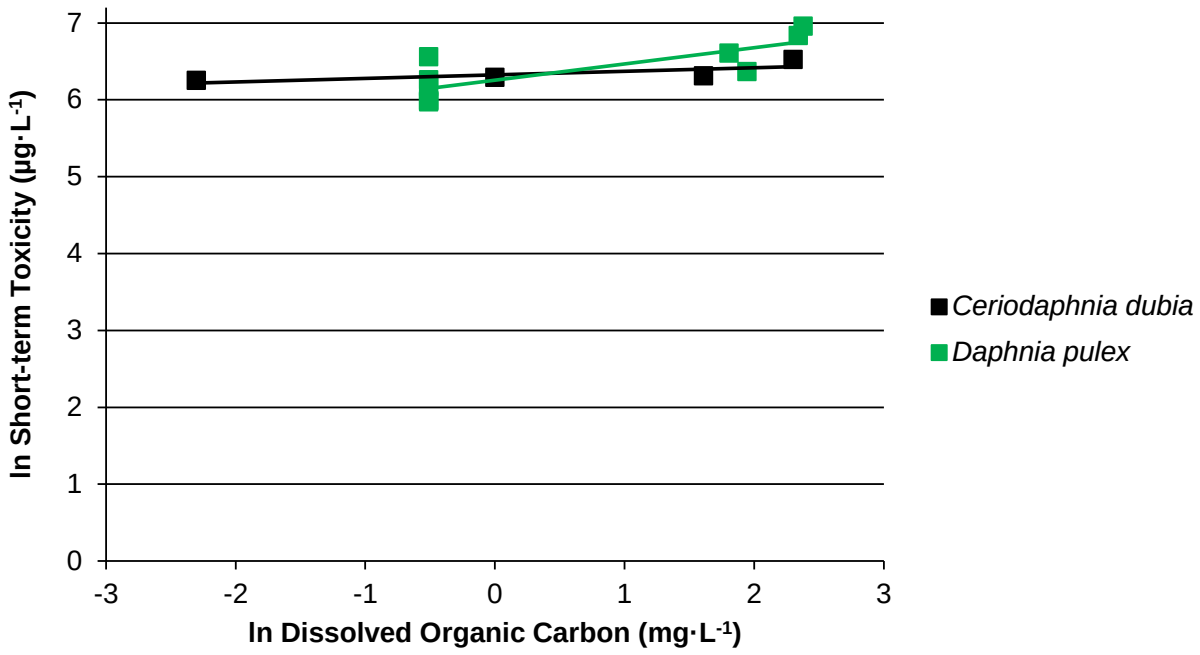


Figure 9.5 DOC-toxicity regressions for short-term data on a natural logarithmic scale.

Endpoints plotted are EC50 for immobility
Source: *Ceriodaphnia dubia* (Hyne *et al.* 2005); *Daphnia pulex* (Clifford and McGeer 2009).

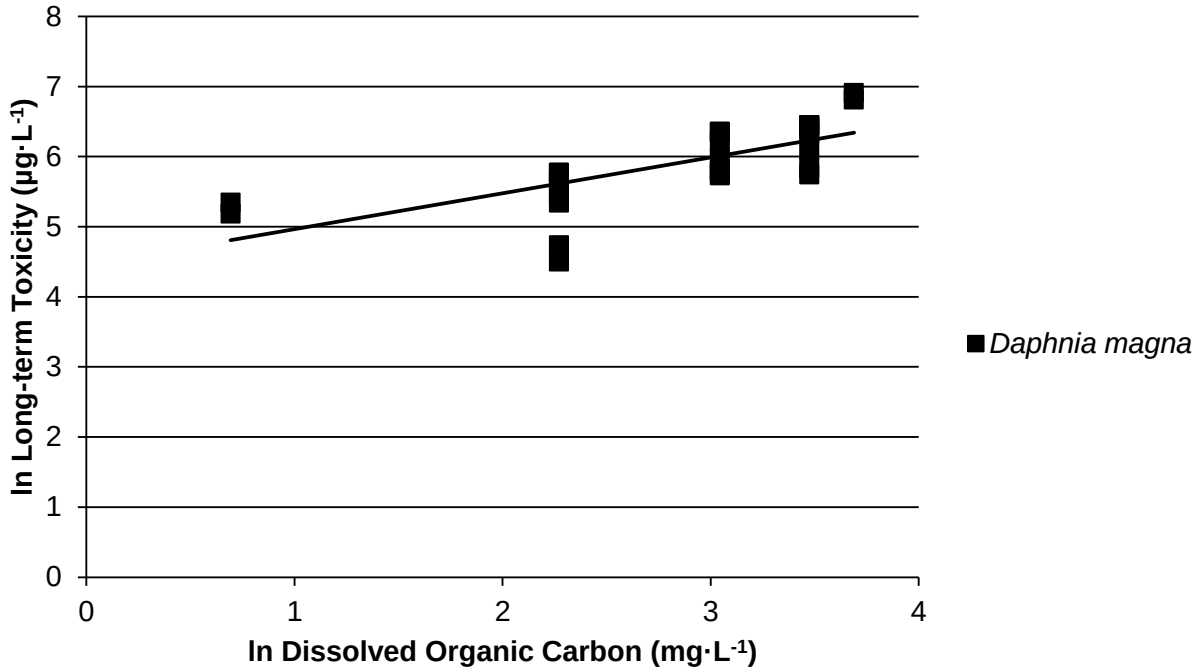


Figure 9.6 DOC-toxicity regression for long-term data on a natural logarithmic scale.

Endpoints plotted include EC10 and NOEC endpoints.
Source: Heijerick et al. (2003).

9.2.5 Suspended Solids

Suspended particles in a water system are usually composed of a mixture of organic and inorganic particles. In general, an increase in suspended particles in the aqueous phase can lead to a decrease in toxicity to aquatic organisms. Most likely, zinc binds or sorbs to the particle surface and consequently becomes less bioavailable and less toxic (Meyer *et al.* 2007). Results published by Hongve *et al.* (1980) directly support this statement. Hongve *et al.* observed that adding 7.6 $mg \cdot L^{-1}$ (in dw) of natural sediments mainly composed of clay particles reduced zinc toxicity to a phytoplankton community. However, in short-term studies with *P. promelas*, Hall *et al.* (1986) reported that although adding 100 to 750 $mg \cdot L^{-1}$ of suspended solids reduced aqueous zinc concentration in test water, a concomitant decrease of zinc toxicity was not observed for all types of sediments tested. The same experiment done with *D. magna* showed that suspended solids decreased zinc toxicity in all tested sediment.

Due to the lack of data demonstrating the influence of suspended solids on zinc toxicity, no correction relationship was developed for suspended solids.

9.2.6 Salinity

Increasing salinity is generally thought to reduce zinc toxicity towards aquatic organisms, because at higher salinities fewer metal free ions are present than at lower salinities (Hall and Anderson 1995). Other studies have shown that interaction of salinity with zinc toxicity could be related to disruption of osmotic regulation (McLusky and Hagerman 1987). Finally, marine organisms could be more susceptible to toxicant such as zinc when exposed at salinities outside their normal salinity range. At conditions under or above an organism's optimal range, salinity could thus become a stressor itself and act synergistically with zinc to enhance zinc toxicity (Beltrame *et al.* 2008; Jones 1975). In this case, no clear relationship between toxicity and salinity could be observed because the lowest toxicity could occur at an intermediate salinity of tested range.

Several studies have reported decreased toxicity of zinc with increasing salinity. In a short-term study involving two estuarine invertebrates, *Corophium volutator* and *Macoma balthica*, Bryant *et al.* (1985) observed that increasing salinity from 5 to 35‰ increased LC_{50} values and survival time for both species. Jones (1975) also reported that salinity was positively related with survival time for the marine species *Idotea balthica, I. neglecta* and *I. emarginata*, and the estuarine species *Jaera albifrons* and *J. nordmanni*. *Nitocra spinipes*, a euryhaline copepod, demonstrated similar results; increasing salinities from 3 to 25‰ resulted in a concomitant increase of 96-h LC_{50} values. However, no clear relationship could be observed between salinity and zinc influence on fecundity in a 13-d experiment with the same species (Bengtsson and Bergström 1987). Beltrame *et al.* (2008) observed that increasing salinity resulted in a decreased zinc toxicity for the South American burrowing crab *Chasmagnathus granulatus* in a short-term study. Finally, Palawski *et al.* (1985) observed that salinity and hardness had similar effects on zinc toxicity on striped bass (*Morone saxatilis*), since 96-h LC_{50} values from hard and saline waters were increased within the same magnitude compared to soft water conditions.

However, other studies reported contrasting results. Cotter *et al.* (1982) observed a longer survival time at lower salinity in toxicity tests with *M. edulis*, even though survivors appeared less healthy at salinity of 22‰ compared to 35‰. Finally, three studies have reported lower zinc toxicity at middle-range salinity with the marine mysid *Praunus flexuosus* (McLusky and Hagerman 1987), the marine polychaete *Nereis diversicolor* (Fernandez and Jones 1990) and the estuarine grass shrimp *Palaemonetes pugio* (McKenny and Neff 1979).

The literature demonstrates that salinity has an effect on zinc toxicity. However, because only a freshwater zinc guideline was developed at this time, salinity was not considered further as a toxicity modifying factor in guideline development. Due to the effect salinity may have on zinc toxicity, it would not be appropriate to apply a freshwater guideline to marine and estuarine environments. Rather, a CWQG derived specifically for marine and estuarine environments would be required.

9.2.7 Temperature

Water temperature can be an important factor affecting chemical toxicity, as most aquatic organisms, except marine mammals, tuna and aquatic birds, are poikilotherms, or cold-blooded organisms (Cairns *et al.* 1975). Because poikilotherms cannot regulate their internal body temperature, variations in water temperature will modify their body temperatures and thus their rates of metabolism. Temperature could be an important variable in lakes or rivers receiving warm industrial effluent (Taylor and Demayo 1980). Temperature increases could lower the ability of aquatic organisms to tolerate toxicants by acting as stress factor synergists (Cairns *et al.* 1975; Khan *et al.* 2006).

Increasing temperature can modify the toxicity of chemicals by increasing an organism's rate of metabolism. The increasing oxygen demand due to higher metabolic demand, combined with the lower oxygen solubility in warmer water, will force the organism to increase its respiratory water inflow through its gills to enhance oxygen uptake, leading to increased exposure to the toxicant (Khangarot and Ray 1987*a*). However, an increase in temperature could also reduce organisms' sensitivity to toxicants by increasing the rate of detoxification mechanisms and excretory processes (Cairns *et al.* 1975).

Several studies have tested the influence of temperature on zinc toxicity to fish, invertebrates and algae during short- and long-term exposures. These studies have observed a wide range of effects because temperature can have multiple effects on zinc toxicity.

Increased zinc toxicity with increasing temperature has been observed in short-term studies of *Carassius auratus* (goldfish), *Lepomis macrochirus* (bluegill), *Notemigonus crysoleucas* (golden shiner) and *Tilapia zilli* (redbelly tilapia) (Cairns *et al.* 1978; Hilmy *et al.* 1987). Furthermore, in a study with *S. salar*, Sprague (1964) observed that a 10°C increase in temperature lowered fish tolerance to zinc, as they survived longer in water at 5°C than at 15°C. However, other studies have shown that rising temperature had no significant influence on zinc toxicity to *Fundulus diaphanus* (banded killifish), *Morone saxatilis* (striped bass), *Lepomis gibbosus* (pumpkinseed), *Morone americana* (white perch), *Anguilla rostrata* (American eel), *Cyprinus carpio* (common carp), *O. mykiss* and *Salvelinus confluentus* (bull trout) (Lloyd 1960; Rehwoldt *et al.* 1971;

Rehwoldt *et al.* 1972; Cairns *et al.* 1978; Hansen *et al.* 2002). In a two-week study, Hodson and Sprague (1975) found that *S. salar* survived for a longer period at lower temperatures when exposed to zinc concentrations between 1 and 25 $mg \cdot L^{-1}$. However, the fish were more tolerant (higher LC_{50}) to zinc at 19°C compared to 3°C.

All invertebrates exposed to zinc at different water temperatures showed a decreasing tolerance to zinc with increasing temperature. Cairns *et al.* (1978) observed that *D. magna, D. pulex* and the annelid *Aeolosoma headleyi* exposed to temperatures ranging from 5 to 25°C showed increasing sensitivity to zinc with increasing temperature. The freshwater snail *Physa heterostropha* also showed a positive correlation between zinc toxicity and temperature in both soft and hard water (Cairns and Scheier 1958). Rathore and Khangarot (2002) have also observed a positive relation between zinc toxicity and water temperature in sludge worms (*Tubifex tubifex*) exposed to temperatures between 15 and 30°C.

Influence of temperature on zinc toxicity to algae has been tested in long-term studies with *S. quadricauda, Chlamydomonas* sp. and *Cyclotella meneghiniana* exposed to temperatures from 5 to 25°C. No clear relationship could be drawn from these data because *S. quadricauda* and *Chlamydomonas* sp. showed reduced zinc toxicity with increasing temperature, while *C. meneghiniana* demonstrated the opposite effect (Cairns *et al.* 1978).

Although it is evident that temperature has an effect on zinc toxicity for aquatic organisms, no general relationship could be derived to adjust or normalize toxicity data for this variable, as the influence of temperature on the toxicity to zinc is clearly species dependent. Therefore, this variable was not considered further for inclusion in guideline development.

9.2.8 Dissolved Oxygen

Low concentrations of dissolved oxygen in water, known as hypoxia, can have important effects on aquatic organisms. Some aquatic organisms, such as fish, have developed physiological abilities to adapt to the short-term stress of hypoxia. One of the most important adaptation mechanisms in fish is the increase of their ventilation rate by increasing stroke volume and breathing frequency, which results in an increase oxygen uptake from water (Hattink *et al.* 2006). In relation to this observation, Lloyd (1960, 1961*a*) presumed that reducing dissolved oxygen in water could lead to increased zinc exposure and uptake by fish gills due to enhanced rate of flow of water. Hypoxia would thus result in an increase of zinc toxicity to fish.

Few studies have tested the assumption that low oxygen concentrations would increase zinc toxicity to aquatic organisms. Lloyd (1960) observed that without fish acclimation to hypoxic conditions, a reduction in the oxygen concentration of water increased the toxicity of zinc to *O. mykiss* in a short-term study. However, when trout were acclimated to low-oxygen conditions, concentration of dissolved oxygen had less effect, if any, on zinc toxicity (Lloyd 1960). In another subacute study, Pickering (1968) obtained contrasting results. They observed increased mortality of *L. macrochirus* as a result of an environmental stress of low dissolved oxygen concentrations, even though the fish were previously acclimated to the hypoxia. Finally, Hattink *et al.* (2006) obtained similar results in a short-term study on carp (*Cyrpinus carpio*). Under

hypoxia, carp were more sensitive to zinc, and survival was about three times shorter than under normal oxygen levels.

Few studies have demonstrated that low oxygen concentration increases toxicity of zinc to fish, so the assumed correlation between oxygen level, ventilation rate and zinc toxicity can not be confirmed. In fact, it has been shown that despite an increased opercular movement and ventilation rate, *C. carpio* and *O. mykiss* exposed to zinc in hypoxic conditions did not accumulate more zinc than fish exposed to zinc in elevated dissolved oxygen concentration in water (Hattink *et al.* 2006; Hughes and Flos 1978). Hattink *et al.* (2006) concluded that the increased toxicity of zinc under hypoxic conditions resulted from the additive effect of the stress of hypoxia and high zinc concentration.

In regard to these results, the additive effect of stress induced by low concentration of dissolved oxygen should be taken into consideration when establishing a water quality guideline or objective for zinc (Taylor and Demayo 1980). However, no general relationship could be derived to adjust or normalize toxicity data for this variable, due to limited data. For this variable to be included in guideline development, multiple taxa representative of the ecosystems and a wide range of the toxicity modifying factors of concern would be needed.

9.2.9 Phosphates

Rai *et al.* (1981) found phosphate significantly reduced the sublethal toxicity of zinc towards the algae *C. vulgaris*, using absorbance after 15 days of exposure as the effect parameter. The range of tested phosphate concentrations was 10,000 to 40,000 $\mu g \cdot L^{-1}$, and at a phosphate concentration of 20,000 $\mu g \cdot L^{-1}$ maximum growth restoration was observed (Rai *et al.* 1981). However, Kamaya *et al.* (2004) found the toxic responses of the algae *P. subcapitata* to zinc appeared similar to all tested concentrations of phosphorus (ranging from 6 to 0.6 μM K_2HPO_4): 72-h IC50 values for zinc at 6.0, 3.0, 1.2 and 0.6 μM P were 44.8, 47.8, 62.0 and 47.7 $\mu g \cdot L^{-1}$, respectively (Kamaya *et al.* 2004). Kuwabara (1985) found phosphorus behaved as a limiting factor in cell yield of the algae *P. subcapitata* and that this yield limitation was exaggerated at higher concentrations of zinc (Kuwabara 1985).

Due to limited data, phosphates were not considered in toxicity modifying factor relationships for the zinc guideline.

9.3 Incorporating Toxicity Modifying Factors into Adjustment Equations

9.3.1 Multiple Linear Regression

The CCME 2007 protocol states that, where possible, it is important to account for exposure and toxicity modifying factors in guideline derivation. This may be done through single or multi-factor equations, matrices or models (CCME 2007). Therefore, multiple linear regression (MLR) analysis was explored as an approach to account for the simultaneous effect of multiple water chemistry variables on zinc toxicity. Section 9.2 describes the independent influence of water chemistry parameters in a subset of data where a single variable was varied while other variables

were kept constant (univariate analysis). Based on these results and advice from current experts, water hardness, pH and DOC were selected as the most important variables to examine through MLR because they are known to affect zinc toxicity.

Forward stepwise MLR analysis was conducted using SYSTAT statistical software (version 13). The analysis determined whether water chemistry parameters could explain a significant portion of variability in zinc toxicity. In forward stepwise MLR, the independent variable (in this case, water hardness, pH or DOC) that explains the greatest amount of the variability in the dependent variable (in this case, zinc toxicity) is entered first. If the relationship between this independent variable and the dependent variable is not significant, the modelling process is considered complete (i.e., no MLR model could be developed). If the relationship is significant, the variable is retained, and the independent variable that explains the greatest proportion of the remaining variability is entered next. If this second variable does not explain a significant additional percentage of the variability, the second variable is removed, and the final model contains only the first independent variable that was entered. If the relationship is significant, the second variable is retained, and the independent variable explaining the next highest proportion of the remaining variability is entered, and so on.

Only data from acceptable studies (primary or secondary) where concentrations of zinc were analytically measured and water chemistry parameters (i.e., hardness, pH and/or DOC) were measured and reported were used in the development of MLR models. MLR analyses were conducted on a species-by-species basis, where toxicity values for a given species were the dependent variables, and the water chemistry parameters were the independent variables. MLR analysis was conducted for a given species if toxicity data were available from tests in which the range of hardness exceeded 100 $mg \cdot L^{-1}$ (with the highest hardness being three times greater than the lowest), the range of DOC exceeded 5 $mg \cdot L^{-1}$ (with the highest DOC being three times greater than the lowest) and the range of pH spanned at least 1.5 units.

The various single-species MLR models were then compared and assessed for their suitability for guideline development. Two criteria were used to choose which species model to use in guideline development: precision and protection. The precision criterion led to selection of a model that was most accurate or explained the highest percentage of the variance in the data. This was examined by plotting model-predicted endpoints versus observed (measured) endpoints and assessing the tightness of the relationship. The protection criterion tested to assure that a final model based on a single, sensitive species was protective of all species in the data set. This was examined by determining the percentage of endpoints in the full acceptable data set (for all species) that fell below the guideline if a particular species MLR model was used to derive the guideline. Note the protectiveness criterion applies only to the long-term guideline, as short-term benchmarks are not meant to meet CCME guiding principles with respect to protection. Rather, short-term benchmarks are meant to estimate severe effects and protect most species against lethality during intermittent and transient events. In contrast, long-term exposure guidelines are meant to protect against all negative effects during indefinite exposures (CCME 2007).

For the long-term guideline, a protection goal of 95% of no- and low-effects endpoints was established, consistent with setting the guideline at the HC_5 level of a no- to low-effects species sensitivity distribution (SSD) curve. Additionally, patterns in protected and unprotected data, as

well as triggers of the CCME protection clause (CCME 2007) (e.g., unprotected severe lethality endpoints, or unprotected species at risk), were examined (Section 11.0) to ensure adequate protection of the guideline.

The process used to select a single species model did not require or rely on the assumption of parallel slopes between various species (i.e., that toxicity modifying factors affected different species in the same way). Rather, the process involved deriving a slope for a single, sensitive species and applying it to all species on the premise that differing responses are not important as long as the model is protective.

9.3.2 Short-term Adjustment Equation

For the short-term MLR analysis, short-term toxicity data were available over the desired range of water hardness, pH and/or DOC for six species: *D. pulex*, *D. magna*, *C. dubia*, *O. mykiss*, *S. trutta*, and *P. promelas*. (See Section 9.5.1 for definitions of short-term exposure for each taxon).

For *D. pulex*, 25 short-term endpoints (48-h EC_{50} values) contained water hardness, pH and DOC data and met the criteria for range of concentrations for these parameters (Clifford and McGeer 2009). For the 25 EC_{50} values included in the analysis, hardness ranged from 18 to 163 $mg \cdot L^{-1}$, pH from 6.32 to 8.01, and DOC from 0.6 to 10.8 $mg \cdot L^{-1}$. In the stepwise MLR analysis, water hardness and DOC were found to be significant parameters ($p < 0.05$), while pH was not (Table 9.1). As shown by the MLR-predicted versus observed short-term EC_{50} values for *D. pulex* (Figure 9-7a), 96% of the predicted *D. pulex* EC_{50} values were within a factor of two of the observed EC_{50} values.

For *D. magna*, seven short-term endpoints (48-h EC_{50} values) contained water hardness, pH and DOC data and met the range of concentrations for these parameters (De Schamphelaere *et al.* 2005). For the seven EC_{50} values included in the analysis, hardness ranged from 13.8 to 250.5 $mg \cdot L^{-1}$, pH from 6.0 to 8.4, and DOC from 0.3 to 17.3 $mg \cdot L^{-1}$. In the stepwise MLR analysis, water hardness and DOC were found to be significant parameters ($p < 0.05$), while pH was not (Table 9.1). As shown by the MLR-predicted versus observed short-term EC_{50} values for *D. magna* (Figure 9-7b), 100% of the predicted *D. magna* EC_{50} values were within a factor of two of the observed EC_{50} values.

Combining the *D. pulex* and *D. magna* data into a single model improved the sample size. The two *Daphnia* species can be combined statistically, as no species effect was detected (i.e., there was no significant difference between the species in the way hardness and DOC affected zinc toxicity), and the pooled model was consistent with the single *Daphnia* species models. The pooled *D. pulex* and *D. magna* model predicted 97% of the *Daphnia* EC_{50} values within a factor of two of the observed values (Figure 9-7c. Table 9.1).

For *C. dubia*, 23 short-term endpoints (48-h EC_{50} values) contained water hardness and pH data and met the range of concentrations for these parameters (Belanger and Cherry 1990; Magliette *et al.* 1995; Muyssen and Janssen 2002*b*; Hyne *et al.* 2005; Cooper *et al.* 2009). Due to the absence of DOC data for this species, the MLR analysis considered only two parameters, hardness and pH. For the 23 EC_{50} values included in the analysis, hardness ranged from 44 to

374 $mg \cdot L^{-1}$ and pH from 5.5 to 8.5. In the stepwise MLR analysis, neither variable was found to be significant (Table 9.1).

For *O. mykiss*, 52 short-term endpoints (96-h to 120-h LC_{50} values) contained water hardness and pH data and met the range of concentrations for these parameters (Solbé 1974; Chapman 1978*b*; Chapman and Stevens 1978; Holcombe and Andrew 1978; Spry and Wood 1984; Cusimano *et al.* 1986; Anadu *et al.* 1989; Alsop and Wood 1999; Hansen *et al.* 2002; Brinkman and Hansen 2004; De Schamphelaere and Janssen 2004; Besser *et al.* 2007; Gündoğdu 2008; Mebane *et al.* 2008; Todd *et al.* 2009). Due to the absence of short-term DOC data for this species, the MLR analysis considered only two parameters, hardness and pH. For the 52 LC_{50} values included in the analysis, hardness ranged from 9.2 to 504 $mg \cdot L^{-1}$ and pH from 4.7 to 8.24. In the stepwise MLR analysis, both hardness and pH were found to be significant parameters ($p < 0.05$) (Table 9.1). As shown by the MLR-predicted versus observed short-term LC_{50} values for *O. mykiss* (Figure 9-7d), 50% of the predicted LC_{50} values were within a factor of two of the observed LC_{50} values. Additional short-term data other than that used to develop the model (e.g., shorter or longer tests, unmeasured zinc concentrations), where relevant water chemistry was measured and reported, were available for this species and were used in model validation (i.e., to assess predicted versus observed LC_{50} values).

For *S. trutta*, 19 short-term endpoints (96-h LC_{50} values) contained water hardness and pH data and met the range of concentrations for these parameters (Everall *et al.* 1989*b*; Davies and Brinkman 1999; Davies *et al.* 2002, 2003). Due to the absence of short-term DOC data for this species, the MLR analysis considered only two parameters, hardness and pH. For the 19 LC_{50} values included in the analysis, hardness ranged from 10.4 to 411 $mg \cdot L^{-1}$ and pH from 3.98 to 9.11. In the stepwise MLR analysis, both hardness and pH were found to be significant parameters ($p < 0.05$) (Table 9.1). As shown by the MLR-predicted versus observed short-term LC_{50} values for *S. trutta* (Figure 9-7e), 17% of the predicted *S. trutta* LC_{50} values were within a factor of two of the observed LC_{50} values. Additional short-term data other than that used to develop the model (e.g., longer tests unmeasured zinc concentrations), where relevant water chemistry was measured and reported, were available for this species and were used in model validation. Due to the poor accuracy of this model in predicting endpoints, it was not considered further.

For *P. promelas*, 19 short-term endpoints (96-h LC_{50} values) contained water hardness and pH data and met the range of concentrations for these parameters (Pickering and Vigor 1965; Mount 1966; Broderius and Smith 1979; Judy and Davies 1979; Parkerton *et al.* 1988; Norberg-King 1989). Due to the absence of short-term DOC data for this species, the MLR analysis considered only two parameters, hardness and pH. For the 19 LC_{50} values included in the analysis, hardness ranged from 45 to 220 $mg \cdot L^{-1}$ and pH from 6 to 8. In the stepwise MLR analysis, pH was found to be a significant variable ($p < 0.05$), while hardness was not. As shown by the MLR-predicted versus observed short-term LC_{50} values for *P. promelas* (Figure 9-7f), 44% of the predicted *P. promelas* LC_{50} values were within a factor of two of the observed LC_{50} values. Additional short-term data other than that used to develop the model (e.g., shorter tests or longer tests, unmeasured zinc concentrations), where relevant water chemistry was measured and reported, were available for this species and were used in model validation. Due to the poor accuracy of this model in predicting endpoints, it was not considered further.

Table 9.1 Summary of MLR analyses for short-term zinc toxicity. See Appendix for data included in analyses

Species	n	MLR model Adj. R^2	p-Values			Model Coefficients (slopes)			y-intercept	% predicted[a]
			ln hardness	pH	ln DOC	ln hardness	pH	ln DOC		
Daphnia pulex	25	0.584	0.000	0.464	0.002	0.845	n/a	0.284	3.196	96
Daphnia magna	7	0.967	0.000	0.487	0.028	0.865	n/a	0.191	3.083	100
Combined *Daphnia pulex* and *Daphnia magna* model	32	0.811	0.000	0.716	0.000	0.833	n/a	0.240	3.224	97
Ceriodaphnia dubia	23	n/a	0.470	0.266	n/a	n/a	n/a	n/a	5.337	n/a
Oncorhynchus mykiss	52	0.486	0.000	0.001	n/a	1.299	-0.905	n/a	7.310	50
Salmo trutta	19	0.481	0.007	0.003	n/a	0.348	-0.347	n/a	7.881	17
Pimephales promelas	19	0.339	0.113	0.005	n/a	n/a	-1.164	n/a	16.872	44

n = sample size

Hardness units were $mg \cdot L^{-1}$

DOC = dissolved organic carbon ($mg \cdot L^{-1}$)

[a] Percent predicted refers to the percentage of endpoints of that species that the model was able to predict within a factor of +/- 2 of the measured value.

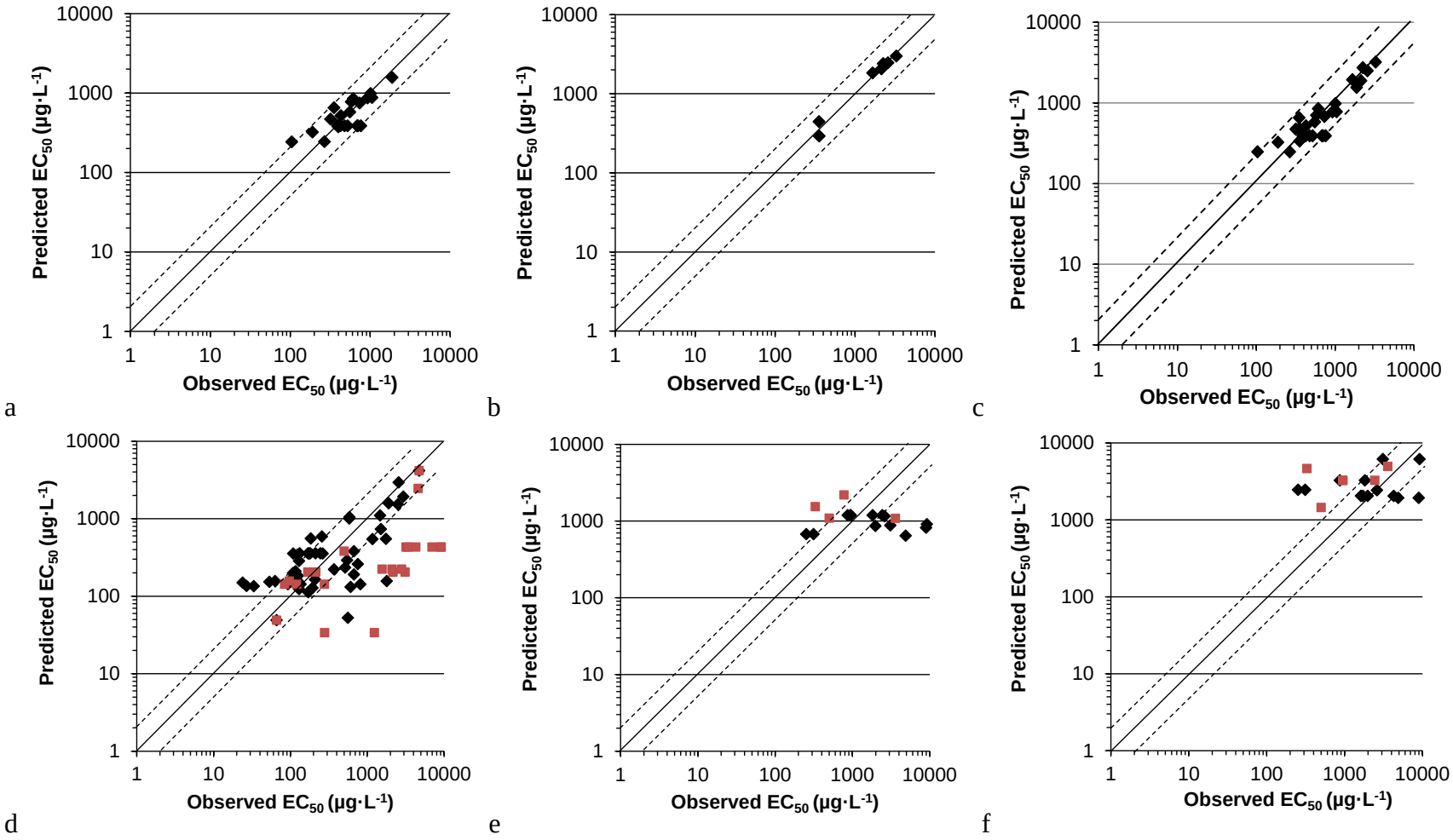


Figure 9.7 Predicted EC_{50} values using the stepwise MLR model for (a) *Daphnia pulex*; (b) *Daphnia magna*; (c) combined *Daphnia pulex* and *Daphnia magna*; (d) *Oncorhynchus mykiss*; (e) *Salmo trutta*; and (f) *Pimephales promelas*

The solid line represents a 1:1 ratio of measured versus predicted EC_{50} values, and the dashed lines represent a factor of +/- 2 from measured values.
◆= data used to develop the MLR model and ■ = additional data available for model validation where relevant water chemistry was measured and reported.

