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Scientific Criteria Document for the Development of the Canadian Water Quality Guidelines for the Protection of Aquatic Life

SILVER

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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 provides the background information and rationale for the development of the Canadian Water Quality Guidelines for silver. They were developed by the National Guidelines and Standards Office of Environment Canada.

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

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LIST OF ACRONYMS

AES	Atomic emission spectrometry
Ag	Silver; unless otherwise specified, Ag represents the total amount of silver present.
ANZECC	Australian and New Zealand Environment and Conservation Council
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
AUM	Autometallography
AVS	Acid volatile sulphides
aw	ash weight
AWWA	American Water Works Association
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BLM	Biotic ligand model
CCME	Canadian Council of Ministers of the Environment
CCREM	Canadian Council of Resource and Environment Ministers
CWQG	Canadian Water Quality Guideline
DOC	Dissolved organic carbon
DL	Detection limit
dw	Dry weight
EC_{50}	The concentration that causes 50% of the experimental biota to show an observed adverse effect. The adverse effect may be immobilization, changes in reproductive potential, growth, or some other ecologically relevant endpoint.
ELS	Early life stage
EQS	Environmental Quality Standards
FIAM	Free Ion Activity Model
fw	Fresh weight
GFAAS	Graphite furnace atomic absorption spectroscopy
GSH	Tripeptide glutathione
HPLC	High performance liquid chromatography
ICP	Inductively-coupled plasma
LC_{50}	The concentration which is lethal to 50% of the experimental biota.
LOEC	Lowest-observed-effect concentration
MATC	Maximum acceptable toxicant concentration; calculated as the geometric mean of the LOEC and the NOEC
MDEC	Minimum detectable effect concentration. The concentration at which the response becomes significantly ($p \leq 0.05$) lower than the control.
MS	Mass spectrometry
MT	Metallothionein
NKA	Sodium Potassium activated adenosinetriphosphatase (or Na+/K+ ATPase)
NGSO	National Guidelines and Standards Office
NOEC	No-observed-effect concentration
NOM	Natural organic matter

NS	Nanosilver, or silver nanoparticles
pH	The negative log of the hydrogen ion activity, -log[H^+]
PHREEQCI	PH (pH), RE (redox), EQ (equilibrium), C (program written in C) and I refers to the interactive version of PHREEQC; a program used to calculate metal speciation in water
SEM	Simultaneously extracted metals
SSD	Species Sensitivity Distribution
UF	Ultrafiltration
US EPA	United States Environmental Protection Agency
WERF	Water Environment Research Foundation
WHAM	Windermere Humic Aqueous Model
WHO	World Health Organization
ww	Wet weight

EXECUTIVE SUMMARY

Data from an up-to-date literature review on silver chemistry, bioaccumulation and toxicity and the recently revised CCME protocol for the derivation of water quality guidelines for protection of aquatic life were used to derive revised water quality guidelines for silver.

Silver occurs in the aquatic environment at concentrations generally in the range of 0.001-0.01 µg/L and is generally bound to particles and colloids or complexed by dissolved ligands. While silver is a naturally occurring element, there are numerous anthropogenic sources to the aquatic environment that include mining and metallurgical operations, the photographic industry, municipal wastewaters and, more recently, its use in nanotechnology. An extremely small fraction of the silver present in natural waters will actually occur as free silver ions. In fresh waters, inorganic and organic sulphide species dominate the chemical speciation of silver, while chloride is a major determinant of the silver chemistry and fate in the marine environment.

Bioaccumulation of silver is dependent on exposure concentrations and chemical speciation and is not associated with physiological effects. There is no evidence of silver biomagnification and bioconcentration and bioaccumulation factors actually decrease with increasing exposure concentrations. Silver uptake is mostly related to free silver ion activity but some neutral complexes such as AgCl have also been associated with silver uptake. Data on silver uptake are available for a number of freshwater and marine species including algae, plant, invertebrates and fish. Dietary exposure to silver has been studied much less than waterborne exposure, however available studies indicate dietary silver toxicity is not a significant concern.

In freshwater fish, silver (as Ag^+) disrupts the functions of two key gill enzymes that are needed for ion regulation. This results in ionoregulatory failure and eventual death. Disruption of ionoregulation has also been observed for freshwater invertebrates. In fresh water, complexation of Ag^+ with ligands and competitive interactions with other cations for gill uptake sites reduce the toxicity of silver to freshwater species. In marine waters, silver is far less acutely toxic, given that silver is largely present in the form of chloride complexes. Although the mechanisms for toxicity in marine species are not entirely clear, the gut and gill are likely targets for silver uptake and toxicity due to the differences in ion regulation.

For long-term exposure in fresh waters, sufficient data were available to meet CCME requirements to derive a 'Type A' guideline based on a species sensitivity distribution (SSD) (Table i). The long-term Canadian Water Quality Guideline (CWQG) for fresh water, derived as the 5^{th} percentile of the SSD, is 0.25 µg/L. For short-term exposure in fresh waters, the 5^{th} percentile of the short-term SSD was 0.22 µg/L. Because the short-term 5^{th} percentile and the long-term CWQG are essentially equal, no designated short-term freshwater benchmark is recommended. For marine exposure, sufficient data were available to derive a short-term SSD and the short-term marine benchmark (Type A) is 7.5 µg/L. There were insufficient data to derive a long-term marine guideline for silver. All values are expressed as total silver.

Revised Canadian water quality guideline for silver[1,2] developed using the 2007 derivation protocol.

Aquatic Environment	Exposure Duration	Type[3]	Concentration (µg Ag/L)	1987 Guideline[8] (µg/L)
Freshwater	Short-term[4]	NRG[6]	NRG	NRG
Freshwater	Long-term[5]	A	0.25	0.1
Marine	Short-term[4]	A	7.5	NRG
Marine	Long-term[5]	NRG[7]	NRG	NRG

NRG = no recommended guideline

[1] This guideline is not applicable to silver nanoparticles.

[2] CWQGs were derived based on the total concentration of Ag.

[3]Type A guidelines were obtained based on species sensitivity distributions (SSD).

[4] Derived with severe-effects data (such as lethality) and are not intended to protect all components of aquatic ecosystem structure and function but rather to protect most species against lethality during severe but transient events (e.g., inappropriate application or disposal of the substance of concern).

[5] Derived with mostly no- and some low-effect data and are intended to protect against negative effects to aquatic ecosystem structure and function during indefinite exposures (e.g., abide by the guiding principle as per CCME 2007).

[6]Because the short-term SSD 5th percentile and the long-term SSD 5th percentile (CWQG) are essentially equal, no designated short-term freshwater benchmark is recommended (see text for details).

[7]There were insufficient data to derive any of A, B1 or B2 guidelines for long-term exposure in marine environments.

[8]CCREM, 1987

RÉSUMÉ

On a utilisé les données provenant d'un récent dépouillement des publications sur la chimie, la bioaccumulation et la toxicité de l'argent ainsi que le Protocole d'élaboration des recommandations pour la qualité des eaux en vue de protéger la vie aquatique révisé dernièrement par le CCME afin d'élaborer des recommandations révisées pour la qualité de l'eau à l'égard de l'argent.

Dans les milieux aquatiques, l'argent est généralement présent en concentrations allant de 0,001 à 0,01 µg/L, et il est le plus souvent lié à des particules et à des colloïdes, ou complexé par des ligands en solution. L'argent est un élément naturel, mais de nombreuses sources anthropiques, comme les activités minières et métallurgiques, l'industrie de la photographie, les eaux usées municipales ainsi que, plus récemment, l'utilisation de l'argent en nanotechnologie, sont aussi responsables de la présence de l'argent dans les milieux aquatiques. Une fraction extrêmement faible de l'argent présent dans les eaux naturelles est sous forme d'ions argent libres. En eau douce, l'argent est surtout présent sous la forme de composés inorganiques et organiques avec le sulfure tandis que les chlorures déterminent en grande partie la chimie et le devenir de l'argent en milieu marin.

La bioaccumulation de l'argent dépend des concentrations d'exposition ainsi que des formes chimiques en présence; elle n'est pas associée à des effets physiologiques. Rien n'indique que l'argent se bioamplifie et, en fait, les facteurs de bioconcentration et de bioaccumulation diminuent à mesure que les concentrations d'exposition augmentent. L'absorption de l'argent est principalement liée à l'activité des ions argent libres, mais certains complexes neutres comme l'AgCl ont également été associés à l'absorption de l'argent. On dispose de données sur l'absorption de l'argent chez un certain nombre d'espèces d'eau douce et d'espèces marines, dont des algues, des plantes, des invertébrés et des poissons. L'exposition à l'argent par voie alimentaire est beaucoup moins étudiée que l'exposition par l'eau; cependant, les études dont on dispose indiquent que la toxicité de l'argent par voie alimentaire n'est pas préoccupante.

Chez les poissons d'eau douce, l'argent (sous forme d'Ag+) perturbe le fonctionnement de deux enzymes clés qui, dans les branchies, sont nécessaires à l'ionorégulation. Cela provoque un dérèglement de l'ionorégulation et, finalement, la mort. On a aussi observé une perturbation de l'ionorégulation chez les invertébrés d'eau douce. En eau douce, la complexation de l'Ag+ avec des ligands ainsi que la concurrence avec d'autres cations aux sites d'absorption sur les branchies réduisent la toxicité de l'argent pour les espèces d'eau douce. La toxicité aiguë de l'argent est beaucoup plus faible en eau salée parce que l'argent y est en majeure partie présent sous forme de complexes avec le chlorure. Bien que les mécanismes de toxicité chez les espèces marines ne soient pas entièrement élucidés, les intestins et les branchies sont selon toute vraisemblance des organes en jeu dans l'absorption et la toxicité de l'argent, vu les différences caractérisant l'ionorégulation dans ce milieu.

Pour l'exposition de longue durée en eau douce, suffisamment de données étaient disponibles pour respecter les exigences du CCME et établir une recommandation de type A en fonction d'une distribution de la sensibilité des espèces (DSE) [tableau i]. La recommandation canadienne pour la qualité des eaux (RCQE) pour l'exposition de longue durée en eau douce, correspondant au

5e centile de la DSE, est de 0,25 µg/l. Pour ce qui est de l'exposition de courte durée en eau douce, le 5e centile de la DSE était de 0,22 µg/l. Puisque le 5e centile de la DSE pour l'exposition de courte durée et la RCQE pour l'exposition de longue durée sont essentiellement égaux, aucune limite n'est recommandée pour l'exposition de courte durée en eau douce. En ce qui a trait à l'exposition en milieu marin, suffisamment de données étaient disponibles pour établir une DSE pour l'exposition de courte durée, et la limite pour l'exposition de courte durée en milieu marin (type A) est de 7,5 µg/l. Les données étaient insuffisantes pour établir une recommandation pour l'exposition de longue durée à l'argent en milieu marin. Toutes les valeurs sont exprimées en concentration totale d'argent.

Recommandations canadiennes révisées pour la qualité des eaux visant l'argent[1,2] selon le protocole d'élaboration de 2007.

Milieu aquatique	Durée de l'exposition	Type[3]	Concentration (µg Ag/L)	Recommandation de 1987[8] (µg/L)
Eau douce	Courte durée[4]	AR[6]	AR	AR
Eau douce	Longue durée[5]	A	0,25	0,1
Eau salée	Courte durée[4]	A	7,5	AR
Eau salée	Longue durée[5]	AR[7]	AR	AR

AR = aucune recommandation.

[1] Cette recommandation ne s'applique pas aux nanoparticules d'argent.

[2] Les RCQE ont été élaborées en fonction de la concentration totale d'Ag.

[3] Les recommandations de type A ont été élaborées d'après la distribution de la sensibilité des espèces (DSE).

[4] Valeurs déterminées d'après les données relatives aux effets graves (comme la létalité) et ne visant pas la protection de toutes les composantes de la structure et de la fonction des écosystèmes aquatiques, mais plutôt la protection de la plupart des espèces contre les effets létaux dans des circonstances graves, mais transitoires (par exemple, application ou élimination inappropriée d'une substance préoccupante).

[5] Valeurs déterminées d'après les données relatives aux concentrations principalement sans effet ou associées à certains effets faibles et visant la protection de la structure et de la fonction des écosystèmes aquatiques pendant des périodes d'exposition illimitées (selon le principe directeur défini dans le protocole de 2007 du CCME).

[6] Puisque le 5e centile de la DSE pour l'exposition de courte durée et le 5e centile de la DSE pour l'exposition de longue durée (RCQE) sont essentiellement égaux, aucune limite n'est recommandée pour l'exposition de courte durée en eau douce (voir le texte).

[7] Les données n'étant pas suffisantes, il a été impossible d'élaborer une recommandation de type A, B1 ou B2 visant l'exposition de longue durée en milieu marin.

[8] CCMRE, 1987.

1.0. INTRODUCTION

Canadian Water Quality Guidelines (CWQGs) collect and integrate aquatic toxicity data on contaminants to provide risk assessors with tools to evaluate water quality and ecosystem health. The protocol (methodology) to develop water quality guidelines was revised in 2007 (CCME, 2007). The revised protocol accounts as much as possible for unique properties of contaminants, environmental factors that influence toxicity and uses species sensitivity distributions to derive guideline values. The new components of the revised protocol include bioaccumulation, bioavailability, toxicity modifying factors and curve fitting techniques for the species sensitivity distribution.

The current report is based on the 2007 *Protocol for the derivation of water quality guidelines for the protection of aquatic life* (CCME, 2007), and provides a revision of the 1987 Canadian Council of Resource and Environment Ministers (CCREM) water quality guideline for silver, which is 0.1 µg/L for freshwater aquatic life. No guideline for silver for marine waters was previously derived (CCREM, 1987). A lot of progress has been made over the last 23 years on the prediction of metal toxicity to aquatic organisms, including silver. To derive the revised water quality guideline for silver, results of an extensive literature search encompassing 30 years of research (1980 – 2013) on silver chemistry and toxicity were combined within the 2007 protocol framework.

1.1. Physical and chemical properties

Silver (symbol Ag, atomic number 47, atomic radius 144 pM) is a transition element with two naturally occurring stable isotopes, ^{107}Ag and ^{109}Ag. There are numerous radioisotopes, none of which occur naturally and most have a short half-life. Properties of silver and some common silver compounds are presented in Table 1.0.

Table 1.0. Physical properties of silver and some common silver compounds (developed from WHO, 2002; FactSage, 2009).

	CAS no.	Formula	Molecular weight (g/mol)	Physical state	Melting point (ºC)	Specific density (g/cm^3)	Water Solubility (@ 20°C)
Silver	7440-22-4	Ag	107.87	Solid metal	961.9	10.49	Insoluble
Silver nitrate	7761-88-8	$AgNO_3$	169.89	Solid crystalline	212	4.352	2160 g/L
Silver sulphide	21548-73-2	Ag_2S	247.80	Grey-black solid	825	7.33	Insoluble
Silver chloride	7783-90-6	AgCl	143.34	White solid	455	5.56	1.9 mg/L
Silver(I) oxide	20667-12-3	Ag_2O	231.74	Solid crystalline	n/a	7.143	22 mg/L (@ 25°C)
Silver(II) oxide	1301-96-8; 35366-11-1	AgO	123.88	Solid crystalline	n/a	7.44	Reacts in water

1.2. Production and uses of silver

Silver is associated with copper, nickel, gold, lead and zinc. In Canada, operations actively producing silver can be found in British Columbia, Saskatchewan, Manitoba, Ontario, Québec and New Brunswick. The top four countries producing silver in 2012 were Mexico, China, Peru and Australia which collectively account for over half of worldwide production. Canada ranked eleventh, with about 3% of total world production (data from The Silver Institute, www.silverinstitute.org).

Silver is a soft, white, lustrous element used in coins, jewellery, tableware, mirrors and photography. Demand for silver is generally restricted to three main uses; industrial and decorative uses, photography, and jewellery and silverware, together accounting for 95% of annual silver consumption. Data from 2012 indicate the majority of silver was used in the industrial sector (466 Moz or 55%), followed by the jewellery market (186 Moz or 22%), the photographic sector (58 Moz or 7%), and finally the silverware market (45 Moz or 5%). Because silver is the best conductor of all metals, its use in electrical applications is widespread. It does not corrode and has low resistance, which makes silver the most safe and reliable material for electrical switches. Other unique characteristics of silver, such as its ductility, strength, its sensitivity to light and high reflectance of light, ability to withstand extreme temperature ranges, and malleability make silver indispensable in a variety of other applications and restrict substitutions (The Silver Institute, www.silverinstitute.org).

1.3. Sources of silver in the environment

Silver is a naturally occurring element that is ubiquitous in all environmental compartments. It is found in the environment in two oxidation states, 0 and 1+. Oxidation states of 2+ and 3+ also can exist but rarely occur in natural environments. The review of Purcell and Peters (1998) provides a summary of sources of silver in the environment; some highlights from this review are outlined below. Naturally occurring concentrations of silver in the environment tend to be low except in and near mineral deposits. Purcell and Peters (1998) estimated that approximately 62% of the total Ag in water comes from natural sources with the remainder coming from anthropogenic inputs. Anthropogenic sources of silver to the environment are diverse and occur along the extraction, manufacture, use, and disposal chain.