Overall, the *D. pulex* and *D. magna* models performed best in terms of predicting measured EC_{50} values, and both represent sensitive species. The combined *D. pulex* and *D. magna* model was selected as the best model to use in developing a short-term benchmark equation, because it had a larger sample size compared to individual *Daphnia* species models, making it more statistically robust. The pooled model made accurate predictions of EC_{50} values, had a high adjusted R^2 value for how well the model described the data, and covered a broad range of hardness and DOC values. The pooled *Daphnia* model passed tests for constant variance (p = 0.417) and normality (Shapiro-Wilk, p = 0 .211) (SigmaPlot, version 13). Multicollinearity of variables was assessed and not detected (tolerance > 0.1).

The pooled *Daphnia* model can be used to standardize toxicity values for all species in the short-term data set to common water chemistry, in this case to a hardness of 50 $mg \cdot L^{-1}$ and a DOC concentration of 0.5 $mg \cdot L^{-1}$. The general equation to standardize toxicity values to a common hardness and DOC is as follows:

$$\text{Standardized } EC_{50} = \exp[[\ln(EC_{50meas}) - DOC_{slope}(\ln[DOC_{meas}] - \ln[DOC_{target}]) - hardness_{slope}(\ln[hardness_{meas}] - \ln[hardness_{target}])]]$$

Where EC_{50meas} = reported EC_{50}; DOC_{slope} = DOC slope from MLR model; DOC_{meas} = test water DOC; DOC_{target} = standardized DOC (0.5 $mg \cdot L^{-1}$); $hardness_{slope}$ = hardness slope from MLR model; $hardness_{meas}$ = test water hardness; $hardness_{target}$ = standardized hardness (50 $mg \cdot L^{-1}$).

Specifically for the pooled *Daphnia* model, the equation to standardize toxicity values to a common hardness and DOC is as follows:

$$\text{Standardized EC50} = \exp[\ln(EC50meas) - 0.240(\ln[DOCmeas] - \ln[DOCtarget]) - 0.833(\ln[hardnessmeas] - \ln[hardnesstarget])]$$

The short-term data set was standardized to common water chemistry (a hardness of 50 $mg \cdot L^{-1}$ and a DOC concentration of 0.5 $mg \cdot L^{-1}$) using the pooled *Daphnia* model equation. An SSD can then be fit to the standardized data set (Section 10).

9.3.3 Long-term Adjustment Equation

For the long-term MLR analysis, long-term toxicity data were available over the desired range of water hardness, pH and/or DOC for three species: including *D. magna*, *O. mykiss* and *P. subcapitata*. (See Section 9.5.2 for definitions of long-term exposure for each taxon).

For *D. magna*, 24 long-term endpoints (21-d EC_{10} values for reproduction) contained water hardness, pH and DOC data and met the range of concentrations for these parameters (De Schamphelaere *et al.* 2005, Heijerick *et al.* 2003). For the 24 EC_{10} values included in the analysis, hardness ranged from 26.5 to 445 $mg \cdot L^{-1}$, pH from 6 to 8.5, and DOC from 0.3 to 40 $mg \cdot L^{-1}$. In the stepwise MLR analysis, DOC was the only significant parameter ($p < 0.05$), while hardness and pH were not (Table 9.2). As shown by the MLR-predicted versus observed long-term EC_{10} values for *D. magna* (Figure 9-8a), 79% of the predicted EC_{10} values were within a

factor of two of the observed EC_{10} values. The model protected 96% of long-term endpoints in the data set (Table 9.2).

For *O. mykiss*, 14 long-term endpoints (30-d LC_{10} values) contained water hardness, pH and DOC data and met the range of concentrations for these parameters (De Schamphelaere and Janssen 2004; De Schamphelaere *et al.* 2005). For the 14 LC_{10} values included in the analysis, hardness ranged from 23.4 to 399 $mg \cdot L^{-1}$, pH from 5.68 to 8.13, and DOC from 0.3 to 22.9 $mg \cdot L^{-1}$. Although there were insufficient data to assess the individual effect of DOC on long-term *O. mykiss* toxicity with other parameters kept constant (univariate analysis), there were sufficient data that measured and reported DOC to include this variable in the MLR analysis for this species. In the stepwise MLR analysis, hardness, pH and DOC were all found to be significant parameters ($p < 0.05$) (Table 9.2). As shown by the MLR-predicted versus observed long-term LC_{10} values for the *O. mykiss* model (Figure 9-8b), 100% of the predicted *O. mykiss* LC_{10} values were within a factor of two of the observed LC_{10} values. The model protected 96% of long-term endpoints in the data set (Table 9.2; see Section 11 for more details).

For *P. subcapitata*, 30 long-term endpoints (72-h EC_{50} values for biomass) contained hardness and pH data and met the range of concentrations for these parameters (De Schamphelaere *et al.* 2004*b*; Heijerick *et al.* 2002*a*). Due to the absence of long-term DOC data for this species, the MLR analysis considered only two parameters, hardness and pH. For the 30 EC_{50} values included in the analysis, hardness ranged from 19.6 to 262.4 $mg \cdot L^{-1}$ and pH from 5.6 to 8.0. In the stepwise MLR analysis, only pH was found to be a significant parameter ($p < 0.05$) (Table 9.2). As shown by the MLR-predicted versus observed long-term EC_{50} values for *P. subcapitata* (Figure 9-8c), 67% of the predicted EC_{50} values were within a factor of two of the observed EC_{50} values. The model protected 95% of long-term endpoints in the data set (Table 9.2). Additional data other than that used to develop the model (e.g., tests with unmeasured zinc concentrations), where relevant water chemistry was measured and reported, were available for this species and were used in model validation.

Table 9.2 Summary of MLR analyses for long-term zinc toxicity. See Appendix for data included in analyses

Species	n	MLR model Adj. R^2	p-Values			Model Coefficients (slopes)			y-intercept	% Predicted[a]	% Protected[b]
			ln hardness	pH	ln DOC	ln hardness	pH	ln DOC			
Daphnia magna	24	0.411	0.565	0.686	0.000	n/a	n/a	0.391	4.643	79	96
Oncorhynchus mykiss	14	0.898	0.000	0.000	0.000	0.947	-0.815	0.398	7.300	100	96
Pseudokirchneriella subcapitata	30	0.488	0.078	0.000	n/a	n/a	-1.122	n/a	11.8	67	95

n = sample size
Hardness units were $mg \cdot L^{-1}$
DOC = dissolved organic carbon ($mg \cdot L^{-1}$)
[a] Percent predicted refers to the percentage of endpoints of that species that the model was able to predict within a factor of +/- 2 of the measured value.
[b] Percent protected refers to the percentage of endpoints in the long-term acceptable data set for all species that are protected if that species MLR model is used to derive a long-term guideline equation.

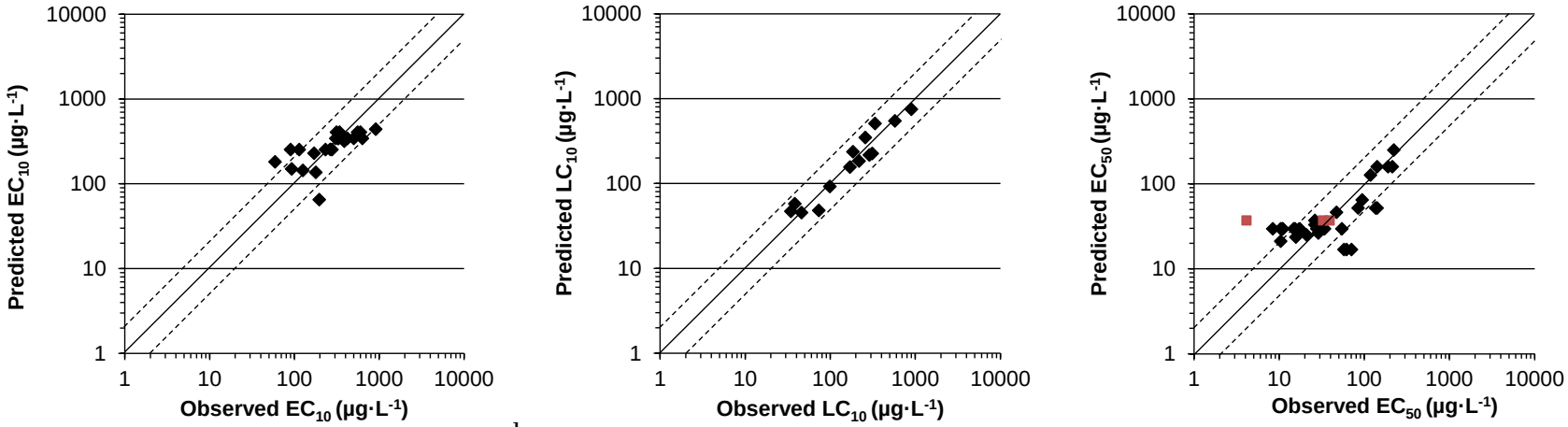


Figure 9.8 Predicted EC/LCx values using the stepwise MLR model for (a) *Daphnia magna*, (b) *Oncorhynchus mykiss* and (c) *Pseudokirchneriella subcapitata*

The solid line represents a 1:1 ratio of measured versus predicted EC/LC_x values and the dashed lines represent a factor of +/- 2 from measured values.
◆ = data used to develop the MLR model and ■ = additional data available for model validation where relevant water chemistry was measured and reported.

The *D. magna* and *P. subcapitata* models were not considered further, because they contained parameters only for DOC and pH, respectively, and not hardness. As seen in the univariate analysis of hardness and long-term toxicity (Section 9.2.1), hardness is an important toxicity modifying factor in long-term exposures for many species. This is also widely supported in the literature. Therefore, a DOC-only or pH-only model would not accurately represent toxicity modifying effects to the range of species in the long-term data set. Therefore, only the *O. mykiss* model was considered further for inclusion in development of a long-term guideline. Additionally, the *O. mykiss* model included a positive slope for hardness (consistent with the literature that organisms are less sensitive to zinc with increasing hardness), as well as a negative slope for pH, which is an important factor for protection of algae, as they demonstrate increased sensitivity to zinc with increasing pH (see Figure 9-4 and De Schamphelaere *et al.* 2003).

Overall, the *O. mykiss* model performed best in terms of predicting measured LC_{10} values. The model had a high adjusted R^2 value for how well the model described the data, and covered a broad range of hardness, pH and DOC values. The model passed tests for constant variance ($p = 0.904$) and normality (Shapiro-Wilk, $p = 0.235$) (SigmaPlot, version 13). Multicollinearity of variables was assessed and not detected (tolerance > 0.1). To assess protectiveness of the *O. mykiss* model, each endpoint in the long-term acceptable data set (n = 606) was compared to what the long-term CWQG value would be at that associated water chemistry if the *O. mykiss* model was used to develop a CWQG equation (see Section 11.0 for additional details). The percentage of unprotected endpoints (i.e., endpoints below the CWQG) was calculated to determine how well the model based on a single species protected all species in the data set. Where a toxicity study did not report hardness, pH or DOC, realistic estimates of water chemistry were imputed using other publications and what is reasonably known about the research facility and type of dilution water used in the test. Additionally, where possible, study authors were contacted for additional information. Where realistic estimates of water chemistry could not be made, standard default values of 50 $mg \cdot L^{-1}$ hardness, 7.5 pH and 0.5 $mg \cdot L^{-1}$ DOC, were used in the calculations to represent standard laboratory conditions. Four percent of acceptable long-term endpoints were below the CWQG, and none were for lethal effects. This aligns with the protection level of deriving guideline values using the 5th percentile of the SSD. A detailed examination of the protectiveness of this model can be found in Section 11.

The *O. mykiss* model can be used to standardize toxicity values for all species in the long-term data set to common water chemistry, in this case to a hardness of 50 $mg \cdot L^{-1}$, a pH of 7.5, and a DOC of 0.5 $mg \cdot L^{-1}$. The general equation to standardize toxicity values to a common hardness, pH and DOC is as follows:

$$\text{Standardized } EC_{10} = \exp[\ln(EC_{10meas}) - DOC_{slope}(\ln[DOC_{meas}] - \ln[DOC_{target}]) - pH_{slope}(pH_{meas} - pH_{target}) - hardness_{slope}(\ln[hardness_{meas}] - \ln[hardness_{target}])]$$

Where EC_{10meas} = reported EC_{10}; DOC_{slope} = DOC slope from MLR model; DOC_{meas} = test water DOC; DOC_{target} = standardized DOC (0.5 $mg \cdot L^{-1}$); pH_{slope} = pH slope from MLR model; pH_{meas} = test water pH; pH_{target} = standardized pH (7.5); $hardness_{slope}$ = hardness slope from MLR model; $hardness_{meas}$ = test water hardness; $hardness_{target}$ = standardized hardness (50 $mg \cdot L^{-1}$).

Specifically for the *O. mykiss* model, the equation to standardize toxicity values to a common hardness, pH and DOC is as follows:

$$\text{Standardized } EC_{10} = \exp[\ln(EC_{10meas}) - 0.398(\ln[DOC_{meas}] - \ln[DOC_{target}]) + 0.815(pH_{meas} - pH_{target})] - 0.947(\ln[hardness_{meas}] - \ln[hardness_{target}]).$$

The long-term data set was standardized to common water chemistry (hardness 50 $mg \cdot L^{-1}$, pH 7.5 and DOC 0.5 $mg \cdot L^{-1}$) using the *O. mykiss* model equation. An SSD can then be fit to the standardized data set (Section 10).

9.3.4 Statistical Considerations of the MLR Approach

Interactions among variables

Interactions among toxicity modifying factors in their effects on zinc toxicity could be present and should be tested for. Therefore, all species-specific models were re-run with the inclusion of interaction terms. For short-term data, only the *C. dubia* model benefitted from the addition of an interaction term. However, the model was found to be unsuitable for deriving the short-term benchmark for four reasons: the model contained a steep, negative slope for hardness, which is in stark contrast to the weight of evidence for this variable; the model explained minimal variability in the data; the model yielded extremely high and low endpoint values when standardized for water chemistry; and the model resulted in unreasonably low short-term benchmark values at all water chemistries.

For long-term data, only the *D. magna* model benefitted from the addition of an interaction term. However, the model was found to be unsuitable for deriving the long-term guideline for three reasons: the model did not include hardness as a variable, which is known to be an important toxicity modifying factor for zinc; the model yielded extremely high and low endpoint values when standardized for water chemistry; and the model resulted in unreasonably low long-term guideline values at all water chemistries. Therefore, interactions were examined and considered but not incorporated into the short-term benchmark or long-term guideline equations.

Differences in species responses

Statistical testing for differences among species was also carried out in a multivariate context (analyses of co-variance). Because responses were not always similar among taxa or species in a multivariate context, combining or averaging data from different species-specific models is problematic. Therefore, the approach of using a model for a single, sensitive species and applying it to all species if sufficiently protective is justified and retained.

Automated stepwise procedures

Automated stepwise procedures can be critiqued when large numbers of independent variables are added to a model, some of which may be correlated, in a data-mining context (i.e., no real hypotheses are being tested). Here, however, hardness, DOC and pH are already known to affect zinc toxicity and were purposefully selected and varied in laboratory experiments to measure their individual and interacting effects on zinc toxicity. In these experiments, the level of each independent variable was varied independently, so issues of collinearity are minimized. The results of the forward stepwise MLR used here were verified using non-automated, nested

analysis of variance testing. Additionally, stepwise procedures (both-ways) were conducted using the Akaike information criterion to determine the retention or elimination of variables from the models. Stepwise results were always the same as non-automated, nested-model testing. Therefore, the results of the automated stepwise procedure are verified.

9.3.5 Toxic Interactions

Cadmium

Attar and Maly (1982) demonstrated cadmium and zinc to be less toxic to *D. magna* in a mixture than individually. In a mixture with equal concentrations of cadmium and zinc, there was a 100- and 33-fold decrease in potency at 72-h and 96-h exposures compared to that expected for the individual metals. The 96-h LC_{50} values were 5 $\mu g \cdot L^{-1}$ for cadmium and 67.91 $\mu g \cdot L^{-1}$ for zinc. A mixture of cadmium and zinc at 8.5 and 53.9 $\mu g \cdot L^{-1}$, respectively, caused 32% mortality at 96-h exposure (Attar and Maly 1982). Similarly, Fargašová (2001) found an antagonistic relationship between zinc at 25,000 and 50,000 $\mu g \cdot L^{-1}$ with cadmium both at 10,000 and 25,000 $\mu g \cdot L^{-1}$ for the larval midge *Chironomus plumosus* (Fargašová 2001). An antagonistic relationship between zinc and cadmium was also observed for the aquatic macrophyte *Lemna trisulca*, as increased levels of zinc mitigated the negative effects of cadmium on plant multiplication rate (Huebert and Shay 1992).

Finlayson and Verrue (1982) found different results; cadmium and zinc interactions were additive in toxicity when tested on juvenile *Oncorhynchus tshawytscha* (Chinook salmon) in a zinc-cadmium ratio of 1:0.008. Lefcort *et al.* (1998) found more than additive effects of cadmium and zinc to tadpoles of the spotted frog *Rana luteiventris*. Zinc and cadmium were found to be more toxic when combined compared to individually; the 96-h LC_{50} for zinc alone was 28,380 $\mu g\ Zn \cdot L^{-1}$ compared to 4,520 $\mu g\ Zn \cdot L^{-1}$ for an equal mixture of cadmium and zinc (Lefcort *et al.* 1998).

Shaw *et al.* (2006) tested four species of daphnids to different concentration combinations of cadmium-zinc mixtures. When zinc was at the LC_{15} value and combined with cadmium at the LC_{50} and LC_{85} value, less than additive interactions were observed for *D. pulex*, *Daphnia ambigua* and *C. dubia*. The exposure of *D. magna* to these concentrations indicated no interaction of the metals. When zinc and cadmium were tested together, both at LC_{50} concentrations, *D. pulex*, *D. ambigua* and *C. dubia* again demonstrated less than additive interactions. However, *D. magna* demonstrated a response predicted by additivity (Shaw *et al.* 2006).

Hansen *et al.* (2002) found that in toxicity tests of a zinc-cadmium mixture (zinc concentrations in the mixture were 100 times those of cadmium) with *S. confluentus* and *O. mykiss*, zinc toxicity dominated. The 120-h LC_{50} for *O. mykiss* exposed to zinc alone ranged from 24 to 53 $\mu g\ Zn \cdot L^{-1}$, while that for the mixture, based solely on the zinc concentration in the mixture, was 32 $\mu g\ Zn \cdot L^{-1}$. Similarly, for *S. confluentus*, the 120-h LC_{50} for zinc alone ranged from 36 to 80 $\mu g\ Zn \cdot L^{-1}$, whereas that for the mixture, based solely on the zinc concentration in the mixture, was 45 $\mu g\ Zn \cdot L^{-1}$ (Hansen *et al.* 2002).

Norwood *et al.* (2003) generated a database from the literature to evaluate the frequency of occurrence of metal mixtures to be less than additive, strictly additive and more than additive.

From a total of 19 tests, binary cadmium-zinc interactions were less than additive in 47% of tests, strictly additive in 26% of tests, and more than additive in 26% of tests.

Copper

Copper-zinc mixtures demonstrated antagonistic toxicity to juvenile *O. tshawytscha*, with the lower copper-zinc ratio (1:12) demonstrating more antagonism compared to the higher ratio (1:3) (Finlayson and Verrue 1982). Similarly, Fargašová (2001) found an antagonistic relationship with zinc at 25,000 $\mu g \cdot L^{-1}$ and copper at 100 and 1,000 $\mu g \cdot L^{-1}$ for the larval midge *C. plumosus*. When the zinc concentration was increased to 50,000 $\mu g \cdot L^{-1}$, a mixture with copper at 100 $\mu g \cdot L^{-1}$ demonstrated again an antagonistic relationship. However, a mixture with copper at 1,000 $\mu g \cdot L^{-1}$ demonstrated a synergistic relationship (Fargašová 2001). Copper and zinc also demonstrated antagonistic toxicity to the relative growth rate of the duckweed *Lemna minor* (Ince *et al.* 1999). Starodub *et al.* (1987*a*) observed an antagonistic relationship between copper and zinc toxicity to the short-term primary productivity of the green algae *S. quadricauda* exposed to mixtures of copper (100 and 200 $\mu g \cdot L^{-1}$) and zinc (250 and 500 $\mu g \cdot L^{-1}$). The long-term exposure of the algae to copper and zinc demonstrated antagonistic toxicity to growth at 100 $\mu g \cdot L^{-1}$ copper and 500 $\mu g \cdot L^{-1}$ zinc, but synergistic toxicity at 200 $\mu g \cdot L^{-1}$ copper and 500 $\mu g \cdot L^{-1}$ zinc (Starodub *et al.* 1987*a*).

For the longfin dace *Agosia chrysogaster*, a copper-zinc mixture demonstrated synergistic toxicity; 96-h LC_{50} values for copper and zinc individually (860 and 790 µg $Zn \cdot L^{-1}$, respectively) were four and three times greater than LC_{50} values of the copper-zinc mixture (210 µg $Cu \cdot L^{-1}$ and 280 µg $Zn \cdot L^{-1}$) (Lewis 1978). Similarly, Fernandez and Jones (1990) found the toxicity of a copper-zinc mixture to the marine worm *N. diversicolor* was greater at salinities of 17.5 and 30‰ compared to the single metals. Additionally, at 6, 12 and 20 °C, the copper-zinc mixture was more toxic than single metals (Fernandez and Jones 1990). In terms of accumulation, the zinc content in worms was greater in the majority of organisms exposed to zinc-copper mixtures compared to those exposed to zinc alone (Fernandez and Jones 1990).

Thompson *et al.* (1980) found the toxicity of a copper-zinc mixture to *L. macrochirus* to be additive in 96-h flow-through exposures. Sprague and Ramsay (1965) also found the lethal threshold for mixtures of copper and zinc to juvenile *S. salar* resulted in an additive effect. In stronger mixtures, however, where toxic units totalled 2 and 5, as opposed to 1, salmon mortality occurred more quickly than would be predicted based on single metal exposure, demonstrating more than additive effects (Sprague and Ramsay 1965).

Norwood *et al.* (2003) generated a metal mixture database from the literature. Through this database, they found that binary copper-zinc interactions were less than additive in 52% of tests, strictly additive in 5% of tests, and more than additive in 43% of tests, from a total of 21 tests.

Other metals

Aluminum: For the larval midge *C. plumosus*, zinc at 25,000 $\mu g \cdot L^{-1}$ demonstrated an antagonistic relationship with aluminum at 25,000 and 50,000 $\mu g \cdot L^{-1}$ (Fargašová 2001). At a zinc concentration of 50,000 $\mu g \cdot L^{-1}$, however, there was a synergistic relationship with aluminum at 25,000 and 50,000 $\mu g \cdot L^{-1}$ (Fargašová 2001). Conversely, Roy and Campbell (1995) observed a

simple additive relationship for exposures of juvenile *S. salar* to mixtures of aluminum and sublethal concentrations of zinc (52.3–111.2 µg $Zn \cdot L^{-1}$).

Cobalt: A cobalt-zinc mixture at concentrations ranging from 100 to 2,000 $\mu g \cdot L^{-1}$ caused a decrease in the relative growth rate of the duckweed *L. minor* compared to zinc and cobalt tested separately. Zinc inhibited cobalt accumulation at each concentration tested. However, the presence of cobalt enhanced zinc accumulation at all concentrations. At 580 $\mu g \cdot L^{-1}$, zinc accumulation was 1,926,000 $\mu g \cdot L^{-1}$ and BCFs were 3,315 $\mu g \cdot L^{-1}$. In comparison, after the addition of 500 $\mu g \cdot L^{-1}$ cobalt, zinc accumulation was 2,875,000 $\mu g \cdot L^{-1}$ and BCFs were 5,751 $\mu g \cdot L^{-1}$ (Dirilgen and Inel 1994*a*). At low concentrations of cobalt and zinc (200–2,000 $\mu g \cdot L^{-1}$), the interaction between the two metals was additive for relative growth rates of *L. minor*, while at higher concentrations the interaction was antagonistic (Ince *et al.* 1999).

Chromium: Ince *et al.* (1999) observed antagonistic interactions between zinc and chromium over a range of concentrations when the effects of binary-metal mixture were observed on the relative growth rate of *L. minor*.

Lead: At zinc concentrations of 250, 300 and 500 $\mu g \cdot L^{-1}$ in mixtures with lead at concentrations of 300, 600, 3,000 and 6,000 $\mu g \cdot L^{-1}$, antagonistic toxicity was observed to the primary productivity of the green algae *S. quadricauda* after short-term exposure. In long-term exposures, both antagonistic and synergistic toxic effects to growth of the algae were observed (Starodub *et al.* 1987*a*).

Multiple-metal mixtures

The tri-metal mixture of copper-zinc-cadmium demonstrated antagonistic toxicity to juvenile *O. tshawytscha*, with the lower copper ratio (1:12:0.08) demonstrating more antagonism compared to the higher copper ratio (1:3:0.02) (Finlayson and Verrue 1982). The tri-metal mixture of lead, copper and zinc behaved antagonistically to the primary production of the green algae *S. quadricauda* in short-term exposures, with copper concentrations of 100 and 200 $\mu g \cdot L^{-1}$, zinc concentrations of 250, 300 and 500 $\mu g \cdot L^{-1}$, and lead concentrations of 300, 600, 3,000 and 6,000 $\mu g \cdot L^{-1}$. In long-term exposures, both antagonistic and synergistic effects were observed towards algal growth (Starodub *et al.* 1987*a*). Enserink *et al.* (1991) found an equitoxic mixture of eight metals—arsenic, cadmium, chromium, copper, mercury, lead, nickel and zinc—had an additive effect on the 21-d LC_{50} and EC_{50} values for the cladoceran *D. magna*. Norwood *et al.* (2007) found bioaccumulation of zinc was not significantly affected by exposure to arsenic, cadmium, cobalt, chromium, nickel, lead or titanium at approximately equitoxic concentrations at the four-week LC_{25} for *H. azteca*.

9.4 Toxicity of Zinc to Freshwater Organisms

The following section presents an overview of the acceptable toxicity values included in the SSDs for short-term and long-term toxicity of zinc to aquatic organisms. The most and least sensitive endpoints are described.

Reported short-term endpoint values were corrected to a hardness of 50 $mg \cdot L^{-1}$ as $CaCO_3$ and a DOC of 0.5 $mg \cdot L^{-1}$ using the pooled *Daphnia* MLR equation. Where endpoints in the data set did not report hardness or DOC, realistic estimates of water chemistry were imputed from other publications and what is reasonably known about the research facility and type of dilution water. Additionally, where possible, study authors were contacted for additional information. The full short-term toxicity data set in the Appendix lists the estimated water chemistry values and notes on how they were derived. Where no reasonable estimate of hardness or DOC could be made, default standard values of 50 $mg \cdot L^{-1}$ for hardness and 0.5 $mg \cdot L^{-1}$ for DOC were used for the calculation to represent standard laboratory conditions. The short-term SSD represents dissolved concentrations of zinc. Data points for total zinc concentrations were plotted in the SSD after being converted to a dissolved concentration using an acute total: dissolved conversion multiplier of 0.978 (US EPA 1996). Where concentrations were not specified as either total or dissolved in the toxicity study, a total concentration was assumed and a conversion factor was applied.

Reported long-term endpoint values were corrected to a hardness of 50 $mg \cdot L^{-1}$, a pH of 7.5 and a DOC concentration of 0.5 $mg \cdot L^{-1}$ using the *O. mykiss* MLR equation. Where endpoints in the data set did not report hardness, pH or DOC, realistic estimates of water chemistry were imputed from other publications and what is reasonably known about the research facility and type of dilution water. Additionally, where possible, study authors were contacted for additional information. The full long-term toxicity data set in the Appendix shows estimated water chemistry values and notes on how they were derived. Where no reasonable estimate of hardness, pH or DOC could be made, default standard values of 50 $mg \cdot L^{-1}$ for hardness, 7.5 for pH and 0.5 $mg \cdot L^{-1}$ for DOC were used for the calculation to represent standard laboratory conditions. The long-term SSD represents dissolved concentrations of zinc. Data points for total zinc concentrations were plotted in the SSD after being converted to a dissolved concentration using a chronic total: dissolved conversion multiplier of 0.986 (US EPA 1996). Where concentrations were not specified as total or dissolved in the toxicity study, a total concentration was assumed and conversion factors were applied.

9.4.1 Short-term Toxicity

Fish

For fish, the effect level for deriving a short-term benchmark is an LC_{50}, and short-term fish tests are generally conducted for 96 hours or less. Short-term LC_{50} values for zinc for inclusion in the SSD were obtained for 34 freshwater fish species. In general, salmonids were found to be more sensitive than other types of fish. The most sensitive endpoint included in the short-term SSD for fish was a geometric mean of six 120-h LC_{50} values for *O. mykiss* of 84.9 µg $Zn \cdot L^{-1}$ (once adjusted to 50 $mg \cdot L^{-1}$ hardness and 0.5 $mg \cdot L^{-1}$ DOC and converted to dissolved zinc) (Hansen *et al.* 2002). The least sensitive fish species included in the SSD was *C. auratus*, with an adjusted 24-h LC_{50} value of 39,517 µg dissolved $Zn \cdot L^{-1}$ (Cairns *et al.* 1978).

Invertebrates

For aquatic invertebrates, the effect level for deriving a short-term benchmark is a short-term LC_{50} or equivalent (for example EC_{50} for immobility). In general, exposure periods of 96 hours or less are considered appropriate for deriving short-term benchmarks, and many invertebrate tests are conducted for 48-h exposure periods. Acceptable short-term LC_{50} or EC_{50} endpoints for

immobility were available for 39 freshwater invertebrate species. The lowest acceptable invertebrate endpoint included in the short-term SSD was a 96-h LC_{50} of 22.7 µg $Zn \cdot L^{-1}$ for the cladoceran *D. magna* (once adjusted to 50 $mg \cdot L^{-1}$ hardness and 0.5 $mg \cdot L^{-1}$ DOC and converted to dissolved zinc) (Attar and Maly 1982), followed by a geometric mean of three 48-h LC_{50} values of 34.0 µg dissolved $Zn \cdot L^{-1}$ for the water flea *C. dubia* (Belanger and Cherry, 1990). The least sensitive invertebrate species were the stonefly Chlorperlidae and the mayflies *Cinygmula* sp. and *Ephemerella* sp., all of which had adjusted 96-h LC_{50} values of >49,058 µg dissolved $Zn \cdot L^{-1}$ (Brinkman and Johnston 2012).

Amphibians

For amphibians, the effect level for deriving a short-term benchmark is an LC_{50}, and tests are generally conducted for 96 hours or less. Short-term LC_{50} values were included in the SSD for four amphibian species. The most sensitive amphibian species included in the short-term SSD was *Bufo boreas* (western toad), with an adjusted 96-h LC_{50} of 535 µg dissolved $Zn \cdot L^{-1}$ (Davies and Brinkman 1999). The least sensitive amphibian endpoint in the SSD was an adjusted 96-hour LC_{50} of 18,947 µg dissolved $Zn \cdot L^{-1}$ for *Xenopus laevis* (African clawed frog) (Dawson *et al.* 1988).

Algae and aquatic plants

Due to a general lack of toxicity data on aquatic plants (for toxicants in general), definitions of aquatic plant exposure data are done on a case-by-case basis. Because of the rapid cell division rate in algae, they usually have high resiliency during short-term exposures. Therefore, algal toxicity tests with exposure periods longer than approximately 24 hours are generally considered inappropriate for inclusion in the derivation of a short-term benchmark. Algal tests with exposure periods of 24 hours or less and severe effects are generally included in the short-term data set. Two algae and two plant studies were included in the short-term SSD. The aquatic plant endpoints included adjusted 96-h IC_{50} values for growth of 2,505 µg dissolved $Zn \cdot L^{-1}$ for *Spirodela polyrrhiza* (greater duckweed) and 2,540 µg dissolved $Zn \cdot L^{-1}$ for *Azolla pinnata* (mosquito fern) (Gaur *et al.* 1994). The algal endpoints included an adjusted 4-h EC_{50} of 36.2 µg dissolved $Zn \cdot L^{-1}$ for growth of *P. subcapitata* (Pardos *et al.* 1998) and an adjusted 24-h EC_{50} of 76.3 µg dissolved $Zn \cdot L^{-1}$ for growth of the green algae *Chlorella pyrenoidosa* (Lin *et al.* 2007).

9.4.2 Long-term Toxicity

Fish

Acceptable long-term zinc toxicity values for fish included endpoints obtained in tests with durations of 21 days or longer for adult or juvenile life stages, and seven days or longer for tests involving fish eggs or larvae. Long-term toxicity data for inclusion in the SSD were obtained for nine species of freshwater fish. The most sensitive fish species in the long-term SSD was *Jordanella floridae* (flagfish), with a 100-d MATC of 27.9 µg $Zn \cdot L^{-1}$ for larval growth (once adjusted to 50 $mg \cdot L^{-1}$ hardness, pH 7.5 and 0.5 $mg \cdot L^{-1}$ DOC and converted to dissolved zinc) (Spehar 1976). The least sensitive species included in the SSD was *Oncorhynchus clarkii pleuriticus* (Colorado River cutthroat trout), with an adjusted 30-d MATC of 169.3 µg dissolved $Zn \cdot L^{-1}$ for swim-up fry survival, which was a geometric mean of two individual MATC values (Brinkman and Hansen 2004).

Invertebrates

For aquatic invertebrates, acceptable long-term data included non-lethal endpoints from test durations of greater than 96 hours for shorter-lived invertebrates, non-lethal endpoints of greater than seven days for longer-lived invertebrates, and lethal endpoints for tests greater than 21 days for longer-lived invertebrates. Lethal endpoints for shorter-lived invertebrates from tests less than 21 days were considered on a case-by-case basis. The most sensitive invertebrate species in the long-term SSD was the midge *Chironomus riparius*, with an adjusted 11-week LOEC for development of 9.89 µg dissolved $Zn \cdot L^{-1}$ (Timmermans *et al.* 1992). The least sensitive invertebrate species was the mayfly *Rhithrogena hageni* nymph, with an adjusted 10-d EC_{10} value for mortality of 1,696 µg dissolved $Zn \cdot L^{-1}$ (Brinkman and Johnson 2008).

Amphibians

For amphibians, long-term exposure periods included tests of 21 days or longer for adult and juvenile life stages, and tests of seven days or longer for egg and larval life stages. Only one acceptable long-term toxicity endpoint for amphibians was available for inclusion in the SSD: an adjusted four-week MATC value of 107.6 µg dissolved $Zn \cdot L^{-1}$ for development of *B. boreas* eggs (Davies and Brinkman 1999).

Algae and aquatic plants

Due to the rapid growth and turnover of algae, all algal toxicity tests longer than 24 hours were considered long-term exposures. Data for other species were evaluated on a case-by-case basis considering the lifespan of the species.

The most sensitive algal species was *P. subcapitata*. The endpoint included in the long-term SSD was a geometric mean of four adjusted 72-h EC_{10} values for growth of 13.8 µg dissolved $Zn \cdot L^{-1}$ (De Schamphelaere *et al.* 2005). The least sensitive algal species was *S. quadricauda*, with an adjusted 5-d EC_{10} value of 1,628 µg dissolved $Zn \cdot L^{-1}$ (Cairns *et al.* 1978).

Two aquatic plant species were included in the long-term SSD. The more sensitive endpoint was an adjusted 7-d EC_{10} for growth of 400 µg dissolved $Zn \cdot L^{-1}$ for *L. minor* (Ince *et al.* 1999). The less sensitive endpoint was for *Ceratophyllum demersum* (hornwort), with an adjusted 15-d LOEC of 1,116 µg dissolved $Zn \cdot L^{-1}$ for changes to chlorophyll content and biomass (Umebese and Motajo 2008).

9.5 Field Studies

9.5.1 Invertebrates

Communities exposed to a low level of zinc (12 $\mu g \cdot L^{-1}$) for four days in experimental streams had significantly reduced numbers of taxa, numbers of individuals and abundance of most dominant taxa (Clements *et al.* 1988). After 10 days, the dominant taxa of control streams were Ephemeroptera and Tanytarsini chironomids, in contrast to streams treated with zinc, which were dominated by Hydropsychidae and Orthocladiini. Similar patterns regarding community structure were observed at the various field sites impacted by zinc concentrations ranging from 6-81 $\mu g \cdot L^{-1}$ (Clements *et al.* 1988).

Clark and Clements (2006) measured zinc concentrations at upstream and downstream stations of the Arkansas River Basin in Colorado. In July 2002, concentrations ranged from 23.3 to 24.0 µg $Zn \cdot L^{-1}$ at upstream stations and from 47.3 to 331.3 µg $Zn \cdot L^{-1}$ at downstream stations. In May they ranged from 13.1 to 263.3 µg $Zn \cdot L^{-1}$ at upstream stations and from 203.8 to 1,030.1 µg $Zn \cdot L^{-1}$ at downstream stations. The experiments found significant mortality of the mayfly *R. hageni* at metal-contaminated sites during the summer, when mayflies were small and in early developmental stages. In contrast, mortality was not significant at metal-contaminated sites during the following spring, when mayflies were larger and more developed. In stream microcosm experiments, zinc concentrations in July 2002 ranged from 25.8 to 1,297.5 µg $Zn \cdot L^{-1}$ and in May 2003 ranged from below detection limits to 932.1 µg $Zn \cdot L^{-1}$. A concentration-response relationship was established between zinc and mayfly density and species richness (Clark and Clements 2006).

In stream microcosm experiments 10-d exposures were conducted with zinc (< 10–792 $\mu g \cdot L^{-1}$), with zinc and cadmium (Zn 9.5–775.2 $\mu g \cdot L^{-1}$, Cd < 0.5–9.8 $\mu g \cdot L^{-1}$) and with zinc, cadmium and copper (Zn < 10–954.3 $\mu g \cdot L^{-1}$, Cd < 0.5–8.8 $\mu g \cdot L^{-1}$, Cu < 5.0–74.9 $\mu g \cdot L^{-1}$). No community measures demonstrated a significant response in the zinc-only exposure (Clements 2004). In the tri-metal mixture, EC_{10} values were 8.1 for total macroinvertebrate abundance and 20.4 for total species richness, based on cumulative criterion units (CCUs)[1] (Clements 2004). For individual taxa, the EC_{10} values for the tri-metal mixture for abundance of Heptageniidae, mayflies and stoneflies were 6.6, 6.7 and 7.5, respectively (Clements 2004). Another study looking at metal mixture (Zn + Cu) exposures calculated EC_{20} values using CCUs for macroinvertebrates in stream microcosms after 34 days. The inhibition threshold 20% effective concentration (EC_{20}) for number of taxa was 15. The most sensitive species was the mollusc *Potamopyrgus antipodarum*, with an EC_{20} of 1.4, followed by a variety of mayfly species with EC_{20} values ranging from 2.1 to 3.1 (Hickey and Golding 2002). Richardson and Kiffney (2000) also explored the toxicity of copper and zinc to invertebrates in a field study, tested at a maximum concentration of approximately 129 µg $Zn \cdot L^{-1}$ for six days. Some species demonstrated significant reduction in abundance: *Ameletus* sp. decreased 93% in highest zinc concentrations, *Baetis* sp. decreased by 60%, and *Zapada cinctipes* and *Zapada haysi* decreased by 58%. Other species demonstrated no significant response, while Dixidae and mites showed a positive correlation between metal concentration and abundance (Richardson and Kiffney 2000).

In situ experiments in the southern basin of Lake Michigan determined responses of plankton and zooplankton communities to the addition of 17.1, 31.2, 62.1 and 89.6 µg $Zn \cdot L^{-1}$. Lake water samples were collected in polyethylene carboys, treated with the different concentrations of zinc (plus a control), and were suspended in the lake. The carboys were retrieved after two weeks, and phytoplankton and zooplankton community responses were assessed. Zooplankton categories consisted of various species of Cladocera and Copepoda, while planktonic categories included various species of Rotifera. Additions of 17.1 µg $Zn \cdot L^{-1}$ for a two-week exposure resulted in significant reductions of chlorophyll *a*, primary productivity, dissolved oxygen, specific zooplankton populations and species diversity (Marshall *et al.* 1983). Therefore, 17.1 µg $Zn \cdot L^{-1}$ was determined to be the LOEC value for the above-mentioned community and population effects. Because the experiment tested a community as a whole, rather than an individual species, endpoints were not included in the SSD.

[1] CCU= ∑[metals]/hardness-adjusted US EPA 1996 chronic criterion value.

A field study in Colorado sampled invertebrates from mining-affected catchments in order to use a critical tissue residue approach and statistical models to estimate linkages between accumulated zinc concentrations and population- and community-level effects. Whole-body zinc concentrations were measured in mayflies *Rhithrogena* spp. and *Drunella* spp., and *Arctopsyche grandis* (caddisflies), and used to predict population and community effects. Whole-body zinc concentration did not show a significant relationship with populations of *A. grandis*. Critical tissue residues of 634 $\mu g \cdot g^{-1}$ for *Drunella* spp. and 267 $\mu g \cdot g^{-1}$ for *Rhithrogena* spp. were associated with a 20% decrease in maximum mayfly densities and with exposure concentrations of 7.0 $Zn \cdot L^{-1}$ for *Drunella* spp. and 3.9 µg $Zn \cdot L^{-1}$ for *Rhithrogena* spp. (Schmidt *et al.* 2011).

Iwasaki and Ormerod (2012) used river macroinvertebrate surveys from the United Kingdom, United States and Japan to derive safe concentrations of zinc. The authors related taxon richness of Ephemeroptera, Plecoptera and Trichoptera to dissolved zinc concentration and defined the safe concentration as the threshold at which effects were apparent. The safe concentration for zinc was estimated at 34 $\mu g \cdot L^{-1}$ (95% confidence interval [CI] 11–307 $\mu g \cdot L^{-1}$).

9.5.2 Fish

In separate artificial stream experiments, juvenile *S. salar* and *Cottus cognatus* (slimy sculpin) were exposed to 0%, 20% and 80% metal mine effluent containing 11 different metals. Zinc concentrations were 54.5 $\mu g \cdot L^{-1}$ at 0%, 116.67 $\mu g \cdot L^{-1}$ at 20% and 341.67 $\mu g \cdot L^{-1}$ at 80%. For *S. salar*, survival at 0% effluent was 88.1%, at 20% effluent was 84.6% and at 80% effluent was 64.2%. In the 80% treatment, length and weight of the fish were significantly reduced. For *C. cognatus*, survival in controls was 69%, at 20% effluent was 56% and at 80% effluent was 25%, (Dubé *et al.* 2005). Growth in both fish was reduced with increasing exposure to metal effluents.

9.5.3 Plants and Algae

Periphyton communities were tested in short-term and long-term exposure to zinc at concentrations ranging from 6,540 to 654,000 µg $Zn \cdot L^{-1}$. The 2-h EC_{50} for algal photosynthesis was 3,662.4 µg $Zn \cdot L^{-1}$. The four-week no-effect concentration (NEC) for periphyton biomass (dw) was 9.81 µg $Zn \cdot L^{-1}$, while the NEC for chlorophyll *a* content was 27.5 µg $Zn \cdot L^{-1}$ (Paulsson *et al.* 2000).

9.6 Development of Resistance Mechanisms to Zinc by Aquatic Organisms

9.6.1 Fish

Significant acclimation was observed in one-month-old *Oncorhynchus nerka* (sockeye salmon) pre-exposed to a zinc concentration of 240 $\mu g \cdot L^{-1}$ in 115-h toxicity exposures of zinc concentrations up to 630 $\mu g \cdot L^{-1}$. Mortality of the non-acclimated group at 630 µg $Zn \cdot L^{-1}$ was 72% (LC_{50} of 447 $\mu g \cdot L^{-1}$) compared to acclimated alevins, which had 0% mortality at 630 µg $Zn \cdot L^{-1}$. In flow-through acute exposures with nine-month-old *O. nerka*, control non-acclimated

fish had a 96-h LC_{50} of 749 µg $Zn \cdot L^{-1}$ compared to that of fish acclimated at 240 µg $Zn \cdot L^{-1}$, which had a 96-h LC_{50} of 1,663 µg $Zn \cdot L^{-1}$ (Chapman 1978*a*).

Juvenile *O. mykiss* acclimated to 50 µg $Zn \cdot L^{-1}$ for seven days had a 4.7-fold increase in zinc tolerance (96-h LC_{50} values were 95 µg $Zn \cdot L^{-1}$ for control fish and 450 µg $Zn \cdot L^{-1}$ for acclimated fish). Similarly, fish acclimated to 80 µg $Zn \cdot L^{-1}$ for seven days had a 2.9-fold increase in zinc tolerance (96-h LC_{50} values were 297 µg $Zn \cdot L^{-1}$ for control fish and 924 µg $Zn \cdot L^{-1}$ for acclimated fish). Acclimation to 100 µg $Zn \cdot L^{-1}$ for nine days resulted in a five-fold increase in tolerance, while acclimation to 300 and 500 µg $Zn \cdot L^{-1}$ for up to 37 days resulted in no further increase in zinc tolerance. Zinc tolerance was rapidly lost when fish were returned to control water, with reversion to control tolerance levels after seven days (Anadu *et al.* 1989).

Short-term changes in zinc tolerance resulting from acclimation were also seen in *P. promelas*. After 14 days of acclimation to 600 µg $Zn \cdot L^{-1}$, tolerance increased 28% over control values, but returned to control levels after 21 days of exposure (Hobson and Birge 1989). Acclimation to 1,800 µg $Zn \cdot L^{-1}$, however, resulted in short-term increased sensitivity to zinc (63 and 74% of controls after 7 and 14 days) and returned to control levels after 21 days of exposure (Hobson and Birge 1989).

Conversely, Alsop and Wood (2000) did not find a significant difference in LC_{50} values between juvenile *O. mykiss* acclimated to 244 µg $Zn \cdot L^{-1}$ for an exposure of 30 days and those of control fish (LC_{50} values were 2,615 µg $Zn \cdot L^{-1}$ for control fish and 3,340 µg $Zn \cdot L^{-1}$ for acclimated fish,). Additionally, no significant difference was observed in total zinc levels of gills between control and acclimated fish (Alsop and Wood 2000).

Alsop *et al.* (1999) found significant acclimation of *O. mykiss* to zinc in hard-water toxicity tests, as demonstrated by a 2.3-fold increase in the 96-h LC_{50} of the group acclimated to 150 µg $Zn \cdot L^{-1}$, and a 2.7-fold increase in the 96-h LC_{50} of those acclimated to 450 µg $Zn \cdot L^{-1}$. In soft-water tests, increases in LC_{50} values were 2.2-fold for fish acclimated to 50 µg $Zn \cdot L^{-1}$, and 3.9-fold for fish acclimated to 120 µg $Zn \cdot L^{-1}$. Three mechanisms have been suggested for increased tolerance to metals for acclimated fish: changes in properties of gill barriers causing a decreased rate of metal entry; enhanced metal storage and detoxification; and enhanced resistance of processes sensitive to metal poisoning (Alsop *et al.* 1999).

9.6.2 Invertebrates

Muyssen and Janssen (2002*b*) acclimated the cladoceran *C. dubia* to zinc concentrations of 3, 13, 50 and 100 $\mu g \cdot L^{-1}$ for 10 generations prior to toxicity testing. The 48-h EC_{50} values for cladocerans acclimated at 3, 13, 50 and 100 µg $Zn \cdot L^{-1}$ were 670, > 800, 507 and 507 $\mu g \cdot L^{-1}$, respectively, with the highest EC_{50} values in the lowest two acclimation concentrations. The results of the chronic test, however, demonstrated increased tolerance with increased zinc acclimation concentration: the 9-d EC_{50} values for cladocerans acclimated at 3, 13, 50 and 100 µg $Zn \cdot L^{-1}$ were 354, 387, 449 and 489 µg $Zn \cdot L^{-1}$, respectively (Muyssen and Janssen 2002*b*).

9.6.3 Plants and Algae

In another study by Muyssen and Janssen (2001), the green algal species *C. vulgaris* was acclimated to 65 µg $Zn \cdot L^{-1}$ for 110 days and demonstrated increased tolerance to zinc in growth and biomass inhibition assays. The 72-h EC_{50} values based on biomass were 105 µg $Zn \cdot L^{-1}$ for acclimated experiments and 34 µg $Zn \cdot L^{-1}$ for control experiments, an increase by a factor of 3.1. The 72-h EC_{50} values based on growth inhibition were 260 µg $Zn \cdot L^{-1}$ for acclimated experiments and 153 µg $Zn \cdot L^{-1}$ for control experiments. The green algae *P. subcapitata* was acclimated to 65 µg $Zn \cdot L^{-1}$ for 100 days and had a 72-h EC_{50} for biomass of 117 µg $Zn \cdot L^{-1}$, compared to 39 µg $Zn \cdot L^{-1}$ for controls, an increase in zinc tolerance of a factor of 3. The 72-h EC_{50} values for growth inhibition were 263 $Zn \cdot L^{-1}$ for acclimated algae and 138 µg $Zn \cdot L^{-1}$ for control algae (Muyssen and Janssen 2001).

10.0 DERIVING THE SHORT-TERM BENCHMARK CONCENTRATION AND THE CANADIAN WATER QUALITY GUIDELINE

10.1 Summary of Existing Water Quality Guidelines

10.1.1 Previous CWQG for the Protection of Aquatic Life for Zinc

In 1980, Taylor and Demayo set a long-term CWQG of 50 $\mu g \cdot L^{-1}$ of total zinc for the protection of freshwater aquatic life in water with hardness lower than 120 $mg \cdot L^{-1}$ (Taylor and Demayo 1980). They observed a linear relationship between the MATC and hardness. Available data were not sufficient to derive an equation, so the guideline was proposed on an interim basis for specific ranges of water hardness (Table 10.1).

Table 10.1 Water Quality Guidelines for Zinc as recommended by Taylor and Demayo (1980)

Hardness $mg \cdot L^{-1}$ as $CaCO_3$	Zinc $\mu g \cdot L^{-1}$ as total zinc
0–120	50
120–180	100
180–300	200
> 300	300

Although the guideline was revised in 1987 to 30 $\mu g \cdot L^{-1}$ of total zinc with no correction for hardness, the guideline remained "tentative" (Canadian Council of Resource and Environment Ministers [CCREM] 1987). This value was recommended because it coincided with the measured NEC for *O. mykiss*) and *P. promelas* and the beginning of growth inhibition in *P. subcapitata* (Brungs 1969; Bartlett *et al.* 1974; Goettl *et al.* 1976). This guideline was not adjusted for water hardness, due to insufficient data showing that chronic toxicity decreases as water hardness increases.

10.1.2 Water Quality Guidelines for the Protection of Aquatic Life for Zinc in Other Jurisdictions

The United States expresses its long-term freshwater guideline as a criteria continuous concentration (CCC) (US EPA 2006). The US EPA uses the following equation, which takes into account water hardness:

$$\text{CCC (dissolved)} = e^{(0.8473\,[\ln(\text{hardness})]+\,0.884)} \cdot 0.986\ (\mu g \cdot L^{-1})$$

The US short-term freshwater guideline or criteria maximum concentration (CMC) is also expressed as an equation:

$$\text{CMC (dissolved)} = e^{(0.8473\,[\ln(\text{hardness})]+\,0.884)} \cdot 0.978\ (\mu g \cdot L^{-1})$$

For a water hardness of 50 $mg \cdot L^{-1}$, the CCC guideline would be 65.7 $\mu g \cdot L^{-1}$ of dissolved zinc, and the CMC guideline would be 65.1 $\mu g \cdot L^{-1}$ of dissolved zinc. The chronic and acute criteria for saltwater are given as numerical values: 81 $\mu g \cdot L^{-1}$ of dissolved zinc for chronic criteria and 90 $\mu g \cdot L^{-1}$ of dissolved zinc for acute criteria, based on US EPA (1987) marine guideline.

Manitoba uses the US EPA (2006) chronic and acute criteria for zinc water quality guidelines (Manitoba Conservation 2011). Québec has adopted the US EPA (1999) criteria for chronic and acute toxicity, using this equation (MDDEP 2007):

$$\text{CCC and CMC (total in } \mu g \cdot L^{-1}\text{)}: e^{(0.8473\,[\ln(\text{hardness})]+\,0.884)}$$

This equation is very similar to the US EPA (2006) equation, except that it provides a guideline in total concentration of zinc instead of dissolved concentration. For a water hardness of 50 $mg \cdot L^{-1}$, the Québec CCC and CMC guideline would be 67 $\mu g \cdot L^{-1}$ of total zinc. Québec also uses the US EPA (1987) chronic and acute criteria for saltwater, which are expressed in numerical limits of 86 µg $Zn \cdot L^{-1}$ for chronic and 95 µg $Zn \cdot L^{-1}$ for acute.

The numerical guideline of 30 µg $Zn \cdot L^{-1}$ derived by CCREM (1987) is used as an objective in Alberta (Alberta Environment and Sustainable Resource Development 2014), Ontario (Ontario Ministry of the Environment and Energy 1994) and Nova Scotia (Nova Scotia Environment and Labour Ministry 2014).

In contrast, British Columbia's (BC) Ministry of Environment and Climate Change Strategy has an interim guideline that says that the average concentration of total zinc should not exceed 7.5 $\mu g \cdot L^{-1}$ when water hardness is lower than or equal to 90 mg $\cdot L^{-1}$ of $CaCO_3$ (Nagpal 1999). This recommended chronic guideline was based on the lowest-observed-effect level (LOEL) of 15 µg $Zn \cdot L^{-1}$ for a population of copepods in a field study (Marshall *et al.* 1983). The BC guideline also provides the following relationship for water hardness exceeding 90 $mg \cdot L^{-1}$:

$$\text{Average concentration } (\mu g \cdot L^{-1}) = 7.5 + 0.75\ (\text{Water hardness} - 90)$$

A maximum concentration of 33 $\mu g \cdot L^{-1}$ of total zinc is proposed in the BC guideline to protect freshwater organisms from acute and lethal effects when water hardness is lower than or equal to 90 $mg \cdot L^{-1}$ of $CaCO_3$ The following equation determined the maximum concentration when water hardness is over that range:

$$\text{Average concentration } (\mu g \cdot L^{-1}) = 33 + 0.75 \text{ (Water hardness } - 0)$$

This recommendation was based on a 96-h LC_{50} of 66 µg $Zn \cdot L^{-1}$ for *O. mykiss* (Cusimano *et al.* 1986). To protect marine aquatic life, the BC guideline recommends the concentration of total zinc should not exceed 10 $\mu g \cdot L^{-1}$ based on the LOELs for marine algae *Schroederella schroedi* and *Skeletonema costatum* (Hollibaugh *et al*. 1980; Kayser 1977).

The European Union proposed a predicted no-effect concentration (PNEC) of 7.8 $\mu g \cdot L^{-1}$ of dissolved zinc for protection of fresh and saltwater organisms and a PNEC of 3.1 µg $Zn \cdot L^{-1}$ for soft water (water hardness less than 24 $mg \cdot L^{-1}$ as $CaCO_3$) based the 5th percentile values of SSD (EU 2006).

Australia and New Zealand have derived guidelines in the form of trigger values using the statistical distribution method with 95% protection. The freshwater high reliability trigger value is 8 $\mu g \cdot L^{-1}$ and applies at hardness of 30 $mg \cdot L^{-1}$. The marine high reliability trigger value is 15 $\mu g \cdot L^{-1}$ (ANZECC 2000).

10.2 Evaluating Toxicological Data for Zinc

All zinc toxicity data were evaluated for scientific acceptability before being considered for or used in the derivation of the short-term benchmark concentration and CWQG. Data from toxicity studies were ranked as primary, secondary or unacceptable in terms of acceptability for guideline derivation. The ranking criteria are described fully in the CCME 2007 protocol and are briefly outlined here.

In order for a toxicity value to be considered primary, the concentration of the toxic substance must be measured at the beginning and end of the exposure period, and the measurement of water quality parameters (hardness, pH, temperature, etc.) must be reported. Adequate replication must be performed, suitable statistical procedures should be used and control mortality should be low (typically less than 10%). Secondary data are those that originate from studies where primary data cannot be generated but are still of acceptable quality and documentation. For example, a study may use calculated (rather than measured) substance concentrations, but the most relevant water quality parameters must be reported. Appropriate test replication is still necessary, but pseudoreplication may be acceptable for secondary studies (e.g., all test organisms in only one aquarium per concentration). Unacceptable data are those that do not meet the criteria of primary or secondary data.

10.3 Adjusting Zinc Toxicity Data for Hardness, pH and DOC

Short-term zinc effect concentrations were adjusted to a hardness of 50 $mg \cdot L^{-1}$ and a DOC concentration of 0.5 $mg \cdot L^{-1}$ using the pooled *Daphnia* MLR normalization equation, as presented in Section 9.3.2. Where endpoints in the data set did not report hardness or DOC, realistic estimates of water chemistry were imputed from other publications and what is reasonably known about the research facility and type of dilution water. Additionally, where possible, study authors were contacted for additional information. The full short-term toxicity data set in the Appendix shows the estimated water chemistry values and notes on how they were derived. Where no reasonable estimate of hardness or DOC could be made, default standard values of 50 $mg \cdot L^{-1}$ for hardness and 0.5 $mg \cdot L^{-1}$ for DOC were used for the calculation to represent standard laboratory conditions.

Long-term zinc effect concentrations were adjusted to a hardness of 50 $mg \cdot L^{-1}$ as $CaCO_3$, a pH of 7.5 and a DOC concentration of 0.5 $mg \cdot L^{-1}$ using the *O. mykiss* MLR normalization equation, as presented in Section 9.3.3. Where endpoints in the data set did not report hardness, pH or DOC, realistic estimates of water chemistry were imputed from other publications and what is reasonably known about the research facility and type of dilution water. Additionally, where possible, study authors were contacted for additional information. The full long-term toxicity data set in the Appendix shows the estimated water chemistry values and notes on how they were derived. Where no reasonable estimate of hardness, pH or DOC could be made, default standard values of 50 $mg \cdot L^{-1}$ for hardness, 7.5 for pH and 0.5 $mg \cdot L^{-1}$ for DOC were used for the calculation to represent standard laboratory conditions.

10.4 Converting Total Zinc Concentrations to Dissolved Concentrations

Conversion factors were used to convert total concentrations of zinc in the SSD toxicity data set to dissolved concentrations for the purpose of developing a benchmark and guideline based on dissolved concentrations, such that it more accurately represents the bioavailable form. Short-term and long-term conversion factors were adopted from the US EPA, and were 0.978 (acute) and 0.986 (chronic) (US EPA 1996). These conversion factors are close to one, indicating that almost all the zinc present in a laboratory toxicity test is in the dissolved form. Several short-term and long-term toxicity studies that measured both total and dissolved concentrations of zinc support the finding that in laboratory tests, dissolved zinc represents the vast majority of total zinc present in solution (Davies *et al.* 2002; Hansen *et al.* 2002; Woodling *et al.* 2002; Clifford and McGeer 2009).

The total:dissolved conversion factors were derived based on laboratory analytical data, so they apply here to laboratory data as well (i.e., to toxicity endpoints based on total concentrations that were included in the SSD). Due to site-specific and seasonal factors as well as complexing agents that can influence the total:dissolved ratio in the field, these conversion factors should not be applied to total concentrations of zinc sampled in the field. Rather, where guideline users have only water samples of total zinc, they should first compare these samples to the dissolved benchmark or guideline, and where there is an exceedance, re-sample for a dissolved concentration.

10.5 Methods Used for Deriving Guidelines (Type A, B1 or B2)

Risk assessors and risk managers require a CWQG for zinc to address zinc use in Canada and potential impacts to aquatic systems. The CCME 2007 protocol includes guideline values for both long- and short-term exposure. The long-term exposure guideline is derived such that it is consistent with the guiding principle of the CWQG, namely to protect all species and all life stages over an indefinite exposure to substance in water. Aquatic life may experience long-term exposure to a substance as a result of continuous release from point or non-point sources, gradual release from soils or sediments, gradual entry through groundwater or runoff, or long-range transport. The short-term exposure value (or benchmark) is derived for use as an additional management tool. It is intended to protect most species against lethality during severe but transient events such as spills or inappropriate use or disposal of the substance in question.

While separate data sets are used to calculate short-term and long-term guidelines, both are derived using one of three approaches. The three approaches are detailed in CCME (2007) and only briefly outlined here. In order of preference, the approaches are:

1. Statistical approach using primary and/or secondary data (Type A or SSD approach)
2. Lowest endpoint approach using only primary data (Type B1)
3. Lowest endpoint approach using primary and/or secondary data (Type B2).

A guideline derived using the statistical approach is called a Type A guideline. An SSD captures the variation in toxicological sensitivity to a contaminant among a set of species. An SSD is a cumulative distribution function (CDF), with effect concentrations plotted on the x-axis and cumulative probability, expressed as a percentage, plotted on the y-axis (Posthuma *et al.* 2002). Short-term, lethal endpoints (e.g., 24-h LC_{50}) make up the data set for short-term guidelines, while long-term exposure with no- or low-effect endpoints (e.g., 21-d EC_{10} for growth) make up the data set for long-term guidelines. From each data set, the guideline value is equal to the concentration on the x-axis that corresponds to 5% cumulative probability on the y-axis. In contrast, the lowest endpoint approaches (Types B1 and B2) use, as the name implies, the lowest acceptable endpoint with a safety factor to estimate the guideline.

Table 10.2 (short-term freshwater exposure guidelines) and Table 10.3 (long-term freshwater exposure guidelines) present the minimum data requirements for application of each of the three methods. If available data are insufficient for deriving a CWQG using the statistical approach, the CWQG is developed using the lowest endpoint approach. Depending on the quantity and quality of data, a Type B1 or Type B2 approach is used. The Type B1 approach uses only acceptable primary toxicity data to derive the guideline, while the Type B2 approach can use acceptable primary and/or secondary data. In every case, a CWQG must be developed using the highest-ranked method that the data allow.

The following sections describe the derivation of the short-term benchmark and long-term CWQG for the protection of freshwater life in surface water for zinc. Note that the long-term SSD-derived CWQG value (the 5th percentile of the SSD) applies only to waters with 50 $mg \cdot L^{-1}$ hardness as $CaCO_3$, pH of 7.5 and DOC concentration of 0.5 $mg \cdot L^{-1}$, since all long-term toxicity data were adjusted to these conditions before being entered into the SSD. A long-term CWQG equation was developed so that CWQGs can be derived for waters of other hardness, pH and

DOC. For short-term data, the SSD-derived benchmark concentration (the 5th percentile of the SSD) applies to waters of hardness 50 $mg \cdot L^{-1}$ as $CaCO_3$ and DOC of 0.5 $mg \cdot L^{-1}$, since all short-term toxicity data were adjusted to these conditions before being entered into the SSD. A short-term benchmark equation was developed so that benchmarks can be derived for waters of other hardness or DOC.

Table 10.2 Minimum data set requirements for the derivation of a short-term exposure guideline for freshwater environments

Group	Guideline		
	Type A	**Type B1**	**Type B2**
Fish	Three species, including at least one salmonid and one non-salmonid.		Two species, including at least one salmonid and one non-salmonid.
Aquatic Invertebrates	Three aquatic or semi-aquatic invertebrates, at least one of which must be a planktonic crustacean. For semi-aquatic invertebrates, the life stages tested must be aquatic. It is desirable, but not necessary, that one of the aquatic invertebrate species be either a mayfly, caddisfly, or stonefly.		Two aquatic or semi-aquatic invertebrates, at least one of which must be a planktonic crustacean. For semi-aquatic invertebrates, the life stages tested must be aquatic. It is desirable, but not necessary, that one of the aquatic invertebrate species be either a mayfly, caddisfly, or stonefly.
Plants	Toxicity data for aquatic plants or algae are highly desirable, but not necessary. However, if a toxicity study indicates that a plant or algal species is among the most sensitive species in the data set, then this substance is considered to be phyto-toxic and two studies on nontarget freshwater plant or algal species are required.		
Amphibians	Toxicity data for amphibians are highly desirable, but not necessary. Data must represent fully aquatic stages.		
Preferred Endpoints	Acceptable LC_{50} or equivalent (e.g., EC_{50} for immobility in small invertebrates).		
Data Quality Requirement	Primary and secondary LC_{50} (or equivalents) data are acceptable to meet the minimum data set requirement. Both primary and secondary data will be plotted. A chosen model should sufficiently and adequately describe data and pass the appropriate goodness-of-fit test.	The minimum data requirement must be met with primary LC_{50} (or equivalents) data. The value used to set the guideline must be primary.	The minimum data requirement must be met with primary LC_{50} (or equivalents) data. Secondary data are acceptable. The value used to set the guideline may be secondary.

Source: CCME (2007).