Mining operations and metals production account for significant releases, particularly to air and soil. The photographic industry has been associated with inputs to the aquatic environment through the use of silver halides in film processing, previously being the major use and disposition. However, with the advent of digital photography in the last decade, this contribution has undoubtedly decreased, dropping to approximately 13% of the market (Wood, 2012). Other anthropogenic sources of Ag to the terrestrial environment are linked to waste water treatment and biosolids disposal (Purcell and Peters, 1998). Atmospheric releases occur primarily from combustion; coal, petroleum, waste incineration, electrical production and cement kilning, which account for 47% of all releases into this compartment. Overall, air emissions only amount to less than 4% of all anthropogenic silver released to the environment. In terms of point sources of

silver to the aquatic environment, effluents from municipal wastewater treatment plants as well as mine and smelter operations represent significant potential sources.

1.4. Silver nanoparticles

The unique bactericidal properties of silver have long been recognised for use in hygienic and medicinal applications (Chen and Schluesener, 2008). Silver nanoparticles or 'nanosilver' (NS) also have unique physio-chemical properties, such as high electrical and thermal conductivity, chemical stability, and catalytic activity, which make them particularly useful in inks, microelectronics, and medical imaging (Fabrega *et al*., 2011). Silver nanoparticles are clusters of silver atoms which are engineered to be between 1 and 100 nm for enhanced interaction due to high specific surface area. The use of NS in consumer products has exploded in recent years, with 313 different products containing NS presently on the market (March, 2011) compared to less than 50 in 2006 according to the Woodrow Wilson Database (www.nanotechproject.org). Consumer goods such as appliances, children's goods, electronics, clothing, cosmetics, and home furnishings are among the many broadly categorized uses of NS. Worldwide NS production is estimated to be 500 tonnes per year (Mueller and Nowack, 2008). This increase in production and usage of NS has the potential to increase the amount of silver ions released into the environment either directly or through wastewater (Blaser *et al.*, 2008).

Since there is no monitoring and little information of NS transport in the environment, it is difficult to get a realistic estimate of the amount of NS that may end up in the receiving environment, whether during production, usage of a particular NS-containing product, or disposal (Fabrega *et al*., 2011). However, attempts at modelling the cumulative aquatic exposure and risk due to NS has been evaluated and, based on estimates of use in Europe by 2010, it has been predicted that 15% of total silver released into the environment will be from biocidal plastics and textiles (Blaser *et al.*, 2008). These modelling exercises are based on established methods to assess the exposure of chemicals to the environment, and therefore these estimates should be viewed with caution. However, due to the increased use and application of NS, there is concern over the potential toxicity to not only microbes but other non-target organisms in both the aquatic and terrestrial environments.

There are a number of studies that have been conducted on fish, invertebrate, algal and microbial organisms, to try to understand the potential environmental impact of NS, specifically related to water quality. Studies on fish include exposures with rainbow trout and zebrafish. Farkas *et al.* (2010) investigated the toxicity of NS on rainbow trout, specifically looking at cytotoxic effects in hepatocytes. They observed adverse effects at low mg/L concentrations. Choi *et al*. (2010) investigated effects of NS on adult zebrafish. They observed increased metallothionein in liver in addition to oxidative stress and apoptosis at concentrations up to 120 mg Ag/L. A 24h LC50 to adult zebrafish of 250mg Ag/L was also reported. This contrasts with a similar study conducted by Griffitt *et al.* (2008) that observed an LC50 of 7.07 mg Ag/L in adult zebrafish. When exposures with zebrafish embryos were conducted, 72-h and 120-h LC50s ranged from 25 to 50 mg Ag/L (Asharani *et al.*, 2008).

Similar to the fish studies reported, invertebrate responses show similar variability. Allen *et al.*, 2010 investigated the effects of a number of silver nanomaterials on *Daphnia magna* neonates.

They found variable toxicity that depended on type of nanomaterial and exposure method. For example, unfiltered $AgNO_3$ had an LC50 of 1.1 µg/L, compared to Sigma Aldrich Ag-nanoparticles that had an LC50 of 31.5 µg/L. Interestingly, the addition of food altered toxicity of the latter, increasing the LC50 to 176.4 µg/L. These results highlight the type of nanomaterial and the potential of food as modifying factors affecting NS toxicity. A similar study, with *Daphnia magna*, assessing biokinetic uptake of NS found that in high concentrations uptake of Ag increased disproportionately, and was potentially associated with ingestion (Zhao *et al*., 2010). The authors point out the importance of food transfer of NS when assessing routes of exposure, which is in contrast to the findings by Allen *et al.* (2010).

Griffitt *et al.* (2008) conducted exposures of metallic nanomaterials to a number of aquatic species, including *Daphnia pulex*. They report a 48-h LC50 of 40 µg Ag/L in adults. The authors discuss the importance of species sensitivity, stating that filter-feeding invertebrates were more susceptible to nano-metal exposures compared with larger organisms such as the zebrafish. In a study with *Chironomus riparius*, Nair *et al*. (2011) observed no larval mortality in exposures up to 2 mg/l of NS. In chronic exposures approximately 50% decreases in pupation and adult emergence were observed in 1 mg/L NS treatments. One study conducted on algae, reported an EC50 (growth) of 0.35 mg Ag/L in *C. reinhardtii* (Navarro *et al*., 2008).

The variability observed between studies highlights the importance of the development of guidance in testing NS. The bioavailability of NS is likely dependant on several factors including the size, shape, chemical composition, charge, and solubility of the nanoparticle. In addition, some of these factors may be more or less relevant depending on the exposure media used (Marambio-Jones and Hoek, 2010). Nanosilver that is produced for commercial use may have 'capping agents', which are often organic compounds (i.e., citrate, cysteine, or starch) that coat the surface of the nanoparticle to promote dispersion, and could affect biological membrane permeability (Fabrega *et al*., 2011).

This document includes information on nanosilver to provide a more complete picture of silver use and toxicity. However, despite the increase in the production and use of NS, and subsequent potential for environmental impacts, this guideline is not applicable to silver nanoparticles, primarily due to the uncertainties associated with measuring the hazard of NS. This guideline is derived based on experimental data with ionic silver, and applies to total silver in the environment which will include, in part, the NS component. While it is not currently possible to separate the toxicity of total silver into its ionic and NS forms, literature indicates that the toxicity of NS is at least in part driven by the dissolution of the particle into silver ions (Angel *et al.*, 2013; Wang *et al.*, 2012; Kennedy *et al.*, 2010; Laban *et al*., 2010). Differences in toxicity due to the special properties of NS are still under investigation and cannot be extrapolated to field conditions. Since the science on NS is still emerging and standardized test methodologies are lacking, it is not possible to include these findings in the derivation of this guideline. However, progress towards establishing standardised testing protocols and further research regarding environmental fate and mechanism of toxicity of NS may allow incorporation of it into future guideline calculations.

1.5. Analytical chemistry and characterization of silver

Common methods for measuring total silver levels in solution include graphite furnace atomic absorption spectroscopy (GFAAS), inductively-coupled plasma mass spectrometry (ICP-MS) and occasionally inductively-coupled plasma atomic emission spectrometry (ICP-AES). When silver concentrations are below 10 to 20 pM, the above methods require preconcentration using techniques such as ammonium pyrrolidinedithiocarbamate or diethylammonium diethyldithiocarbamate extraction (Kramer *et al.*, 2002). Since ICP-MS is also able to discriminate isotopes, tracer studies using ^{109}Ag and ^{107}Ag are possible. Advances in detection techniques are being pursued, including MS instruments (e.g., laser ablation, multicollector magnetic sector), MS coupled to chromatography (e.g., High Performance Liquid Chromatography (HPLC-ICP-MS)), and spectrofluorometry (Kramer *et al*., 2002).

Clean techniques are essential for analysis of trace metals, especially for silver (e.g., Wen *et al.*, 2002). Important components of clean techniques include; (1) sample collection and storage in containers low in trace metals (e.g., low or high-density polyethylene or Teflon containers, acid-cleaned in a filtered-air environment), (2) avoidance of laboratory contamination of samples using ultra-pure reagents and working under filtered-air environments (e.g., Class 100 clean room), and (3) prevent "loss" of silver (or other metals) in samples via sample preservation by acidification with nitric acid immediately after sample collection. If sample acidification is not done within 24 h or less, silver losses may be irreversible, even with subsequent acidification. In cases where acidification is not feasible, as for example in speciation studies, storage in glass containers is recommended (Kramer *et al.*, 2002).

Understanding chemical speciation of silver in water is essential in delineating its environmental fate. However, most analytical methods for silver determine the total mass in a sample. Sample pre-treatment prior to analysis can be used to characterize different fractions. Examples include filtration and ultrafiltration to characterize the dissolved (less than 10 kDa) and colloidal (greater than 10 kDa and less than 0.45 µm) fractions, respectively, and ion selective electrodes can be used to measure the free ion activity. Ward and Kramer (2002) and Ward *et al.* (2006a and 2006b) successfully used ion-selective electrodes to measure silver ion activity in marine chronic toxicity studies. The use of ion-selective electrodes for silver in fresh water is more challenging and subjected to interferences, notably with natural organic matter. A less commonly applied method is to measure the labile fraction of Ag using anodic stripping voltammetry (Labar and Lamberts, 1997). However, it is currently challenging to directly measure extremely low Ag^+ concentrations typically found in aquatic systems.

Clean ultrafiltration (UF) methods for the fractionation of silver indicate that the majority of the silver in aquatic systems is colloid-bound. A wide range of UF media (cellulose-based, polysulphone derivatives) and UF designs (spiral-wound, hollow-fiber, plates) are available. However, artifacts are a hindrance for UF methods including losses due to sorption and ion rejection of the UF media. As the size cut-offs become smaller (which can be as low as 1 Kda), the artifacts tend to increase. Also, quantification is difficult because changes in ionic strength may affect the size and shape of colloids, especially organic colloids. This Ag fraction passes through the 0.45 µm filter (the cut-off size for particulate phase) so routine analysis of colloidal silver has not been incorporated into exposure characterization.

Sulphides have a key role in the chemical behaviour of silver. Bianchini and Bowles (2002) studied the effect of reactive sulphide on silver toxicity. Sulphide protected against silver toxicity when based on measured data, however, once filtered, toxicity was similar in the presence and absence of sulphide. The authors attributed this difference to chemisorptions of the metal sulphide onto membrane filter and provided evidence that the toxic fraction of silver is that which is unbound to sulphide (Bianchini and Bowles, 2002). Other research demonstrated large losses of sulphide and silver (70% and 59% respectively) in exposures of durations as short as 24-48 hours, and loss of silver was greater in the absence of sulphide compared to in its presence (Bowles *et al.*, 2002). Hence, it is important to measure both silver and reduced sulphides in a sample. However, techniques that measure less than 0.01 µg/L silver and less than micromolar levels of reduced sulphides are lacking. Since storage longer than two hours tends to cause sulphides and metal sulphides to adsorb on container walls, there is a need for better storage protocols (Kramer *et al.*, 2002). Silver is also known to form a sulphide mineral known as acanthite (Ag_2S) in sediments. A technique for measuring metals in sediments, known as SEM-AVS (Simultaneously Extracted Metals – Acid volatile sulphides), was approved by the USEPA (2000) and has been applied to silver (Ankley *et al.*, 1996). The general concept of the method is that the activity of the metal in sediments is determined by the amount of AVS present in the sediment matrix. The fraction of metals that may bind to sulphides and thus will be captured by sediments can be estimated. Sulphide is measured using cold acid distillation with 1 M HCl, known as acid volatile sulphide. Silver can displace iron in the FeS complex (Allen, 2000). The unbound metals are the "SEM", represented as µmol/g dw of sediment. A disadvantage of the SEM-AVS approach is that the release of sulphide by 1 M HCl from monovalent metals rather than divalent metals may not be quantitative within a short time period. To overcome this obstacle, 1 M H_2SO_4 may be used instead of HCl since silver sulphate is soluble (Kramer *et al.*, 2002). An alternative approach may be a two-step analysis to obtain total sulphide and silver. A subsample can then be analyzed by acidic Cr(II) reduction of the sediment which releases sulphide from FeS, FeS_2 and any silver associated with these complexes (Rozan and Luther III, 2001). An additional subsample can be analyzed by nitric acid digestion which dissolves FeS and FeS_2 and releases the silver bound to these sulphides (Howarth and Merkel, 1984). If the released silver is less than the total sulphide measured, it indicates that the silver is bound to insoluble metal sulphides. However, these approaches need to be studied further before they can be implemented into routine analysis for silver.

Many studies also use geochemical equilibrium modelling to provide an estimate of Ag speciation in the exposure medium (e.g., Unsworth *et al.*, 2006; Lee *et al.*, 2004; Peng *et al.*, 2002; Fortin and Campbell, 2000; Playle, 1998). These models apply thermodynamic stability constants (Log*K*) to evaluate the effect of water chemistry on speciation of silver or other trace metals. For example, speciation modelling is a key component of the biotic ligand model (BLM) approach, which estimates metal speciation and metal binding to biologically active sites based on water chemistry parameters (Paquin *et al.*, 2002). Common geochemistry modelling software that can be used for silver include MINEQL+, MINTEQA2 and CHESS. These models do not include stability constants for silver complexation by sulphide in their default databases. Of the currently popular models, WHAM incorporates the effect of total and dissolved organic carbon (TOC and DOC) in calculating silver speciation but does not include stability constants for silver in its default database. The performance of all of the geochemical modeling software programs

and the ability to contribute meaningful speciation data is directly related to the amount and quality of the input data. While recent studies are more complete in this respect, it is sometimes difficult to estimate speciation for older studies because of the lack of available water chemistry.

2.0. ENVIRONMENTAL CONCENTRATIONS, FATE AND BEHAVIOUR

2.1. General considerations on properties of metals

The environmental chemistry of silver is of critical importance in the estimation of potential toxic effects in aquatic ecosystems. It is important to highlight that silver and silver compounds have unique characteristics and it is necessary to account for these in assessing potential environmental impacts. Many of the characteristics of organic chemicals and substances do not apply to metals, including silver. As outlined in the framework for metals risk assessment from the US EPA (USEPA 2007):

> "Metals are neither created nor destroyed by biological or chemical processes; they are transformed from one chemical form to another. Native (zero valence) forms of most metals and some metal compounds are not readily soluble, and as a result, toxicity tests based on soluble salts may overestimate the bioavailability and toxicity of these substances."

The guidance also discusses the fact that all metals occur naturally in all environmental media and therefore organisms have evolved over time in the presence of metals.

2.2. Environmental concentrations

For naturally occurring elements such as silver, ambient concentrations (which include natural background levels as well as anthropogenic inputs) need to be considered in the application of the generic water quality guideline. The current silver guideline derivation is based on toxicity data derived in laboratory waters and does not consider actual silver natural background or ambient concentrations. Background concentrations can be higher than the generic criteria (CWQG) and in some instances could be used as site-specific water quality criteria in place of the generic CWQG (CCME, 2003).

2.3. Natural background concentrations in water

The abundance of silver on Earth is low in comparison to other metals. The average crustal concentration is estimated to be 0.07 mg/kg and is mostly concentrated in basalt (0.1 mg/kg) and igneous rocks (0.07 mg/kg; CICAD, 2002). In crude oil and water from hot springs and steam wells, the concentrations are naturally elevated. Until the mid-1980s, levels of silver in the environment were believed to be much higher than is known today. Application of clean

techniques and the developments in analytical technology have provided new information on silver levels in the environment (Table 2.0). In general, silver levels in the environment are found at picomolar to nanomolar levels (0.001 to 0.1 µg/L) (Kramer *et al.*, 2002). In marine waters, the background levels of total silver are 0.0001 to 0.0022 µg/L, and in fresh waters 0.00054 to 0.0054 µg/L (Kramer *et al.*, 2002).

Table 2.0. Concentrations of silver in the environment (at sites considered to be relatively far from point source discharge) (adapted from Kramer *et al.*, 2002)

Environment	**Concentration (µg/L)**	
	Filtered (0.2 – 0.45 µm)	**Unfiltered**
Ocean (Depth < 1 km)	0.00004 – 0.0011	< 0.0000026 – 0.00060
Ocean (Depth > 1 km)	0.0011 – 0.0025	0.0002 – 0.0058
Estuaries and bays	---	0.00063
Coastal waters	0.0003 – 0.0012	0.0004 – 0.0018
Rivers	< 0.00001 – 0.052	< 0.00001 – 0.151.
Lakes	---	0.0002 – 0.0072

2.4. Ambient concentrations in surface waters (freshwater and marine)

Silver concentrations in rivers, lakes and estuaries are usually about 0.010 µg/L in unpolluted areas (Ratte, 1999). In urban and industrialized areas, silver concentrations in surface water can range from 0.010 to 0.10 µg/L (Ratte, 1999). Even in highly polluted systems, total silver concentrations rarely exceed ~0.10 µg/L. Exceptions might be areas such as effluent treatment mixing zones, where levels may reach up to 10.0 µg/L (Kramer *et al*., 2002). Specifically in Canada, total silver concentration data measured in thousands of samples of surface waters between 2008 and 2013 were available from several regional managers (Environment Canada, 2013). These regional data span across a full range of pH and hardness levels, as well as other unreported physical and chemical parameters. Readers should also be aware that these data do not distinguish concentrations measured in pristine versus anthropogenically affected sites, and some of the raw data have not been validated (Environment Canada, 2013). In the British Columbia and Yukon, concentrations in a variety of rivers ranged from below detection (0.001 µg/L) to 10 µg/L with a mean of 0.005 µg/L. In the Prairie and Northern region, including Alberta, Manitoba, Saskatchewan and Northwest Territories, concentrations in rivers and creeks ranged from below detection (0.001 µg/L) to 0.69 µg/L with a mean of 0.005 µg/L. In the Atlantic region, including Prince Edward Island, Nova Scotia, Newfoundland, and New Brunswick, concentrations in lakes, ponds, and streams ranged from below detection (0.001 µg/L) to 1.13 µg/L, with a majority of reported measurements at or below detection limit. Dissolved (< 0.45 µm) silver concentrations measured in 42 Québec rivers (N = 342) between 2008 and 2011 ranged from < 0.001 to 0.032 µg/L and total recoverable silver concentrations ranged from < 0.001 to 0.085 µg/L (Hébert, 2012).