Table 10.3 Minimum data set requirements for the derivation of a long-term exposure guideline for freshwater environments

Group	Guideline		
	Type A	**Type B1**	**Type B2**
Fish	Three species, including at least one salmonid and one non-salmonid.		Two species, including at least one salmonid and one non-salmonid.
Aquatic Invertebrates	Three aquatic or semi-aquatic invertebrates, at least one of which must be a planktonic crustacean. For semi-aquatic invertebrates, the life stages tested must be aquatic. It is desirable, but not necessary, that one of the aquatic invertebrate species be either a mayfly, caddisfly, or stonefly.		Two aquatic or semi-aquatic invertebrates, at least one of which must be a planktonic crustacean. For semi-aquatic invertebrates, the life stages tested must be aquatic. It is desirable, but not necessary, that one of the aquatic invertebrate species be either a mayfly, caddisfly, or stonefly.
Aquatic Plants	At least one study on a freshwater vascular plant or freshwater algal species. If a toxicity study indicates that a plant or algal species is among the most sensitive species in the data set, then this substance is considered to be phyto-toxic and three studies on nontarget freshwater plant or algal species are required.		Toxicity data for plants are highly desirable, but not necessary. If a toxicity study indicates that a plant or algal species is among the most sensitive species in the data set, then this substance is considered to be phyto-toxic and two studies on nontarget freshwater plant or algal species are required.
Amphibians	Toxicity data for amphibians are highly desirable, but not necessary. Data must represent fully aquatic stages.		Toxicity data for amphibians are highly desirable, but not necessary. Data must represent fully aquatic stages.

Group	Guideline		
	Type A	**Type B1**	**Type B2**
Preferred Endpoints	The acceptable endpoints representing the no-effects threshold and EC_{10}/IC_{10} for a species are plotted. The other, less preferred, endpoints may be added sequentially to the data set to fulfill the minimum data requirement condition and improve the result of the modelling for the guideline derivation if the more preferred endpoint for a given species is not available. The preference ranking is done in the following order: Most appropriate EC_x/IC_x representing a no-effects threshold > EC_{10}/IC_{10} > EC_{11-25}/IC_{11-25} > MATC > NOEC > LOEC > EC_{26-49}/IC_{26-49} > nonlethal EC_{50}/IC_{50}. Multiple comparable records for the same endpoint are to be combined by the geometric mean of these records to represent the averaged species effects endpoint.	The most preferred acceptable endpoint representing a low-effects threshold for a species is used as the critical study; the next less preferred endpoint will be used sequentially only if the more preferred endpoint for a given species is not available. The preference ranking is done in the following order: Most appropriate EC_x/IC_x representing a low-effects threshold > EC_{15-25}/IC_{15-25} > LOEC > MATC > EC_{26-49}/IC_{26-49} > nonlethal EC_{50}/IC_{50} > LC_{50}.	
Data Quality Requirement	Primary and secondary no-effects and low-effects level data are acceptable to meet the minimum data set requirement. Both primary and secondary data will be plotted. A chosen model should sufficiently and adequately describe data and pass the appropriate goodness-of-fit test.	The minimum data requirement must be met with primary data. The value used to set the guideline must be primary. Only low-effect data can be used to fulfill the minimum data requirement.	Secondary data are acceptable. The value used to set the guideline may be secondary. Only low-effect data can be used to fulfill the minimum data requirement.

Source: CCME (2007).

10.6 Freshwater Zinc Guidelines

10.6.1 Short-term Benchmark Concentration

In total, 1,093 short-term freshwater toxicity data points were obtained for zinc. Of these, 581 were deemed of acceptable data quality following the criteria in CCME (2007). Of the acceptable data, 81 species were included in a short-term SSD. Other data points were omitted to avoid including low- or no-effects endpoints (i.e., any endpoint other than an LC_{50} or equivalent) or multiple data points for a single species in the SSD. The CCME 2007 protocol requires that only one short-term endpoint (i.e., LC_{50} value or equivalent) can be included in the short-term SSD for each species. In some cases, there were several values for a given species and life stage with the same duration (e.g., several 96-h LC_{50} values for juveniles of a species). These values, however, were not identical; this variation may be the result of differences in experimental conditions, species strain and/or bioassay protocol.

Numerous methods can be applied to account for multiple similar data points for a single species (Duboudin *et al.* 2004). For the derivation of the short-term SSD for zinc, this intra-species variability was accounted for by taking the geometric mean of the toxicity values when multiple data points were obtained for the same species, life stage, duration, effect and test water quality (CCME 2007). Geometric means were taken only if exposure-water conditions were consistent (e.g. consistent temperature, pH). An exception was if the exposure conditions varied, but that particular variable was accounted for in the MLR adjustment equation. For example, if two data entries had the same exposure conditions except for differences in hardness or DOC, a geometric mean could still be calculated because endpoint values were standardized to the same hardness and DOC using the MLR normalization equation. In some cases, more than one toxicity value was available for a given species, but the duration and/or life stages differed, meaning that the geometric mean of the values could not be taken. In these cases, the most sensitive data point (or geometric mean value) was selected for inclusion in the short-term SSD. For full details regarding short-term data point selection, see CCME (2007).

The values reported in Table 10.4 for the short-term SSD represent effect concentrations standardized to a hardness of 50 $mg \cdot L^{-1}$ as $CaCO_3$ and a DOC concentration of 0.5 $mg \cdot L^{-1}$ using the pooled *Daphnia* MLR normalization equation. All values included in the SSD were originally conducted at test hardness and DOC concentrations within the range of the data used to derive the MLR equation (i.e., application of the MLR standardization equation was not extrapolated beyond the water chemistry of the data from which it was derived). The values in the SSD represent dissolved concentrations of zinc. Total concentrations were converted to dissolved concentrations using a total:dissolved conversion factor of 0.978 (US EPA 1996), and dissolved concentrations were plotted directly. The 5th percentile of the SSD (HC5 value) represents the short-term benchmark concentration for waters with a hardness of 50 $mg \cdot L^{-1}$ and a DOC of 0.5 $mg \cdot L^{-1}$.

Table 10.4 Toxicity data points used in the SSD to determine the short-term benchmark concentration for zinc. Endpoint concentrations have been standardized to a hardness of 50 mg·L^{-1} as $CaCO_3$ and a DOC concentration of 0.5 mg·L^{-1}. Total concentrations have been converted to dissolved concentrations using a total: dissolved conversion factor

SSD rank order	Species	Endpoint	Life stage	Data quality	Measured effect concentration (µg·L^{-1}) (variation)	Reference	Adjusted effect concentration (µg·L^{-1})
1	*Daphnia magna* (cladoceran)	96-h LC_{50}	Juvenile	1	67.91	Attar and Maly (1982)	22.7
2	*Ceriodaphnia dubia* (water flea)	48-h LC_{50}	Less than 24 h	-	Geometric mean	-	34.0
3	*Pseudokirchneriella subcapitata* (green algae)	4-h EC_{50} (growth)	Not reported	2	97 (25, 75 percentiles: 70, 111)	Pardos *et al.* (1998)	36.2
4	*Ceriodaphnia reticulata* (water flea)	48-h LC_{50}	Less than 4 h	2	76 (61–93)	Mount and Norberg (1984)	67.2
5	*Chlorella pyrenoidosa* (green algae)	24-h EC_{50} (growth)	Not reported	2	57	Lin *et al.* (2007)	76.3
6	*Oncorhynchus mykiss* (rainbow trout)	5-d LC_{50}	Juvenile	-	Geometric mean	-	84.9
7	*Daphnia pulex* (water flea)	48-h LC_{50}	Less than 24 h	2	107 (76–151)	Mount and Norgberg (1984)	94.6
8	*Oncorhynchus tshawytscha* (Chinook salmon)	96-h LC_{50}	Juvenile	2	84 (75% CI +/- 41)	Finlayson and Verrue (1982)	99.6
9	*Oncorhynchus clarkii virginalis* (Rio Grande cutthroat trout)	96-h LC_{50}	Fry	2	142 (95% CI 128–157)	Brinkman and Johnston (2012)	120
10	*Cottus bairdi* (mottled sculpin)	96-h LC_{50}	Newly emerged	1	156 (95% CI 125–193)	Woodling *et al.* (2002)	121
11	*Salvelinus confluentus* (bull trout)	5-d LC_{50}	Juvenile	-	Geometric mean	-	123
12	*Morone saxatilis* (striped bass)	96-h LC_{50}	35–80 d	2	120 (95% CI 80–170)	Palawski *et al.* (1985)	141
13	*Salmo trutta* (brown trout)	96-h LC_{50}	Yearling	1	640 (95% CI 520–780)	Everall *et al.* (1989*b*)	147
14	*Daphnia ambigua* (cladoceran)	48-h LC_{50}	Neonate	1	304.76 (95% CI 223.67–402.21)	Shaw *et al.* (2006)	150
15	*Agosia chrysogaster* (longfin dace)	96-h LC_{50}	Juvenile	1	790 (400–1,500)	Lewis (1978)	152
16	*Thymallus arcticus* (Arctic grayling)	96-h LC_{50}	Juvenile	-	Geometric mean	-	171
17	*Lampsilis rafinesqueana* (Neosho mucket)	48-h EC_{50} (survival)	Juvenile	1	134 (95% CI 115–157)	Wang *et al.* (2010)	175

SSD rank order	Species	Endpoint	Life stage	Data quality	Measured effect concentration ($\mu g \cdot L^{-1}$) (variation)	Reference	Adjusted effect concentration ($\mu g \cdot L^{-1}$)
18	*Pimephales promelas* (fathead minnow)	96-h TL_m	Fry	2	870 (95% CI 790–1,010)	Pickering and Vigor (1965)	194
19	*Daphnia longispina* (cladoceran)	48-h EC_{50} (immobility)	Less than 48 h	-	Geometric mean	-	210
20	*Daphnia carinata* (cladoceran)	48-h LC_{50}	Neonate	1	339.8 (95% CI 263.4–438.6)	Cooper *et al.* (2009)	224
21	*Oncorhynchus clarkii pleuriticus* (Colorado River cutthroat trout)	96-h LC_{50}	Fry	-	Geometric mean	-	245
22	*Simocephalus vetulus* (cladoceran)	48-h EC_{50} (immobility)	Less than 48 h	-	Geometric mean	-	246
23	*Daphnia galeata* (cladoceran)	48-h EC_{50} (immobility)	Less than 48 h	2	1,001 (SD 82)	Bossuyt *et al.* (2005)	262
24	*Simocephalus exspinosus* (cladoceran)	48-h EC_{50} (immobility)	Less than 48 h	-	Geometric mean	-	307
25	*Prosopium williamsoni* (mountain whitefish)	96-h LC_{50}	Fry	-	Geometric mean	-	327
26	*Oncorhynchus clarkii stomias* (greenback cutthroat trout)	96-h LC_{50}	Fry	-	Geometric mean	-	328
27	*Acroperus elongatus* (cladoceran)	48-h EC_{50} (immobility)	Less than 48 h	2	1,614 (SD 837)	Bossuyt *et al.* (2005)	423
28	*Chydorus ovalis* (cladoceran)	48-h EC_{50} (immobility)	Less than 48 h	2	1,627 (SD 963)	Bossuyt *et al.* (2005)	426
29	*Ceriodaphnia pulchella* (cladoceran)	48-h EC_{50} (immobility)	Less than 48 h	-	Geometric mean	-	443
30	*Lampsilis siliquoidea* (fatmucket clam)	96-h EC_{50} (survival)	Juvenile	-	Geometric mean	-	470
31	*Chydorus sphaericus* (cladoceran)	48-h EC_{50} (immobility)	Less than 48 h	-	Geometric mean	-	516
32	*Ptychocheilus lucius* (Colorado pikeminnow)	96-h LC_{50}	Swim-up fry	2	1,700 (95% CI 1,000–5,100)	Hamilton (1995)	533
33	*Bufo boreas* (western toad)	96-h LC_{50}	Egg	1	840 (95% CI 760–929)	Davies and Brinkman (1999)	535
34	*Oncorhynchus nerka* (sockeye salmon)	115-h LC_{50}	Alevin	2	447 (95% CI 385–544)	Chapman (1978)	717
35	*Oncorhynchus kisutch* (coho salmon)	96-h LC_{50}	Alevin	2	727 (95% CI 507–1,042)	Buhl and Hamilton (1990)	834

SSD rank order	Species	Endpoint	Life stage	Data quality	Measured effect concentration ($\mu g \cdot L^{-1}$) (variation)	Reference	Adjusted effect concentration ($\mu g \cdot L^{-1}$)
36	*Culicoides furens* (midge)	96-h LC_{50}	Larva	2	1,200	Vedamanikam and Shazilli (2008*a*)	888
37	*Chironomus plumosus* (midge)	96-h LC_{50}	Larva	2	1,350	Vedamanikam and Shazilli (2008*a*)	999
38	*Physa heterostropha* (snail)	96-h LC_{50}	Not reported	-	Geometric mean	-	1,021
39	*Moina macrocopa* (cladoceran)	48-h LC_{50}	Neonate	2	1,170 (95% CI 1,020–1,320)	Wong (1992)	1,144
40	*Tubifex* (sludge worm)	96-h LC_{50}	Not reported	2	5,650 (95% CI 4,280–6,910)	Rathore and Khangarot (2002)	1,145
41	*Xyrauchen texanus* (razorback sucker)	96-h LC_{50}	Swim-up fry	2	4,100 (95% CI 3,400–5,200)	Hamilton (1995)	1,286
42	*Physa gyrina* (snail)	96-h LC_{50}	Adult	2	1,274	Nebeker *et al.* (1986)	1,356
43	*Rhinichthys cataractae* (longnose dace)	96-h LC_{50}	Fry	2	1,900 (95% CI 1,700–2,120)	Brinkman and Johnston (2012)	1,382
44	*Brachionus havanaensis* (rotifer)	24-h LC_{50}	Neonate	2	2,271 (95% CL +/- 404.4)	Juárez-Franco *et al.* (2007)	1,428
45	*Gila elegans* (bonytail chub)	96-h LC_{50}	Swim-up fry	2	4,800 (95% CI 2,100–7,100)	Hamilton (1995)	1,505
46	*Lymnaea luteola* (snail)	96-h LC_{50}	Adult	2	5,000	Khangarot and Ray (1987*a*)	1,542
47	*Salvelinus fontinalis* (brook trout)	96-h LC_{50}	Juvenile	-	Geometric mean	-	1,713
48	*Platygobio gracilis* (flathead chub)	96-h LC_{50}	Fry	2	2,590 (95% CI 2,150-3,130)	Brinkman and Johnston (2012)	1,809
49	*Hydra viridissima* (green hydra)	96-h LC_{50}	Not reported	2	935 (SE 46.5)	Holdway *et al.* (2001)	2,003
50	*Lirceus alabamae* (isopod)	96-h LC_{50}	Not reported	2	8,300 (95% CI 7,200–9,570)	Bosnak and Morgan (1981)	2,077
51	*Cyprinus carpio* (common carp)	96-h LC_{50}	Juvenile	2	9,744.6 (5,951.4–20,731.8)	Hattink *et al.* (2006)	2,496

SSD rank order	Species	Endpoint	Life stage	Data quality	Measured effect concentration ($\mu g \cdot L^{-1}$) (variation)	Reference	Adjusted effect concentration ($\mu g \cdot L^{-1}$)
52	*Spirodela polyrrhiza* (greater duckweed)	4-d IC_{50} (growth)	Adult	2	935 (+/- 6.5 [SD])	Gaur *et al.* (1994)	2,505
53	*Azolla pinnata* (mosquito fern)	4-d IC_{50} (growth)	Adult	2	948 (+/- 6.5 [SD])	Gaur *et al.* (1994)	2,540
54	*Catostomus commersoni* (white sucker)	96-h LC_{50}	Adult	2	2,200	Duncan and Klaverkamp (1983)	2,688
55	*Lepomis macrochirus* (bluegill)	96-h LC_{50}	Not reported, likely juvenile (32–67 mm)	2	3,200 (2,100–4,600)	Thompson *et al.* (1980)	3,155
56	*Catostomus latipinnis* (flannelmouth sucker)	24-h LC_{50}	Larva	2	8,890 (95% CI 7,020–10,900)	Hamilton and Buhl (1997)	3,604
57	*Corbicula fluminea* (bivalve)	96-h LC_{50}	Not reported	2	6,040 (95% CI 4,720–7,260)	Rodgers *et al.* (1980)	3,696
58	*Brachydanio rerio* (zebrafish)	96-h LC_{50}	Adult	2	8,062	Xiong *et al.* (2011)	3,761
59	*Caecidotea bicrenata* (isopod)	96-h LC_{50}	Not reported	2	20,220 (95% CI 12,140–33,660)	Bosnak and Morgan (1981)	3,897
60	*Gambusia holbrooki* (eastern mosquitofish)	96-h LC_{50}	0.39 g	-	Geometric mean	-	4,192
61	*Rana hexadactyla* (green pond frog)	96-h LC_{50}	Tadpole	2	2,100 (95% CI 1,670–3,030)	Khangarot *et al.* (1985)	4,404
62	*Hydra vulgaris* (pink hydra)	96-h LC_{50}	Not reported	2	2,300 (SE 147.2)	Holdway *et al.* (2001)	4,928
63	*Bufo melanostictus* (Asian toad)	96-h LC_{50}	Tadpole	2	19,860 (95% CI 17,680–23,900)	Khangarot and Ray (1987*b*)	4,945
64	*Morone americana* (white perch)	48-h TL_m	Adult	2	10,100	Rehwoldt *et al.* (1972)	5,253
65	*Ptychocheilus oregonensis* (northern pikeminnow)	96-h LC_{50}	Juvenile	-	Geometric mean	-	5,420
66	*Hydra oligactis* (brown hydra)	72-h LC_{50}	Not reported	2	20,000	Karntanut and Pascoe (2002)	5,928
67	*Lumbriculus variegatus* (blackworm)	96-h LC_{50}	Not reported	2	6,300 (95% CI 5,600–7,200)	Bailey and Liu (1980)	7,293

SSD rank order	Species	Endpoint	Life stage	Data quality	Measured effect concentration ($\mu g \cdot L^{-1}$) (variation)	Reference	Adjusted effect concentration ($\mu g \cdot L^{-1}$)
68	*Anguilla rostrata* (American eel)	96-h TL_m	Likely adult (> 20 cm)	2	14,500	Rehwoldt *et al.* (1972)	7,542
69	*Notemigonus crysoleucas* (golden shiner)	24-h LC_{50}	Juvenile	2	7,760	Cairns *et al.* (1978)	7,666
70	*Baetis tricaudatus* (mayfly)	96-h LC_{50}	Nymph	2	10,100 (95% CI 7,480–13,400)	Brinkman and Johnston (2012)	8,429
71	*Fundulus diaphanus* (banded killifish)	96-h TL_m	Adult	2	19,200	Rehwoldt *et al.* (1972)	9,987
72	*Lepomis gibbosus* (pumpkinseed)	96-h TL_m	Adult	2	20,100	Rehwoldt *et al.* (1972)	10,455
73	*Aeolosoma headleyi* (annelid)	48-h LC_{50}	Not reported	2	13,500	Cairns *et al.* (1978)	11,076
74	*Xenopus laevis* (African clawed frog)	4-d LC_{50}	Embryo	2	34,500 (95% CI +/- 1,200)	Dawson *et al.* (1988)	18,947
75	*Lepidostoma* sp. (caddisfly)	96-h LC_{50}	Nymph	2	48,500	Brinkman and Johnston (2012)	35,215
76	*Carassius auratus* (goldfish)	24-h LC_{50}	Juvenile	2	40,000	Cairns *et al.* (1978)	39,517
77	*Rhithrogena hageni* (mayfly)	96-h LC_{50}	Nymph	1	50,500 (95% CI 39,100–65,300)	Brinkman and Johnston (2008)	40,479
78	*Drunella doddsi* (mayfly)	96-h LC_{50}	Nymph	2	64,000	Brinkman and Johnston (2012)	46,625
79	*Chloroperlidae* (stonefly)	96-h LC_{50}	Nymph	2	68,800	Brinkman and Johnston (2012)	49,058
80	*Cinygmula* sp. (mayfly)	96-h LC_{50}	Nymph	2	68,800	Brinkman and Johnston (2012)	49,058
81	*Ephemerella* sp. (mayfly)	96-h LC_{50}	Nymph	2	68,800	Brinkman and Johnston (2012)	49,058

[1] Geometric mean values were taken from studies with the same species, endpoint and duration, and similar life-stage and test-water quality parameters. Geometric means were also calculated from studies with varying hardness and/or DOC because the short-term pooled *Daphnia* MLR normalization equation standardized endpoint values for these variables. For details on which individual studies were used to calculate geometric means, as well as additional details on all studies, see the Appendix.

[2] Adjusted effect concentrations were calculated using the pooled *Daphnia* MLR normalization equation: Standardized $EC_{50} = \exp[\ln(EC_{50meas}) - 0.240(\ln[DOC_{meas}] - \ln[DOC_{target}]) - 0.833(\ln[hardness_{meas}] - \ln[hardness_{target}])]$. Total concentrations were converted to dissolved concentrations using a total:dissolved conversion factor of 0.978 (US EPA 1996).

Each species for which appropriate short-term toxicity data were available was ranked according to sensitivity, and its centralized position on the SSD (Hazen plotting position) was determined using the following standard equation (Aldenberg *et al.* 2002; Newman *et al.* 2002):

$$\frac{i - 0.5}{N}$$

where

i = the species rank based on ascending EC_{50} values and LC_{50} values
N = the total number of species included in the SSD derivation

These positional rankings, along with their corresponding EC_{50} and LC_{50} values, were used to derive the SSD. The software "SSD Master" (version 3.0; developed by Intrinsik, available from Environment and Climate Change Canada, Gatineau QC, mailto:ec.rqe-eqg.ec@canada.ca) was used to fit SSDs to the data set. Several CDFs (normal, logistic, extreme value, Weibull and Gumbel) were fit to the data using regression methods. Evaluation goodness of fit of the various models included examining probability-probability plots, quantile-quantile plots, residual plots, Anderson-Darling goodness-of-fit test, sum of residual error, mean sum of squared error terms in the lower tail, width of confidence intervals (CI), and overall visual assessment of model fit.

The normal model provided the best fit of the models tested by most goodness-of-fit measures. While the Gumbel model visually had better fit in the lower tail of the distribution, it had poorer overall fit, a higher Anderson-Darling test statistic, higher mean sum of squared error terms in the lower tail, wide uncertainty and a higher sum of residual error, and it yielded an HC5 value almost two times higher than the normal model. Due to this uncertainty, the normal model was selected as the best model to err on the side of conservatism (Anderson-Darling statistic (A^2) = 0.388). The equation of the normal model is of the form:

$$f(x) = \frac{1}{2}\left(1 + erf\ \left(\frac{x-\mu}{\sigma\sqrt{2}}\right)\right)$$

where, for the fitted model: x = log (concentration), μ = 3.051 and σ = 0.900. The functional response, $f(x)$, is the proportion of taxa affected at the given concentration. The location and scale parameters, μ and σ, are the mean and standard deviation of the theoretical population, respectively, and *erf* is the error function.

Figure 10-1 shows the short-term SSD. Table 10.5 shows summary statistics for the short-term SSD. The 5th percentile on the short-term SSD is 37.27 µg Zn·L^{-1}. The lower confidence limit (5%) on the 5th percentile is 34.63 µg·L^{-1}, and the upper confidence limit (95%) on the 5th percentile is 40.12 µg·L^{-1}.

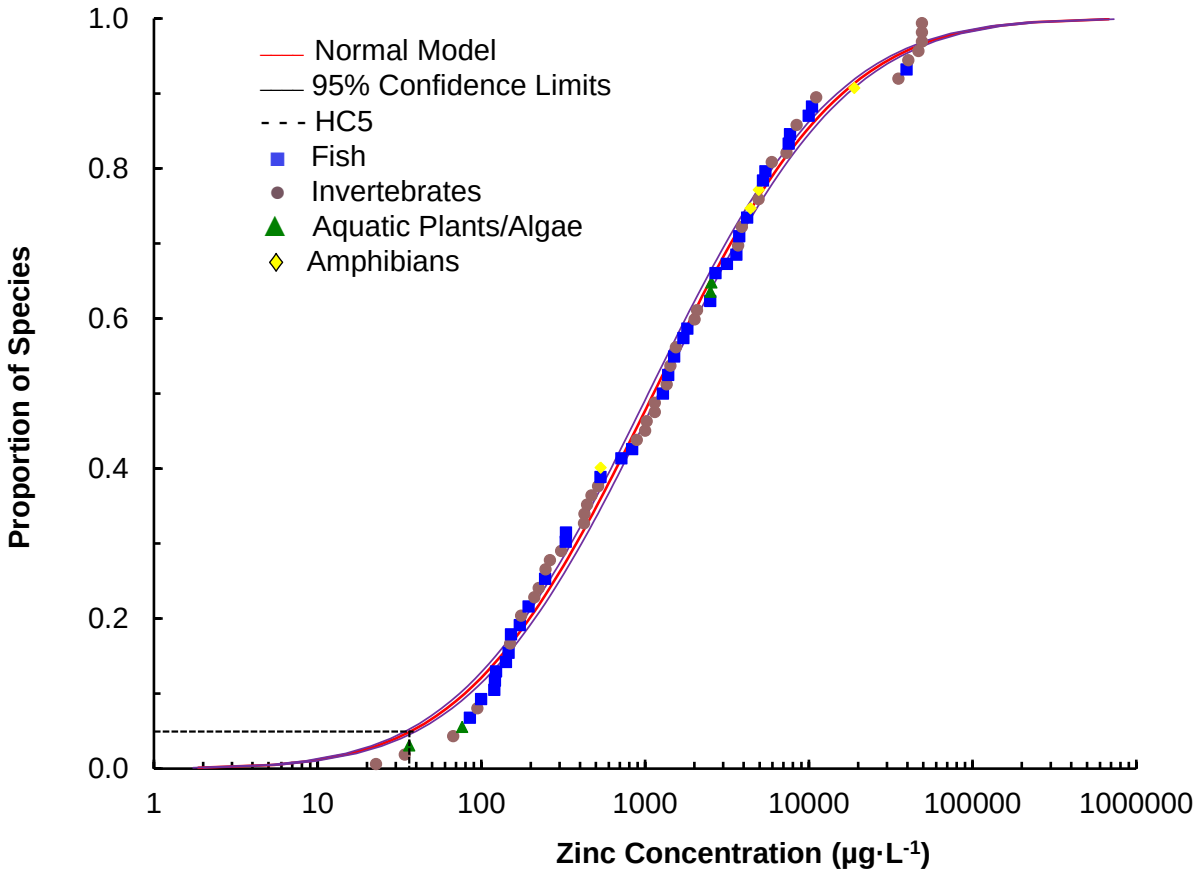


Figure 10.1 Short-term SSD for zinc in freshwater derived by fitting the normal model to the short-term data points of 81 aquatic species versus Hazen plotting position

The intercept of the 5th percentile of the fitted curve (benchmark value) was determined to be 37.27 µg·L^{-1} zinc, with 95% confidence intervals of 34.63 and 40.12 µg·L^{-1}. Due to the number of data points, legible species labels could not be added.

Table 10.5 Short-term benchmark concentration for zinc resulting from the Type A SSD approach at 50 mg·L^{-1} water hardness and 0.5 mg·L^{-1} DOC concentration

	Zinc concentration[1] (µg·L^{-1})
Short-term benchmark concentration, SSD 5th percentile	37.27
Short-term benchmark concentration, SSD 5th percentile, 95% Lower CL	34.63
Short-term benchmark concentration, SSD 5th percentile, 95% upper CL	40.12

CL = confidence limit
[1] Dissolved concentration

Three data points on the short-term SSD curve fell below the 5th percentile value. The likelihood of a data point on an SSD falling below the 5th percentile increases with sample size, and is therefore inherent in the SSD calculation. Since the short-term guideline is meant to protect a

specified fraction of organisms from severe effects and to provide guidance on the impacts of severe, transient events, this 5th percentile concentration is acceptable (CCME 2007).

10.6.2 Long-term CWQG

In total, 831 long-term freshwater toxicity data points were obtained for zinc. Of these, 606 were deemed of acceptable data quality according to CCME (2007). Of these acceptable data, long-term data points were considered appropriate for a long-term SSD if they were low- or no-effects endpoints, and inappropriate if they were severe endpoints (e.g., median lethal [LC_{50}] values). For details, see CCME (2007).

Of the remaining data points, 29 species were included in the long-term SSD. Other data points were omitted in order to avoid including multiple data points for the same species in the SSD. Numerous methods can be applied to account for multiple similar endpoints for a single species (Duboudin *et al.* 2004). For the derivation of the long-term SSD for zinc, this intra-species variability was accounted for by taking the geometric mean of the toxicity values when multiple data points were obtained for the same species, life stage, duration, effect, endpoint and experimental conditions. Geometric means were taken only if exposure-water conditions were consistent. An exception was if the exposure conditions varied but that particular variable was accounted for in the MLR adjustment equation. For example, if two data entries had the same exposure conditions except for differences in hardness, pH and/or DOC, a geometric mean could still be calculated because endpoint values were standardized to the same hardness, pH and DOC using the MLR normalization equation.

In some cases, more than one toxicity value was available for a given species, but the life stage, duration, effect or endpoint type differed, meaning that the geometric mean of the values could not be taken. According to the CCME 2007 protocol if there is more than one long-term endpoint type (e.g., an EC_{10} and an NOEC) for a given species and effect, the most preferred endpoint will be selected for inclusion in the SSD.

The preferred rank order of endpoints for a long-term SSD (CCME 2007) is as follows:

1. most appropriate EC_x/IC_x representing a no-effects threshold
2. EC_{10}/IC_{10}
3. $EC_{11\text{-}25}/IC_{11\text{-}25}$
4. MATC
5. NOEC
6. LOEC
7. $EC_{26\text{-}49}/IC_{26\text{-}49}$
8. non-lethal EC_{50}/IC_{50}

If more than one toxicity value (or geometric mean) is available for a given species, effect and endpoint, but the duration and/or life stage differs, the most sensitive data point (or geometric mean value) will be selected for inclusion in the long-term SSD. For full details regarding long-term endpoint selection, see CCME (2007).

The values reported in Table 10.6 for the long-term SSD represent effect concentrations standardized to a hardness of 50 $mg \cdot L^{-1}$ as $CaCO_3$, a pH of 7.5 and a DOC of 0.5 $mg \cdot L^{-1}$ using the *O. mykiss* MLR normalization equation. All values included in the SSD were conducted at hardness, pH and DOC values within the range of the data used to derive the MLR equation (i.e., the MLR standardization equation was not extrapolated beyond the water chemistry of the data from which it was derived). The values in the SSD represent dissolved concentrations of zinc. Total concentrations were converted to dissolved concentrations using a total:dissolved conversion factor of 0.986 (US EPA 1996), and dissolved concentrations were plotted directly. The 5th percentile of the SSD (HC5 value) represents the long-term CWQG concentration for waters with a hardness of 50 $mg \cdot L^{-1}$, a pH of 7.5 and a DOC concentration of 0.5 $mg \cdot L^{-1}$.

Table 10.6 Toxicity data points used in the SSD to determine the long-term CWQG for zinc. Endpoint concentrations have been standardized to a hardness of 50 mg·L^{-1} as $CaCO_3$, a pH of 7.5, and a DOC concentration of 0.5 mg·L^{-1}. Total concentrations have been converted to dissolved using a total: dissolved conversion factor.

SSD rank order	Species	Endpoint	Life stage	Data quality	Measured effect concentration(µg·L^{-1})	Reference	Adjusted effect concentration (µg·L^{-1})
1	*Chironomus riparius* (harlequin fly)	11-week LOEC (development)	1st instar	2	100	Timmermans *et al.* (1992)	9.89
2	*Ceriodaphnia dubia* (water flea)	7-d MATC (reproduction)	Neonate	1	18.1	Cooper *et al.* (2009)	11.3
3	*Pseudokirchneriella subcapitata* (green algae)	72-h EC_{10} (growth rate)	Exponential phase	-	Geometric mean	-	13.8
4	*Daphnia magna* (cladoceran)	21-d EC_{10} (reproduction)	Newborn juvenile	-	Geometric mean	-	15.0
5	*Potamopyrgus jenkinsi* (New Zealand mud snail)	12-week MATC (growth)	Juvenile	2	91	Dorgelo *et al.* (1995)	19.1
6	*Jordanella floridae* (flagfish)	100-d MATC (growth)	Larva	2	36	Spehar (1976)	27.9
7	*Cottus bairdi* (mottled sculpin)	30-d EC_{10} (mortality)	Less than 2 months	1	155.7	Brinkman and Woodling (2005)	31.5
8	*Brachionus havanaensis* (rotifer)	18-d EC_{10} (population growth inhibition)	Adults and juveniles	2	78.2	Juárez-Franco *et al.* (2007)	36.5
9	*Phoxinus phoxinus* (Eurasian minnow)	150-d LC_{10} (mortality)	Yearling	2	102	Bengtsson (1974)	51.0
10	*Dreissena polymorpha* (zebra mussel)	10-week LC_{10} (mortality)	Adult	2	517	Kraak *et al.* (1994*b*)	51.1
11	*Pimephales promelas* (fathead minnow)	7-d IC_{10} (growth)	Larva	2	83.9	Norberg and Mount (1985)	68.2
12	*Brachionus calyciflorus* (rotifer)	48-h EC_{10} (intrinsic rate of population increase)	Less than 2 hours	-	Geometric mean	-	73.0
13	*Oncorhynchus mykiss* (rainbow trout)	30-d LC_{10} (mortality)	Juvenile	-	Geometric mean	-	101
14	*Lampsilis siliquoidea* (fatmucket clam)	28-d IC_{10} (length)	Juvenile	1	55 (95% CI 24–181)	Wang *et al.* (2010)	104
15	*Bufo boreas* (western toad)	4-week MATC (development)	Egg	1	264	Davies and Brinkman (1999)	108
16	*Lymnaea stagnalis* (great pond snail)	28-d EC_{10} (growth)	21 days	-	Geometric mean	-	113

SSD rank order	Species	Endpoint	Life stage	Data quality	Measured effect concentration(µg·L^{-1})	Reference	Adjusted effect concentration (µg·L^{-1})
17	*Salmo trutta* (brown trout)	58-d MATC (weight)	Early life stage	1	196	Davies *et al.* (2002)	130
18	*Prosopium williamsoni* (mountain whitefish)	90-d IC_{10} (biomass)	Eyed egg to fry	1	380	Brinkman and Vieira (2008)	133
19	*Salvelinus fontinalis* (brook trout)	24-week IC_{10} (egg fragility)	Egg	2	200	Holcombe *et al.* (1979)	161
20	*Oncorhynchus clarkii pleuriticus* (Colorado river cutthroat trout)	30-d MATC (biomass)	Swim-up fry	-	Geometric mean	-	169
21	*Chlorella* sp. (green algae)	48-h IC_{50} (growth rate)	Exponential growth	-	Geometric mean	-	225
22	*Physa gyrina* (snail)	30-d NOEC/L (mortality)	Adult	2	570	Nebeker *et al.* (1986)	344
23	*Lemna minor* (common duckweed)	7-d EC_{10} (growth)	Not reported	2	1,379.05	Ince *et al.* (1999)	400
24	*Lyngbya* sp. (cyanobacteria)	18-d EC_{10} (growth rate)	Population	2	2,438	Cairns *et al.* (1978)	415
25	*Cyclotella meneghiniana* (diatom)	5-d EC_{10} (growth rate)	Population	2	2,803	Cairns *et al.* (1978)	477
26	*Ceratophyllum demersum* (hornwort)	15-d LOEC (chlorophyll content and biomass)	Not reported	2	3,000	Umebese and Motajo (2008)	1,116
27	*Chlamydomonas* sp. (green algae)	10-d EC_{10} (growth rate)	Population	2	8,381	Cairns *et al.* (1978)	1,428
28	*Scenedesmus quadricauda* (green algae)	5-d EC_{10} (growth rate)	Population	2	9,559	Cairns *et al.* (1978)	1,628
29	*Rhithrogena hageni* (mayfly)	10-d EC_{10} (mortality)	Nymph	1	2,069.2	Brinkman and Johnston (2008)	1,696

[1] Geometric mean value taken from studies with same species, endpoint and duration, and similar life stage and test water quality parameters. Geometric means were also calculated from studies with varying hardness, pH and/or DOC because the long-term *O. mykiss* MLR normalization equation standardized endpoint values for these variables. For details on which individual studies were used to calculate geometric means, as well as additional details on all studies, see the Appendix.

[2] Adjusted effect concentrations were calculated using the *O. mykiss* MLR normalization equation: Standardized $EC_{10} = \exp[\ln(EC_{10meas}) - 0.398(\ln[DOC_{meas}] - \ln[DOC_{target}]) + 0.815(pH_{meas} - pH_{target})] - 0.947(\ln[hardness_{meas}] - \ln[hardness_{target}])$. Total concentrations were converted to dissolved concentrations using a total:dissolved conversion factor of 0.986 (US EPA 1996).

Each species for which appropriate long-term toxicity data were available was ranked according to sensitivity, and its centralized position on the SSD (Hazen plotting position) was determined using the following standard equation (Aldenberg *et al.* 2002; Newman *et al.* 2002):

$$\frac{i - 0.5}{N}$$

where

i = the species rank based on ascending toxicity values (e.g., EC_x values)
N = the total number of species included in the SSD derivation

These positional rankings, along with their corresponding toxicity values (e.g., EC_x values), were used to derive the SSD. The software "SSD Master" (version 3.0; developed by Intrinsik, available from Environment and Climate Change Canada, Gatineau QC, mailto:ec.rqe-eqg.ec@canada.ca) was used to fit SSDs to the data set. Several CDFs (normal, logistic, extreme value, Weibull and Gumbel) were fit to the data using regression methods. Evaluation of goodness of fit of the various models included examination of probability-probability plots, quantile-quantile plots, residual plots, Anderson-Darling goodness-of-fit test, mean sum of squared error terms in the lower tail, and overall visual assessment of model fit.

The logistic model provided the best fit of the models tested (Anderson-Darling statistic (A^2) = 0.247). The equation of the logistic model is of the form:

$$F(x) = \frac{1}{1 + e^{-\frac{x-\mu}{s}}}$$

where, in the case of the fitted model, x = log (concentration), μ= 2.026 and s= 0.402.

Figure 10-2 shows the long-term SSD, and Table 10.7 shows the summary statistics for the long-term SSD. The 5th percentile on the long-term SSD is 6.97 $\mu g \cdot L^{-1}$. The lower confidence limit (5%) on the 5th percentile is 5.14 $\mu g \cdot L^{-1}$, and the upper confidence limit (95%) on the 5th percentile is 9.43 $\mu g \cdot L^{-1}$.

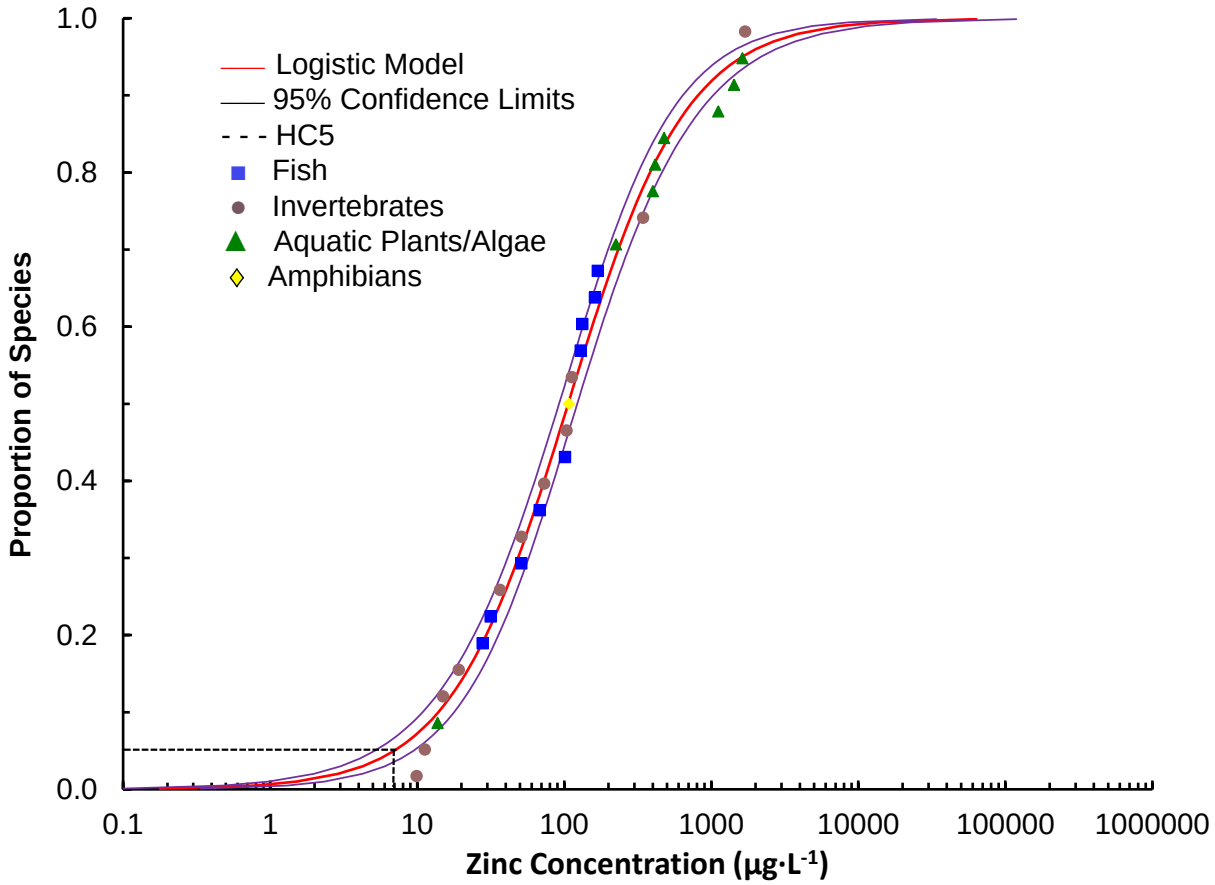


Figure 10.2 Long-term SSD for zinc in fresh water derived by fitting the logistic model to the long-term data points of 29 aquatic species versus Hazen plotting position

The intercept of the 5th percentile of the fitted curve (guideline value) was determined to be 6.97 µg·L^{-1} zinc, with 95% confidence intervals of 5.14 and 9.43 µg·L^{-1}. Due to the number of data points, legible species labels could not be added.

Table 10.7 Long-term CWQG for zinc resulting from the Type A SSD approach at water hardness of 50 mg·L^{-1}, pH of 7.5 and DOC of 0.5 mg·L^{-1}

	Zinc concentration[1] (µg·L^{-1})
Long-term CWQG, SSD 5th percentile	6.97
Long-term CWQG, SSD 5th percentile, 95% lower CL	5.14
Long-term CWQG, SSD 5th percentile, 95% upper CL	9.43

CL= confidence limit

[1] Dissolved concentration

No data points fell below the 5th percentile value on the long-term SSD curve. The CWQG for zinc was assessed for protectiveness (Section 11.0) and was found to achieve the intended level of protection.

10.7 Deriving Guideline Equations for Zinc that Incorporate Toxicity Modifying Factors

The first steps in developing short-term benchmarks and long-term CWQGs were discussed above. Short-term and long-term toxicity data sets were normalized to a common set of water chemistry using the short-term pooled *Daphnia* and long-term *O. mykiss* MLR models, respectively. SSDs were run with normalized data sets, and HC5 values at standard water chemistry were derived. The next step is to derive benchmark and CWQG equations into which local water hardness, DOC and/or pH can be entered in order to produce an appropriate site-specific benchmark or CWQG.

10.7.1 Short-term Benchmark Equation

Based on the 5th percentile of the short-term SSD at a hardness of 50 $mg \cdot L^{-1}$ and a DOC concentration of 0.5 $mg \cdot L^{-1}$ (i.e., 37.27 µg $Zn \cdot L^{-1}$; Table 10.5), and given the slope of the relationship between the natural logarithms of hardness and short-term toxicity values (0.833; Table 9.1) and the slope of the relationship between natural logarithms of DOC concentrations and short-term toxicity values (0.240; Table 9.1), the y-intercept can be calculated in order to derive an MLR-based short-term benchmark equation:

$$\text{y-intercept} = \ln(5^{th}\text{ percentile}) - [\text{Hardness slope} \times \ln(\text{Hardness})] - [\text{DOC slope} \times \ln(\text{DOC})]$$
$$= \ln(37.27) - [0.833 \times \ln(50)] - [0.240 \times \ln(0.5)]$$
$$= 0.526$$

Therefore, the resulting equation for deriving a short-term benchmark for zinc is:

$$\text{Benchmark} = \exp(0.833[\ln(\text{hardness})] + 0.240[\ln(\text{DOC})] + 0.526)$$

where the benchmark is in $\mu g \cdot L^{-1}$ dissolved zinc, hardness is measured as $CaCO_3$ equivalents in $mg \cdot L^{-1}$ and DOC concentration is in $mg \cdot L^{-1}$. Users can enter site-specific water hardness and DOC measurements to calculate what short-term benchmark concentration would apply to that particular water chemistry. The short-term benchmark equation is valid at hardness between 13.8 and 250.5 $mg \cdot L^{-1}$ as $CaCO_3$ and DOC between 0.3 and 17.3 $mg \cdot L^{-1}$. Upper limits are placed on the hardness and DOC values entered into the equation in order to retain accuracy of the benchmark. The short-term benchmark equation was derived from data with a hardness range of 13.8 to 250.5 $mg \cdot L^{-1}$ as $CaCO_3$ and a DOC range of 0.3 to 17.3 $mg \cdot L^{-1}$. The MLR model accurately predicted toxicity (within a factor of ±2) at these range limits. Therefore, the maximum hardness that can be entered into the equation is 250.5 $mg \cdot L^{-1}$ as $CaCO_3$, and the maximum DOC concentration that can be entered is 17.3 $mg \cdot L^{-1}$. At hardness and DOC concentrations greater than these values, the upper limit would apply and be used in the equation to calculate the short-term benchmark. Accordingly, the short-term benchmark cannot exceed 334 $\mu g \cdot L^{-1}$, regardless of the hardness and DOC concentrations of the site. For hardness below 13.8 mg $CaCO_3 \cdot L^{-1}$ or DOC below 0.3 $mg \cdot L^{-1}$, where users want a more stringent benchmark, they should extrapolate with caution and contact their local authority for advice.

Figure 10-3 shows short-term zinc benchmark concentrations plotted as a function of hardness ($mg \cdot L^{-1}$ as $CaCO_3$) and DOC ($mg \cdot L^{-1}$). Table 10.8 gives examples of short-term benchmark concentrations in fresh water for various levels of hardness and DOC.

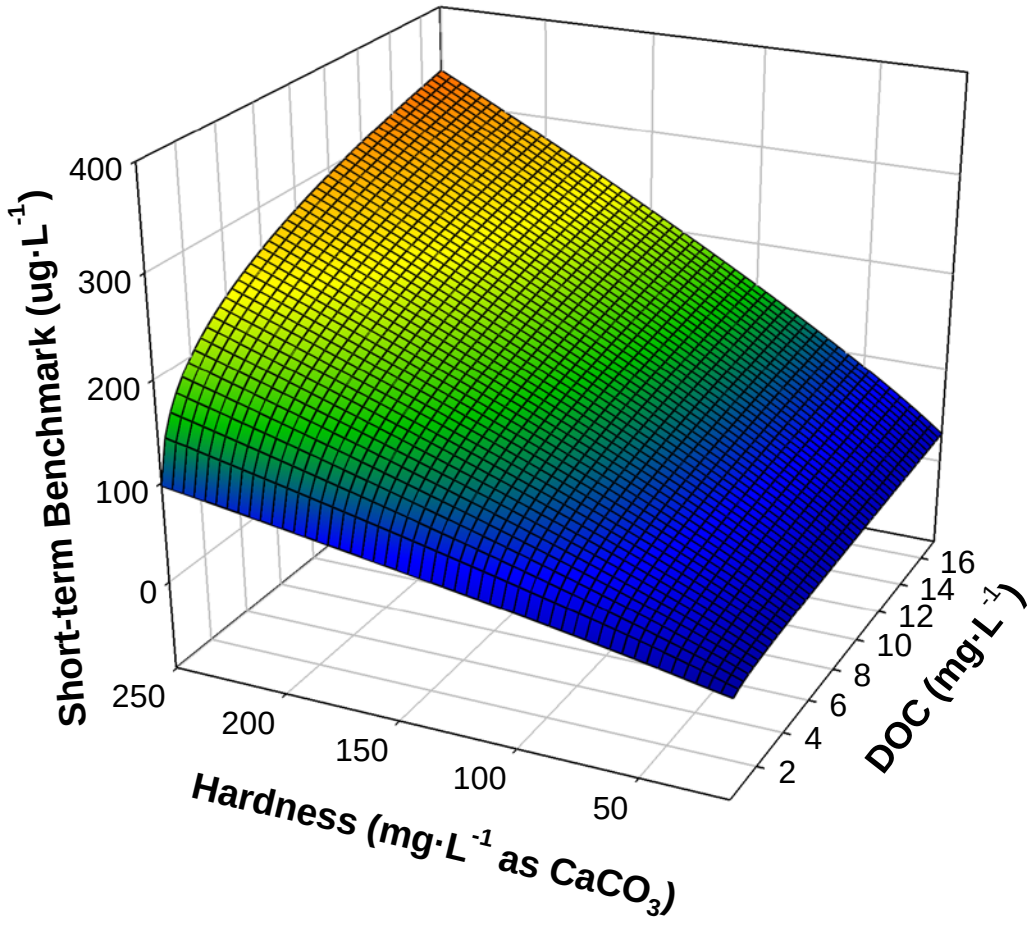


Figure 10.3 Short-term benchmark concentrations for dissolved zinc as a function of hardness and DOC based on the pooled *Daphnia* MLR modelling approach

Table 10.8 Example short-term benchmark concentrations ($\mu g \cdot L^{-1}$) for dissolved zinc at various levels of water hardness and DOC

DOC ($mg \cdot L^{-1}$)	Hardness (mg $CaCO_3 \cdot L^{-1}$)							
	15	**25**	**50**	**75**	**100**	**150**	**200**	**250.5 (upper limit)**
0.5	14	21	37	52	66	93	118	143
2	19	29	52	73	93	130	165	199
5	24	36	65	91	115	162	206	248
10	28	43	77	107	136	191	243	293
17.3 (upper limit)	32	49	87	122	155	218	277	334 (maximum)

10.7.2 Long-term CWQG Equation

Based on the 5th percentile of the long-term SSD at a hardness of 50 $mg \cdot L^{-1}$, a pH of 7.5 and a DOC concentration of 0.5 $mg \cdot L^{-1}$ (i.e., 6.97 µg $Zn \cdot L^{-1}$; Table 10.7), and given the slope of the relationship between the natural logarithms of hardness and long-term toxicity values (0.947; Table 9.2), the slope of the relationship between pH and the natural logarithms of long-term toxicity values (–0.815; Table 9.2), and the slope of the relationship between DOC and the natural logarithms of long-term toxicity values (0.398; Table 9.2), the y-intercept can be calculated in order to derive an MLR-based long-term CWQG equation:

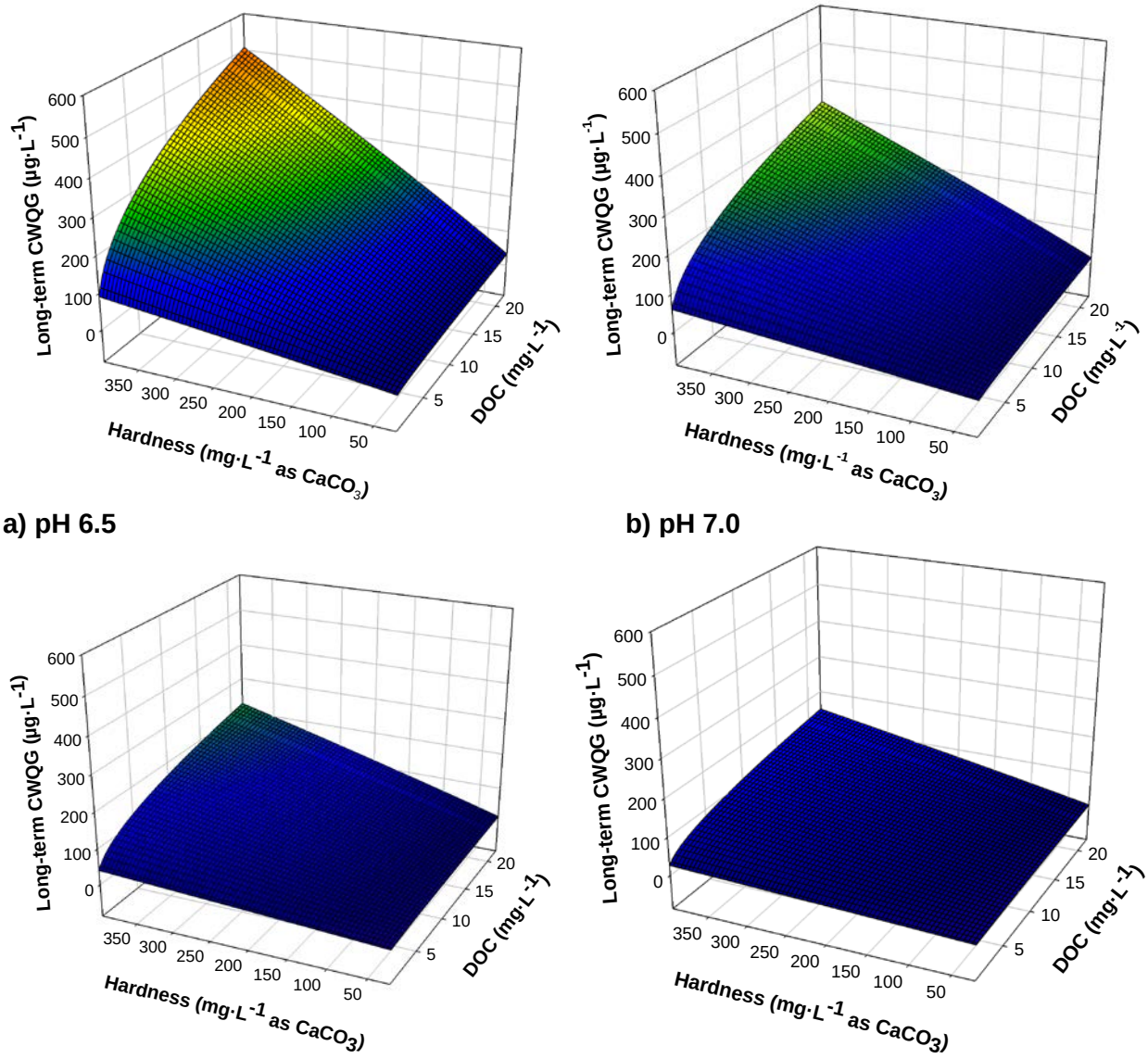
$$\text{y-intercept} = \ln(\text{5th percentile}) - (\text{Hardness slope} \times \ln(\text{Hardness})] - (\text{pH slope} \times \text{pH}] - (\text{DOC slope x } \ln(\text{DOC})]$$
$$= \ln(6.97) - [0.947 \times \ln(50)] - [-0.815 \times 7.5] - [0.398 \times \ln(0.5)]$$
$$= 4.625$$

Therefore, the resulting equation for deriving a long-term CWQG for zinc is:

$$\text{CWQG} = \exp(0.947[\ln(\text{hardness})] - 0.815[\text{pH}] + 0.398[\ln(\text{DOC})] + 4.625)$$

where the CWQG is in $\mu g \cdot L^{-1}$ dissolved zinc, hardness is measured as $CaCO_3$ equivalents in $mg \cdot L^{-1}$, pH is in standard units and DOC is in $mg \cdot L^{-1}$. Users can enter site-specific water hardness, pH and DOC measurements to calculate what long-term CWQG would apply to that particular water chemistry. The long-term CWQG equation is valid at hardness 23.4– 399 mg $CaCO_3 \cdot L^{-1}$, pH 6.5–8.13 and DOC 0.3–22.9 $mg \cdot L^{-1}$. Limits are placed on the hardness, pH and DOC values that can be entered into the guideline equation to ensure the equation is accurate and the CWQG is protective. The long-term CWQG equation was derived from data with a range of hardness of 23.4 to 399 $mg \cdot L^{-1}$ as $CaCO_3$, pH of 5.68 to 8.13, and DOC of 0.3 to 22.9 $mg \cdot L^{-1}$. The model accurately predicted toxicity (within a factor of ±2) within these ranges. Regarding pH, the lower range of pH (i.e., 5.68) is outside the CCME freshwater pH guideline of 6.5 to 9 (CCREM 1987). Therefore, the lower limit of the CCME pH guideline (6.5) is set as the lower pH limit for the long-term CWQG equation. At hardness concentrations greater than 399 $mg \cdot L^{-1}$ as $CaCO_3$, at pH lower than 6.5 and at DOC concentrations greater than 22.9 $mg \cdot L^{-1}$, these upper and lower limits apply, and they are to be used in the equation to calculate the long-term CWQG. Accordingly, the CWQG could not exceed 516 $\mu g \cdot L^{-1}$ dissolved zinc, regardless of the hardness concentration, pH and DOC concentration of the site. For hardness below 23.4 mg $CaCO_3 \cdot L^{-1}$, pH above 8.13, or DOC below 0.3 $mg \cdot L^{-1}$, where users want a more stringent WQG, they should extrapolate with caution and contact their local authority for advice.

Figure 10-4 shows long-term zinc CWQGs plotted as a function of hardness ($mg \cdot L^{-1}$ as $CaCO_3$), pH and DOC ($mg \cdot L^{-1}$). Table 10.9 gives examples of long-term CWQG concentrations in freshwater for various levels of hardness, pH and DOC.



a) pH 6.5

b) pH 7.0

c) pH 7.5

d) pH 8.0

Figure 10.4 Long-term CWQGs for dissolved zinc as a function of hardness, pH and DOC based on the *Oncorhynchus mykiss* MLR modelling approach

Table 10.9 Example CWQGs (µg·L^{-1}) for dissolved zinc for the protection of aquatic life at various levels of water hardness, pH and DOC

pH 6.5						
DOC (mg·L^{-1})	**Hardness (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0.5	8.2	16	23	30	59	113
2	14	27	40	53	102	195
5	20	39	58	76	146	281
10	27	52	76	100	193	371
22.9	37	72	106	139	268	516 (**maximum**)
pH 7.0						
DOC (mg·L^{-1})	**Hardness (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0.5	5.4	10	15	20	39	75
2	9.4	18	27	35	68	130
5	14	26	38	50	97	187
10	18	35	51	67	128	247
22.9	25	48	70	93	178	343
pH 7.5						
DOC (mg·L^{-1})	**Hardness (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0.5	3.6	7.0	10	13	26	50
2	6.3	12	18	23	45	87
5	9.0	17	26	34	65	125
10	12	23	34	44	85	164
22.9	17	32	47	62	119	228
pH 8.0						
DOC (mg·L^{-1})	**Hardness (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0.5	2.4	4.6	6.8	8.9	17	33
2	4.2	8.1	12	16	30	58
5	6.0	12	17	22	43	83
10	7.9	15	22	29	57	109
22.9	11	21	31	41	79	152

10.8 Marine Guidelines

CCME did not derive a marine water quality guideline for zinc at this time and hence no marine value is recommended. It is not appropriate to apply the zinc freshwater guideline to marine or estuarine environments.

11.0 ASSESSING THE PROTECTION OF THE LONG-TERM CANADIAN WATER QUALITY GUIDELINE FOR ZINC

To determine whether the long-term zinc guideline value is sufficiently protective, results of acceptable aquatic toxicity studies in which toxic effects were observed at concentrations below the long-term zinc guideline value were examined. The CCME 2007 protocol includes a section called the “protection clause,” which applies only to the long-term guideline:

> The protection clause may be invoked if an acceptable single (or, if applicable, geometric mean) no-effect or low-effect level endpoint (e.g., EC_x for growth, reproduction, survival, or behaviour) for a species at risk (as defined by the Committee on the Status of Endangered Wildlife in Canada [COSEWIC] is lower than the proposed guideline (i.e., is below the 5^{th} percentile intercept to the fitted curve), then that endpoint becomes the recommended guideline value. If this endpoint is a moderate- or severe-effect level endpoint for a species at risk (i.e., EC_x $x \geq 50\%$, or a lethality endpoint [LC_x]), then the guideline value shall be determined on a case-by-case basis (e.g., by using an appropriate safety factor) (Chapman *et al.* 1998).
>
> Similarly, if an acceptable single (or, if applicable, geometric mean) lethal-effects endpoint (i.e., LC_x, where x is $\geq 15\%$) for any species is lower than the proposed guideline (i.e., is below the 5th percentile intercept to the fitted curve), then that endpoint becomes the recommended guideline value.
>
> Furthermore, special consideration will be required if multiple endpoints for a single taxon (e.g., fish, invertebrates, or plants/algae) and/or an elevated number of secondary studies are clustered around the 5th percentile. Best scientific judgment should be used in deciding whether this situation is present (e.g., due consideration should be given to the percentage of data points in question to the whole data set) and in determining the best path forward to address this situation. (CCME 2007, p. 5)

The protectiveness of the long-term CWQG based on the *O. mykiss* MLR model containing adjustment parameters for water hardness, pH and DOC was evaluated. Long-term CWQGs were calculated for each of the 606 long-term toxicity values in the acceptable data set. The calculated CWQG was then compared to the reported measured long-term toxicity value at that associated water chemistry, and a ratio of the measured toxicity value to the CWQG was calculated. A ratio of greater than one indicates that the CWQG is protective of the toxicity value in that particular test. A ratio of less than one indicates that the measured toxicity value in the particular test is below the CWQG, and hence not protected. The ratio was greater than one in 96% of the long-term toxicity values (Figure 11.1). The individual test results where the ratio was less than one were further examined.

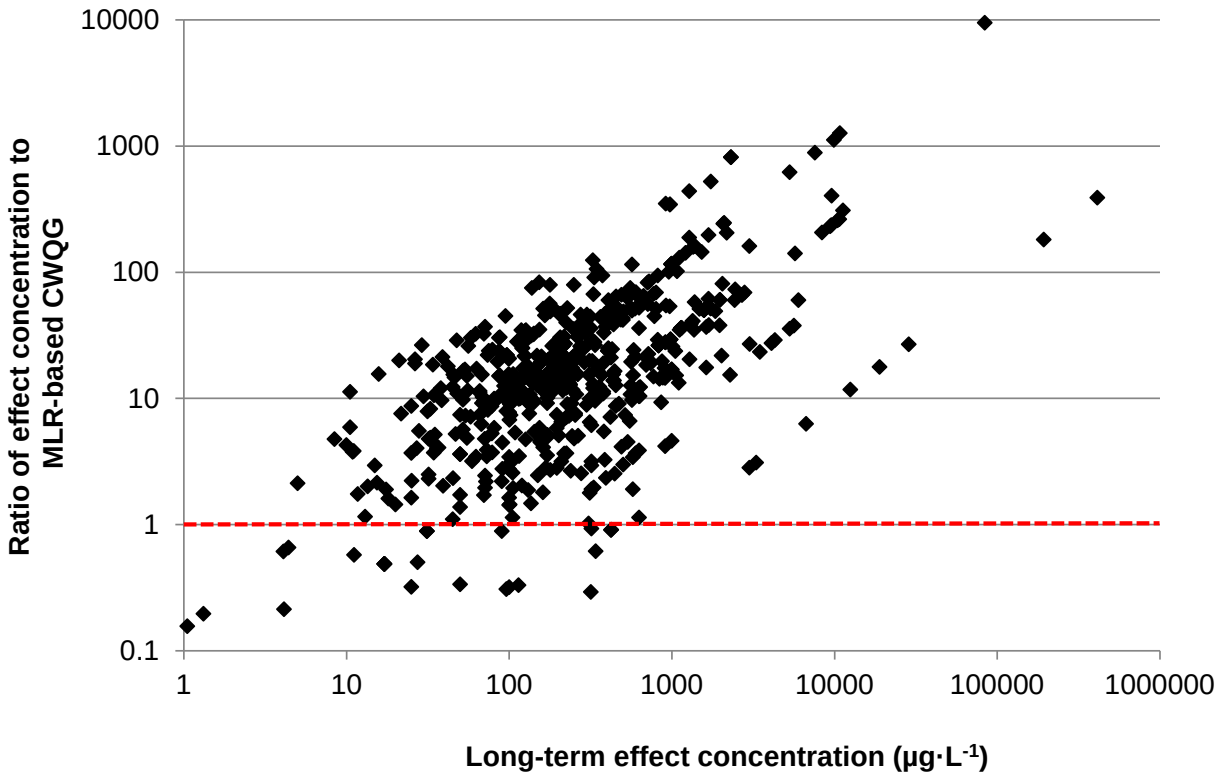


Figure 11.1 Ratio of long-term effect concentration for zinc (µg·L^{-1}) to CWQGs calculated using the *O. mykiss* MLR model containing hardness, pH and DOC

The dotted red line indicates a ratio of 1:1.

Out of 606 long-term toxicity values, 24 (< 4%) were below the ratio of one (hence were below the CWQG). Once the limits of the long-term CWQG equation are imposed (maximum hardness of 399 mg·L^{-1}, maximum DOC of 22.9 mg·L^{-1} and minimum pH of 6.5), 20 endpoints fall below the CWQG. This represents greater than 96% protection of the data set and aligns with the derivation of guideline values using the HC5 of the SSD. The endpoints that were below the ratio of one (after imposing limits on hardness, pH and DOC) included endpoints for *P. subcapitata* (n = 7), *C. dubia* (n = 2), *D. magna* (n = 4), *L. minor* (n = 1) and a mixed community (n = 6), discussed below.