2.5. Speciation and partitioning in aquatic ecosystems

Over 94% of silver released to the environment is expected to remain in the soil or wastewater sludge at the site of discharge. In freshwater environments, Ag will adsorb to sediments or suspended particles (Ratte, 1999). As mentioned in Section 1.5, silver in aquatic systems can be partitioned based on size, e.g., with the particulate phase being >0.45 µm, colloids being greater than 10 kDa and less than 0.45 µm, and truly dissolved at less than 10 kDa. Adams and Kramer (1999) studied a municipal wastewater effluent, receiving waters and pore waters from an anoxic lake sediment that were sampled from Dundas and Burlington (Ontario, Canada). They observed that a significant portion of silver occurred in the colloidal (30-35%) and dissolved (15-20%) phases. Dissolved silver concentrations were similar in effluent and receiving waters, suggesting that silver in the dissolved phase is strongly complexed by ligands and is not affected by aggregation or sorption processes such as those present in waste water treatment plants (Adams and Kramer, 1999).

Dissolved silver can occur in different chemical species. These species include free ions and complexes:

$$\sum (Ag^{+} + Ag(OH)_2^{-} + AgOH_{(aq)} + AgCl_2^{-} + AgCl_{(aq)} + AgCl_3^{-2} + AgCl_4^{-3} + AgNO_3 + AgSO_4^{-} + \text{etc.})$$

where “etc.” refers to additional complexes that may form depending on the underlying geochemical makeup of the aqueous system. For example, within natural systems complexation to dissolved organic carbon (DOC; also commonly referred to as natural organic matter (NOM)) and to sulphides is of key importance because Ag^{+} binds strongly to these ligands. In natural waters very little, if any, of the Ag_{Total} will be in the free ion Ag^{+} form (Kramer *et al.*, 2007, see below). Several geochemical parameters can alter Ag speciation. Examples of Ag speciation at an Ag_{Total} of 100 nM (~11 µg/L), 500 nM (~54 µg/L) and 1000 nM (~108 µg/L) in typical soft fresh water over a range of chloride concentrations are shown in Figure 2.2. Ag speciation is also affected by the presence of cations which compete for complexation sites, for example Na^{+}. Given that CWQGs are derived based on the total concentration of Ag and given that only a limited number of species of Ag are associated with toxicity (acute toxicity is primarily associated with Ag^{+}, with AgCl contributing in some cases) guideline values can represent extremely conservative estimates of potential impacts.

Consideration of inorganic and organic sulphides is essential to understand silver speciation in natural waters. Sulphide ligands (inorganic and organic) can be stable in oxic waters for a significant time at concentrations of <1-100 nM (e.g., Kramer *et al.*, 2007). Kramer *et al.*, (2007) hypothesized that metal–sulphide complexes stabilize sulphide ligands. The metal-sulphide complexes are associated with organic matter by multi-ligand binding or in nano-pore encapsulations in organic matter (Figure 2.2). These interactions may create a correlation between sulphide ligands and organic matter and are in general, considered mixed metal-sulphides. As a result, a group B metal (transition metals found in the middle of the periodic table) would replace or sorb to a sulphide ligand in natural organic matter (NOM; Kramer *et al.*, 2007). Adams and Kramer 1999 and Kramer *et al.*, 2007 suggested that almost all silver in natural conditions are complexed by sulphides, leaving negligible levels of free ionic silver. This

is due to the strong binding affinity of silver to sulphides (Log*K* ~ 13) and the fact that concentrations of sulphides in the environment are typically 200-15000 times higher than silver concentrations. Silver affinity to sulphides out-competes all other trace metals, except mercury. Similar binding affinities with organic sulphide compounds such as thiols have also been reported, however, they were found in much lower concentrations in surface waters and do not play a significant role in silver speciation. With compounds containing oxygen and nitrogen groups (e.g., ethylenediaminetetraacetic acid [EDTA]), silver has an affinity less than half of that with sulphides (Log*K* ~ 6; Adams and Kramer, 1999).

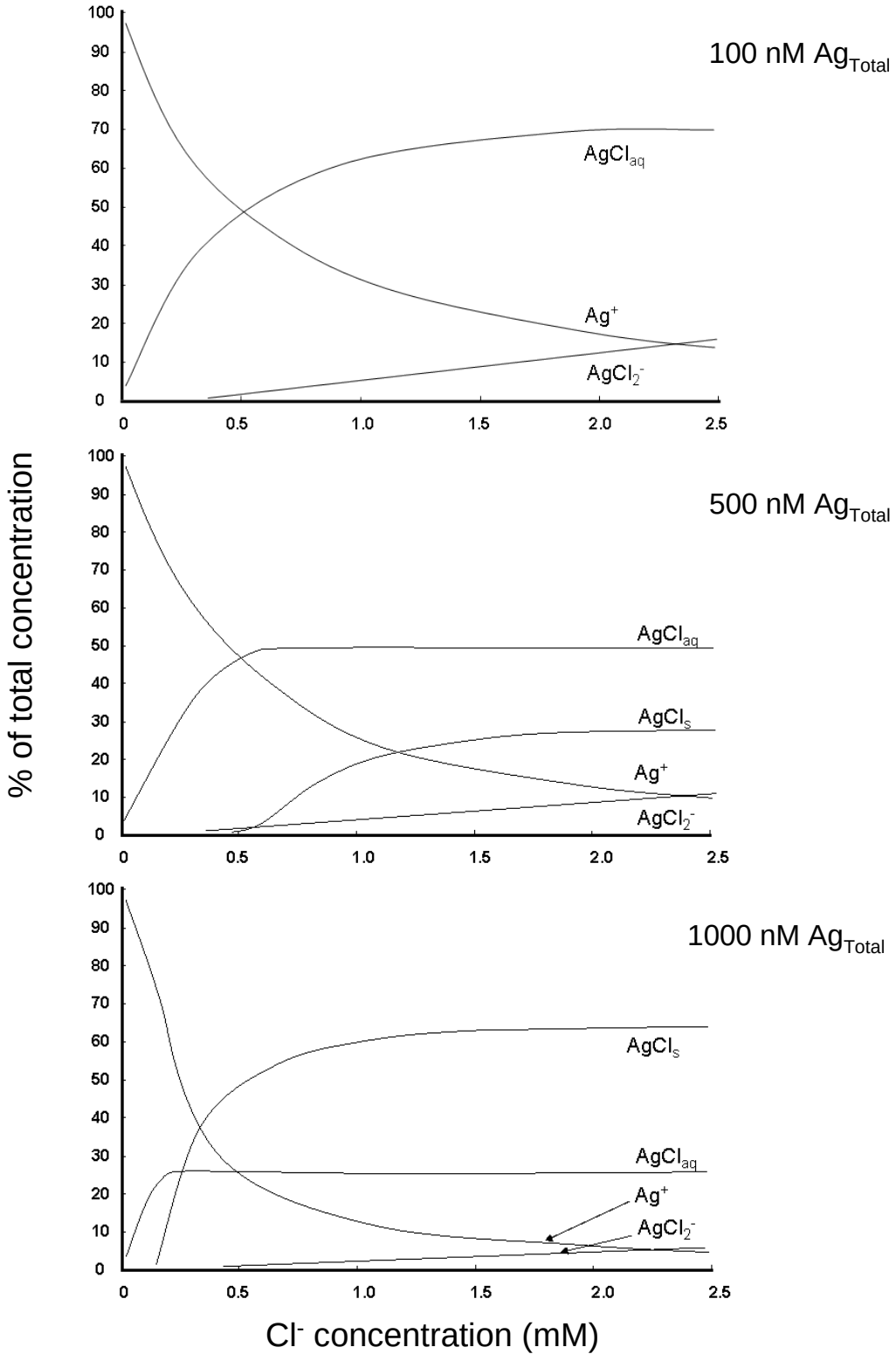

Figure 2. 1 The influence of varying water chloride concentration on the speciation of silver in soft water. The composition of soft water was taken from US EPA, 1985. Speciation was calculated using MINEQL+ version 4.5.

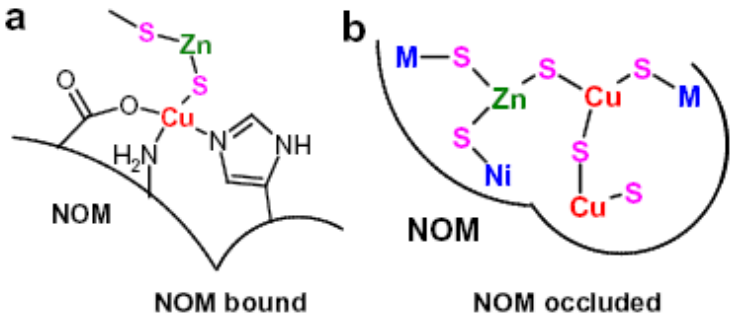

Figure 2.2 Schematic of two possible associations of metals and sulphide groups with NOM. (a) Incorporation of sulphide ligands within NOM by multi-ligand association with an intermediate metal (b) Encapsulation of larger multi-metal-sulphide complexes in nanopores of NOM via electrostatic forces (Kramer *et al.*, 2007).

In estuarine and marine environments, chlorides play a key role in Ag speciation. Even under conditions of low ionic strength, silver can precipitate as AgCl(s) known as ceragyrite (Luoma *et al.*, 1995). As a result of the formation of chloro-complexes, salinity is of key importance in estuarine and marine environments. Thermodynamic models confirm that strong chloro-complexes dominate speciation in such environments. As salinity increases, from fresh water to estuaries and finally the ocean, colloid-bound silver dissociates and silver complexation with chloride occurs. Luoma *et al.* (1995) investigated Ag sorption to natural sediments in estuarine environments (San Francisco Bay) and found that formation of stable chloro-complexes favours the dispersal of silver as well as the sequestration by sediments (relatively slow kinetics). Most silver in estuarine systems is deposited to sediments (Ratte, 1999), but there is also potential for chloride-bound silver to remain in solution when silver concentrations are low (below the threshold for ceragyrite formation).

It should be noted based on the above that most toxicity testing with silver involves exposures that are likely not relevant for natural environmental conditions. Testing is often done with reconstituted or filtered waters with little or no DOC or sulphides and using highly soluble salts, such as $AgNO_3$, in relatively dilute waters. These test scenarios result in Ag being primarily as free ion form (Ag^+) in the exposure media. There are two important considerations in linking silver and silver containing products/substances to effects in aquatic environments. The first is that natural conditions in the environment rarely, if ever, mimic those of lab tests. For example, as discussed above, free ion Ag (Ag^+) is seldom present in natural waters for any period of time and in appreciable quantities. The second factor for consideration is that silver (i.e., silver metal) and the form of silver contained in the various substances and materials containing silver have significantly different properties compared to silver nitrate. Most of these are only sparingly soluble in water (see Table 1.0). In spite of this, lab-based standardized toxicity tests with soluble metal salts (e.g., $AgNO_3$) remain the primary source of data for deriving water quality guidelines. Therefore, in interpreting guideline values in the context of natural environments it is important to understand the properties of the substances in relation to transformation and dissolution as well as the site specific factors that may influence exposure, bioavailability and impact relationships in aquatic biota.

3.0. BIOACCUMULATION AND BIOCONCENTRATION

3.1. Bioaccumulation concepts as applied to metals

Bioaccumulation is the process of the uptake of a substance across an external biological membrane (or other interface exposed to the environment). It is defined as the net accumulation in a tissue (or whole body) as a result of exposure (McGeer *et al.*, 2003). It integrates uptake across respiratory, gastrointestinal and other surfaces, as well as elimination. Bioaccumulation is measured on a tissue wet weight or dry weight basis. The application of assessment criteria for bioaccumulation of organic substances is well established however, metals have unique properties and processes and these need to be considered when evaluating the bioaccumulation of metal substances. In relation to metals, bioaccumulation is affected by the specific chemistry of the metal, the way the metal reacts with the environment, the physiological and ecological traits specific to a species, and the route of exposure (Luoma and Rainbow, 2011).

All discussions on metal bioaccumulation need to address the general issues surrounding the inherent complexities and uncertainties. Knowledge on bioaccumulation is highly relevant to understanding the potential toxic impacts because metal has to be taken up into an organism to have effects. As such, criteria based on bioaccumulation account for the variability associated with exposure geochemistry. However, the ability to derive relationships between bioaccumulated metal (silver included) and adverse effects is limited, except on a case by case basis. Accumulated metal concentrations may interact at sites where toxicity is expressed but may also be sequestered and/or eliminated, or stored in detoxified forms (McGeer *et al.*, 2003; Adams *et al.*, 2011). Therefore whole body and (perhaps to a lesser extent) tissue residue measurements characterize a concentration that is made up of unknown proportions of various stored and detoxified forms as well as metal that may cause effects. Tissue residue concentrations have the advantage of indicating the bioavailability of a metal, but the linkage between tissue concentration and toxicity is not clear, and varies greatly between species and within a species in different environments because of induced detoxification mechanisms. Adams *et al.* (2011) point out that data interpretation is complicated by the fact that there are no standard methodologies or test species. Therefore, bioaccumulation of silver is not a useful tool for application in water quality guidelines or criteria unless linkages between accumulation and the mechanism of toxicity are clearly elucidated.

3.2. Biomagnification, bioaccumulation factors and bioconcentration factors

The most common measures of bioaccumulation are the bioaccumulation factor (BAF) and the bioconcentration factor (BCF). In Section 3.1, bioaccumulation was defined as the net accumulation of a substance in tissue following exposure to various media (e.g., water, food, sediment). Bioconcentration, on the other hand, is defined as the net accumulation of a substance in tissue following exposure to water only. Both BAF and BCF are expressed as the ratio, at steady state, of the substance concentration in the organism (or tissue) to the substance exposure concentration. Therefore BCF traditionally are calculated during lab studies whereas BAFs are calculated from field data.

Biomagnification is another measure that is often used to describe the bioaccumulation of substances. It is defined as an increase in the concentration in an organism from a lower trophic level to a higher trophic level within the same food web (McGeer *et al.,* 2003). It is calculated as the ratio of organism concentrations from higher to lower trophic level and is assumed to result from dietary exposure (prey to predator). When the biomagnification factor is higher than 1, then biomagnification is said to have occurred. Inorganic metals rarely biomagnify across three or more trophic levels, although this can be seen with certain organic forms, for example methyl mercury (McGeer *et al.,* 2004). Trophic transfer is conceptually similar to biomagnification in that it characterizes the transfer of bioaccumulated substances from prey to predator. It is important when considering the potential for direct dietary toxicity.

While it is possible that high levels of silver can accumulate in aquatic organisms, the link between bioaccumulation and effect are lacking. Hogstrand *et al.,* (1996) exposed trout to 30,000 µg Ag /L as thiosulphate $Ag(S_2O_3)_n^-$ which produced a liver tissue concentration of 73,150 µg Ag/kg but with few physiological differences compared with unexposed controls (also see section 3.3 below). There is no evidence for the biomagnification of silver (Terhaar *et al.,* 1977; Ratte, 1999; McGeer *et al.,* 2003). BCFs and BAFs for silver were reviewed by McGeer *et al.,* (2003) and were found to be inversely related to exposure concentrations. As a result of the inverse correlations as well as the unique features of metals, it was concluded that these measures should not be used as criteria for metals. The EPA guidance on the use of BCFs and BAFs for metals limits their use to site-specific risk assessments where details of exposure and impacts can be closely linked to bioaccumulation (USEPA, 2007). In this context it is recognized that if impacts are being assessed then bioaccumulation is likely to be complementary except as related to understanding mechanisms of impact.

3.3. Bioavailability and accumulation of silver

Organisms in aquatic systems are directly exposed to their environment via respiratory, gastrointestinal or other epithelial surfaces. The potential uptake of metals across these surfaces is related to the structure and function of the surface, the geochemical forms (i.e., metal speciation) in the exposure medium and interactions that occur at the interface of the tissue with its environment. In fish, for example, silver uptake occurs at cells that are specialized for physiological functions related to nutrient uptake and ionic regulation, in the gastrointestinal tract and in the gills.

The free Ag^+ ion is the most acutely toxic and bioreactive Ag species, though other small neutral Ag-complexes are bioavailable. For example, 7-day exposures of juvenile rainbow trout to silver thiosulphate (AgS_2O_3) showed accumulation in the liver to a level that was 335-fold higher than control fish, however, little toxicity was observed (Hogstrand *et al.,* 1996). Similarly, while 10 ug/L of ionic silver caused a variety of internal disturbances in rainbow trout, silver complexed with thiosulphate at 3000-fold greater concentrations had minor effects, despite accumulating in the gill, plasma and liver 2 to 4-fold greater than in the ionic exposures (Wood *et al.,*1996). Using treatments with varying ratios of AgCl and Ag^+, McGeer and Wood (1998) showed that AgCl was accumulated in the gills of rainbow trout after 48 h of exposure but did not have the same impact on the physiological mechanism of toxicity (inhibition of NKA, see section 5.2) as

did Ag^+. The data on AgCl are equivocal as Erickson *et al.*, (1998) concluded that neutral AgCl complexes were contributing to acute silver toxicity in fathead minnow, most probably because the neutral complexes are more readily transported across the membranes to the site of action in fathead minnows.