Seven individual endpoints for *P. subcapitata* had ratios less than one. These endpoints included three EC_{10} values, two IC_{50} values and three EC_{50} values for effects to growth and biomass. There were 64 individual endpoints in the acceptable data set for this species that had ratios above one (i.e., above the CWQG). The species mean ratio for *P. subcapitata* (i.e., the geometric mean of all long-term ratios of toxicity value to CWQG for this species) is 8.1, meaning the species mean ratio is more than eight times the CWQG. Therefore, the weight of evidence suggests this species is adequately protected by the CWQG. For *C. dubia*, two endpoints had ratios less than one. These included two LOECs for effects to reproduction. There were 22 other endpoints in the acceptable data set for this species with ratios greater than one. The species mean ratio for *C. dubia* is 2.8. For *D. magna*, four endpoints had ratios less than one. These included three EC_{10} values and one NOEC for effects to reproduction. There were 109 other

endpoints in the acceptable data set for this species with ratios greater than one. The species mean ratio for *D. magna* is 7.0. For *L. minor,* one endpoint had a ratio less than one, which was an IC_{10} value for effects to growth. There were five other endpoints in the acceptable data set for this species with ratios greater than one, and the species mean ratio for *L. minor* is 54. There were six endpoints for a mixed community that had ratios less than one, with a mean ratio of 0.6. These endpoints were derived from an *in situ* community study and included LOECs for effects to community similarity, species diversity and population reduction of crustacean zooplankton and rotifer plankton. These endpoints were not included in the SSD because they did not represent controlled effects on an individual species, and the abscence of other toxicants in the natural water was not confirmed.

None of the long-term endpoints below the ratio of one were for a species at risk, or for lethal effects equal to or above a level of 15%. There was no demonstration of taxon clustering below the CWQG.

Short-term toxicity data were also compared to the long-term CWQG calculated at the associated water chemistry. Only one endpoint in the acceptable short-term data set out of a total of 581 was below the long-term CWQG. This endpoint was a 48-h LC_{50} value for *C. dubia*. For *C. dubia*, there were 25 other short-term LC_{50} values above the CWQG. This suggests the long-term CWQG is providing sufficient protection from acute lethality.

Overall examination of the available data as discussed above suggests the long-term CWQG equation is protective and that the protection clause is not applicable to the long-term guideline for zinc.

12.0 CONSIDERATION FOR USES OF THE SHORT-TERM BENCHMARK CONCENTRATION AND CANADIAN WATER QUALITY GUIDELINE

A short-term benchmark concentration provides guidance for short-term exposures, and a CWQG provides guidance for long-term exposures. The short-term exposure value intends to protect most species against lethality during severe but transient events such as spills or inappropriate use or disposal of the substance in question. Long-term guidelines are intended to protect the most sensitive species and life stages indefinitely. Aquatic life may be chronically exposed to a substance as a result of gradual release from soils or sediments and gradual entry through groundwater or runoff, emissions from industrial processes, and long-range transport.

Before using the zinc short-term benchmark concentration or CWQG, it should be taken into consideration that the main purpose of this process was to develop Canada-wide water quality guidelines based on existing scientific information. The short-term benchmark concentration and CWQG for zinc are two of many tools for assessing and interpreting zinc monitoring data in water. The effect of zinc on aquatic organisms can vary greatly among sites because the species composition, physicochemical characteristics, and presence of other toxicants that could interact additively or synergistically with zinc may differ through ecosystems (CCME 2007). These guidelines should thus be used as a basis for deriving site-specific guidelines and objectives

when needed. For example, at sites with high natural background concentrations of zinc, a guideline exceedance does not necessarily guarantee an adverse effect of zinc toxicity, as local aquatic organisms may have developed mechanisms to tolerate and even adapt to high zinc concentrations

For more information on the procedure for deriving site-specific water quality guidelines, please refer to CCME (2003).

13.0 GUIDELINES SUMMARY

The following short-term benchmark and long-term CWQG equations are recommended for the protection of aquatic life for zinc.

CWQG for the Protection of Aquatic Life for Zinc

	Short-term exposure[a] ($\mu g \cdot L^{-1}$)	Long-term exposure[b] ($\mu g \cdot L^{-1}$)
Freshwater	37[c]	7.0[d]
Marine	Not assessed	Not assessed

[a] The short-term exposure benchmark is meant to estimate severe effects and to protect most species against lethality during intermittent and transient events (e.g., spills, infrequent releases of short-lived/non-persistent substances).

[b] The long-term exposure guideline is meant to protect against all negative effects during indefinite exposures.

[c] The short-term benchmark is for dissolved zinc and is calculated using the equation:

benchmark = exp(0.833[ln(hardness mg·L^{-1})] + 0.240[ln(DOC mg·L^{-1})] + 0.526). The value given in the table is for surface water of 50 mg $CaCO_3 \cdot L^{-1}$ hardness and 0.5 mg·L^{-1} DOC. The benchmark equation is valid between hardness 13.8 and 250.5 mg $CaCO_3 \cdot L^{-1}$ and DOC between 0.3 and 17.3 mg·L^{-1}, which is the range of data used to derive the hardness and DOC slopes. Extrapolations should not be made above the upper hardness limit of 250.5 mg $CaCO_3 \cdot L^{-1}$ or above the upper DOC limit of 17.3 mg·L^{-1}. For hardness below 13.8 mg $CaCO_3 \cdot L^{-1}$ or DOC below 0.3 mg·L^{-1}, where users want a more stringent benchmark, they should extrapolate with caution and contact their local authority for advice.

[d] The long-term CWQG is for dissolved zinc and is calculated using the equation:

CWQG = exp(0.947[ln(hardness)] − 0.815[pH] + 0.398[ln(DOC)] + 4.625). The value given in the table is for surface water of 50 mg $CaCO_3 \cdot L^{-1}$ hardness, pH of 7.5 and 0.5 mg·L^{-1} DOC. The CWQG equation is valid for hardness between 23.4 and 399 mg $CaCO_3 \cdot L^{-1}$, pH between 6.5 and 8.13, and DOC between 0.3 to 22.9 mg·L^{-1}, which is the range of data used to derive the hardness, pH and DOC slopes. Extrapolations should not be made above the upper hardness limit of 399 mg $CaCO_3 \cdot L^{-1}$, above the upper DOC limit of 22.9 mg·L^{-1} or below the lower pH limit of 6.5. For hardness below 23.4 mg $CaCO_3 \cdot L^{-1}$, DOC below 0.3 mg·L^{-1} or pH above 8.13, where users want a more stringent WQG, they should extrapolate with caution and contact their local authority for advice.

Note: The freshwater benchmark and CWQG equations must be used in order to obtain a site-specific benchmark and CWQG, respectively, based on the DOC, pH and hardness of the water body of interest (see tables below for examples of benchmark and guideline values at various levels of water chemistry).

The short-term benchmark and long-term guideline are for dissolved concentrations of zinc. Where guideline users have only water sample concentrations of total zinc, they should first compare these samples to the dissolved guideline, and where there is an exceedance, re-sample for a dissolved concentration.

Marine guidelines were not derived at this time, so no marine value is recommended. Note that it is not appropriate to apply this zinc freshwater guideline to marine or estuarine environments.

Example short-term benchmark concentrations (µg·L^{-1}) for dissolved zinc in fresh water at various levels of water hardness and DOC

DOC (mg· L^{-1})	Hardness (mg· L^{-1})							
	15	**25**	**50**	**75**	**100**	**150**	**200**	**250.5 (upper limit)**
0.5	14	21	37	52	66	93	118	143
2	19	29	52	73	93	130	165	199
5	24	36	65	91	115	162	206	248
10	28	43	77	107	136	191	243	293
17.3 (upper limit)	32	49	87	122	155	218	277	334 **(maximum)**

Example long-term CWQGs for the protection of aquatic life (µg·L^{-1}) for dissolved zinc in fresh water at various levels of water hardness, pH and DOC

pH 6.5						
DOC (mg·L^{-1})	**Hardness (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0.5	8.2	16	23	30	59	113
2	14	27	40	53	102	195
5	20	39	58	76	146	281
10	27	52	76	100	193	371
22.9	37	72	106	139	268	516 (**maximum)**
pH 7.0						
DOC (mg·L^{-1})	**Hardness (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0.5	5.4	10	15	20	39	75
2	9.4	18	27	35	68	130
5	14	26	38	50	97	187
10	18	35	51	67	128	247
22.9	25	48	70	93	178	343
pH 7.5						
DOC (mg·L^{-1})	**Hardness (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0.5	3.6	7.0	10	13	26	50
2	6.3	12	18	23	45	87
5	9.0	17	26	34	65	125
10	12	23	34	44	85	164
22.9	17	32	47	62	119	228
pH 8.0						
DOC (mg·L^{-1})	**Hardness (mg $CaCO_3$·L^{-1})**					
	25	50	75	100	200	399
0.5	2.4	4.6	6.8	8.9	17	33
2	4.2	8.1	12	16	30	58
5	6.0	12	17	22	43	83
10	7.9	15	22	29	57	109
22.9	11	21	31	41	79	152

14.0 REFERENCES

References below include citations from the Scientific Criteria Document and references listed in the Appendix.

Adamo, P., Dudka, S., Wilson, M.J., and McHardy, W.J. 2002. Distribution of trace elements in soils from Sudbury smelting area (Ontario, Canada). Water, Air, and Soil Pollut. **137**(1–4): 95–116.

Alberta Environment and Sustainable Resource Development. 2014. Environmental quality guidelines for Alberta surface waters. Water Policy Branch, Policy Division, Edmonton, AB.

ATSDR (Agency for Toxic Substances and Disease Registry). 2005. Toxicological profile: Zinc [online]. Available from https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=302&tid=54 [accessed 14 March 2018].

Ahluwalia, A.S., and Kaur, M. 1988. Effect of some heavy metal compounds on growth and differentiation in a blue-green and a green alga. Microbios **53**: 37–45.

Alam, M.K., and Maughan, O.E. 1992. The effect of malathion, diazinon and various concentrations of zinc, copper, nickel, lead, iron, and mercury on fish. Biol. Trace Elem. Res. **34**(3): 225–236.

Alam, M.K., and Maughan, O.E. 1995. Acute toxicity of heavy metals to common carp (*Cyrpinus carpio)*. J. Environ. Sci. Health, Part A: Environ. Sci. Eng. **30**(8): 1807–1816.

Aldenberg, T., Jaworska, J.S., and Traas, T.P. 2002. Normal species sensitivity distributions and probabilistic ecological risk assessment. *In* Species sensitivity distributions in ecotoxicology. *Edited by* L. Posthuma, G. W. I. Suter, and T. Traas. CRC Press, Boca Raton, FL. pp. 49–102.

Alsop, D.H., and Wood, C.M. 1999. Influence of waterborne cations on zinc uptake and toxicity in rainbow trout, *Oncorhynchys mykiss*. Can. J. Fish. Aquat. Sci. **56**: 2112–2119.

Alsop, D.H., and Wood, C.M. 2000. Kinetic analysis of zinc accumulation in the gills of juvenile rainbow trout: effects of zinc acclimation and implications for biotic ligand modeling. Environ. Toxicol. Chem. **19**(7): 1911–1918.

Alsop, D.H., McGreer, J.C., McDonald, G., and Wood, C.M. 1999. Costs of chronic waterborne zinc exposure and the consequences of zinc acclimation on the gill/zinc interactions of rainbow trout in hard and soft water. Environ. Toxicol. Chem. **18**(5): 1014–1025.

Anadu, D.I., Chapman, G.A., Curtis, L.R., and Tubb, R.A. 1989. Effect of zinc exposure on subsequent acute tolerance to heavy metals in rainbow trout. Bull. Environ. Contam. Toxicol. **43**: 329–336.

Anderson, B.G. 1950. The apparent thresholds of toxicity to *Daphnia magna* for chlorides of various metals when added to Lake Erie water. Trans. Am. Fish. Soc. **78**: 96–113.

Anderson, R.L., Walbridge, C.T., and Fiandt, J.T. 1980. Survival and growth of *Tanytarsus dissimilis* (Chironomidae) exposed to copper, cadmium, zinc and lead. Arch. Environ. Contam. Toxicol. **9**: 329–335.

Andros, J.D., and Garton, R.R. 1980. Acute lethality of copper, cadmium, and zinc to northern squawfish. Trans. Am. Fish. Soc. **109**: 235–238.

Australian and New Zealand Environment and Conservation Council. 2000. National water quality management strategy: An introduction to the Australian and New Zealand guidelines for fresh and marine water quality. ANZECC, Canberra.

Arambasic, M.B., Bjelic, S., and Subakov, G. 1995. Acute toxicity of heavy metals (copper, lead, zinc), phenol and sodium on *Allum cepa, Lepidium sativum*, and *Daphnia magna*: Comparative investigations and the practical applications. Water Research **29**(2): 497–503.

Arnot, J.A., and Gobas, F.A.P.C. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environmental Review. **14**: 257–297.

Aruoja, V., Dubourguier, H-C., Kasemets, K., and Kahru, A. 2009. Toxicity of nanoparticles of CuO, ZnO, and TiO_2 to microalgae *Pseudokirchneriella subcapitata.* Sci. Total Environ. **407**: 1461–1468.

Attar, E.N., and Maly, E.J. 1982. Acute toxicity of cadmium, zinc, and cadmium-zinc mixtures to *Daphnia magna*. Arch. Environ. Contam. Toxicol. **11**: 291–296.

Bailey, H.C., and Liu, D.H.W. 1980. *Lumbriculus variegates*, a benthic oligochaete, as a bioassay organism. *In* Aquatic ecotoxicology, ASTM STP 707. *Edited by* J. G. Eaton, P.R. Parrish, and A.C. Hendricks. American Society for Testing Material, Philadelphia, PA. pp. 205–215.

Bailey, H.C., Elphick, J.R., Potter, A., and Zak, B. 1999. Zinc toxicity in stormwater runff from sawmills in British Columbia. Water Research **33**(11): 2721–2725.

Ball, I.R. 1967. The relative susceptibilities of some species of fresh-water fish to poisons—II. Zinc. Water Research **1**: 777–783.

Barata, C., Baird, D.J., and Markich, S.J. 1998. Influence of genetic and environmental factors on the tolerance of *Daphnia magna* Straus to essential and non-essential metals. Aquat. Toxicol. **42**: 115–137.

Barron, M.G., and Albeke, S. 2000. Calcium control of zinc uptake in rainbow trout. Aquat. Toxicol. **50**(3): 257–264.

Bartlett, L., Rabe, F.W., and Funk, W.H. 1974. Effects of copper, zinc and cadmium on *Selenastrum capriconutum*. Water Research **8**: 179–185.

Basile, A., Sorbo, S., Conte, B., Castaldo Cobianchi, R., Trinchella, F., Capasso, C., and Carginale, V. 2012. Toxicity, accumulation, and removal of heavy metals by three aquatic macrophytes. Int. J. Phytorem. **14**: 374–387.

Baudouin, M.F., and Scoppa, P. 1974. Acute toxicity of various metals to freshwater zooplankton. Bull. Environ. Contam. Toxicol. **12**: 745–751.

BC MOE (British Columbia Ministry of the Environment). 2004. State of the environment for British Columbia. Previously available from http://ecoinfo.org/updates/updates_e.cfm#coast.

BC MOE 2010. Protocol 4 for Contaminated Sites. Determining Background Soil Quality. October 12, 2010.

Beach, M.J., and Pascoe, D. 1998. The role of *Hydra vulgaris* in assessing the toxicity of freshwater pollutants. Water Research **32**(1): 101–106.

Bechard, K.M., Gillis, P.L., and Wood, C.M. 2008. Acute toxicity of waterborne Cd, Cu, Pb, Ni, and Zn to first-instar *Chironomus riparius* larvae. Arch. Environ. Contam. Toxicol. **54**:454–459.

Bednarz, T., and Warkowska-Dratnal, H. 1983/1984. Toxicity of zinc, cadmium, lead, copper and their mixture for *Chlorella pyrenoidosai.* Acta Hydrobiol **25/26**(3/4): 389–400.

Belabed, W., Kestali, N., Semsari, S., and Gaid, A. 1994. Évaluation de la toxicité de quelques métaux lourds à l'aide du test daphnie (Toxicity study of some heavy metals with Daphnia test). Tech., Sci., Methodes **6**: 331–335.

Belanger, S.E., and Cherry, D.S. 1990. Interacting effects of pH acclimatation, pH, and heavy metals on acute and chronic toxicity to *Ceriodaphnia dubia* (Cladocera). J. Crustacean Biol. **10**: 225–235.

Beltrame, M.O., De Marco, S.G., and Marcovecchio, J.E. 2008. Cadmium and zinc in Mar Chiquita Coastal Lagoon (Argentina): Salinity effects on lethal toxicity in juveniles of the burrowing crab *Chasmagnathus granulatus*. Arch. Environ. Contam. Toxicol. **55**: 78–85.

Bengtsson, B.-E. 1974. Effect of zinc on growth of the minnow *Phoxinus phoxinus*. Oikos **25**: 370-373.

Bengtsson, B.-E., and Bergström, B. 1987. A flowthrough fecundity test with *Nitocra spinipes* (Harpacticoidea crustacea) for aquatic toxicity. Ecotoxicol. Environ. Saf. **14**: 260–268.

Benoit, D.A., and Holcombe, G.W. 1978. Toxic effects of zinc on fathead minnows *Pimephales promelas* in soft water. J. Fish Biol. **13**: 701–708.

Berglind, R., and Dave, G. 1984. Acute toxicity of chromate, DDT, PCP, TPBS, and zinc to *Daphnia magna* cultured in hard and soft water. Bull. Environ. Contam. Toxicol. **33**: 63–68.

Besser, J.M., Mebane, C.A., Mount, D.R., Ivey, C.D., Kunz, J.L., Greer, I.E., May, T.W., and Ingersoll, C.G. 2007. Sensitivity of mottled sculpins (*Cottus bairdi*) and rainbow trout (*Oncorhynchus mykiss*) to acute and chronic toxicity of cadmium, copper and zinc. Environ. Toxicol. Chem. **26**(8): 1657–1665.

Biesinger, K.E., and Christensen, G.M. 1972. Effects of various metals on survival, growth, reproduction and metabolism of *Daphnia magna*. J. Fish. Res. Board Can. **29**: 1691–1700.

Biesinger, K.E., Christensen, G.M., and Fiandt, J.T. 1986. Effects of metal salt mixtures on *Daphnia magna* reproduction. Ecotoxicol. Environ. Saf. **11**:9–14.

Bolten, J.L., Stehr, C.M., Boyd, D.T., Burrows, D.G., Tkalin, A.V., and Lishavskaya, T.S. 2003. Organic and trace metal contaminants in sediments and English Sole tissues from Vancouver Harbour, Canada. Mar. Environ. Res. **57**: 19–36.

Borgmann, U., Norwood, W.P., and Clarke, C. 1993. Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*. Hydrobiologia **259**: 79–89.

Borgmann, U., Couillard, Y., Doyle, P., and Dixons, D.G. 2005. Toxicity of sixty-three metals and metalloids to *Hyalella azteca* at two levels of water hardness. Environ. Toxicol. Chem. **24**(3): 641–652.

Bosnak, A.D., and Morgan, E.L. 1981. Acute toxicity of cadmium, zinc, and total residual chlorine to epigean and hypogean isopods (Asellidae). NSS Bull. **43**: 12–18.

Bossuyt, B.T.A., Muyssen, B.T.A., and Janssen, C.R. 2005. Relevance of generic and site-specific species sensitivity distributions in the current risk assessment procedures for copper and zinc. Environ. Toxicol. Chem. **24**(2): 470–478.

Bowmer, C.T., Hooftman, R.N., Hanstveit, A.O., Venderbosch, P.W.M., and van der Hoeven, N. 1998. The ecotoxicity and the biodegradability of lactic acid, alkyl lactate esters and lactate salts. Chemosphere **28**(7): 1317–1333.

Bradley, R.W., and Morris, J.R. 1986. Heavy metals in fish from a series of metal-contaminated lakes near Sudbury, Ontario. Water, Air, Soil Pollut. **27**: 341–354.

Bradley, R.W., and Sprague, J.B. 1985. The influence of pH, water hardness, and alkalinity on the acute lethality of zinc to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. **42**: 731–736.

Bringolf, R.B., Morris, B.A., Boese, C.J., Santore, R.C., Allen, H.E., and Meyer, J.S. 2006. Influence of dissolved organic matter on acute toxicity of zinc to larval fathead minnows (*Pimephales promelas*). Arch. Environ. Contam. Toxicol. **51**: 438–444.

Brinkman S.F, and Hansen D. 2004. Effect of hardness on zinc toxicity to Colorado River cutthroat (*Onchorhynchus clarkii pleuriticus*) and rainbow trout (*Oncorhynchus mykiss*) embryos and fry. Water Pollution Studies, Federal Aid in Fish and Wildlife Restoration Project F-243-R11. Colorado Division of Wildlife, Fort Collins, CO.

Brinkman, S.F., and Johnston, W.D. 2008. Acute toxicity of aqueous copper, cadmium, and zinc to the mayfly *Rhithrogena hageni*. Arch. Environ. Contam. Toxicol. **54**: 466–472.

Brinkman, S.F., and Johnston, W.D. 2012. Acute toxicity of zinc to several aquatic species native to the Rocky Mountains. Arch. Environ. Contam. Toxicol. **62**: 272–281.

Brinkman, S., and Vieira, N. 2008. Water pollution studies: federal aid project. Division of Wildlife, Fish Research Section, Fort Collins, CO.

Brinkman, S., and Woodling, J. 2005. Zinc toxicity to the mottled sculpin (*Cottus bairdi*) in high-hardness water. Environ. Toxicol. Chem. **24**(6): 1515–1517.

Broderius, S.J., and Smith, L.L., Jr. 1979. Lethal and sublethal effects of binary mixtures of cyanide and hexavalent chronium, zinc, or amonia to the fatheadminnow, (*Pimephales promelas*), and rainbow trout, (*Salmo gairdneri*). J. Fish. Res. Board Can. **36**: 164–172.

Brown, J.R., and Chow, L.Y. 1977. Heavy metal concentrations in Ontario fish. Bull. Environ. Contam. Toxicol. **17**(2): 190–195.

Brown, R.J., Rundle, S.D., Hutchinson, T.H., Williams, T.D., and Malcolm, B.J. 2009. A microplate freshwater copepod bioassay for evaluating acute and chronic effects of chemicals. Environ. Toxicol. Chem. **24**(6): 1528–1531.

Brungs, W.A. 1969. Chronic toxicity of zinc to the fathead minnow, *Pimephales promelas* Rafinesque. Trans. Am. Fish. Soc. **98**: 272–279.

Bryant, V., Newbery, D.M., McLusky, D.S., and Campbell, R. 1985. Effect of temperature and salinity on the toxicity of nickel and zinc to two estuarine invertebrates (*Corophium volutator, Macoma balthica*). Mar. Ecol.: Prog. Ser. **24**: 139–153.

Budavari, S. 1996. The Merck Index. Merck & Co., Inc., Rahway, NJ.

Buhl, K.J. and Hamilton, S.J. 1990. Comparative toxicity of inorganic contaminants released by placer mining to early life stages of salmonids. Ecotoxicol. Environ. Saf. **20**: 325–342.

Buhl, K.J., and Hamilton, S.J. 1996. Toxicity of inorganic contaminants, individually and in environmental mixtures, to three endangered fishes (Colorado Squawfish, Bonytail, and Razorback Sucker). Arch. Environ. Contam. Toxicol. **30**: 84–92.

Burton, D.T., Morgan, E.L., and Cairns, J., Jr. 1972. Mortality curves of bluegills (*Lepomis macrochirus* Rafinesque) simultaneously exposed to temperature and zinc stress. Trans. Am. Fish. Soc. **101**(3): 435–441.

Caffrey, P.B., and Keating, K.I. 1997. Results of zinc deprivation in daphnid culture. Environ. Toxicol. Chem. **16**: 572–575.

Cairns, J., Jr., and Messenger, D. 1974. An interim report on the effects of prior exposure to sublethal concentrations of toxicants upon the tolerance of snails to thermal shock. Archiv fur Hydrobiologie **74**: 441–447.

Cairns, J., Jr., and Scheier, A. 1957. The effects of temperature and hardness of water upon the toxicity of zinc to the common bluegill (*Lepomis macrochirus* Raf.). Not. Nat. Acad. Nat. Sci. Philadelphia **299**: 1–12.

Cairns, J., Jr., and Scheier, A. 1958. The effects of temperature and hardness of water upon the toxicity of zinc to the pond snail, *Physa heterostropha* (Say). Not. Nat. Acad. Nat. Sci. Philadelphia **308**: 1–11.

Cairns, J., Jr., Waller, W.T., and Smrchek, J.C. 1969. Fish bioassays contrasting constant and fluctuating input of toxicants. Rev. Biol. **7**: 75–91.

Cairns, J., Jr., Bahns, T.K., Burton, D.K., Dickson, K.L., Sparks, R.E., and Waller, W.T. 1971. The effects of pH, solubility and temperature upon the acute toxicity of zinc to the bluegill sunfish (*Lepomis macrochirus* Raf.). Trans. Kans. Acad. Sci. **74**(1): 81–92.

Cairns, J., Jr., Heath, A.G., and Parker, B.C. 1975. The effects of temperature upon toxicity of chemicals to aquatic organisms. Hydrobiologia **47**(1): 135–171.

Cairns, J., Jr., Buikema, A.L, Jr., Heath, A.G., and Parker, J.I. 1978. Effects of temperature on aquatic organisms to selected chemicals. Virginia Water Resources Research Center, Virginia Polytechnic Institute and State University. Bulletin 106. Blacksburg, VA.

Cairns, J., Jr., Garton, R.R., and Tubb, R.A. 1982. Use of fish ventilation frequency to estimate chronically safe toxicant concentrations. Trans. Am. Fish. Soc. **111**: 70–77.

Callahan, M.A., Slimak, N.W., May, I.P., Fowler, C.F., Freed, J.R., Jennings, P., Durfee, R.L., Whitmore, F.C., Maestri, B., Mabey, W.R., Holt, B.R., and Gould, C. 1979. Water-related environmental fate of 129 priority polluants. Vol, 1: Introduction and technical background, metals and inorganics, pesticides and PCBs. Versar, Inc., Springfield, VA.

Campbell, P.G.C., and Stokes, P.M. 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. **42**: 2034–2049.

Canli, M. 2005. Dietary and water-borne Zn exposures affect energy reserves and subsequent Zn tolerance of *Daphnia magna*. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. **141**: 110–116.

Carlson, A.R., and Roush, T.H. 1985. Site-specific water quality studies of the Straight River, Minnesota: Complex effluent toxicity, zinc toxicity, and biological survey relationships. US EPA Environmental Research Lab, Duluth, MN.

Carson, W.G., and Carson, W.V. 1972. Toxicity of copper and zinc to juvenile atlantic salmon in the presence of humic acid and lignosulfonates. Report Series No. 1181. Fisheries Research Board of Canada, Biological Station, St. Andrews, NB.

Canadian Council of Ministers of the Environment. 2003. Canadian water quality guidelines for the protection of aquatic life: Guidance on the site-specific application of water quality guidelines in Canada: Procedures for deriving numerical water quality objectives. *In* Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment, 1999. CCME, Winnipeg, MB.

Canadian Council of Ministers of the Environment. 2007. A protocol for the derivation of water quality guidelines for the protection of aquatic life. *In* Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment, 1999. CCME, Winnipeg, MB. Available from www.ccme.ca.

CCREM (Canadian Council of Resource and Environment Ministers) 1987. Canadian water quality guidelines. Prepared by the Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers, Toronto, ON.

Chapman, G.A. 1978a. Effects of continuous zinc exposure on sockeye salmon during adult-to-smolt freshwater residency. Trans. Am. Fish. Soc. **107**: 828–836.

Chapman, G.A. 1978b. Toxicities of cadmium, copper and zinc to four juvenile stages of chinok salmon and steelhead. Trans. Am. Fish. Soc. **107**: 841–847.

Chapman, G.A., and Stevens, D.G. 1978. Acutely lethal levels of cadmium, copper and zinc to adult male coho salmon and steelhead. Trans. Am. Fish. Soc. **107**: 837–840.

Chapman, G.A., Ota, S., and Recht, F. 1980. Effects of water hardness on the toxicity of metals to *Daphnia magna*. Status Report-January 1980. Corvallis Environmental Research Laboratory. Office of Research and Development. U.S. EPA. Corvallis, OR.

Chapman, P.M., Fairbrother, A., and Brown, D. 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environ. Toxicol. Chem. **17**(1): 99–108.

Chen, C.-Y., and Lin, K.-C. 1997. Optimization and performance evaluation in the contniuous algal toxicity test. Environ. Toxicol. Chem. **16**(7): 1337–1344.

Chen, C.-Y., Lin, K.-C., and Yang, D.-T. 1997. Comparison of the relative toxicity relationships based on batch and continuous algal toxicity tests. Chemosphere **35**(9): 1959–1965.

Chiaudani, G., and Vighi, M. 1978. The use of *Selenastrum capricornutum* batch cultures in toxicity studies. Mitt. Int. Ver. Limnol. **21**: 316–329.

Chito, D., Weng, L., Galceran, J., Companys, E., Puy, J., van Riemskijk, W.H., and van Leeuwen, H.P. 2012. Determination of free Zn^{2+} concentration in synthetic and natural samples with AGNES (absence of gradients and Nernstian equilibrium equilibrium stripping) and DMT (Donnan membrane technique). Sci. Total Environ. **421–422**: 238–244.

Clark, J.L., and Clements, W.H. 2006. The use of in situ and stream microcosm experiments to assess population- and community-level responses to metals. Environ. Toxicol. Chem. **25**(9): 2306–2312.

Clement Associates. 1989. Toxicological profile for zinc. Agency for toxic substances and disease registry, U.S. Public Health Service, Rockville, ML.

Clements, W.H. 2004. Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community responses. Ecological Applications **14**(3): 954–967.

Clements, W.H., Cherry, D.S., and Cairns, J. 1988. Impact of heavy metals on insect communities in streams: A comparison of observational and experimental results. Can. J. Fish. Aquat. Sci. **45**(11): 2017–2025.

Cleven, R.F.M.J., Janus, J.A., Annema, J.A., and Smith, B.D. 1993. Integrated criteria document - Zinc. National Institute of Health and Environemntal Protection, Bilthoven, The Netherlands.

Clifford, M., and McGeer, J.C. 2009. Development of a biotic ligand model for the acute toxicity of zinc to *Daphnia pulex* in soft waters. Aquat. Toxicol. **91**: 26–32.

Coale, K.H. and Flegal, A.R. 1989. Copper, zinc cadmium and lead in surface waters of Lakes Erie and Ontario. Sci. Total Environ. **87/88**: 297–304.

Collyard, S.A., Ankley, G.T., Hoke, R.A., and Goldenstein, T. 1994. Influence of age on the relative sensitivity of *Hyalella azteca* to Diazinon, Alkylphenol ethoxylates, copper, cadmium and zinc. Arch. Environ. Contam. Toxicol. **26**: 110–113.

Cooper, N.L., Bidwell, J.R., and Kumar, A. 2009. Toxicity of copper, lead, and zinc mixtures to *Ceriodaphnia dubia* and *Daphnia carinata*. Ecotoxicol. Environ. Saf. **72**: 1523–1528.

Cotter, A.J.R., Phillips, D.J.H., and Ahsanullah, M. 1982. The significance of temperature, salinity and zinc as lethal factors for the mussel *Mytilus edulis* in a polluted estuary. Marine Biology **68**: 135–141.

Cusimano, R.F., Brakke, E.F., and Chapman, G.A. 1986. Effects of pH on the toxicities of cadmium, copper and zinc to steelhead trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. **43**: 1497–1503.

Dave, G., Damgaard, B., Grande, M., Martelin, J.E., Rosander, B., and Viktor, T. 1987. Ring test of an embryo-larval toxicity test with zebrafish (*Brachydanio rerio*) using chromium and zinc as toxicants. Environ. Toxicol. Chem. **6**: 61–71.

Davies, P.H., and Brinkman, S. 1999. Water pollution studies. Federal Aid Project #F-243R-6. Colorado Division of Wildlife, Fort Collins, CO.

Davies, P.H., Brinkman, S., and Hansen, D. 2002. Water pollution studies. Federal Aid Project #F-243R-9. Colorado Division of Wildlife, Fort Collins, CO.

Davies, P.H., Brinkman, S., and Hansen, D. 2003. Water pollution studies. Federal Aid Project #F-243-R10. Colorado Division of Wildlife, Fort Collins, CO.

Dawson, D.A., Stebler, S.L., Burks, S.L., and Bantle, J.A. 1988. Evaluation of the developmental toxicity of metal-contaminated sediments using short-term fathead minnow and frog embryo-larval assays. Environ. Toxicol. Chem. **7**: 27–34.

De Filippis, L.F., and Ziegler, H. 1993. Effect of sublethal concentrations of zinc, cadmium, and mercury on the photosynthetic carbon reduction cycle of *Euglena*. Journal of Plant Physiology **142**: 167–172.

De Schamphelaere, K.A.C., and Janssen, C.R. 2002. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium and pH. Environ. Sci. Technol. **36**: 48–54.

De Schamphelaere, K.A.C., and Janssen, C.R. 2004. Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (*Oncorhynchus mykiss*): Comparison with other fish species and development of a biotic ligand model. Environ. Sci. Technol. **38**: 6201–6209.

De Schamphelaere, K.A.C., and Janssen, C.R. 2010. Cross-phylum extrapolation of the *Daphnia magna* chronic biotic ligand model for zinc to the snail *Lymnaea stagnalis* and the rotifer *Brachionus calyciflorus*. Sci. Total Environ. **408**: 5414–5422.

De Schamphelaere, K.A.C., Heijerick, D.G., and Janssen, C.R. 2003. Development and validation of biotic ligand models for predicting chronic zinc toxicity to fish, daphnids and algae. University of Ghent, Gent, Belgium.

De Schamphelaere, K.A.C., Canli, M., Van Lierde, V., Forrez, I., Vanhaecke, F., and Janssen, C.R. 2004a. Reproductive toxicity of dietary zinc to *Daphnia magna*. Aquat. Toxicol. **70**: 233–244.

De Schamphelaere, K.A.C., Heijerick, D.G., and Janssen, C.R. 2004b. Comparison of the effect of different pH buffering techniques on the toxicity of copper and zinc to *Daphnia magna* and *Pseudokirchneriella subcapitata*. Ecotoxicology **13**: 697–705.

De Schamphelaere, K.A.C., Lofts, S., and Janssen, C.R. 2005. Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids, and fish in natural surface waters. Environ. Toxicol. Chem. **24**(5): 1190–1197.

DeForest, D.K., and Van Genderen, E.J. 2012. Application of U.S. EPA guidelines in a bioavailability-based assessment of ambient water quality criteria for zinc in freshwater. Environ. Toxicol. Chem. **31**(6): 1264–1272.

DeForest, D.K., Brix, K.V., and Adams, W.J. 2007. Assessing metal bioaccumulation in aquatic environments: The inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. Aquat. Toxicol. **84**: 236–246.

Delgado, M., Bigeriego, M., and Guardiola, E. 1993. Uptake of Zn, Cr and Cd by water hyacinths. Water Research **27**(2): 269–272.

Deniseger, J., and Pommen, L.W. 1995. Water quality assessment and objectives for Tsolum River basin, Vancouver Island. Water Quality Branch, Environmental Protection Department, Ministry of Environment, Lands, and Parks, Victoria, BC.

Deniseger, J., Erickson, L.J., Austin, A., Roch, M., and Clark, M.J.R. 1990. The effects of decreasing heavy metal concentrations on the biota of Buttle Lake, Vancouver Island, British Columbia. Water Research **24**(4): 403–416.

Desrosiers, M., Gagnon, C., Masson, C., Martel, L., and Babut, M.P. 2008. Relationships among total recoverable and reactive metals and metalloid in St. Lawrence River sediment: Bioaccumulation by chironomids and implications for ecological risk assessment. Sci. Total Environ. **389**: 101–114.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., and Santore, R.C. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. **20**: 2383–2396.

Dirilgen, N., and Inel, Y. 1994a. Cobalt-copper and cobalt-zinc effects on duckweed growth and metal accumulation. J. Environ. Sci. Health, Part A: Environ. Sci. Eng. **29**(1): 63–81.

Dirilgen, N., and Inel, Y. 1994b. Effects of zinc and copper on growth and metal accumulation in duckweed, *Lemna minor*. Bull. Environ. Contam. Toxicol. **53**: 442–449.

Dorgelo, J., Meester, H., and Velzen C.V. 1995. Effects of diet and heavy metals on growth rate and fertility in the deposit-feeding snail *Potamopyrgus jenkinsi* (Smith) (Gastropoda: Hydrobiidae). Hydrobiologia **316**: 199–210.

Dragos, N., Bercea, V., Nicoara, A., and Chiorean, A. 1988. Toxic effects of zinc, cadmium and their mixtures on the growth of two unicellular green algae. Rev. Roum. Biol., Ser. Biol. Veg. **33**(2): 103–110.

Drost, W., Matzke, M., and Backhaus, T. 2007. Heavy metal toxicity to *Lemna minor*: studies on the time dependence of growth inhibition and the recovery after exposure. Chemosphere **67**: 36–43.

Duarte, S., Pascoal, C., and Cassio, F. 2009. Functional stability of stream-dwelling microbial decomposers exposed to copper and zinc stress. Freshwater Biol. **54**: 1683–1691.

Dubé, M.G., MacLatchy, D.L., Kieffer, J.D., Glozier, N.E., Culp, J.M., and Cash, K.J. 2005. Effects of metal mining effluent on Atlantic salmon (*Salmo salar*) and slimy sculpin (*Cottus cognatus*): Using artificial streams to assess existing effects and predict future consequences. Sci. Total Environ. **343**: 135–154.

Duboudin, C., Ciffroy, P., and Maugaud, H. 2004. Effects of data manipulation and statistical methods on species sensitivity distributions. Environ. Toxicol. Chem. **23**: 489–499.

Duncan, D.A., and Klaverkamp, J.F. 1983. Tolerance and resistance to cadmium in white suckers (*Catostomus commersoni*) previously exposed to cadmium, mercury, zinc or selenium. Can. J. Fish. Aquat. Sci. **40**: 128–138.

Eaton, J.G. 1973. Chronic toxicity of a copper, cadmium and zinc mixture to the fathead minnow (*Pimephales promelas* Rafinesque). Water Research **7**: 1723–1736.

Eisenhauer, J.B., Brown Sullivan, K., and Lydy, M.J. 1999. Response of genotypes of *Hyalella azteca* to zinc toxicity. Bull. Environ. Contam. Toxicol. **63**: 125–132.

Eisler, R. 1993. Zinc hazards to fish, wildlife, and invrtebrates: A synoptic review. Biological report 10 – Contaminant hazard reviews report 26. Department of the Interior Fish and Wildlife Service, Washington, DC.

El Hendy, H.A., Yousef, M.I., and El-Naga, N.I.A. 2001. Effect of dietary zinc deficiency on hematological and biochemical parameters and concentrations of zinc, copper and iron in growing rats. Toxicology **167**: 163–170.

Ellgaard, E.G., Tusa, J.E., and Malizia, A.A. 1978. Locomotor activity of bluegill *Lepomis Macrochirus*: hyperactivity induced by sublethal concentrations of cadmium, chromium and zinc. J. Fish Biol. **12**(1): 19–23.

Enserink, E.L., Maas-Diepeveen, J.L., and Van Leeuwen, C.J. 1991. Combined effects of metals; An ecotoxicological evaluation. Water Research **25**(6): 679–687.

Environment Canada and Ministère du Développement durable, de l'Environnement et des Parcs du Québec. 2007. Criteria for the assessment of sediment quality in Quebec and application frameworks: Prevention, dredging and remediation. Environment Canada, Ottawa, ON.

Errécalde, O., Seidl, M., and Campbell, P.G.C. 1998. Influence of a low molecular weight metabolite (citrate) on the toxicity of cadmium and zinc to the unicellular green alga *Selenastrum capricornutum*: An exception to the free-ion model. Water Research. **32**(2): 419–429.

European Union. 2006. Draft risk assessment report - zinc metal (Final draft of September 2006). Ministry of Housing, Spatial Planning and the Environment, European Union.

Evans, L.J. 2000. Fractionation and aqueous speciation of zinc in a lake polluted by mining activities, Flin Flon, Canada. Water, Air, Soil Pollut. **122**(3-4): 299–316.

Everall, N.C., Macfarlane, N.A.A., and Sedgwick, R.W. 1989a. The effects of water hardness upon the uptake, accumulation and excretion of zinc in the brown trout *Salmo trutta*. J. Fish Biol. **35**(6): 881–892.

Everall, N.C., Macfarlane, N.A.A., and Sedgwick, R.W. 1989b. The interactions of water hardness and pH with the acute toxicity of zinc to the brown trout, *Salmo trutta* L. J. Fish Biol. **35**: 27–36.

Fargašová, A. 2001. Winter third- to fourth-instar larvae of *Chironomus plumosus* as bioassay tools for assessment of acute toxicity of metals and their binary combinations. Ecotoxicol. Environ. Saf. **48**: 1–5.

Farris, J.L., Belanger, S.E., Cherry, D.S., and Cairns Jr., J. 1989. Cellulolytic activity as a novel approach to assess long-term zinc stress to *Corbicula*. Water Research **23**(10): 1275–1283.

Fernandez, T.V., and Jones, N.V. 1990. Studies on the toxicity of zinc and copper applied singly and jointly to *Nereis diversicolor* at different salinities and temperatures. Trop. Ecol. **31**(1): 47–55.

Finlayson, B.J., and Verrue, K.M. 1982. Toxicities of copper, zinc, and cadmium mixtures to juvenile chinook salmon. Trans. Am. Fish. Soc. **111**(5): 645–650.

Florence, T.M. 1977. Trace metal species in fresh waters. Water Research **11**: 681–687.

Francis, J.C. and Harrison, F.W. 1988. Copper and zinc toxicity in *Ephydatia fluviatilis* (Porifera, Spongillidae). Trans. Am. Microsc. Soc. **107**(1): 67–78.

Galceran, J., Huidobro, C., Companys, E., and Alberti, G. 2007. AGNES: A technique for determining the concentration of free metal ions. The case of Zn(II) in coastal Mediterranean seawater. Talanta **71**:1 795–1803

Gaur, J.P., Noraho, N., and Chauhan, Y. S. 1994. Relationship between heavy metal accumulation and toxicity in *Spirodela polyrhiza* (L.) Schleid. and *Azolla pinnata* R. Br. Aquat. Bot. **49**(2-3): 183–192.

Gehrke, P.C. 1988. Acute cardiorespiratory responses of spangled perch, Leiopotherapon-unicolor (Gunther 1859), to sublethal concentrations of zinc, temephos and 2,4-D. Aust. J. Mar. Freshwater Res. **39**(6): 767–774.

Gobas, F.A., and Morrison, H.A. 2000. Bioconcentration and biomagnification in the aquatic environment. *In* Handbook of property estimation methods for chemicals, environmental and health sciences. *Edited by* R.S. Boethling and D. Mackay D. CRC Press, Boca Raton, FL. pp. 189–231.

Gobeil, C., Rondeau, B., and Beaudin, L. 2005. Contribution of municipal effluents to metal fluxes in the St. Lawrence River. Environ. Sci. Technol. **39**: 456–464.

Goettl, J. P., Davies, P.H., and Sinley, J.R. 1976. Water pollution studies. Review No. 8. Colorado Division of Wildlife, Fort Collins, CO.

Goodarzi, F., Sanei, H., Garrett, R.G., and Duncan, W.F. 2002. Accumulation of trace elements on the surface soil around the Trail smelter, British Columbia, Canada. Environ. Geol. **43**: 29–38.

Gündoğdu, A. 2008. Acute toxicity of zinc and copper for rainbow trout (*Oncorhynchus mykiss*). J. FisheriesSciences.com. **2**(5): 711–721.

Gupta, A.K., and Sharma, S.K. 1994. Bioaccumulation of zinc in *Cirrhinus mrigala* fingerlings during short-term static bioassay. J. Environ. Biol. **15**(3): 231–237.

Hall, L.W., and Anderson, R.D. 1995. The influence of salinity on toxicity of various classes of chemicals to aquatic biota. Crit. Rev. Toxicol. **25**(4): 281–346.

Hall, W.S., Dickson, K.L., Saleh, F.Y., Rodgers, J.H., Jr., Wilcox, D., and Entazami, A. 1986. Effects of suspended solids on the acute toxicity of zinc to *Daphnia magna* and *Pimephales promelas*. Water Resour. Bull. **22**: 913–920.

Hamilton, S.J. 1995. Hazard assessment of inorganics to three endangered fish in the green river, Utah. Ecotoxicol. Environ. Saf. **30**: 134–142.

Hamilton, S.J., and Buhl, K.J. 1990. Safety assessment of selected inorganic elements to fry of Chinook salmon (*Oncorhynchus tshawytscha*). Ecotoxicol. Environ. Saf. **20**: 307–324.

Hamilton, S.J. and Buhl, K.J. 1997. Hazard evaluation of inorganics, singly and in mixtures, to flannelmouth sucker *Catostomus Iatipinnis* in the San Juan River, New Mexico. Ecotoxicol. Environ. Saf. **38**: 296–308.

Hansen, J.A., Welsh, P.G., Lipton, J., Cacela, D., and Dailey, A.D. 2002. Relative sensitivity of bull trout (*Salvelinus confluentus*) and rainbow trout (*Oncorhynchus mykiss*) to acute exposures of cadmium and zinc. Environ. Toxicol. Chem. **21**(1): 67–75.

Hatakeyama, S. 1989. Effect of copper and zinc on the growth and emergence of Epeorus-latifolium (Ephemeroptera) in an indoor model stream. Hydrobiologia **174**(1): 17–27.

Hattink, J., De Boeck, G., and Blust, R. 2006. Toxicity, accumulation, and retention of zinc by carp under normoxic and hypoxic conditions. Environ. Toxicol. Chem. **25**(1): 87–96.

Hedtke, J.L., Robinson-Wilson, E., and Weber, L.J. 1982. Influence of body size and developmental stage of coho salmon (*Oncorhynchus kisutch*) on lethality of several toxicants. Fundam. Appl. Toxicol. **2**: 67–72.

Heijerick, D.G., De Schamphelaere, K.A.C., and Janssen, C.R. 2002a. Biotic ligand model development predicting Zn toxicity to the algae *Pseudokirchneriella subcapitata:* Possibilities and limitations. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. **133**: 207–218.

Heijerick, D.G., De Schamphelaere, K.A.C., and Janssen, C.R. 2002b. Predicting acute zinc toxicity for *Daphnia magna* as a function of key water chemistry characteristics: Development and validation of a biotic ligand model. Environ. Toxicol. Chem. **21**(6): 1309–1315.

Heijerick, D.G., Janssen, C.R., and De Coen, W.M. 2003. The combined effects of hardness, pH and dissolved organic carbon on the chronic toxicity of Zn to *D. magna* : Development of a surface response model. Arch. Environ. Contam. Toxicol. **44**: 210–217.

Heijerick, D.G., De Schamphelaere, K.A.C., Van Sprang, P.A., and Janssen, C.R. 2005. Development of a chronic zinc biotic ligand model for *Daphnia magna*. Ecotoxicol. Environ. Saf. **62**: 1–10.

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.-C., and Kahru, A. 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus.* Chemosphere **71**: 1308–1316.

Hem, J.D. 1972. Chemistry and occurence of cadmium and zinc in surface water and groundwater. Water Resour. Res. **8**(3): 661–679.

Henderson, P.J., McMartin, I., Hall, GE., Percival, J. B., and Walker, D.A. 1998. The chemical and physical characteristics of heavy metals in humus and till in the vicinity of the base metal smelter at Flin, Flon, Manitoba, Canada. Environ. Geol. **34**(1): 39–58.

Hickey, C.W,. and Golding, L.A. 2002. Response of macroinvertebrates to copper and zinc in a stream mesocosm. Environ. Toxicol. Chem. **21**(9): 1854–1863.

Hickey, C.W., Blaise, C., and Costan, G. 1991. Microtesting appraisal of ATP and cell recovery toxicity end points after acute exposure of *Selenastrum capricornutum* to selected chemicals. Environ. Toxicol. Water Qual. **6**: 383–403.

Hilmy, A.M., El-Domiaty, N.A., Daabees, A.Y., and Abdel Latife, H.A. 1987. Toxicity in *Tilapia zilli* and *Clarias lazera* (pisces) induced by zinc, seasonally. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. **86**(2): 263–265.

Hiltibran, R.C. 1971. Effects of cadmium, zinc, manganese, and calcium on oxygen and phosphate metabolism of bluegill liver mitochodria. J. - Water Pollut. Control Fed. **43**(5): 818–823.

Hobson, J.F., and Birge, W J. 1989. Acclimation-induced changes in toxicity and induction of metallothionein-like proteins in the fathead minnow following sublethal exposure to zinc. Environ. Toxicol. Chem. **8**: 157–169.

Hodson, P.V., and Sprague, J.B. 1975. Temperature-induced changes in aute toxicity of zinc to Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. **32**: 1–10.

Hogstrand, C., and Wood, C.M. 1995. Mechanisms for zinc acclimation in freshwater rainbow trout. Mar. Environ. Res. **39**(1-4): 131–135.

Hogstrand, C., Wilson, R.W., Polgar, D., and Wood, C.M. 1994. Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. J. Exp. Biol. **186**: 55–73.

Hogstrand, C., Verbost, P.M., Wendelaar Bonga, S.E., and Wood, C.M. 1996. Mechanisms of zinc uptake in gills of freshwater rainbow trout: Interplay with calcium transport. Am. J. Physiol. **270**(5 Pt 2): R1141–R1147.

Hogstrand, C., Webb, N., and Wood, C.M. 1998. Covariation in regulation of affinity for branchial zinc and calcium uptake in freshwater rainbow trout. J. Exp. Biol. **201**(11): 1809–1815.

Holcombe, G.W. and Andrew, R.W. 1978. The acute toxicity of zinc to rainbow and brook trout: Comparisons in hard and soft water. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

Holcombe, G.W., Benoit, D.A., and Leonard, E.N. 1979. Long-term effects of zinc exposures on brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. **108**: 76–87.

Holdway, D.A., Lok, K., and Semaan, M. 2001. The acute and chronic toxicity of cadmium and zinc to two hydra species. Environ. Toxicol. **16**(6): 557–565.
Hollibaugh, J.T., Siebert, D.L.R., and Thomas, W.H. 1980. A comparison of the acute toxicities of ten heavy metals to phytoplankton from Saanich Inlet, B. C., Canada. Estuarine Coastal Mar. Sci. **10**: 93–105.
Hongve, D., Skogheim, O.K., Hindar, A., and Abrahamsen, H. 1980. Effects of heavy metals in combination with NTA, humic acid, and suspended sediment on natural phytoplankton photosynthesis. Bull. Environ. Contam. Toxicol. **25**: 594–600.
Huebert, D.B., and Shay, J.M. 1992. Zinc toxicity and its interaction with cadmium in the submerged aquatic macrophyte *Lemna trisulca*. Environ. Toxicol. Chem. **11**: 715–720.
Hughes, G.M., and Flos, R. 1978. Zinc content of the gills of rainbow trout (*S. gairdneri*) after treatment with zinc solutions under normoxic and hypoxic conditions. J. Fish Biol. **13**: 717–728.
Hung, G.A., and Chmura, G.L. 2007. Metal accumulation in surface salt marsh sediments of the Bay of Fundy, Canada. Estuaries Coasts. **30**(4): 725–734.
Hyne, R.V., Pablo, F., Julli, M., and Markich, S.J. 2005. Influence of water chemistry on the acute toxicity of copper and zinc to the cladoceran *Ceriodaphnia* CF *dubia*. Environ. Toxicol. Chem. **24**(7): 1667–1675.
Iwasaki, Y., and Ormerod, S.J. 2012. Estimating safe concentrations of trace metals from inter-continental field data on river macroinvertebrates. Environ. Pollut. **166**: 182–186.
Ince, N.H., Dirilgen, N., Apikyan, I.G., Tezcanli, G., and Ustün, B. 1999. Assessment of toxic interactions of heavy metals in binary mixtures: A statistical approach. Arch. Environ. Contam. Toxicol. 36: 365–372.
Janssen, C.R., and Persoone, G. 1993. Rapid toxicity screening tests for aquatic biota. 1. Methodology and experiments with Daphnia magna. Environ. Toxicol. Chem. **12**(4): 711–717.
Jenner, H.A., and Janssen-Mommen, J.P.M. 1993. Duckweed *Lemna minor* as a tool for testing toxicity of coal residues and polluted sediments. Arch. Environ. Contam. Toxicol. **25**: 3–11.
Johns, C., and Timmerman, B.E. 1998. Total cadmium, copper and zinc in two Dreissenid mussles, *Dreissena polymorpha* and *Dreissena bugensis*, at the outflow of Lake Ontario. J. Great Lakes Res. **24**(1): 55–64.
Jones, M.B. 1975. Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine and estuarine isopods (crustacea). Marine Biology **30**: 13–20.
Juárez-Franco, M.F., Sarma, S.S.S., and Nandini, S. 2007. Effect of cadmium and zinc on the population growth of *Brachionus havanaensis* (Rotifera: Brachionidae). J. Environ. Sci. Health, Part A: Environ. Sci. Eng. **42**: 1489–1493.
Judy, R.D., Jr., and Davies, P. H. 1979. Effects of calcium addition as $Ca(NO_3)_2$ on zinc toxicity to fathead minnow, *Pimephales promelas* Rafinesque. Bull. Environ. Contam. Toxicol. **22**: 88–94.
Kamaya, Y., Takada, T., and Suzuki, K. 2004. Effect of medium phosphate levels on the sensitivity of *Selenastrum capricornutum* to chemiclas. Bull. Environ. Contam. Toxicol. **73**: 995–1000.
Karntanut, W., and Pascoe, D. 2002. The toxicity of copper, cadmium and zinc to four different *Hydra* (Cnidaria: Hydrozoa). Chemosphere **47**: 1059–1064.
Kashian, D.R., Prusha, B.A., and Clements, W.H. 2004. Influence of total organic carbon and UV-B radiation on zinc toxicity and bioaccumulation to aquatic communities. Environ. Sci. Technol. **38**(6371): 6376.
Kayser, H. 1977. Effect of zinc sulfate on the growth of mono- and multispecies cultures of some marine plankton algae. Helgoländer wiss Meeresunters **30**: 682–696.
Keller, A.E. and Zam, S.G. 1991. The acute toxicity of selected metals to the freshwater mussel *Anodonta imbecilis*. Environ. Toxicol. Chem. **10**: 539–546.
Khan, M.A.Q., Ahmed, S.A., Catalin, B., Khodadoust, A., Ajayi, O., and Vaughn, M. 2006. Effect of temperature on heavy metal toxicity to juvenile crayfish, *Orconectes immunis*. Environ. Toxicol. **21**(5): 513–520.
Khangarot, B.S., and Ray, P.K. 1987a. Zinc sensitivity of a freshwater snail *Lymnaea luteola* in relation to seasonal variations in temperature. Bull. Environ. Contam. Toxicol. **39**: 45–49.
Khangarot, B.S., and Ray, P.K. 1987b. Sensitivity of toad tadpoles, *Bufo melanostictus* to heavy metals. Bull. Environ. Contam. Toxicol. **38**: 523–527.
Khangarot, B.S. and Ray, P.K. 1989. Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea *Daphnia magna* Straus. Ecotoxicol. Environ. Saf. **18**: 109–120.
Khangarot, B.S., Sehgal, A., and Bhasin, M.K. 1983. "'Man and the biosphere"' – Studies on Sikkim Himalayas. Part 1: Acute toxicity of copper and zinc to common carp *Cyprinus carpio* (Linn.) in soft water. Acta Hydrochim. Hydrobiol. **11**: 667–673.
Khangarot, B.S., Sehgal, A., and Bhasin, M.K. 1985. "Man and biosphere" – Studies on the Sikkim Himalayas. Part 5: Acute toxicity of selected heavy metals on the tadpoles of *Rana hexadactyla*. Acta Hydrochim. Hydrobiol. **2**: 259–263.

Khangarot, B.S., Rathore, R.S., and Singh, B.B. 2003. pH-dependent toxicity to heavy metals to a freshwater sludgeworm *Tubifex tubifex* Müller. Bull. Environ. Contam. Toxicol. **71**: 283–289.

Knauer, K., Behra, R., and Sigg, L. 1997. Effects of free Cu2+ and Zn2+ ions on growth and metal accumulation in freshwater algae. Environ. Toxicol. Chem. **16**(2): 220–229.

Kraak, M.H.S., Toussaint, M., Lavy, D., and Davids, C. 1994a. Short-term effects of metals on the filtration rate of the zebra mussel *Dreissena polymorpha*. Environ. Pollut. **84**: 139–143.

Kraak, M.H.S., Wink, Y.A., Stuijfzand, S.C., Buckert-de Jong, M.C., De Groot, C.J., and Admiraal, W. 1994b. Chronic ecotoxicity of Zn and Pb to the zebra mussel *Dreissena polymorpha*. Aquat. Toxicol. **30**: 77–89.

Kungolos, A., Samaras, P., Tsiridis, V., Petala, M., and Sakellaropoulos, G. 2006. Bioavailability and toxicity of heavy metals in the presence of natural organic matter. J. Environ. Sci. Health, Part A: Environ. Sci. Eng. **41**: 1509–1517.

Kuwabara, J.S. 1985. Phosphorus-zinc interactive effects on growth by *Selenastrum capricornutum* (chlorophyta). Environ. Sci. Technol. **19**(5): 417–421.

Lahive, E., O'Halloran, J., and Jansen, M.A.K. 2011. Differential sensitivity of four *Lemnaceae* species to zinc sulphate. Environ. Exp. Bot. **71**: 25–33.

Lalande, M., and Pinel-Alloul, B. 1986. Acute toxicity of cadmium, copper, mercury and zinc to Tropocyclops prasinus mexicanus (Cyclopoida, copepoda) from three Quebec lakes. Environ. Toxicol. Chem. **5**(1): 95–102.

Lazorchak, J.M., and Smith, M.E. 2007. Rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) 7-day survival and growth test method. Arch. Environ. Contam. Toxicol. **53**: 397–405.

LeBlanc, G.A. 1982. Laboratory investigation into the development of resistance of Daphnia magna (Straus) to environmental pollutants. Environ. Pollut., Ser. A **27**(4): 309–322.

Lefcort, H., Meguire, R.A., Wilson, L.H., and Ettinger, W.F. 1998. Heavy metals alter the survival, growth, metamorphosis, and antipredatory behaviour of Columbia Spotted Frog (*Rana luteiventris*) tadpoles. Arch. Environ. Contam. Toxicol. **35**: 447–456.

Lewis, M. 1978. Acute toxicity of copper, zinc and manganese in single and mixed salt solutions to juvenile longfin dace, *Agosia chrysogaster*. J. Fish Biol. **13**: 695–700.

Lide, D.R. 2006. CRC handbook of chemistry and physics. CRC Press, Boca Raton, FL.

Lin, K.-C., Lee, Y.-L., and Chen, C.-Y. 2007. Metal toxicity to *Chlorella pyrenoidosa* assessed by a short-term continuous test. J. Hazard. Mater. **142**: 236–241.

Lindsay, W.L. 1979. Chemical equilibria in soils. John Wiley & Sons, New York, NY.

Lloyd, R. 1960. The toxicity of zinc sulphate to rainbow trout. Ann. Appl. Biol. **48**: 84–94.

Lloyd, R. 1961a. Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo Gairdnerii* Richardson). J. Exp. Biol. **38**(2): 447–455.

Lloyd, R. 1961b. The toxicity of mixtures of zinc and copper sulfates to rainbow trout (*Salmo gairdnerii* Richardson). Ann. Appl. Biol. **49**: 535–538.

Lum, K.R., Kaiser, K.L.E., and Jaskot, C. 1991. Distribution and fluxes of metals in the St.Lawrence River from the outflow of Lake Ontario to Québec City. Aquat. Sci. **53**(1): 1–19.

Macdonald, A., Silk, K., Schwartz, M., and Playle, RC. 2002. A lead-gill binding model to predict acute lead toxicity to rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. **133**: 227–242.

Magdaleno, A., Gomez, C.E., Velez, C.G., and Accorinti, J. 1997. Preliminary toxicity tests using the green alga *Ankistrodesmus falcatus.* Environ. Toxicol. Water Qual. **12**: 11–14.

Magliette, R.J., Doherty, F.G., McKinney, D., and Venkataramani, E.S. 1995. Need for environmental quality guidelines based on ambient freshwater quality criteria in natural waters – case study "zinc." Bull. Environ. Contam. Toxicol. **54**: 626–632.

Malle, K.G. 1992. Zinc in the environment. Acta Hydrochim. Hydrobiol. **20**(4): 196–204.

Marshall, J.S., Parker, J.I., Mellinger, D.L., and Lei, C. 1983. Bioaccumulation and effects of cadmium and zinc in a Lake Michigan plankton community. Can. J. Fish. Aquat. Sci. **40**: 1469–1479.

Martin, T.R., and Holdich, D.M. 1986. The acute lethal toxicity of heavy metals to peracarid crustaceans (with particular reference to fresh-water asellids and gammarids). Water Research **20**: 1137–1147.

Masters, J.A., Lewis, M.A., Davidson, D., and Bruce, R.D. 1991. Validation of a four-day *Ceriodaphnia* toxicity test and statistical considerations in data analysis. Environ. Toxicol. Chem. **10**: 47–55.

Manitoba Conservation. 2011. Manitoba water quality standards, objectives, and guidelines. Water Science and Management Branch, Manitoba Water Stewardship, Winnipeg, MB.

McGeer, J.C., Szebedinszky, C., McDonald, D.G., and Wood, C. M. 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout 2: Tissue specific metal contamination. Aquat. Toxicol. **50**: 245–256.

McGeer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J., and Green, A. 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: Implications for hazard assessment of metals in the aquatic environment. Environ. Toxicol. Chem. **22**(5): 1017–1037.

McHardy, B.M., and George, J.J. 1990. Bioaccumulation and toxicity of zinc in the green alga, *Cladophora glomerata*. Environ. Pollut. **66**: 55–66.

McKenny, C.L. and Neff, J.M. 1979. Individual effects and interactions of salinity, temperature, and zinc on larval development of the grass shrimp *Palaemonetes pugio*. I. Survival and development duration through metamorphosis. Marine Biology **52**: 177–188.

McLeay, D.J. 1976. A rapid method for measuring the acute toxicity of pulp mill effluent and other toxicants to salmonid fish at ambiant room tempreature. J. Fish. Res. Board Can. **33**: 1303–1311.

McLusky, D.S., and Hagerman, L. 1987. The toxicity of chromium, nickel and zinc: Effects of salinity and temperature and the osmoregulatory consequences in the mysid *Praunus flexuosus*. Aquat. Toxicol. **10**: 225–238.

MDDEP (Ministry of Sustainable Development, Environment and Parks) 2007. Critères de qualité de l'eau de surface au Québec [online]. Available from: http://www.mddep.gouv.qc.ca/eau/criteres_eau/index.htm [accessed 14 March 2018].

Mebane, C.A., Hennessy, D.P., and Dillon, F.S. 2008. Developing acute-to-chronic toxicity ratios for lead, cadmium and zinc using rainbow trout, a mayfly and a midge. Water, Air, Soil Pollut. **188**: 41–66.

Meyer, J.S., Clearwater, S.J., Doser, T.A., Rogaczewski, M.J., and Hansen, J.A. 2007. Effects of water chemistry on bioavailability and toxicity of waterborne cadmium, copper, nickel, lead, and zinc to freshwater organisms. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL.

Mills, W.L. 1976. Water quality bioassay using selected protozoa, II. The effects of zinc on population growth of *Euglena gracilis*. J. Environ. Sci. Health, Part A: Environ. Sci. Eng. **A11**(8/9): 567–572.

Mirenda, R.J. 1986. Acute toxicity and accumulation of zinc in the crayfish, *Orconectes virilis* (Hagen). Bull. Environ. Contam. Toxicol. **37**(3): 387–394.

Morel, F.M.M., and Hering, J. G. 1993. Principles and applications of aquatic chemistry. John Wiley & Sons, New York.

Mount, D. I., 1966. The effect of total hardness and pH on acute toxicity of zinc to fish. Int. J. Air Water Pollut. **10**: 49–56.

Mount, D.I., and Norberg, T.J. 1984. A seven-day life-cycle cladoceran toxicity test. Environ. Toxicol. Chem. **3**: 425–434.

Mudroch, A., and Duncan, G. A. 1986. Distribution of metals in different size fractions of sediment from the Niagra River. J. Great Lakes Res. **12**(2): 117–126.

Mudroch, A., Sarazin, L., and Lomas, T. 1988. Summary of surface and background concentrations of selected elements in the Great Lakes sediments. J. Great Lakes Res. **14**(2): 241–251.

Münzinger, A., and Monicelli, F. 1991. A comparison of the sensitivity of three *Daphnia magna* populations under chronic heavy metal stress. Ecotoxicol. Environ. Saf. **22**: 24–31.