Silver accumulation of non-charged lipophilic complexes with some organic ligands also can occur, crossing through the cell membrane via passive diffusion. Such ligands include diethyl-ditho-carbamates, 8-hydroxy-quinoline, and xanthates (Ratte, 1999), which are used in the mining industry as flotation agents in the mineral extraction process. Uptake of neutral hydrophilic complexes may also occur if silver is bound to low molecular weight organic compounds. There are no data on silver, however, copper complexation with low molecular weight organic ligands (e.g., glycine, alanine, glutamine acid, citrate and ethylenediamine) has been shown to increase uptake and toxicity (Ratte, 1999).

As discussed in Section 2.3, reduced sulphur is a strong complexing agent for the Ag^+ ion in natural waters and Kramer *et al.*, (2002) suggests that there likely exist uptake pathways that cause sulphide-bound silver into aquatic organisms. The most likely mechanism of this transfer is through ligand-exchange (Kramer *et al.*, 2002):

$$Ag\text{-}S_1 + S_2 = S_1 + Ag\text{-}S_2$$

where S_1 is an aquatic sulphide ligand and S_2 is a biomolecule composed of a sulphydryl group(s). The exchange kinetics will depend on the binding affinities of the ligands with Ag(I) and on their concentrations.

Bianchini *et al.*, (2005) showed elevated Ag accumulation in *Daphnia magna* exposed to Ag in the presence of reactive sulphide compared to organisms exposed to Ag without reactive sulphide present. They were able to demonstrate that this Ag is incorporated into the organism, primarily in the digestive tract, and not simply adsorbed on the exoskeleton. This accumulated AgS is not retained by the daphnid once moved into clean water, implying that although higher amounts of Ag are ingested in the presence of reactive sulphide this Ag is quickly excreted as feces and Ag levels are returned to similar concentrations as found in organisms exposed to $AgNO_3$. This indicates that, although reactive, sulphide very effectively complexes Ag in the aquatic environment, this AgS complex is taken into the gastro-intestinal tract and could be potentially bioavailable and/or bioreactive.

3.4. Partitioning of accumulated silver

Once taken up from the medium, silver is distributed throughout the organism and in the case of fish, is sequestered in tissues including liver, intestine, gills, and carcass (Galvez *et al.*, 2002). The fate of bioaccumulated silver is not entirely understood but binding to biomolecules will occur. Many of these biomolecules contain sulphydryl moieties that bind silver and significantly reduce the possibility for toxic interactions. Examples include amino acids (e.g., cysteine), tripeptide glutathione (GSH), metallothionein and phytochelatin (Kramer *et al.*, 2002). Metallothionein (MT) is one of the proteins believed to play a large role in metabolism and

detoxification of silver. MTs are normally found at trace levels in tissues such as brain, gill, gonad, kidney or liver. Metals, especially silver, are known to induce MT synthesis (Hogstrand *et al.*, 1996; Mayer *et al.*, 1996). MT binds strongly to silver with a Log*K* of 11.7 (Kägi and Shäffer, 1988), and the complexation involves 10-12 atoms of silver per MT molecule (Mason and Jenkins, 1995). Another route for bioaccumulation is through the tripeptide, GSH (gg-glutamylcysteinylglycine). GSH is often found in tissues at high concentrations, up to 10 µM. It has a strong binding affinity to silver (Log*K* = 12.3; Adams and Kramer, 1999) with a 1:1 complexation.

A common technique for studying intracellular localization of trace metals is autometallography (AUM) in which metal sulphide or selenide accumulations can be enhanced with Ag ions and made visible under light or electron microscope (Domouhtsidou and Dimitriadis, 2000). This cytochemical technique was used to study localization of metals, including silver, in gills and digestive gland of the common mussel (*Mytilus galloprovincialis*) after a 98-day exposure to a nominal solution of 0.1 mg/L of Hg, 0.1 mg/L of Ag, 0.1 mg/L of Pb, and 0.1 mg/L of Cu (Domouhtsidou and Dimitriadis, 2000). Accumulation was observed in the apical and basal part of the cells. Greater accumulation was observed in the frontal part of the gill filament for silver. In the digestive gland, silver localization occurred in the heterolysosomes and the residual bodies of the digestive cells, as well as the dense bodies of the basophilic cells. Overall, metal exposure affected the gross morphology of the studied tissues and resulted in the fusion of residual bodies in the digestive cells, the fragmentation or vacuolization of the rough endoplasmic reticulum, and the increase in the number of granules in the basophilic cells. Additionally, in gills, the fusion of gill filaments was observed (Domouhtsidou and Dimitriadis, 2000). A study by Bustamante *et al.*, (2000) examined localization of 12 trace metals, including silver, in a cephalopod species (*Nautilus macromphalus*). The study found greatest accumulation of silver in the renal and pericardial appendages (Bustamante *et al.*, 2000).

Galvez *et al.*, 2002 exposed juvenile rainbow trout to a 2-day radioactive pulse of ^{110m}Ag at 11.9 µg/L (as $AgNO_3$), followed by a 19-day exposure to 3.8 µg/L non-radioactive Ag. The distribution of ^{110m}Ag in the gills, liver, intestine, kidney, brain and remaining carcass was investigated over the 19-day period and showed the intestine accumulated the highest proportion of the ^{110m}Ag burden (34%). By day 8, however, less than 5% of the total radioactivity remained in this tissue and the majority of the ^{110m}Ag eliminated from the intestine appeared to distribute to the liver eventually accounting for 65% of the total radioactivity in the fish. Aside from the liver and intestine, only the gills and carcass contained any significant amounts (>5%) of the total body ^{110m}Ag. Liver and gill samples were fractionated using differential centrifugation techniques to identify the subcellular distribution of ^{110m}Ag. Between days 8 and 19, the ^{110m}Ag levels in the liver cell cytosol increased from 35% to 72% of the total cellular burden. In the gills, ^{110m}Ag was predominantly found in a membrane compartment termed the nuclear fraction (approx. 60% of the total) and did not change over time (day 8 to day 19). Using size-exclusion chromatography, approximately 70% of the ^{110m}Ag content in the liver cytosol eluted at a molecular weight indicative of metallothionein. The cytosolic distribution of ^{110m}Ag in gills was quite diffuse, occurring primarily in the heavy molecular weight fractions. This study made no attempt to link the accumulation patterns of silver with the effects of silver.

Hogstrand *et al.*, (2003) exposed rainbow trout and European eel to Ag(I) added as $^{110m}AgNO_3$ in water with low or high chloride content to test speciation (low chloride promoted formation of Ag^+ while high-chloride promoted formation of $AgCl_{aq}$). Following a 24-h exposure to $^{110m}Ag(I)$ tissue radioactivity was monitored for a 67-day period. The liver was the dominant compartment for $^{110m}Ag(I)$ accumulation in both species and for both exposures; ranging from 41 to 97% for trout and from 18 to 77% for eel over the 67-day depuration period. Changing the speciation of Ag(I) did not effect whole body loads but did effect internal distributions. Trout exposed to $AgCl_{aq}$ eliminated $^{110m}Ag(I)$ from the kidney more quickly that trout exposed as Ag^+. In eel, exposure to $AgCl_{aq}$ hastened elimination of $^{110m}Ag(I)$ from mid and posterior intestine and increased retention in the kidney (Hogstrand *et al.*, 2003).

3.5. Secondary poisoning

Much of the research to date has focussed on waterborne exposures of Ag and its toxic impact to biota, however, under field conditions organisms will also encounter Ag (and other metals) via ingestion of Ag associated with food and other particulate matter. A study by Baines *et al.*, (2002) estimated assimilation and retention of trace metals by fish from food in order to help understand the linkage between toxicity and biogeochemical cycling of the metals through the aquatic food chain. The study used a pulse-chase radiotracer technique to estimate the assimilation of silver and other trace metals in 43- and 88-day-old juvenile striped bass (*Morone saxatilius*). The fish were fed various crustacean species that were fed with radiolabeled diatoms. Assimilation efficiencies were higher in older fish presumably because of longer gut passage times for larger fish.

Galvez *et al.*, (2001) investigated the physiological effects of biologically incorporated Ag (3.1 µg/g) to juvenile rainbow trout, by exposing fish to Ag-thiosulphate and including these Ag-contaminated fish into a trout meal which was fed as a pelletted form. During, and at the end of, the 126-d exposure to biologically incorporated dietary Ag, no significant changes in mortality, specific growth rates, food consumption rates, and food conversion efficiencies. Further, dietborne silver did not affect Na^+ influx or plasma Na^+ concentrations or intestinal NKA activity and no physiological signs of stress were exhibited. Silver did accumulate in the tissues of exposed fish. Significant Ag accumulation was seen in the intestine and liver, and after liver-Ag concentrations reached a plateau, elevated Ag was seen in plasma, gills, and kidney. No metallothionein induction was seen, however, the authors theorized that levels already present in the liver were sufficient to bind all silver.

Similar results to the Galvez *et al.*, 2001 study was seen by Mann *et al.*, 2004a in their work with the American Red Crayfish (*Procambarus clarkii*). They fed crayfish biologically incorporated Ag (as Ag-thiosulphate exposed trout) and saw high levels of trophic assimilation of Ag, with the majority of Ag accumulating in the hepatopancreas, but with no apparent physiological effects. In crayfish, the mode of waterborne Ag^+ acute toxicity appears to be the same as in trout, i.e., a disturbance of Na balance along with gill NKA impairment (see Section 5.3). These studies highlight the importance of exposure route when addressing Ag toxicity. The osmoregulatory effect of Ag appears to be caused by waterborne Ag^+ while dietary exposure to biologically

incorporated Ag (as would be encountered in the field by predatory organisms) is relatively benign for these organisms.

The responses of marine and freshwater crustacean zooplankton to both dietary and waterborne dissolved silver imply that the dietary route of exposure is potentially of sublethal toxicological importance to some species (Hook and Fisher, 2001a). Effects (decreased egg production and total egg protein content) were only seen following Ag assimilation from food. Silver accumulated in cladocerans and copepods by both routes of exposure, though higher whole-body Ag levels were observed following waterborne exposure. However, it appears that Ag accumulated by waterborne exposure is mainly kept in the exoskeleton and Ag assimilated from the food is distributed to more sensitive tissues within the organism and therefore able to cause reproductive effects. Effects on egg production were seen in marine copepods and freshwater cladocerans when food was exposed to 0.108 µg Ag/L and 0.027-0.054 µg/L, respectively. The study of Hook and Fisher (2001a) has generated considerable interest since publication, particularly the freshwater cladocercan data. Kolts *et al.* (2009) provides a comprehensive examination of the effects of dietborne silver on reproduction and survival in *Ceriodaphnia dubia* with particular reference to a repetition of previous studies (including Hook and Fisher 2001a). Dietary silver generally did not impact survival or reproduction beyond the effects caused by waterborne Ag alone. The contrary nature of the results of Hook and Fisher (2001a) compared to the results of the replicate study (Kolts *et al.*, 2009) make interpretation equivocal.

3.6. Bioaccumulation in fresh waters

3.6.1. Algae

Phytoplankton plays a vital role in the biogeochemical cycling of metals in the aquatic environment because of their place at the base of the food chain. It rapidly accumulates dissolved metals (<0.2 µm particle size) including Cu, Zn, Se, Hg and Ag. Silver has a biological elimination half-life of 115 h and although its accumulation in algae was thought to be due to adsorption onto the cell surface rather than uptake (Ratte, 1999), there is evidence of enhanced uptake as AgS_2O_3 by anion transport (Fortin and Campbell, 2001). An experiment by Garnier and Baudin (1989) revealed rapid accumulation of silver (^{110m}Ag) by *Scenedesmus obliquus* (green alga), reaching equilibrium within 24 hours. The observed BCF levels were high at 1.8-25 x 10^5 dw or 3-42 x 10^4 ww. Thus, algae could be the single most important source of metal introduction into the aquatic food chain.

3.6.2. Aquatic Plants

Jones *et al.* (1985) studied Ag levels in three aquatic bryophytes (*Scapania undulata*, *Hygrohypnum luridum* and *Polytrichum commune*) from lead mining streams in Wales, England. All the species had higher silver levels in their tissues than in the exposure water. A strong correlation between tissue silver levels and tissue levels of lead, zinc, copper and cadmium was found in *S. undulata*, deeming it a suitable biomonitor for pollution. Wells *et al.* (1980) conducted a study on 23 aquatic plant species in Saginaw Bay, Lake Huron. They measured

metal levels in these plants using neutron activation analysis and found Ag levels ranging from non-detectable (<0.1 ppm) to 66.9 ppm. The two highest Ag concentrations measured (56.3 and 66.9 ppm) were found in two species of bulrush (*Scirpus validus* and *S. acutus*, respectively). Both the green alga, *Cladophora* sp, and cat tail (*Typha angustifolia*) had relatively high silver levels; 25.7 and 39.2 ppm, respectively. All other species collected and analyzed for silver had levels less than 1.4 ppm.

3.6.3. Invertebrates

Uptake of metals occurs in several ways in benthic organisms such as by direct contact of the external surface of the body with contaminated sediment particles, from the interstitial water, and from sediment particles being ingested and digested in the intestine (Ratte, 1999).

Silver accumulation in zebra mussels, which possess a rapid filtration mechanism, was studied by Roditi and Fisher (1996) using microcosms and radiolabeled isotopes. Several metals, including silver, were studied in the presence of different DOC levels. The study showed correlation between tissue concentration and environmental concentrations, suggesting that Zebra mussels are appropriate indicators of metal contamination in the environment. Another study conducted by Ribeiro Guevara *et al.* (2005) on Ag levels in impacted and reference lakes in Argentina, found that Ag intake by the native mussel, *Diplodon chilensis* is associated with clastic particulate, which is ruled by sediment intake. They hypothesized that Ag bioaccumulation is related to grain size selection, i.e., smaller grain size would equal higher Ag intake.

Brown *et al.* (2003) assessed the effects of silver at contaminated estuary sites in San Francisco Bay to the clam *Potamocorbula amurensis*. Monthly sampling of the reproductive cycle of the clams compared to Ag tissue concentrations revealed that the proportion of reproductive clams was less than 60% when Ag tissue concentrations were high (generally greater than 2 ug/g). Comparatively, when Ag tissue concentrations were lower (≤1 ug/g) the proportion of reproductive clams was 80-100% (Brown *et al.*, 2003).

Freshwater oligochaetes (*Lumbriculus variegatus*) exposed to silver sulphide contaminated sediments (444 mg Ag/kg) for a 28-day cycle, showed no effect on reproduction or dry weight. Little silver was accumulated by the organisms (BCF of 0.18; Hirsch, 1998) and what was accumulated was thought to be mainly due to surface adsorption. This finding suggests that sulphide-bound silver in contaminated sediments may not be bioavailable to oligochaetes, due to the low solubility of the silver sulphide.

Lam and Wang (2006) showed that Ag uptake by *Daphnia magna* is directly proportional to aqueous Ag concentration. Their calculated uptake rate constant (k_u) for Ag was much higher than has been previously reported for Cd and Zn, but is lower than that of Hg or MeHg, making Ag second only to Hg as having the highest potential for accumulation in daphnids. Furthermore, there was no indication that Ag saturation occurs over their Ag concentration range of 0.008-0.88 µg/L. In this study they also examined dietary Ag assimilation and found that with higher food concentrations (food density), the Ag assimilation efficiency was lowest. This effect of

density of algal food has also been documented in the marine copepod, *Acartia spinicauda* (Xu and Wang, 2004). It is suggested that Ag is not easily released from the food source when it enters the gut of the animal because it has a high particle reactivity. They go further to suggest that in water with high food biomass, Ag accumulation by daphnids will be substantially diminished.

3.6.4. Fish

Freshwater fish (*Micropterus salmoides* and *Lepomis macrochirus*) were observed to accumulate silver for only the first two months of exposure during a six month study, after which equilibrium between silver in the exposure water and in the tissue of the fish had been attained (Coleman and Cearley, 1974). Silver accumulation was found to be greatest in the gills, followed by the internal organs and the rest of the fish body. However, total silver concentrations in the internal organs (600 µg/kg) were found to be greater than in the gills (370 µg/kg) or the rest of the body (17 µg/kg). Also, the uptake of zinc was found to decrease as silver uptake increased. However, the unusually high levels of chloride are a confounding factor for the interpretation of the results (Davies and Goettl, 1978).

Fish sampled from Ag impacted and reference lakes in Patagonia, Argentina (Ribeiro Guevara *et al.*, 2005) showed high inter-species variability in Ag accumulation. The velvet catfish, *Diplomistes viedmensis,* a bottom dweller, accumulated the least amount of Ag overall, however, it showed a spatial distribution of Ag accumulation, i.e., Ag tissue concentrations decreased as sampling points were farther from Ag input. The reduced mobility of this fish species compared with the other fish sampled makes it a better indicator species for Ag since it better reflects the surrounding environment. The other fish species, salmonids and perch, showed relatively high Ag content in the livers, with the highest concentrations reaching 1-29 µg/g dry weight. There was evidence of food chain effects on Ag bioaccumulation in this study, where the top predators (salmonids) generally had higher tissue Ag burden than the creole perch (also a piscivore, but a lower trophic level species). Of the three species of salmonids sampled, brown trout exhibits the highest level of piscivoury (and highest levels of Ag in the liver) compared to brook trout and rainbow trout which both include fish and macrozoobenthic organisms in their diet. This study also considered seasonal variability in Ag accumulation and found no indication of seasonal effect.

Bury and Wood (1999) investigated uptake of silver through the gills of freshwater rainbow trout. At higher $AgNO_3$ concentrations (>36 nM), uptake and accumulation of silver was rapid, and at low concentration exposures (<36 nM), uptake increased very gradually. Increasing water sodium levels (0.05 mM to 21 mM) significantly reduced silver uptake. In contrast, increasing calcium and potassium levels (up to 10 mM) did not have an effect on silver uptake. Mechanistic studies indicated the presence of proton-coupled Na^+ channels in the apical membrane of the gills through which Ag^+ is able to enter (Bury and Wood, 1999).

3.7. Bioaccumulation in marine organisms

3.7.1. Algae

In phytoplankton, species variation does not appear to affect the rate of silver accumulation (Sanders and Abbe, 1989). Silver uptake was rapid in phytoplankton compared to other organisms and was inversely proportional to salinity. Bioavailability appeared to be controlled by free silver ion (Ag^+) and possibly silver chloride complexes.

Xu and Wang (2004) studied the effect of macronutrient level on Ag bioaccumulation in the marine diatom (*Thalassiosira pseudonana*). Diatoms exhibited a higher Ag accumulation with increasing nitrate, ammonium and phosphate levels. The uptake rate of Ag increased by 1.4 to 16-fold over the concentrations used in their study (5.88 to 176 µM for nitrate and ammonium, 0.24 to 7.24 for phosphate), which is in agreement with studies on other metals (Cd, Zn and Fe) though Ag uptake appears to be the most affected. The authors report that at lower nutrient levels, the protein content of diatoms is reduced, however, when nutrient levels are higher, protein synthesis occurs, which may lead to the increased uptake of Ag due to its high affinity for S-containing ligands. This would be a plausible explanation for increased uptake with higher nitrate and ammonium levels, but does not explain the increased uptake when higher levels of phosphorus were present. They theorize that a general increase in cell growth rate with higher nutrient levels would account for increased metal uptake. Even though Ag is not an essential metal, increasing growth rate would result in increased essential metal uptake, and Ag could be 'accidentally' transported into the cell. Lastly, an increased growth rate would result in numerous smaller sized cells, which would increase the surface-area-to-volume ratio and therefore higher Ag uptake.

3.7.2. Invertebrates

In molluscs, silver concentrations can vary to a great extent between different but related species. Most of the existing data are for the digestive gland and kidneys. Season and latitude of habitat affects silver levels in benthic organisms. For example, American oysters from Chesapeake Bay showed seasonal variations of silver in whole body measurements. Concentrations were lower in the summer and under high salinity, and higher at sites near human activity. In addition, the bioavailable species were the free monovalent ion (Ag^+) and the uncharged AgCl (Sanders *et al.*, 1990; Daskalakis, 1996). Other factors affecting silver concentrations in oyster tissues include sex, age, size, reproductive stage, general health, metabolism, water temperature, dissolved oxygen, turbidity, chemical speciation and interaction (Presley *et al.*, 1990). Temporal variations are also observed due to differences in the surrounding activities. For example, California mussel (*Mytilus californicus*) showed a significant decrease in silver concentration in tissues between 1977 and 1990, during which time the body burden decreased from 10-70 mg/kg dw to <2 mg/kg dw. A major cause of this change was deemed to be the reduction in emission rates notably by wastewater treatment centres (Stephenson and Leonard, 1994).

Cain and Luoma (1985) and Yoo *et al.* (2004) studied accumulation of silver in clams (*Macoma balthica*). Cain and Luoma (1985) studied two populations, one already existing in a

contaminated site and the other transplanted from a pristine site. The clams already resident in the contaminated site accumulated twice the amount of silver in hard and soft tissues as the transplanted clams. However, the transplanted clams retained 90% of the accumulated silver, while the resident clams lost as much silver from the soft tissue as was gained. In addition, shell closure occurred earlier in the transplanted clams. Yoo *et al.* (2004) found *M. balthica* accumulated Ag proportionally to the weak-acid extractable Ag in the sediments (Ag-SEM) and was unrelated to the oxidation state of the sediment (i.e., AVS levels). Difference in AVS concentrations (oxic versus anoxic) had no significant influence on Ag bioaccumulation. Somewhat surprisingly, *M. balthica* accumulated Ag even when Ag-SEM was much less than AVS. Further, tissue Ag in *M. balthica* fed an Ag-contaminated diet did not significantly differ from individuals fed uncontaminated food. This was not the case for other species tested in the experiment demonstrating that bioaccumulation is influenced by biology (Yoo *et al.*, 2004).

Another study examined the long-term effects of silver exposure on growth, bioaccumulation and histopathology of blue mussels (*Mytilus edulis*). Laboratory raised mussels exposed to 10 µg/L silver showed significant accumulation only after 12 months, while all (including 1 and 5 µg/L exposure) showed measurable accumulation after 18 and 21 months (Calabrese *et al.*, 1984).

Metayer *et al.* (1990) found species differences in silver bioaccumulation in molluscs. For example, scallops and oysters were found to accumulate more silver than mussels. Silver was exposed to the test species in food (phytoplankton) and/or in exposure water. Exposure through water only or water and food resulted in significantly higher silver uptake than through food only exposure. A study by Majorta *et al.* (1988) found that silver body burden in oysters (*Crassostrea gigas*) reached a plateau after 14 days of exposure. The majority of the silver was stored in the low toxicity sulphide form in amoeboscytes and basal membranes of tissues and organs (Majorta *et al.*, 1988).

Silver accumulation in polychaete worms (*Neanthes=Nereis virens*) was observed to increase with ambient silver levels and exposure time (Pereira and Kanungo, 1981) when transplanted from a pristine environment to silver contaminated test water (1.0 µg/L). Hence, the amount of silver accumulated in the transplanted worms was more than twice that of the worms which had inhabited a local polluted area. The transplanted worms showed a decrease in oxygen consumption when silver body burden was greater than 113 mg/kg and a decrease in water content when body burden was greater than 88 mg/kg. The worms from the polluted water did not show any adverse effects. In contrast, only low levels of silver accumulation were observed in a different species of worms, *Nereis diversicolor* by Bryan and Hummerstone (1977).

Measurements of silver in invertebrate species dwelling in southern California wetlands (Mugu Lagoon, Malibu Lagoon and Ballona Wetlands) ranged in concentration from <0.3 to 5.9 µg/g. The highest concentration of silver (5.9 µg/g) was found in bivalve *Tagelus californianus* at Ballona Lagoon. Silver was present at sufficiently high levels at all three sites to be considered an environmental health hazard (Cohen *et al.*, 2001).

In estuarine environments, silver is more often bound to inorganic material such as chloride. Also, as salinity of the water increases, less silver is bound to organic compounds. In

crustaceans, such as shrimp (*Palaemonetes pugio*), silver uptake correlated more with silver chloride concentration (r^2 of 0.90) than with any other silver species. This may be because silver chloride complexes can readily pass through the cell membrane due to their neutral charge. However, uptake of total silver and chloride complexes decreased when salinity increased (Warrington, 1996). A study by Cain and Luoma (1985) examined silver body burden in spot prawn (*Pandalus platyceros*), specifically in the abdomen and carapace. No correlation was found between silver accumulation and size, age or sex of the shrimp. The majority of the accumulation occurred in the hepatopancreas (8.27 µg/g), followed by the carapace tissue (1.16 µg/g) and the abdomen (0.80 µg/g). Silver behaves differently from the other trace metals in that the speciation reactions that enhance Ag solubility also enhance its bioavailability and dispersion in estuarine or marine environments (Luoma *et al.*, 1995).

A study by Carvalho *et al.* (1999) examined uptake of silver and other metals from colloidally bound species. Their study on silver uptake in shrimp (*Penaeus aztecus*) found that colloidally bound metals, including silver, were bioavailable to shrimp. The hepatopancreas in the shrimp appeared to have the highest accumulation for both free ion and colloidally complexed samples. However, the total radiotracer activity was highest in the abdomen for shrimp exposed to the free-ionic metals (i.e., Ag^+) and highest in hepatopancreas for shrimp exposed to colloidally complexed metal samples (Carvalho *et al.*, 1999).

In the marine copepod, *Temora longicornus*, metal adsorption to the exoskeleton does not appear to account for the high uptake rate of Ag (Wang and Fisher, 1998). They found that 30 to 70% of the Ag body burden is likely to come from aqueous uptake and not from the dietary route and that Ag is retained by the organism less efficiently when accumulated from food.

3.7.3. Fish

There is limited information on the bioaccumulation of Ag by marine fish. Accumulation of Ag by Dover sole (*Microstomus pacificus*), a sediment-dwelling flatfish, was studied near a waste water control plant's outfall system and no overall pattern of metal accumulation was observed in the flesh, gonads and livers of sampled fish. Further analysis of trace elements, including Ag, in flesh, liver, gonads, kidney, heart, brain, and gill arches of diseased and healthy Dover sole showed no significant accumulation of Ag (McDermott *et al.*, 1976).

In fish sampled from the Northwest Atlantic ocean, two studies by Hellou *et al.* (1992a, 1992b) reported metal concentrations in Bluefin tuna (*Thunnus thynnus*) and cod (*Gadus morhua*), respectively. Silver concentrations in tuna muscle ranged from 0.001 to 0.008 µg/g dry weight (dw). In cod, Ag concentrations (µg/g dw) in liver, muscle and ovarian tissue ranged from 0.49 to 0.83, below detection to 0.1, and 0.11 to 0.17, respectively.

4.0. TOXICITY OF SILVER AND SILVER COMPOUNDS TO AQUATIC LIFE

4.1. Essentiality

There is no evidence to date that silver has any essential biological function in aquatic systems.

4.2. Mode of action and toxicokinetics

Much of the understanding of silver toxicity has arisen through studies on fish, particularly rainbow trout. The free ion form of silver (Ag^+) disrupts ion balance via competition at the Na^+ specific uptake channel. Ag^+ is an ionoregulatory toxicant to the gill that reduces Na^+ and Cl^- levels, disrupts fluid volume, and causes circulatory failure (Wood, 2012). Silver accumulation leads to a poisoning of the basolateral Na^+K^+-adenosinetriphosphatase (Na^+/K^+ ATPase or NKA) in the chloride cells of the gill epithelium. NKA is a multisubunit protein embedded in the basolateral (blood) side of the cell and is one of the key proteins driving the uptake of Na^+ (and indirectly other cations and Cl^-). Its function is essential for ionoregulation. The cause of Ag^+ toxicity to aquatic organisms through this mechanism is the interference of the function of NKA by binding to a Mg site on one of the subunits (Morgan *et al*., 1997). This binding irreversibly inhibits activity of the enzyme, resulting in ionoregulatory disturbance. If sufficient ion transport capacity is lost, the fish will die. Morgan *et al.* (1997) showed a dose dependent inhibition of Na^+ and Cl^- uptake by silver, and inhibition of NKA has been shown in a number of studies.

Another site of action for acute toxicity is inhibition of the enzyme carbonic anhydrase in the branchial ionocytes (Wood, 2012). Carbonic anhydrase catalyzes hydration of carbon dioxide to form acidic and basic counterions (H^+ and HCO^-_3), against which uptake of the ions Na^+ and Cl^- are exchanged at the apical surface (Evans *et al*., 2005 as reported in Wood, 2012).

With respect to mechanisms of chronic toxicity, interference with Na^+ and Cl^- regulation is similar to that seen in acute toxicity where whole-body levels of the two ions gradually decrease (Wood, 2012). Physiological disturbances associated with the decrease are also similar to acute toxicity including decreases in whole-body Na^+ uptake and Na^+/K^+ -ATPase activity and increases in whole-body cortisol and ammonia levels (Brauner and Wood, 2002b; Brauner *et al*., 2003 as reported in Wood, 2012). Furthermore, in fish, silver may induce the detoxifying protein metallothionein, potentially causing decreases in growth, hatching or survival due to increased metabolic costs. In some invertebrate species, silver may inhibit reproduction by disrupting the synthesis of vitellogenin (Wood, 2012).

In marine fish, the main toxicity mechanism appears to involve osmoregulatory failure, as seen in freshwater fish. However, while this failure can be attributed to only one organ (gills) and one function (branchial ionoregulation) in freshwater fish, marine teleosts have two possible target organs (gills and gut) and two possible target functions (branchial ionoregulation and gastrointestinal ionoregulation) (Wood, 2012). This is due to marine fish drinking the aquatic medium continually to remain hypotonic and therefore bringing Ag from the water column in direct contact with the gut surface (Wood, 2012).

4.3. Aquatic toxicity and toxicity modifying factors: an understanding through speciation

Much of the research discussed previously is based on the effects of Ag in laboratory water, often with low ionic strength and typically low in dissolved organic carbon (DOC) or other complexing ligands. In such laboratory testing, $AgNO_3$, which readily dissociates into the free Ag^+ ion, was found to be one of the most toxic of the Ag forms to freshwater species (Bury *et al.*, 1999; Karen *et al.*, 1999; Rodgers *et al.*, 1997). However, under field conditions the levels of free Ag^+ would be very low. Also, it has been well documented that Ag accumulation and toxicity is mitigated by organic and inorganic complexation, and by competing cations for binding sites on the biotic ligand (i.e., fish gills). It is therefore recognized that a generic guideline value with no modifying factors will be conservative and silver complexation as well as the presence of competing cations must be considered to appropriately assess the potential toxicity of silver in natural waters.

In the past, this influence of water chemistry was recognized in the form of a 'hardness correction' where limits were expressed as a function of total water hardness. This relationship was based on limited data, and its shortcomings have been recognized. Today's understanding is that hardness cations (Ca^{2+} and Mg^{2+}) are relatively ineffective in acute studies at reducing Ag accumulation and toxicity (Karen *et al.*, 1999). Hardness has been determined to have a weak protective effect (Davies *et al*., 1978; Erickson *et al*., 1998; Bury *et al*., 1999, as reported in Wood, 2012) and it appears to be mainly attributed to Ca^{2+} rather than Mg^{2+} (Schwartz and Playle, 2001 as reported in Wood, 2012).

Sodium (Na^+) has a protective effect against Ag toxicity. Janes and Playle (1995) showed that Na (added as NaOH) decreased gill Ag^+ accumulation. This effect was seen at relatively high amounts of Na (~37 mg/L), but similar results were found by Morgan *et al.* (1997), and Paquin *et al.* (2002) at levels in the range of 1-37 mg/L. Efforts to investigate the effect of alkalinity on Ag toxicity to *C. dubia* resulted in an increase in LC50 (0.15 to 0.2 µg/L) that was later attributed to the presence of additional Na in the medium (Naddy *et al.*, 2007b). In this study, alkalinity was adjusted using $NaHCO_3$ from 100 to 200 mg/L as $CaCO_3$, which increased the level of Na from 36 to 72 mg/L. Speciation calculations determined that the change in alkalinity did not affect Ag speciation since Ag does not form complexes with CO_3, however the increase in Na led to an increase in LC50. Lam and Wang (2006) showed a clear negative relationship between Ag uptake and Na concentration (0.05 – 10 mM) in exposures with *D. magna*.

Protons (H^+) have also been considered as a possible competing cation for Ag uptake. Janes and Playle (1995) showed that H^+ ions do not compete with Ag^+ for gill binding sites in rainbow trout over a pH range of 4.5-6.8 in ion-poor, low dissolved organic carbon (DOC) water. Though seemingly inconsistent results have been seen with respect to H^+ competition, it is expected that any effect of pH would be due to interactions with DOC. That is, as pH is raised, less H^+ is available to bind to DOC leaving sites open for Ag^+ complexation. Because pH does not have an appreciable effect on inorganic Ag speciation, it is thought that pH changes would have very little effect of Ag bioavailability in low DOC conditions (for a more detailed discussion on DOC, see below).

In comparison to $AgNO_3$, silver inorganic complexes such as silver thiosulphate, silver chloride and silver sulphide were found to exert very low toxicity (LeBlanc *et al.*, 1984; Hogstrand *et al.*, 1996), indicating the effect of complexation on silver toxicity. As a Group-B metal, inorganic speciation of Ag is expected to be controlled by complexation by sulphides and chlorides. As discussed previously, reduced sulphur is a strong inorganic ligand binding Ag with a Log*K*Ag-S of ~13. Studies by Bianchini and Bowles (2002) and Bianchini and Wood (2008) demonstrate protective effects of sulphide on silver toxicity, and highlight the need to include reduced sulphur in the Biotic Ligand Model (BLM). Bianchini and Bowles (2002) demonstrate the importance of sulphide as a ligand, even in fully oxygenated aquatic systems in which reduced sulphur tends to be unstable.