Muyssen, B.T.A. and Janssen, C.R. 2001. Zinc acclimation and its effect on the zinc tolerance of *Raphidocelis subcapitata* and *Chlorella vulgaris* in laboratory experiments. Chemosphere **45**: 507–514.

Muyssen, B.T.A. and Janssen, C.R. 2002a. Accumulation and regulation of zinc in *Daphnia magna*: Links with homeostasis and toxicity. Arch. Environ. Contam. Toxicol. **43**: 492–496.

Muyssen, B.T.A. and Janssen, C.R. 2002b. Tolerance and acclimation to zinc of *Ceriodaphnia dubia*. Environ. Pollut. **117**: 301–306.

Muyssen, B.T.A., Janssen, C.R., and Bossuyt, B.T.A. 2002. Tolerance and acclimation to zinc of field-collected *Daphnia magna* populations. Aquat. Toxicol. **56**: 69–79.

Muyssen, B.T.A., Bossuyt, B.T.A., and Janssen, C. R. 2005. Inter- and intra-species variation in acute zinc tolerance of field-collected cladoceran populations. Chemosphere **61**: 1159–1167.

Muyssen, B.T.A., De Schamphelaere, K.A.C., and Janssen, C.R. 2006. Mechanisms of chronic waterborne Zn toxicity in *Daphnia magna*. Aquat. Toxicol. **77**(4): 393–401.

Nagpal, N.K. 1990. Campbell River area, Oyster River basin, water quality assessment and objectives. Technical Appendix. Water Quality Branch, Environmental Protection Department, Ministry of Environment, Lands, and Parks, Victoria, BC.

Nagpal, N.K. 1999. Ambient water quality for zinc – Technical report. Water Management Branch, Victoria, BC.

Nasu, Y., and Kugimoto, M. 1981. *Lemna* (Duckweed) as an indicator of water pollution. I. The sensitivity of *Lemna paucicostata* to heavy metals. Arch. Environ. Contam. Toxicol. **10**: 159–169.

National Pollutant Release Inventory (NPRI). 2014. National pollutant release and air pollutant emission summaries and trends downloadable datasets for 2013. Facility data [online]. Available from https://www.ec.gc.ca/inrp-npri/default.asp?lang=en&n=0EC58C98- [accessed 18 February 2015].

NRCan (Natural Resources Canada). 2007a. Mineral use [online]. Previously available from http://mmsd1.mms.nrcan.gc.ca/mmsd/consumption/default_e.asp.

NRCan 2007b. Minerals and metals: A world to discover [online]. Previously available from http://www.nrcan.gc.ca/mms/scho-ecol/main_e.htm#zinc.

NRCan 2007c. Mines, quarries, pits, bogs, mills and concentrators in Canada – Zinc [online]. Previously available from http://mmsd1.mms.nrcan.gc.ca/mmsd/producers/commodityCompany_e.asp?nId=29&minetype=metal.

NRCan 2008. Production of Canada's leading minerals monthly publication [online]. Previously available from http://mmsd1.mms.nrcan.gc.ca/mmsd/data/default_e.asp.

NRCan 2012a. Production of Canada's leading minerals. Monthly statistics [online]. Previously available from http://mmsd.mms.nrcan.gc.ca/stat-stat/prod-prod/mon-men-eng.aspx.

NRCan 2012b. Mineral production of Canada, by province and territory. Annual statistics [online]. Previously available from http://mmsd.mms.nrcan.gc.ca/stat-stat/prod-prod/ann-ann-eng.aspx.

NRCan 2012c. Mineral use. Annual statistics [online]. Previously available from http://mmsd.mms.nrcan.gc.ca/stat-stat/use-util/sta-sta-eng.aspx.

Naylor, C., Pindar, L., and Calow, P. 1990. Inter- and intraspecific variation in sensitivity to toxins; the effects of acidity and zinc on the freshwater crustaceans *Asellus aquaticus* and *Gammarus pulex*. Water Research **24**(6): 757–762.

Nebeker, A.V., Savonen, C., Baker, R.J., and McCrady, J.K. 1984. Effects of copper, nickel and zinc on the life cycle of the caddisfly *Clistoronia magnifica* (Limnephilidae). Environ. Toxicol. Chem. **3**: 645–649.

Nebeker, A.V., Stinchfield, A., Savonen, C., and Chapman, G.A. 1986. Effects of copper, nickel and zinc on three species of Oregon freshwater snails. Environ. Toxicol. Chem. **5**: 807–811.

Nehring, R.B. 1976. Aquatic insects as biological monitors of heavy metal pollution. Bull. Environ. Contam. Toxicol. **15**(2): 147–154.

Nehring, R.B., and Goettl, J. P. 1974. Acute toxicity of a zinc-polluted stream to four species of salmonids. Bull. Environ. Contam. Toxicol. **12**(4): 464–469.

Newhook, R., Hirtle, H., Byrne, K., and Meek, M.E. 2003. Releases from copper smelters and refineries and zinc plants in Canada: Human health exposure and risk characterization. Sci. Total Environ. **301**: 23–41.

Newman, M.C., Ownby, D.R., Mezin, L.C.A., Powell, D.C., Christensen, T.R.L., Lerberg, S.B., Anderson, B.-A., and Padma, T.V. 2002. Species sensitivity distributions in ecological risk assessment: Distributional assumptions, alternate bootstrap techniques, and estimation of adequate number of species. *In* Species sensitivity distributions in ecotoxicology. *Edited by* L. Posthuma, G.W.I. Suter, and T. Traas. CRC Press, Boca Raton, FL. pp. 119–132.

Niyogi, S., and Wood, C.M. 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. Environ. Sci. Technol. **38**(23): 6177–6192.

Norberg, T.J. and Mount, D.I. 1985. A new fathead minnow (*Pimephales promelas*) subchronic toxicity test. Environ. Toxicol. Chem. **4**: 711–718.

Norberg-King, T.J. 1989. An evaluation of the fathead minnow seven-day subchronic test for estimating chronic toxicity. Environ. Toxicol. Chem. **8**: 1075–089.

Norwood, W.P., Borgmann, U., Dixon, D.G., and Wallace, A. 2003. Effects of metal mixtures on aquatic biota: A review of observations and methods. Hum. Ecol. Risk Assess. **9**(4): 795–811.

Norwood, W.P., Borgmann, U., and Dixon, D.G. 2007. Interactive effects of metals in mixtures on bioaccumulation in the amphipod *Hyalella azteca*. Aquat. Toxicol. **84**: 255–267.

Nova Scotia Environment. 2008. Surface water management programs; Surface water monitoring and reporting.

Nova Scotia Environment and Labour. 2004. Guide to surface water withdrawal approvals. Previously available from http://www.gov.ns.ca/enla/water/docs/guideToSurfaceWaterWithdrawalApprovals.pdf

Nriagu, J.O., Lawson, G., Wong, H.K.T., and Cheam, V. 1996. Dissolved trace metals in Lakes Superior, Erie and Ontario. Environ. Sci. Technol. **30**: 178–187.

Oikari, A., Kukkonen, J., and Virtanen, V. 1992. Acute toxicity of chemicals to *Daphnia magna* in humics waters. Sci. Total Environ. **117/118**: 367–377.

Ontario Ministry of Environment and Energy. 1994. Policies, guidelines, provincial water quality objectives [online]. Available from https://www.ontario.ca/page/water-management-policies-guidelines-provincial-water-quality-objectives [accessed 14 March 2018].

OMOE (Ontario Ministry of the Environment). 2011. Rationale for the development of soil and groundwater standards for use at contaminated Sites in Ontario. Prepared by the Standards Development Branch, Ontario Ministry of the Environment. PIBS 7386e01.

Palawski, D., Hunn, J.B., and Dwyer, F.J. 1985. Sensitivity of young striped bass to organic and inorganic contaminants in fresh and saline waters. Trans. Am. Fish. Soc. **114**: 748–753.

Pantani, C., Pannunzio, G., De Cristofaro, M., Novelli, A.A., and Salvatori, M. 1997. Comparative acute toxicity of some pesticides, metals, and surfactants to Gammarus italicus Goedm. and Echinogammarus tibaldii Pink. and Stock (Crustacea: Amphipoda). Bull. Environ. Contam. Toxicol. **59**(6): 963–967.

Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., Delos, C.G., Di Toro, D.M., Dwyer, R.L., Galvez, F., Gensemer, R.W., Goss, G.G., Hogstrand, C., Janssen, C.R., McGeer, J.C., Naddy, R.B., Playle, R.C., Santore, R.C., Schneider, U., Stubblefield, W.A., Wood, C.M., and Wu, K.B. 2002. The biotic ligand model: A historical overview. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. **133** (1–2): 3–35.

Pardos, M., Benninghoff, C., and Thomas, R.L. 1998. Photosynthetic and population growth response of the test alga *Selenastrum capricornutum* Printz to zinc, cadmium and suspended sediment elutriates. J. Appl. Phycol. **10**: 145–151.

Pardue, W.J., and Wood, T.S. 1980. Baseline toxicity data for freshwater Bryoza exposed to copper, cadmium, and zinc. J. Tenn. Acad. Sci. **55**: 27–31.

Parkerton, T.F., Stewart, S.M., Dickson, K.L., Rodgers, J.H., Jr., and Saleh, F.Y. 1988. Evaluation of the indicator species procedure for deriving site-specific water quality criteria for zinc. *In* Aquatic toxicology and hazard assessment: 10th volume, ASTM STP 971. *Edited by* W.J. Adams, G.A. Chapman, and W.G. Landis. American Society for Testing and Materials, Philadelphia, PA. pp. 423–435.

Parsons, M.B., and Cranston, M.B. 2006. Influence of lead smelter emissions on the distribution of metals in marine sediments from Chaleur Bay, eastern Canada. Geochem.: Explor., Environ., Anal. **6**(2–3): 259–276.

Patrick, R., Cairns, J., Jr., and Scheier, A. 1968. The relative sensitivity of diatoms, snails, and fish to twenty common constituents of industrial wastes. The Progressive Fish-Culturist **30**(3): 137–140.

Paulauskis, J.D., and Winner, R.W. 1988. Effects of water hardness and humic acid on zinc toxicity to *Daphnia magna* Straus. Aquat. Toxicol. **12**: 273–290.

Paulsson, M., Nyström, B., and Blanck, H. 2000. Long-term toxicity of zinc to bacteria and algae in periphyton communities from the river Göta Alv, based on a microcosm study. Aquat. Toxicol. **47**: 243–257.

Pestana, J.L.T., Ré, A., Nogueira, A.J.A., and Soares, A.M.V.M. 2007. Effects of cadmium and zinc on the feeding behaviour of two freshwater crustaceans: *Atyaephyra desmarestii* (Decapoda) and *Echinogammarus meridionalis* (Amphipoda). Chemosphere **68**: 1556–1562.

Phipps, G.L., Mattson, V.R., and Ankley, G.T. 1995. Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. Arch. Environ. Contam. Toxicol. **28**: 281–286.

Pickering, Q.H. 1968. Some effects of dissolved oxygen concentration upon the toxicity of zinc to the bluegill, *Lepomis macrochirus*, raf. Water Research **2**: 187–194.

Pickering, Q.H. and Henderson, C. 1966. The acute toxicity of some heavy metals to different species of warmwater fishes. Int. J. Air Water Pollut. **10**: 453–463.

Pickering, Q.H. and Vigor, W.N. 1965. The acute toxicity of zing to eggs and fry of the fathead minnow. The Progressive Fish-Culturist **27**: 153–157.

Pierson, K.B. 1981. Effects of chronic zinc exposure on the growth, sexual maturity, reproduction, and bioaccumulation of the guppy, *Poecilia reticulata*. Can. J. Fish. Aquat. Sci. **38**(1): 23–31.

Playle, R.C. 1998. Modelling metal interaction at fish gills. Sci. Total Environ. **219**: 147–163.

Posthuma, L., Suter, G.W.I., and Traas, T.E. 2002. Species sensitivity distribution in ecotoxicology. CRC Press, Boca Raton FL.

Pourkhabbaz, A., Kasmani, M.E., Kiyani, V., and Hosynzadeh, M.H. 2011. Effects of water hardness and Cu and Zn on LC50 in *Gambusia holbrooki*. Chem. Speciation and Bioavailability **23**(4): 224–228.

Pugh, R.E., Dick, D.G., and Fredeen, A.L. 2002. Heavy metal (Pb, Zn, Cd, Fe, and Cu) contents of plant foliage near the Anvil Range lead/zinc mine, Faro, Yukon Territory. Ecotoxicol. Environ. Saf. **52**: 273–279.

PWQMN [Provincial (Stream) Water Quality Monitoring Network]. 2016. PWQMN dataset for 2014 [online]. Available from https://www.ontario.ca/data/provincial-stream-water-quality-monitoring-network [accessed 2 May 2016].

Pynnönen, K. 1995. Effect of pH, hardness and maternal pre-exposure on the toxicity of Cd, Cu and Zn to the glochidial larvae of a freshwater clam *Anodonta cygnea*. Water Research **29**: 247–254.

Qureshi, S.A., and Saksena, A.B. 1980. The acute toxicity of some heavy metals to *Tilapia mossambica* (Peters) Aqua **1**:19–20.

Rachlin, J.W., and Perlmutter, A. 1968. Response of an inbred strain of platyfish and fathead minnow to zinc. The Progressive Fish-Culturist **30**: 203–207.

Rai, L.C., Gaur, J.P., and Kumar, H.D. 1981. Protective effects of certain environmental factors on the toxicity of zinc, mercury and methylmercury to *Chlorella vulgaris*. Environ. Res. **25**: 250–259.

RAMP (Regional Aquatics Monitoring Program). 2016. RAMP monitoring database for water quality for 2014 [online]. Available from http://www.ramp-alberta.org/data/Water/water.aspx [accessed 2 May 2016].

Rathore, R.S., and Khangarot, B.S. 2002. Effects of temperature on the sensitivity of sludgeworm *Tubifex tubifex* Müller to selected heavy metals. Ecotoxicol. Environ. Saf. **53**: 27–36.

Rathore, R.S. and Khangarot, B. S. 2003. Effects of water hardness and metal concentration on a freshwater *Tubifex tubifex* Muller. Water, Air, Soil Pollut. **142**(1–4): 341–356.

Reader, J.P., Everall, N.C., Sayer, M.D.J., and Morris, R. 1989. The effects of eight trace metals in acid soft water on survival, mineral uptake and skeletal calcium deposition in yolk-sac fry of brown trout, *Salmo trutta* L. J. Fish Biol. **35**: 187–198.

Rehwoldt, R., Bida, G., and Nerrie, B. 1971. Acute toxicity of copper, nickel and zinc ions to some Hudson River fish species. Bull. Environ. Contam. Toxicol. **6**(5): 445–448.

Rehwoldt, R., Menapage, L.W., Nerrie, B., and Alessandrello, D. 1972. The effect of increased temperature upon the toxicity of some heavy metal ions. Bull. Environ. Contam. Toxicol. **8**: 91.

Rehwoldt, R., Lasko, L., Shaw, C., and Wirhowski, E. 1973. The acute toxicity of some heavy metal ions toword benthic organisms. Bull. Environ. Contam. Toxicol. **10**: 291–294.

Reimann, C., and De Caritat, P. 1998. Chemical elements in the environment – Factsheets for the geochemist and environmental scientist. Springer, Verlag Berlin Heidelberg, Germany.

Reimer, P., and Duthie, H. C. 1993. Concentrations of zinc and chromium in aquatic macrophytes from the Sudbury and Muskoka regions of Ontario, Canada. Environ. Pollut. **79**: 261–265.

Richardson, J.S., and Kiffney, P.,M. 2000. Responses of a macroinvertebrate community from a pristine, southern British Columbia, Canada stream to metals in experimental mesocosms. Environ. Toxicol. Chem. **19**(3): 736–743.

Richelle, E., Degoudenne, Y., Dejonghe, L., and Van de Vyver, G. 1995. Experimental and field studies on the effect of selected heavy metals on three freshwater sponge species: *Ephydatia fluviatilis*, *Ephydatia muelleri*, and *Spongilla lacustris*. Arch. Hydrobiol. **135**(2): 209–231.

Rodgers, J.H., Jr., Cherry, D., Graney, R.L., Dickson, K.L., and Cairns, J., Jr. 1980. Comparison of heavy metal interactions in acute and artificial stream bioassay techniques for the Asiatic clam (*Corbicula fluminea*). *In* Aquatic toxicology, ASTM STP 707. *Edited by* J.G. Eaton, P.R. Parrish, and A.C. Hendricks. American Society for Testing and Materials, Philadelphia, PA. pp. 266–280.

Rondeau, B., Cossa, D., Gagnon, P., Pham, T.T., and Surette, C. 2005. Hydrological and biogeochemical dynamics of the minor and trace elements in the St. Lawrence River. Appl. Geochem. **20**: 1391–1408.

Roy, R., and Campbell, P.G.C. 1995. Survival time modeling of exposure of juvenile Atlantic salmon (*Salmo salar*) to mixtures of aluminum and zinc in soft water at low pH. Aquat. Toxicol. **33**: 155–176.

Samant, H.S., Doe, K.G., and Vaidya, O.C. 1990. An integrated chemical and biological study of the bioavailability of metals in sediments from two contaminated harbours in New Brunswick, Canada. Sci. Total Environ. **96**: 253–268.

Sanei, H., Goodarzi, F., and Hilts, S. 2007. Site-specific natural background concentrations of metals in topsoil from the Trail region, British Columbia, Canada. Geochem.: Explor., Environ., Anal. **7**(1): 41–48.

Santore, R.C., Mathew, R., Paquin, P.R., and Di Toro, D. 2002. Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow and *Daphnia magna*. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. **133**: 271–85.

Sayer, M.D.J., Reader, J.P., and Morris, R. 1989. The effect of calcium concentration on the toxicity of copper, lead and zinc to yolk-sac fry of brown trout, *Salmo trutta* L., in soft, acid water. J. Fish Biol. **35**: 323–332.

Schmidt, T.S., Clements, W.H., Zuellig, R.E., Mitchell, K.A., Church, S.E., Wanty, R.B., San Juan, C.A., Adams, M., and Lamothe, P.J., 2011. Critical tissue residue approach linking accumulated metals in aquatic insects to population and community-level effects. Environ. Sci. Technol. **45**(16): 7004–7010.

Schubauer-Berigan, M.K., Dierkes, J.R., Monson, P.D., and Ankley, G.T. 1993. pH-dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia, Pimephales promelas, Hyalella azteca* and *Lumbriculus variegatus*. Environ. Toxicol. Chem. **12**: 1261–1266.

Sehgal, R., and Saxena, A.B. 1986. Toxicity of zinc to a viviparous fish, *Lebistes reticulatus* (Peters). Bull. Environ. Contam. Toxicol. **36**(1): 888–894.

Sela, M., Garty, J., and Telor, E. 1989. The accumulation and the effect of heavy metals on the water fern *Azolla filiculoides*. New Phytol. **112**(1): 7–12.

Sellers, C.M., Jr., Heath, A.G., and Bass, M.L. 1975. The effect of sublethal concentrations of copper and zinc on ventilatory activity, blood oxygen and pH in rainbow trout (*Salmo gairdneri*). Water Research **9**(4): 401–408.

Sharma, M.S., and Selvaraj, C.S. 1994. Zinc, lead and cadmium toxicity to selected freshwater zooplankters. Pollut. Res. **13**(2): 191–201.

Shaw, J.R., Dempsey, T.D., Chen, C.Y., Hamilton, J.W., and Folt, C.L. 2006. Comparative toxicity of cadmium, zinc and mixtures of cadmium and zinc to daphnids. Environ. Toxicol. Chem. **25**(1): 182–189.

Shazili, N.A.M., and Pascoe, D. 1986. Variable sensitivity of rainbow trout (*Salmo gairdneri*) eggs and alevins to heavy metals. Bull. Environ. Contam. Toxicol. **36**(3): 468–474.

Shukla, G.S., Murti, R., and Omkar. 1983. Cadmium and zinc toxicity to an aquatic insect, *Ranatra elongata* (Fabr). Toxicol. Lett. **15**:39–41.

Sigg, L., Behra, P., and Stumm, W. 2000. Chimie des milieux aquatiques: chimie des eaux naturelles et des interfaces dans l'environnement. Dunod, Paris.

Sinley, J.R., Goettl, J.P., and Davies, P.H. 1974. The effect of zinc on rainbow trout (*Salmo gairdneri*) in hard and soft water. Bull. Environ. Contam. Toxicol. **12**(2): 193–201.

Skidmore, J.F. 1970. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J. Exp. Biol. **52**: 481–494.

Skidmore, J.F., and Tovell, W.A. 1972. Toxic effects of zinc sulphate on the gills of rainbow trout. Water Research **6**: 217–230.

Smith, I.M., Hall, K.J., Lavkulich, L.M., and Schreir, H. 2007. Trace metal concentrations in an intensive agricultural watershed in British Columbia, Canada. J. Am. Water Resour. Assoc. **43**(6): 1455–1467.

Solbé, J.F. De L.G. 1974. Toxicity of zinc-sulfate to rainbow-trout in very hard water. Water Research **8**: 389–391.

Solbé, J.F. De L.G., and Flook, V.A. 1975. Studies on the toxicity of zinc sulphate and of cadmium sulphate to stone loach *Noemacheilus barbatulus* (L.) in hard water. J. Fish Biol. **7**: 631–637.

Sorvari, J., and Sillanpaa, M. 1996. Influence of metal complex formation on heavy metal and free EDTA and STPA acute toxicity determined by *Daphnia magna*. Chemosphere **33**(6): 1119–1127.

Spear, P.A. 1981. Zinc in the aquatic environment: chemistry, distribution and toxicology. NRCC 17589. National Research Council of Canada, Ottawa, ON.

Spehar, R.L. 1976. Cadmium and zinc toxicity to flagfish *Jordanella floridae*. J. Fish. Res. Board Can. **33**: 1939–1945.

Sprague, J.B. 1964. Lethal concentrations of copper and zinc for young Atlantic salmon. J. Fish. Res. Board Can. **21**: 17–26.

Sprague, J. B., and Ramsay, B. A. 1965. Lethal levels mixed copper-zinc solutions for juvenile salmon. J. Fish. Res. Board Can. **22**(2): 425–432.

Spry, D. J., and Wood, C. M. 1984. Acid-base, plasma ion and blood gas changes in rainbow trout during short-term toxic zinc exposure. J. Comp. Physiol., B **154**: 149–158.

Spry, D.J., and Wood, C.M. 1985. Ion flux, acid-base status, and blood gases in rainbow trout, Salmo gairdneri, exposed to toxic zinc in natural soft water. Can. J. Fish. Aquat. Sci. **42**(8): 1332–1341.

Spry, D.J., Hodson, P.V., and Wood, C.M. 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri.* Can. J. Fish. Aquat. Sci. **45**: 32–41.

Srivastava, M., Rawat, M.M.S., and Srivastava, A.K. 1991. Effect of different concentrations of zinc on the survival and growth behaviour of *Chlorella vulgaris.* Pollut. Res. **10**(1): 49–51.

Starodub, M.E., Wong, P.T.S., and Mayfield, C.I. 1987a. Short-term and long-term studies on individual and combined toxicities of copper, zinc and lead to *Scenedesmus quadricauda*. Sci. Total Environ. **63**: 101–110.

Starodub, M.E., Wong, P.T.S., Mayfield, C.I., and Chau, Y.K. 1987b. Influence of complexation and pH on individual and combined heavy metal toxicity to a freshwater green alga. Can. J. Fish. Aquat. Sci. **44**: 1173–1180.

Stone, M., and Droppo, I. G. 1996. Distribution of lead, copper and zinc in size-fractionated river bed sediment in two agricultural catchments of southern Ontario, Canada. Environ. Pollut. **93**(3): 353–362.

Stumm, W., and Morgan, J. J. 1981. Aquatic chemistry – An introduction emphasizing chemical equilibria in natural waters. John Wiley & Sons, New York, NY.

Swain, L.G. 1990. Okanagan area, Similkameen River sub-basin, water quality assessment and objectives. Technical appendix, first update. Water Quality Branch, Environmental Protection Department, Ministry of Environment, Lands, and Parks, Victoria, BC.

Taylor, M.C. and Demayo, A. 1980. Zinc. *In* Guidelines for surface water quality. Vol. 1: Inorganic chemical substances. Water Quality Branch, Inland Water Directorate, Environment Canada, Ottawa, ON.

Thompson, K.W., Hendricks, A.C., and Cairns, J. 1980. Acute toxicity of zinc and copper singly and in combination to the bluegill (*Lepomis macrochirus*). Bull. Environ. Contam. Toxicol. **25**: 122–129.

Timmermans, K.R., Peeters, W., and Tonkes, M. 1992. Cadmium, zinc, lead and copper in *Chironomus riparius* (Meigen) larvae (Diptera, Chironomidae): uptake and effects. Hydrobiologia **241**(2): 119–134.

Todd, A.S., Brinkman, S., Wolf, R.E., Lamothe, P.J., Smith, K.S., and Ranville, J.F. 2009. An enriched stable-isotope approach to determine the gill-zinc binding properties of juvenile rainbow trout (*Oncorhynchus mykiss*) during acute zinc exposures in hard and soft waters. Environ. Toxicol. Chem. **28**(6): 1233–1243.

Turner, D.R., Whitfield, M., and Dickson, K. L. 1981. The equilibrium speciaction of dissolved component in freshwater and seawater at 25ºC and 1 atm pressure. Geochimica et Cosmochimica Acta 45: 855-881.

Umebese, C.E., and Motajo, A.F. 2008. Accumulation, tolerance and impact of aluminium, copper, and zinc on growth and nitrate reductase activity of *Ceratophyllum demersum* (Hornwort). J. Environ. Biol. **29**(2): 197–200.

Upadhyay, R., and Panda, S.K. 2010. Zinc reduces copper toxicity induced oxidative stress by promoting antioxidant defense in freshly grown aquatic duckweed *Spirodela polyrhiza* L. J. Hazard. Mater. **175**(1-3): 1081–1084.

US EPA (US Environmental Protections Agency). 1987. Ambient water quality criteria for zinc – 1987. EPA-440/5-87-003. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

US EPA. 1996. The metals translator: Guidance for calculating a total recoverable permit limit from a dissolved criterion. EPA 823-B-96-007.. Office of Water, U.S Environmental Protection Agency, Washington, DC.

US EPA. 1999. National recommended water quality criteria –Correction. EPA 822-Z-99-001. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

US EPA. 2001. 2001 update of ambient water quality criteria for cadmium. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

US EPA. 2006. National recommended water quality criteria. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

van der Werff, M., and Pruyt, M.J. 1982. Long-term effects of heavy metals on aquatic plants. Chemosphere **11**(8): 727–739.

Van Sprang, P.A., Verdonck, F.A.M., Van Assche, F., Regoli, L., and De Schamphelaere, K.A.C. 2009. Environmental risk assessment of zinc in European freshwaters: A critical approach. Sci. Total Environ. **407**: 5373-5391.

Vardy, D.W., Tompsett, A.R., Sigurdson, J.L., Doering, J.A., Zhang, X., Giesy, J.P., and Hecker, M. 2011. Effects of subchronic exposure of early life stages of white sturgeon (*Acipenser transmontanus*) to copper, cadmium, and zinc. Environ. Toxicol. Chem. **30**(11): 2497–2505.

Vedamanikam, V.J., and Shazilli, N.A.M. 2008a. Comparative toxicity of nine metals to two Malaysian aquatic dipterian larvae with reference to temperature variation. Bull. Environ. Contam. Toxicol. **80**: 516–520.

Vedamanikam, V.J., and Shazilli, N.A.M. 2008b. The effect of multi-generational exposure to metals and resultant change in median lethal toxicity tests values over subsequent generations. Bull. Environ. Contam. Toxicol. **80**:63–67.

Wagemann, R., Innes, S., and Richard, P.R. 1996. Overview and regional and temporal differences of heavy metals in Arctic whales and ringed seals in the Canadian Arctic. Sci. Total Environ. **186**(1–2): 41–66.

Wang, W. 1986. Toxicity tests of aquatic pollutants by using common duckweed. Environ. Pollut., Ser. B **11**: 1–14.

Wang, N., Ingersoll, C.G., Ivey, C.D., Hardesty, D.K., May, T.W., Augspurger, T., Roberts, A.D., Van Genderen, E., and Barnhart, M.C. 2010. Sensitivity of early life stages of freshwater mussels (Unionidae) to acute and chronic toxicity of lead, cadmium, and zinc in water. Environ. Toxicol. Chem. **29**(9): 2053–2063.

Wani, G.P. 1986. Toxicity of heavy metals to embryonic stages of *Cyprinus carpio communis*. Pollut. Res **5**(2): 47–51.

Ward, R.E. 1990. Metal concentrations and digestive gland lysosomal stability in mussels from Halifax inlet, Canada. Mar. Pollut. Bull. **21**(5): 237–240.

Watson, T.A., and McKeown, B.A. 1976. The activity of Δ5-3β hydroxysteroid dehydrogenase enzyme in the interrenal tissue of rainbow trout (*Salmo gairdneri* Richardson) exposed to sublethal concentrations of zinc. Bull. Environ. Contam. Toxicol. **16**(2): 173–181.

Wayland, M., and Crosley, R. 2006. Selenium and other trace elements in aquatic insects in coal mine-affected streams in the Rockey Mountains of Alberta, Canada. Arch. Environ. Contam. Toxicol. **50**(4): 511–522.

Wetzel, R.G. 2001. Limnology: Lake and river ecosystems. Academic Press, San Diego, CA.

WHO (World Health Organization). 2001. Zinc (Environmental health criteria; 221). World Health Organisation, Geneva.

Widianarko, B., Kuntoro, F.X.S., Van Gestel, C.A.M., and Van Straalen, N.M. 2001. Toxicokinetics and toxicity of zinc under time-varying exposure in the guppy (*Poecilia reticulata*). Environ. Toxicol. Chem. **20**(4): 763–768.

Wilde, K.L., Stauber, J.L., Markich, S.J., Franklin, N.M., and Brown, P.L. 2006. The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga (*Chlorella* sp.). Arch. Environ. Contam. Toxicol. **51**: 174–185.

Willis, M. 1988. Experimental studies of the effects of zinc on *Ancylus fluviatilis* (Muller) (Mollusca; Gastropoda) from the Afon Crafnant, N. Wales. Arch. Hydrobiol. **112** (2): 299–316.

Willis, M. 1989. Experimental studies on the effects of zinc on *Erpobdella Octulata* (L.) (Annelida: Hirudinea) from the Afon Crafnant, N. Wales. Archiv fur Hydrobiologie **116**(4): 449–469.

Winner, R.W. 1981. A comparison of body length, brood size, and longevity as indices of chronic copper and zinc stresses in *Daphnia magna*. Environ. Pollut., Ser. A **26**: 33–37.

Winner, R.W., and Gauss, J.D. 1986. Relationship between chronic toxicity and bioaccumulation of copper, cadmium and zinc as affected by water hardness and humic acid. Aquat. Toxicol. **8**: 149–161.

Wong, C.K. 1992. Effects of chromium, copper, nickel, and zinc on survival and feeding of the cladoceran Moina macrocopa. Bull. Environ. Contam. Toxicol. **49**(4): 593–599.

Wong, C.K. 1993. Effects of chromium, copper, nickel, and zinc on longevity and reproduction of the cladoceran *Moina macrocopa*. Bull. Environ. Contam. Toxicol. **50**: 633–639.

Woodling, J., Brinkman, S., and Albeke, S. 2002. Acute and chronic toxicity of zinc to the mottled sculpin *Cottus bairdi*. Environ. Toxicol. Chem. **21**(9): 1922–1926.

Wurtz, C.B., and Bridges, C.H. 1961. Preliminary results from macroinvertabrate bioassays. Proceedings of the Pennsylvania Academy of Sciences **35**: 51–56.

Xiong, D., Fang, T., Yu, L., Sima, Z., and Zhu, W. 2011. Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. Sci. Total Environ. **409**: 1444–1452.

Xue, H.B., and Sigg, L. 1994. Zinc speciation in lakes waters ant its determination by ligand exchange with EDTA and differential pulse anodic stripping voltammetry. Anal. Chim. Acta **284**: 505–515.

Yeats, P.A., and Bewers, J.M. 1982. Discharge of metals from the St. Lawrence River. Can. J. Earth Sci. **19**(5): 982–992.

Zitko, P., Carson, W. V., and Carson, W. G. 1973. Prediction of incipient lethal levels of copper to juvenile Atlantic salmon in the presence of humic acid by cupric electrode. Bull. Environ. Contam. Toxicol. **10**(5): 265–271.

Zou, E., and Bu, S. 1994. Acute toxicity of copper, cadmium and zinc to the water flea *Moina irrasa*. Bull. Environ. Contam. Toxicol. **52**: 742–748.