The presence of natural organic matter (NOM; measured as DOC, in mg C/L) in natural waters is probably the most important complexing ligand, other than sulphide, for Ag and most other metals. In contrast to AgCl complexes, Ag-NOM complexes are large in size and as such, their ability to passively diffuse through a target membrane is limited. Studies with Aldrich humic acid (commonly used as a surrogate for NOM) have revealed a large protective effect against Ag toxicity. For example, work with fathead minnows exposed to Ag in the presence or absence of DOC has resulted in ~4-fold increase in LC50 (Karen *et al.*, 1999; Bury *et al.*, 1999; Erickson *et al.*, 1998). Similarly, Ag toxicity in invertebrates is also greatly reduced in the presence of DOC (Karen *et al.*, 1999; Glover *et al.*, 2005a; 2005b; Naddy *et al.*, 2007b). Since Aldrich humic acid is low in sulphide, there is some question as to whether some laboratory results underestimate the impact of DOC in natural waters. Therefore, efforts have been made recently to use NOM sourced from natural waters, concentrated from large volumes of water and reconstituted in lab water (Glover *et al.*, 2005a). An interesting characteristic of NOM is its variability depending on its source. This variability also translates into its effectiveness in reducing Ag toxicity. Currently, it appears that relatively simple optical measurements that reflect the aromaticity of NOM, such as fluorescence index, may correlate to reduction in metal toxicity, including Ag (Schwartz *et al.*, 2004; Glover *et al.*, 2005b). An interesting indirect effect of Ca^{2+} to Ag^{+} toxicity is the possibility that Ca^{2+} can compete with Ag^{+} for available binding sites on DOC, thus reducing the protective effect of DOC against Ag. This is supported by results with *D. magna* done by Karen *et al.*, 1999 where increasing Ca^{2+} concentrations led to an actual decrease in Ag LC50. Recent work by Naddy *et al.*, 2007b with *Ceriodaphnia dubia* are consistent with this result as well. In natural waters, however, levels of Ag would rarely exceed the capacity for sulphide complexation (Adams and Kramer, 1999), and if this were to occur, NOM would be present to bind up any additional Ag, making any effects of Ca^{2+} described above unlikely.

Inorganic complexation by Cl^{-} can effectively reduce the bioavailability of Ag and its toxicity, however, it depends on which Ag-chloro-complex is present and varies among aquatic life species. A number of different chloro-complexes will form, with AgCl being the dominant species when Cl ranges from 10 to 300 mg/L and the $AgCl_2^{-}$ complex predominant at higher Cl^{-} concentrations. Though Cl^{-} levels do not often exceed 100 mg/L in fresh water, the exception would be near wastewater discharge points or in areas where fresh water meets an estuary. The complicating factor with Cl^{-} is that the small, neutral AgCl complex is readily taken up by aquatic organisms by passive diffusion, though its contribution to toxicity appears to be minimal. Many studies that have tried to clarify the mitigating effect of Cl^{-} on Ag toxicity have been

confounded by the accompanying cation, i.e., many have used NaCl as the source of Cl^-. Therefore, any reduction in toxicity seen is likely partially due to the Na present in solution. Investigations on the protective effect of Cl^- (0.3 to 43 mg/L) with various species of freshwater fish (*P. promelas*, *F. heteroclitus* and *D. rerio*; Bielmyer *et al.* (2007) and the European Eel (*Anguilla anguilla*; Grosell *et al.*, 2000) found that Cl^- offers little protection, which is inconsistent with studies using rainbow trout (Galvez and Wood, 1997; McGeer and Wood, 1998). It appears that, with fish, only rainbow trout are protected from Ag toxicity by Cl^-. Invertebrates (*C. dubia* and *D. magna*), however, seem to benefit from Cl^- but any protective effects are only seen when Cl^- concentrations are higher than 50 mg/L (Karen *et al.*, 1999; Naddy *et al.*, 2007a).

Water quality criteria that are based on total metal concentrations regardless of water chemistry cannot account for the toxicity modifying factors discussed above. The acute toxicity of Ag is largely based on the concentrations of free Ag^+, and the key characteristics that determine this are DOC, reduced sulphide and Cl^-. A tool with the most promise of incorporating these determinants is the Biotic Ligand Model (BLM). The model is based on the early Free Ion Activity Model (FIAM; Morel, 1983) which focussed on cationic metal binding to critical sites, and recognized the importance of DOC complexation. However, in addition to complexation, the BLM recognizes competitive effects of other cations present in water.

Several BLMs have been developed for predicting the acute toxicity of silver in freshwater species. The first was developed by Hydroqual (Paquin *et al*., 1999) and integrated acute toxicity data for rainbow trout and fathead minnow with 2-hour gill binding data, and was adapted for daphnids by downwardly adjusting the lethal accumulation (LA50) value (Wood, 2012). The model was able to predict within a factor of two the variations in 96-h LC50 values based on water levels of Cl^-, Ca^{2+}, and DOC. However, this version of the BLM uses an assumed LA50 value that is higher than what has since been measured, and a lower log *K* value (Wood, 2012). These factors may explain why the model has tended to under predict toxicity to fathead minnows in soft water, as shown by a validation study in natural waters by Bielmeyer *et al*. (2007). The second acute BLM version (McGeer *et al.*, 2000) is physiologically based and predicts toxicity in fish from inhibition of gill Na^+/K^+-ATPase rather than based on the total Ag burden at the gill. It was tested over a range of water chemistry parameters including Cl^-, Ca^{2+}, Na^+, pH and DOC, and the vast majority of predicted values were within a factor of two of measured values (Wood, 2012). Finally, there is an acute BLM developed by Bury *et al*. (2002) that is directly based on daphnid toxicity data. Currently there is no BLM for predicting the chronic toxicity of silver to freshwater organisms (Wood, 2012). The use of BLMs in the derivation of CWQGs is currently under examination and will be a consideration for future guidelines.

4.4. Toxicity interactions with other substances and metals

Until recently, much focus has been placed on the effects of single metals in laboratory exposures. However, aquatic biota undoubtedly face metal mixtures in the field, especially at impacted sites, and thus the toxicity of mixtures containing metals and other substances is a

relatively new and valuable area of research. To date, there is limited information available on the effect of interactions of potential toxicants.

Bertram and Playle (2002) studied the effect of silver exposure to fed and unfed rainbow trout (*Oncorhynchus mykiss*). No significant physiological differences were found between the fed and unfed fish. This study concluded that once Ag enters the fish, the subsequent elimination is not affected by food-related process such as increased metabolic rate or food debris in water.

A study examining the interactions of five trace elements on bioaccumulation in zebrafish (*Brachydanio rerio*) in 12-d waterborne exposures revealed that Ag bioaccumulation is proportional to water Ag concentration and Ag has a negative effect (approx. 30% decrease) on Hg accumulation (as MeHg; Ribeyre *et al.*, 1995). Mercury concentrations in the fish decrease when the concentration of Ag increases, in a nonlinear fashion. This antagonistic effect of Ag on Hg accumulation is not likely due to competition between ions at metallothionein binding sites since the affinity of MT for methyl-Hg (MeHg) is very low. The study also concluded that Zn, Cu and Se had a significant positive effect on Ag bioaccumulation, increasing Ag concentrations by 10% when compared with Ag accumulation in isolated Ag exposures. They made no attempt at reconciling accumulation with toxicity.

In model simulations with multiple metals using MINEQL+, Playle (2004) showed that in conditions where metal concentrations are low (total concentration of all metals are less than 1 toxic unit (TU), or the expected level that would result in <50% mortality) there would be greater than strict additivity or a "synergism" in metal accumulation. When total metal concentrations are 'intermediate', i.e., total metal concentration would yield 50% mortality or 1 TU, the model predicts strict additivity, whereas at high metal concentrations (> 1 TU) the expected result is less than strict additivity, or an "antagonistic" effect on accumulation. Generally, this is accounted for by the nonlinearity of the BLM, where at low concentrations, there is 'easy' binding to the biotic ligand (high affinity sites) and at higher metal concentrations the high affinity sites are filled first and subsequently the lower affinity sites are filled.

To date, there are not enough data to be able to incorporate synergistic or antagonistic effects of other substances into a revised CWQG for silver.

4.5. Short-term and long-term toxicity of silver

A number of studies have elucidated the long-term impacts of Ag on early life stages of rainbow trout. These studies investigated the accumulation and distribution in eggs or whole body, the mechanisms of toxicity, relative sensitivities of different life stages, and the effect of water chemistry on Ag toxicity.

Guadagnolo *et al.* (2001) showed that the stage of development in the rainbow trout embryo is an important factor in sensitivity to Ag. The chorion of the rainbow trout embryo is a protein-rich membrane (~14% total protein), containing amino acids such as proline, glutamic acid and cysteine. Sulphydryl-rich cysteine plays a particularly important role in defending the embryo against Ag toxicity by limiting the rate by which Ag enters the egg (Guadagnolo *et al.*, 2000).

The majority of the silver accumulated in the egg was in the chorion (65-85%). The long-term physiological effect of Ag exposure to rainbow trout embryos and larvae appear to be similar to that of short-term effects, where there is an impairment in the organism's ability to regulate Na^+ and Cl^- balance, by a reduction in NKA activity (Brauner and Wood, 2002a).

Physiological effects in juvenile rainbow trout to long-term exposure of silver nitrate in moderately hard fresh water were studied by Galvez *et al.* (1998). Exposure length of the study was 28 days for two different concentrations of silver (0.5 and 2.0 µg/L) added to dechlorinated tap water. At the lower concentration of silver exposure (0.5 µg/L) fish showed a small increase in food consumption (15%) and no change in growth rates while the 2.0 µg/L Ag exposure resulted in a 29% decrease in food consumption and 43% reduction in growth rate. Sodium and chloride levels in the plasma decreased significantly at day 16 and day 7 of exposure for the low and high silver exposure, respectively, but recovered thereafter. For the high silver exposure, accumulation of silver increased gradually in the liver up to day 15 when the wet weight of Ag reached 39.7 µg/g (285-fold higher than controls). Also, MT levels increased by 81% by day 7, and mortality reached 15% by the end of the exposure period (Galvez *et al.*, 1998).

Galvez and Wood (2002) investigated effect of silver exposure on juvenile rainbow trout, during a 23-day exposure to 0, 0.1, 1, 3 and 5 µg/L of silver nitrate. The 5 µg/L exposure showed significant toxic effects in terms of growth rate, food consumption, food-conversion efficiency and the critical swimming speed. Although plasma levels of Na^+ and Cl^- decreased initially, the levels came back to normal by the end of the exposure period. Based on their results, they concluded that changes in Na^+ transport at gills cause physiological acclimation and may eventually lead to toxicological acclimation (Galvez and Wood, 2002).

Invertebrates tend to be more sensitive to metals when compared to fish. *Ceriodaphnia dubia* and *D. magna* are listed as the two most acutely sensitive species in the 1998 U.S. EPA draft silver Ambient Water Quality Criteria (AWQC) update, with genus mean acute values of ~1 and 2 µg/L, respectively. Because of their high sensitivities, these crustacea are important organisms to study, however their small size has been a limiting factor for detailed physiological studies. On the basis of long-term toxicity, it appears that relative sensitivity to Ag and the toxicokinetics of Ag toxicity is similar. In one study with *D. magna*, neonates (newly hatched offspring) were exposed to 2 µg/L dissolved Ag over 21-days and various reproductive and physiological endpoints were observed, such as survival, growth, time to first brood, mean young produced per adult, number of broods produced, mean number of young per brood, whole body Na^+ and NKA activity. There was a 20% mortality in the Ag-exposed group and a 14% decrease in neonates produced per adult per productive day. The remaining reproductive endpoints were not affected. Ionoregulation in *D. magna* was affected, however, with an 81% inhibition of Na^+ influx and a 65% decrease in whole body Na^+ content despite a 60% increase in whole body NKA activity. This overall increase in NKA activity is suspected to be due to acclimation, i.e., an increased synthesis of NKA in response to decreasing Na^+ levels. One main difference in the ionoregulatory response of invertebrates is the lack of Cl^- disturbance (Bianchini and Wood, 2003). In this regard, the response of crustaceans to Ag appears to differ from rainbow trout.

It is worth noting that an eminent researcher in the aquatic toxicity of silver (Wood 2012) observed that the differences in testing procedures for acute versus chronic testing using

invertebrates (organisms were not fed during acute exposures) resulted in the perception that acutely lethal responses occurred at silver concentrations the same as or lower than those causing chronic sublethal toxicity. This would be contrary to the usual acute-to-chronic ratios for most substances. In fact, he said, the results with fish confirmed that chronic sublethal effects occurred at about one tenth the concentrations than caused acute lethality. This has resulted in some undue regulatory concern. This fact was considered in detail in deciding not to publish the short-term benchmark for silver in fresh water (see below).

4.6. Marine life

There is a marked difference in the toxicity of silver in brackish and marine waters compared with fresh water. Until recently there has been little effort put forth to study Ag toxicity in marine environments, most likely because of the relatively high short- and long-term toxicity of Ag in fresh water compared to saltwater environments.

To discuss Ag toxicity in marine settings, it is important to understand two key differences from Ag toxicity in freshwater environments. First, is the physiological difference between freshwater fish, for example, and marine fish. The site of Ag toxicity in freshwater fish is the gill, since this is the principal site of ionoregulation. In fresh water, fish are constantly pumping ions into the blood to combat losses due to differences in osmolality between the blood and surrounding water. Thus, when Ag disrupts ionoregulation, ion-loss will occur resulting in eventual death. In contrast, marine teleosts experience osmostically driven water loss across the gill, due to the higher osmolality in the ambient water compared to the blood. To combat water loss, marine fish need to take in water to replace lost fluid. This is achieved by actively transporting ions in the gut so water follows by osmosis. They then excrete excess ions at the gills and kidney. This fundamental difference leads to Ag exposure in the gut rather than the gills (Wood *et al.*, 1999).

The second consideration when discussing Ag toxicity in marine organisms is the speciation of Ag. Silver is highly influenced by Cl^- in brackish and marine waters, while complexation with organic matter, Br^- and I^- are negligible. In fresh water, Ag has a high affinity for particulates, however, in waters with higher salinity, the vast majority (>90%) of Ag is complexed by Cl^- and very little is contained within the filterable organic colloids and particles (Pedroso *et al.*, 2007). Though the small, neutral AgCl complex is known to enter biological membranes via passive diffusion, the formation of higher order chloro-complexes will occur, to a point where in full strength seawater (35‰ salinity), 83% of Ag is present as $AgCl_3^{2-}$ (0.0003% as Ag^+; Ward and Kramer, 2002). This Ag-chloro-complexation appears to reduce toxicity. The 96-h LC50 values for *Americamysis bahia* calculated from 28-d and 7-d toxicity tests initiated with 7-d old mysids were 260 µg/L Ag at 20‰. Shaw *et al*. (1998) report a 96-h Ag LC50 of 3.07 – 6.2 µM (331-669 µg/L) for tidepool sculpins (*Oligocottus maculosus*) over a salinity range of 25 to 32‰. Results from Nichols *et al.* (2006) demonstrate a constant decrease in Ag accumulation and the reduced importance of NOM to Ag toxicity in gulf toadfish (*Opsanus beta*) with increasing salinity. Work by Pedroso *et al.* (2007) reported a 20-fold increase in LC50s (7.1-156.7 µg/L dissolved Ag) with the euryhaline copepod, *Acartia tonsa*, with increasing salinity (5-30‰).

5.0. DERIVATION OF CANADIAN WATER QUALITY GUIDELINE FOR SILVER

5.1. Guidelines currently used in other jurisdictions

The last published CWQG for silver in fresh waters was 0.1 µg/L (CCREM 1987). The U.S. EPA is revising the criteria for silver. Currently, the U.S. EPA's national recommended acute water quality criteria (1980) for silver in fresh water is based on water hardness and calculated using the equation; $e^{[1.72\ (\ln \text{hardness})-6.52]}$

Since 1992, this acute value is defined as; $e^{[1.72\ (\ln \text{hardness})-6.52]} / 2$

For marine water, the chronic criterion is 1.9 µg/L.

The British Columbia Ministry of Environment has recommended criteria for the protection of marine and freshwater life that are also related to water hardness. For fresh water with a hardness less than or equal to 100 mg $CaCO_3$/L, the 30-day mean criterion for Ag is 0.05 µg/L with a maximum of 0.1µg/L. Fresh water with a hardness greater than 100mg/L, the 30-day mean criterion for Ag is 1.5 µg/L, with a maximum of 3µg/L. The criterion for marine waters are the same as fresh waters with > 100 mg/L hardness (Warrington, 1996). The acute freshwater guidelines for Alberta and Québec are based on the U.S. EPA hardness corrected criteria. For the chronic value, Québec used the CCME value. In Australia and New Zealand, the approach is based on the level of ecosystem protection that is desired. The highest level of protection used in their approach is the protection of 99% of species. At this level of protection, the 'trigger' values for Ag are 0.02 and 0.8 µg/L in fresh and marine waters, respectively (ANZECC, 2000). The UK non-statutory environmental quality standards (EQS) for protection of aquatic life are based on total dissolved silver. The freshwater annual average should not exceed 0.05 ug/L, and the freshwater maximum acceptable concentration is 0.1 ug/L. The marine EQS values are interim, with an annual average not to be exceeded of 0.5 ug/L, and a marine maximum acceptable concentration of 1.0 ug/L (UK Environment Agency, 2011).

5.2. Data summary and guideline derivation

Type A guidelines employ the use of a regression-based approach called a species sensitivity distribution (SSD). Toxicity data are collected and, the most preferred and/or sensitive endpoint per species is plotted as per the Protocol (CCME, 2007) to ensure only one data point per species is represented on the SSD. To account for intra-species variability, species mean values can be calculated (geometric mean of similar toxicity data points) where applicable and if experimental conditions are comparable. This was not the case with the silver datasets, as factors affecting metal toxicity were either variable or unreported between endpoints, and hence the most sensitive endpoint per species was plotted in the SSD. The data are plotted in an SSD and one of five regression models (Normal, Logistic, Extreme Value, Gumbel and Weibull) are chosen as the best fit to the distribution using statistical and graphical techniques. The model chosen as the best fit to the data is based on goodness-of-fit and model feasibility, including 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, as well as overall visual assessment of model fit. The guideline for both short- and long-term exposure is defined as the intercept of the 5th percentile of the y-axis with the fitted SSD curve. The software package, "SSD Master version 3.0" (CCME, 2013) was used to generate the SSDs, and included five cumulative distribution functions (Normal, Extreme Value, Weibull, Logistic and Gumbel).

Available toxicity data for Ag in fresh water are compiled in Appendix A. For both short- and long-term freshwater guidelines, the available data met the minimum requirements for the derivation of a Type A guideline (e.g., derived using a species sensitivity distribution) (Tables 5.0 and 5.1). Toxicity data for marine waters are found in Appendix A. Minimum data requirements were also met to derive a Type A short-term marine water guideline, however there were insufficient data to generate a guideline for long-term effects in marine water (Tables 5.2 and 5.3). This is due to a lack of available fish data.

Table 5.0. Minimum data set requirements for the generation of a short-term benchmark concentration for freshwater environments (CCME 2007).

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 non-target 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	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.

	adequately describe data and pass the appropriate goodness-of-fit test.		

Table 5.1. Minimum data set requirements for the generation of a long-term guideline value for freshwater environments (CCME 2007).

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	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 non-target 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 non-target 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	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 preferred endpoint for a	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.	

Group	Guideline		
	Type A	**Type B1**	**Type B2**
	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 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.

Table 5.2. Minimum data set requirements for the generation of a short-term benchmark concentration for marine environments (CCME 2007).

Group	Guideline		
	Type A	**Type B1**	**Type B2**
Fish	At least three studies on three or more marine fish species, at least one of which is a temperate species.		At least two studies on two or more marine fish species, at least one of which is a temperate species.
Aquatic Invertebrates	At least two studies on two or more marine species from different classes, at least one of which is a temperate species.		At least two studies on two or more marine species.
Plants	At least one study on a temperate marine vascular plant or marine 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 two studies on non-target marine plant or algal species are required.		Toxicity data for marine 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 non-target marine plant or algal species are required.
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.

Table 5.3. Minimum data set requirements for the generation of a long-term guideline value for marine environments (CCME 2007).

Group	Guideline		
	Type A	**Type B1**	**Type B2**
Fish	At least three studies on three or more marine fish species, at least one of which is a temperate species.		At least two studies on two or more marine fish species, at least one of which is a temperate species.
Aquatic Invertebrates	At least two studies on two or more marine species from different classes, at least one of which is a temperate species.		At least two studies on two or more marine species.
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 non-target freshwater plant or algal species are required.	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 two studies on non-target freshwater plant or algal species are required.	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 non-target freshwater plant or algal species are required
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}.	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}.	

Group	Guideline		
	Type A	**Type B1**	**Type B2**
	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.		
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.

5.2.1. Evaluation of toxicological data

Data were categorized as primary or secondary based on the guidance given in the CCME draft protocol for derivation of a water quality guideline (CCME, 2007). Primary data, at a minimum, included measured Ag concentrations, and included measurements of relevant water quality variables such as Ca, Mg, Na, pH, dissolved oxygen, dissolved organic matter (as DOC).

Table 5.4. Data summary for the derivation of short- and long-term guidelines for marine and fresh water.

Guideline	No. endpoints examined	No. endpoints selected	No. fish species req'd for 'Type A' guideline	No. fish species included	No. invertebrate species req'd for 'Type A' guideline	No. invertebrate species included	No. plant/algal species req'd for 'Type A' guideline	No. plant/algal species included
Short-term Freshwater	355	198	3	6	3	10	0*	2
Long-term Freshwater	52	13	3	4	3	4	1	1
Short-term Marine	81	60	3	7	2	10	1	2

*none are *required*, but data is highly desirable

5.2.2. Derivation of the short-term benchmark concentration (fresh water)

The short-term benchmark concentration is intended to protect most species against lethality during severe, but transient events such as spills or inappropriate use/disposal of the substance in

question. To generate a Type A short-term freshwater benchmark concentration, data must include three species of fish, at least one salmonid and one non-salmonid, and three aquatic (or semi-aquatic) invertebrate species. Data for aquatic plants or algae, as well as for amphibians are desirable, but not mandatory (Table 5.4). Available data for the short-term freshwater SSD included 10 invertebrate species, 6 fish species, and 2 algae species.

The 5th percentile of the short-term freshwater SSD is 0.22 µg Ag/L (Table 5.5). Various models were fit to the data including the Normal, Logistic, Extreme-Value and Gumbel models. 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, as well as overall visual assessment of model fit. Using these methods, the Logistic model (below) was the best fit and the Anderson-Darling goodness-of-fit Statistic was 0.141. The equation of the Logistic model is;

$$f(x) = \frac{1}{1 + e(-\frac{x - \mu}{\sigma})}$$

where in the case of the fitted model, $\mu = 1.026$, and $\sigma = 0.574$ (Figure 5.1). Each species for which appropriate toxicity data were available was ranked according to sensitivity (from lowest to highest), and its centralized position on the SSD (Hazen plotting position) was determined using the following equation (Alderberg *et al.*, 2002; Newman *et al.*, 2002):

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

Where

i = the species rank based on ascending toxicity values
N = the total number of species included in the SSD.

Table 5.5. Final aquatic toxicity data* selected for short-term freshwater SSD.

Rank	Common Name	Scientific Name	Life Stage	Reported Endpoint	Concentration (µg/L Ag) (Variation)	Reference
1	Cladoceran	*Ceriodaphnia dubia*	<24-h old	48-h LC50	0.16	Naddy *et al*. 2007c
2	Cladoceran	*Daphnia magna*	<24-h old	48-h LC50	0.26	Bianchini *et al*., 2002a
3	Green algae	*Chlamydomonas reinhardtii*	n/a	6-h EC50 (Growth) ‡	1.29 (95% CI 1.19-1.4)	Lee *et al*., 2005
4	Rainbow trout	*Oncorhynchus mykiss*	Juvenile	96-h LC50	1.48	Karen *et al*., 1999
5	Fathead minnow	*Pimephales promelas*	Larvae	96-h LC50	1.99	Bielmyer *et al*., 2007
6	Green algae	*Pseudokirchneriella subcapitata*	n/a	6-h EC50 (Growth) ‡	2.8 (95% CI 2.27-3.34)	Lee *et al*., 2005
7	Scud	*Gammarus pseudolimnaeus*	0.67 cm	48-h LC50	4.7 (95% CI 3.8-5.8)	Lima *et al*., 1982
8	Ciliate	*Spirostomum ambiguum*	n/a	48-h LC50	8.8 (± SD 3.7)	Nalecz-Jawecki and Sawicki, 1998
9	Flagfish	*Jordanella floridae*	30-d old	96-h LC50	10.7	Lima *et al.,* 1982
10	Bluegill	*Lepomis macrochirus*	Juvenile	96-h LC50	13 (95% CI 9-20)	Holcombe *et al.,* 1987
11	Channel catfish	*Ictalurus punctatus*	Juvenile	96-h LC50	17.3 (95% CI 15.5-19.2)	Holcombe *et al.,* 1983
12	Cladoceran	*Simocephalus sp.*	n/a	48-h LC50	27	Hook and Fisher, 2001a
13	Leech	*Nephelopsis obscura*	1.3 g	96-h LC50	29 (95% CI 20-42)	Holcombe *et al.,* 1987
14	Eel	*Anguilla anguilla*	Adult	96-h LC50	34.4 (95% CI 31.07-39.05)	Grosell *et al.,* 2000
15	Crayfish	*Cambarus diogenes diogenes*	Adult	96-h LC50	65.9	Bianchini *et al.,* 2002b
16	Snail	*Aplexa hypnorum*	Adult	96-h LC50	83	Holcombe *et al*., 1987
17	Midge	*Tanytarsus dissimilis*	Larvae	48-h LC50	420	Holcombe *et al..,* 1987
18	Crayfish	*Orconectes immunis*	Adult	96-h LC50	560 (95% CI 450-690)	Holcomble *et al.,* 1987

* All studies utilized $AgNO_3$ salt.

‡This study used a shorter than normal test duration to limit Ag depletion in the test media. Their EC50 values are comparable to, but among the lowest, reported EC50s in the literature based on nominal Ag concentrations.

Additional study details can be found in Appendix A.

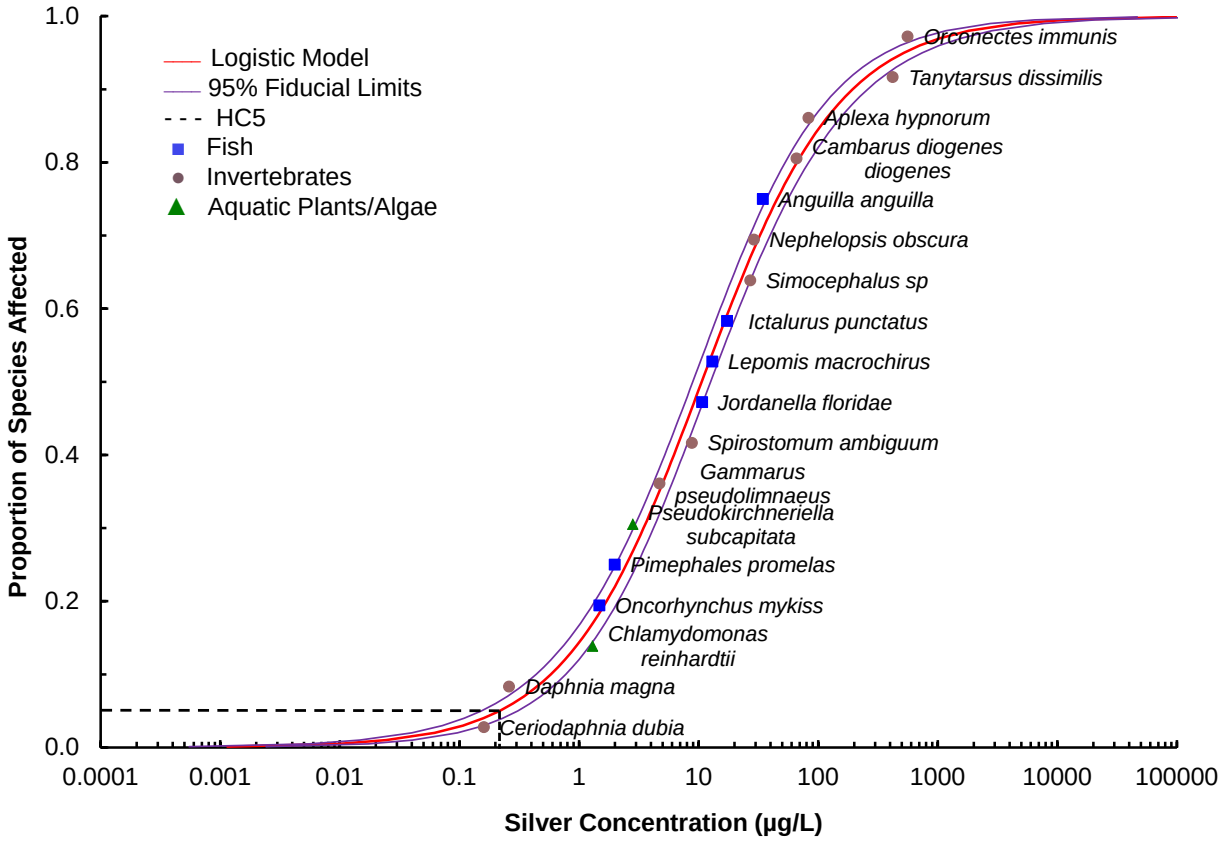

Figure 5.1 Species sensitivity distribution for short-term Ag toxicity in fresh water derived by fitting the logistic model to the logarithm of acceptable toxicity data for 18 aquatic species versus Hazen plotting position (proportion of species affected). The dashed line denotes the 5th percentile.

Table 5.6. Short-term freshwater 5th percentile[1] for silver resulting from the SSD Method. (LFL=lower fiducial limit; UFL=upper fiducial limit)

	Ag Concentration (µg/L)
SSD 5th percentile	0.22
SSD 5th percentile, 95% LFL	0.15
SSD 5th percentile, 95% UFL	0.31

[1] The short-term freshwater 5th percentile was derived based on the total concentration of Ag.

The data used to derive the short-term freshwater SSD shows both algal species and the cladocera, *C. dubia* and *D. magna*, are among the most sensitive. One might note that the *C. dubia* effective concentration falls below the HC5. In fact, 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. Short-term benchmarks are meant to protect a specified fraction of organisms from severe effects and to provide guidance on the impacts of severe, transient events (CCME, 2007). Note that meeting the long-term guideline will protect from severe as well a more subtle toxic effects.

Based on the SSD 5th percentile concentration, no short-term freshwater benchmark is recommended for silver. Because the short-term SSD 5th percentile (0.22 µg/L) and the long-term SSD 5th percentile and CWQG (0.25 µg/L) (see Section 5.2.4) are essentially equal, no designated short-term benchmark concentration is recommended. Generally, one expects the short-term benchmark to be higher than the long-term guideline, as shorter exposure durations for most chemicals require higher concentrations to cause an effect. The closeness of the short-term and long-term 5th percentile values can be explained by low endpoints included in the short-term SSD that are from experiments conducted in reconstituted waters and in the absence of food. Reconstituted waters reflect highly bioavailable conditions (with limited complexing of silver) not seen in natural waters, and which result in low endpoint values. The absence of food during short-term exposures also represents highly bioavailable conditions with limited complexing. In contrast, long-term exposures necessitate feeding of test organisms, which results in complexation of silver by food particles, and consequently, reduced toxicity. While this concept is true for all metals, it is especially relevant for silver due to the strong relationship between binding affinity and toxicity.

5.2.3. Derivation of the short-term benchmark concentration (marine)

The short-term benchmark concentration is intended to protect most species against lethality during severe, but transient events such as spills or inappropriate use/disposal of the substance in question. To derive a Type A marine short-term guideline, at least three marine fish species must be included, one of which must be temperate species. Two species of marine invertebrates and one species of a marine vascular plant or algae are also required. Data for the Type A short-term marine guideline included 7 fish species, 10 invertebrate species, and 2 algal species. Since the presence of chloride can affect Ag toxicity (see sections 4.3, 4.6) the available data were categorized into salinity ranges. Our salinity categorization was based on guidance provided in

Environment Canada's Biological Test Methods (e.g., EPS 1/RM 10, July 1990). Full strength marine water has a total dissolved salt concentration of >20‰, and brackish water is defined as having a salt concentration of 10 – 20‰. Here, we define salt concentrations of <10‰ as 'dilute brackish'. The calculated short-term benchmark concentration is 7.51 µg Ag/L using the species sensitivity distribution approach (Table 5.8). Various models were fit to the data including the Normal, Logistic, Extreme-Value and Gumbel models. 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, as well as overall visual assessment of model fit. Using these methods, the Normal model (below) was the best fit and the Anderson-Darling goodness-of-fit Statistic was 0.349. The equation of the Normal model is;

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

where in the case of the fitted model, $\mu = 2.014$, $\sigma = 0.692$, and *erf* is the error function (Figure 5.2). Each species for which appropriate toxicity data were available was ranked according to sensitivity (from lowest to highest), and its centralized position on the SSD (Hazen plotting position) was determined using the following equation (Alderberg *et al.*, 2002; Newman *et al.*, 2002):

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

Where

i = the species rank based on ascending toxicity values

N = the total number of species included in the SSD.

Table 5.7. Final aquatic toxicity data* selected for short-term marine SSD.

Rank	Common Name	Scientific Name	Life Stage	Reported Endpoint	Concentarion (µg/L Ag) (Variation)	Reference
1	Eastern oyster	*Crassostrea virginica*	Embryo	48-h LC50	5.8	Calabrese *et al.,* 1977
2	Pacific oyster	*Crassostrea gigas*	Larvae	48-h LC50	19	Dinnel *et al.,* 1983
3	Hard clam	*Mercenaria mercenaria*	Embryo	42-48-h LC50	21	Calabrese *et al.,* 1977
4	Dinoflagellate	*Gymnodinium splendens*	n/a	48-h LC50	21	Wilson and Freeburg, 1980
5	Purple-spined sea urchin	*Arbacia punctulata*	Adult	96-h EC50	40	Ward *et al.,* 2006a
6	Copepod	*Acartia tonsa*	Adult	48-h LC50	43.2	Hook and Fisher, 2001
7	Copepod	*Acartia hudsonica*	Adult	48-h LC50	43.2	Hook and Fisher, 2001
8	Mysid	*Americamysis bahia*	<48-h old	96-h LC50	65	Schimmel 1981
9	Haptophyte	*Isochrysis galbana*	n/a	48-h LC50	81	Wilson and Freeburg, 1980
10	Copepod	*Tigriopus brevicornis*	Adult	96-h LC50	95 ±2	Pavillon *et al*., 2002
11	Spiny dogfish	*Squalus acanthus*	Adult	96-h LC50	100	DeBoeck *et al*., 2001
12	Polychaete	*Neanthes areanaceodentata*	Adult	96-h LC50	145	Pesch and Hoffman 1983
13	Tidepool sculpin	*Oligocottus maculosus*	Juvenile	96-h LC50	331	Shaw *et al*., 1998
14	Shiner perch	*Cymatogaster aggregata*	Adult	96-h EC50 (Immobility)	356 (95% FL 282-452)	Dinnel *et al*., 1989
15	Rainbow trout	*Oncorhynchus mykiss*	Juvenile	96-h LC50	401.5 (95% CL 343.9-473.2)	Ferguson and Hogstand, 1998
16	Sheepshead minnow	*Cyprinodon variegatus*	Juvenile	96-h LC50	441	Schimmel 1981
17	Coho salmon	*Oncorhynchus kisutch*	Adult	96-h EC50 (Immobility)	488 (95% FL 405-590)	Dinnel *et al*., 1989
18	Polychaete	*Hediste diversicolor*	Adult	96-h LC50	647	Mouneyrac *et al.,* 2003
19	English sole	*Paraphrys vetulus*	Juvenile	96-h EC50	800	Dinnel *et al.,* 1983

* All studies utilized $AgNO_3$ salt.
Additional study details can be found in Appendix A.

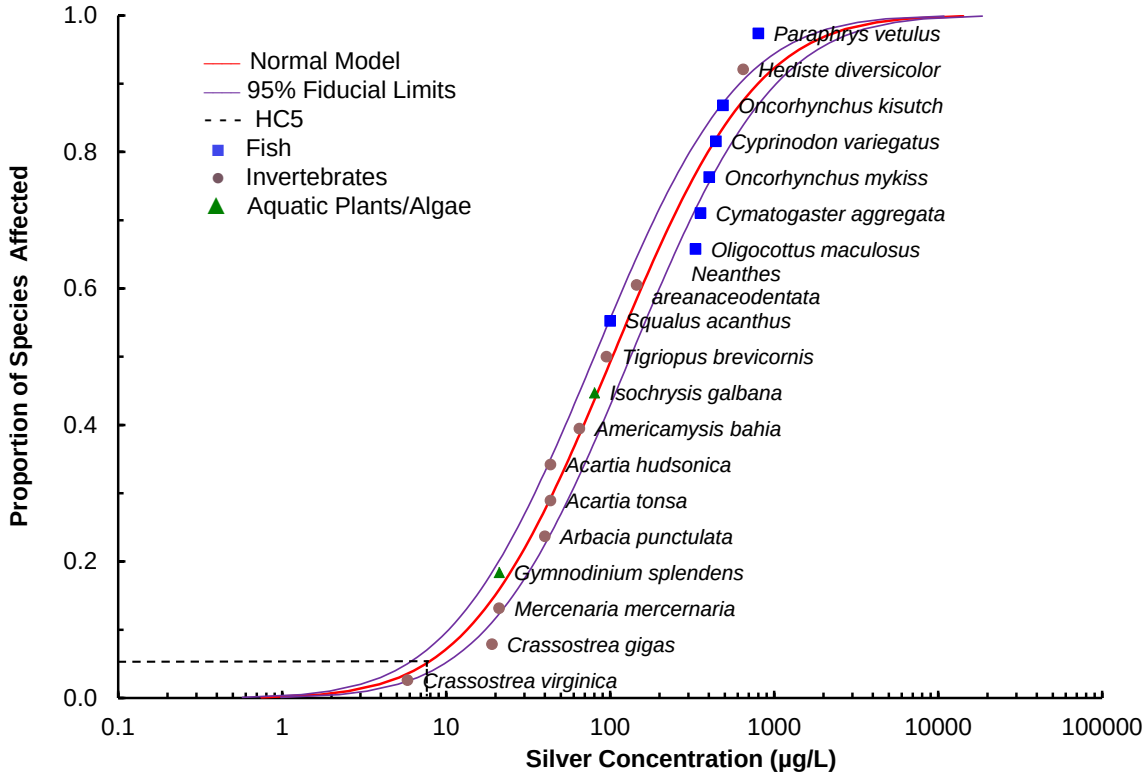

Figure 5.2 Species sensitivity distribution for short-term Ag toxicity data in marine environments derived by fitting the Normal model to the logarithm of acceptable toxicity data for 19 aquatic species versus Hazen plotting position (proportion of species affected). The dashed line at the bottom of the graph denotes the 5th percentile and the corresponding short-term benchmark concentration value.

As can be noted in Figure 5.2, it appears that, in marine environments, invertebrate and algal species tend to be more sensitive to Ag than fish species. This ranking is similar to that found in fresh water, where fish appear more tolerant. One data point (for *Crassostrea virginica*) falls below the short-term marine benchmark concentration of 7.51. 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 benchmark is meant to protect a specified fraction of organisms from severe effects and to provide guidance on the impacts of severe, transient events, this benchmark concentration is acceptable (CCME, 2007). Note that meeting the long-term guideline will protect from severe effects.

Table 5.8. Short-term marine 5th percentile = benchmark concentration[1] for silver resulting from the SSD Method. (LFL= lower fiducial limit; UFL= upper fiducial limit)

	Ag Concentration (µg/L)
SSD 5th percentile	7.5
SSD 5th percentile, 95% LFL	5.8
SSD 5th percentile, 95% UFL	9.7

[1] The short-term marine benchmark concentration was derived based on the total concentration of Ag.

5.2.4. Long-term guideline derivation (fresh water)

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 the 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/sediments and gradual entry through groundwater/runoff, and long range transport. Data requirements for Type A long-term freshwater guidelines are similar to the long-term marine requirements. For both, 3 species of fish and 1 plant or algal species are required. One salmonid fish species and 1 non-salmonid are required for fresh water, and at least one temperate species is needed for the marine guideline. For fresh water, 3 invertebrate species are required, whereas for the marine guideline 2 are needed. Primary data were preferentially used in deriving the guideline. Table 5.9 summarizes the endpoints included in the derivation of the long-term freshwater guideline.

Table 5.9. Summary of endpoints used for the long-term freshwater SSD.

Environment	LC_{1-10}	IC/EC_{11-25}	MATC	NOEC	IC/EC_{25-49}	IC/EC50
Fresh water	2	1	4	2	0	0

The calculated long-term freshwater guideline is 0.25 µg Ag/L (Table 5.11), using the species sensitivity distribution approach presented in Figure 5.3. Various models were fit to the data including the Normal, Logistic, Extreme-Value and Gumbel models. 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, as well as overall visual assessment of model fit. Using these methods, the Gumbel model (below) was the best fit and the Anderson-Darling goodness-of-fit Statistic was 0.181. The equation of the Gumbel model is;

$$f(x) = e^{-e^{\frac{(L-x)}{s}}}$$

Where in the fitted Gumbel model, L = 0.007, and s= 0.548 (Figure 5.3). Each species for which appropriate toxicity data were available was ranked according to sensitivity (from lowest to highest), and its centralized position on the SSD (Hazen plotting position) was determined using the following equation (Alderberg *et al.*, 2002; Newman *et al.*, 2002):

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

Where

i = the species rank based on ascending toxicity values

N = the total number of species included in the SSD.

Data for the long-term freshwater guideline included 4 invertebrate species, 4 fish species and 1 plant species.

Table 5.10. Final aquatic toxicity data[1] selected for long-term freshwater SSD.

Rank	Common Name	Scientific Name	Life Stage[2]	Reported Endpoint	Concentration (µg/L Ag) (Variation)	Reference
1	Rainbow trout	*Oncorhynchus mykiss*	ELS	3-month MATC growth	0.24	Davies *et al*., 1978
2	Duckweed	*Lemna gibba*	n/a	7-d MATC frond number	0.63	Bian *et al*., 2013
3	Cladoceran	*Ceriodaphnia dubia*	<24-h old	10-d MATC reproduction	0.78	Rodgers *et a*l., 1997
4	Fathead minnow	*Pimephales promelas*	ELS	28-d MATC growth	0.83	Holcombe *et al.,* 1983
5	Channel catfish	*Ictalurus punctatus*	ELS	9-d LC10	1.9	Birge *et al*., 2000
6	Cladoceran	*Daphnia magna*	Neonate	21-d LC20	2.12 (95% CI 2.05-2.18)	Bianchini and Wood, 2008
7	Amphipod	*Hyalella azteca*	2-3 wk old	10-d NOEC survival	4	Rodgers *et al.,* 1997
8	Midge	*Chironomus tentans*	Larvae	10-d NOEC dry weight	13	Call *et al*., 1999
9	Largemouth bass	*Micropterus salmoides*	ELS	8-d LC10	23	Birge *et al.,* 2000

[1]All studies utilized $AgNO_3$ salt.
[2]ELS = Early Life Stage
Additional study details can be found in Appendix A.

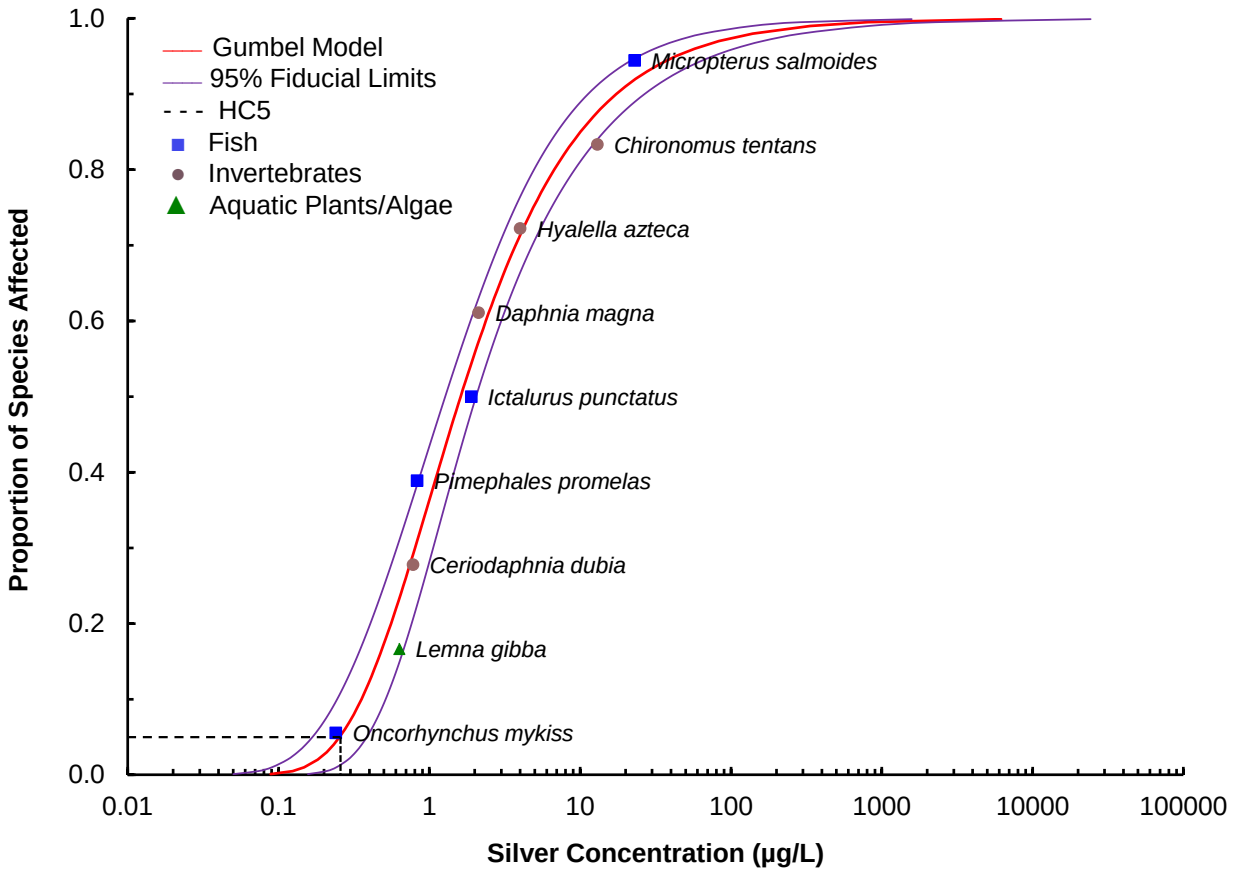

Figure 5.3 Species sensitivity distribution for long-term Ag toxicity in fresh water derived by fitting the Gumbel model to the logarithm of acceptable toxicity data for 9 aquatic species versus Hazen plotting position (proportion of species affected). The dashed line at the bottom of the graph denotes the 5th percentile which equals the corresponding long-term water quality guideline.

Table 5.11. Long-term freshwater 5th percentile and guideline concentration for silver resulting from the SSD Method. (LFL= lower fiducial limit; UFL= upper fiducial limit)

	Concentration (µg/L)
SSD 5th percentile	0.25
SSD 5th percentile, 95% LFL	0.17
SSD 5th percentile, 95% UFL	0.39

[1] The long-term freshwater SSD was derived based on the total concentration of Ag.

Fish were relatively the most sensitive taxon to silver in the long-term freshwater SSD, with the exception of the most tolerant species, *Micropterus salmoides*. One data point on the long-term SSD curve fell below the long-term freshwater guideline of 0.25 µg/L, a 3-month MATC for

growth effects to rainbow trout of 0.24 µg/L. The likelihood of a datapoint on an SSD falling below the 5th percentile increases with sample size, and is therefore inherent in the SSD calculation.

In cases where an endpoint falls below the HC5 (or guideline) value in a long-term SSD, consideration may be given to applying the 'protection clause' only if there is a strong reason to question whether the guideline value is achieving the intended level of protection. The CCME protocol for guideline derivation (CCME, 2007) states that "the protection clause may be invoked if an acceptable single (or, if applicable, geometric mean) no-effect or low-effect level endpoint for a species at risk is lower than the proposed guideline…". Or, in the case of a species other than a species at risk the protection clause will be used "if an acceptable single (or, if applicable, geometric mean) lethal-effects endpoint (i.e., LCx, where $x \geq 15\%$) for any species is lower than the proposed guideline…". Another potential reason to implement the protection clause is if the data point falling below the HC5 value is for a species of commercial or recreational importance, or for an 'ecologically' important species, then water managers may choose to use that endpoint as the guideline value on a site-specific basis.

In the case of the long-term silver guideline, there are some lethality endpoints from the short-term acceptable dataset for the species *Ceriodaphnia dubia* that are below the CWQG of 0.25 µg/L. However, there are a total of 35 LC50s for *C. dubia* in the acceptable short-term data set, the majority of which are above the CWQG, and they range from 0.16 to 2.88 µg/L with a geometric mean of 0.68 µg/L. All of the LC50s for *C. dubia* that are below the CWQG are from a single study (Naddy *et al.*, 2007) where most or all silver is in the dissolved phase using very pure water (whereas the guideline is based on total silver). Other LC50s for *C. dubia* from a different study (Bielmyer *et al.*, 2007) which were conducted in natural water or tap water ranged from 0.34 to 9.52 µg/L, which is above the CWQG. There are no data points for any other species in the acceptable short-term dataset that are below 0.25 µg/L.

In considering the long-term acceptable data set, the *C. dubia* data point plotted in the long-term SSD is a 30-d MATC of 0.78 µg/L for effects on reproduction. Therefore, there is a sensitive, non-lethal endpoint for *C. dubia* above 0.25 µg/L. Furthermore, the long-term dataset does not contain any acceptable endpoints below the CWQG that represent lethal effects or effects to species at risk. Based on these findings, the protection clause was not invoked as there was no strong reason to question the long-term CWQG in achieving the intended level of protection.

5.3. Guideline summary

There were sufficient long-term data to derive a Type A guideline for freshwater environments. In total, 9 freshwater species were included in the long-term SSD and the Gumbel model was used to derive the long-term freshwater CWQG. There were insufficient data to derive any type of guideline (A, B1 or B2) for long-term exposure in marine environments.

Data for short-term toxicity in both freshwater and marine environments met the requirements for derivation of SSDs. The short-term marine dataset contained 19 species and the Normal model was the best fit to determine the short-term marine benchmark. The short-term freshwater

dataset contained 18 species and the Logistic model was the best fitting model. Because the short-term freshwater 5th percentile was essentially equal to the long-term 5th percentile (CWQG), no designated short-term benchmark for fresh waters was recommended.

Table 5.12. Summary of CWQGs[1] for silver.

Aquatic Environment	Exposure Duration	Type	Guideline (µg/L)
Freshwater	Short-term	n/a	n/a
Freshwater	Long-term	A	0.25
Marine	Short-term	A	7.5
Marine	Long-term	n/a	n/a

[1] The CWQGs were derived based on the total concentration of Ag.

The guidelines apply to total silver and are not applicable to nano particles (see Section 1.4). Short-term guidelines are in place to protect most species from severe toxicological effects of silver, during transient events, such as spills to receiving environments, while long-term guidelines are meant to protect all species from negative effects at all times. Since these guidelines are not corrected for any toxicity modifying factors, they are generic and will not account for site-specific factors. In the event a site-specific guideline is needed, CCME has outlined several procedures to implement site-specific factors.

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Appendix A Summary of toxicity data evaluated for short-term benchmark concentration and long-term Canadian Water Quality Guideline derivation (See separate Microsoft *Excel file, located at* http://www.ccme.ca/files/Resources/supporting_scientific_documents/CWQG - Silver-SCD-Appendix.xlsx)