WATER QUALITY GUIDELINE SERIES

Molybdenum Water Quality Guidelines for the Protection of Freshwater Aquatic Life, Livestock, Wildlife and Irrigation

Technical Report

Ministry of Environment and Climate Change Strategy

Water Protection & Sustainability Branch

BRITISH COLUMBIA

No. WQG-07

The Water Quality Guideline Series is a collection of British Columbia (B.C.) Ministry of Environment and Climate Change Strategy water quality guidelines. Water quality guidelines are developed to protect a variety of water values and uses: freshwater aquatic life, drinking water sources, recreation, livestock watering, irrigation, and wildlife. The Water Quality Guideline Series focuses on publishing water quality guideline technical reports and guideline summaries using the best available science to aid in the management of B.C.'s water resources. For additional information on B.C.'s approved water quality parameter specific guidelines, visit:

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A discharge pipe in B.C.
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EXECUTIVE SUMMARY

The British Columbia Ministry of Environment and Climate Change Strategy (ENV) develops province-wide ambient Water Quality Guidelines (WQGs) for substances or physical attributes that are important for managing both fresh and marine surface waters of British Columbia (B.C.). WQGs provide a basis for water quality assessments and inform decision-making in the natural resource sector. WQGs may be created for the protection of designated values, including aquatic life, wildlife, agriculture, drinking water sources, and recreation. This document presents updated molybdenum (Mo) WQGs for the protection of aquatic life, wildlife and agricultural uses (livestock watering and irrigation).

The development of WQGs reflects the guiding principle that all forms of life and all stages of their life cycle are to be protected during indefinite exposure to the substance of interest. Both long-term chronic and short-term acute WQGs may be recommended if enough toxicological data are available.

Mo is an essential trace element for all organisms; however, elevated concentrations can adversely affect aquatic and terrestrial life. While background Mo concentrations in B.C. are generally lower than the threshold for adverse effects to biota, anthropogonic activities such as mining can increase Mo concentrations to levels that can be harmful.

In general, aquatic organisms are relatively tolerant of Mo during both short-term and long-term exposures. However, low level Mo exposures can be toxic to livestock and wildlife, particularly ruminants, where Mo may inhibit copper uptake resulting in various effects including inhibited reproduction, reduced growth, and death. Terrestrial plants (e.g., agricultural crops) are also susceptible to Mo toxicity; chronic exposures may cause a reduction in yield, growth and root elongation of plants.

The B.C. Mo WQGs were first established in 1986 (ENV 1986). Since then, new toxicity data have been produced to better characterize species' sensitivity to Mo and this update reflects this new information.

The updated Mo WQGs apply to aquatic life, wildlife, livestock and crops and are based on a thorough review of the current scientific literature on Mo toxicity. They provide flexibility for assessing impacts to ecosystems and agriculture systems depending upon the values and uses of the water body. The WQGs for ruminant livestock and ruminant wildlife have the lowest numerical values (Table E.1) given the sensitivity of the ruminant digestive tract to Mo. Toxicity test results show that aquatic life is relatively tolerant of Mo and, therefore, the aquatic life WQG has the highest numerical value and is four to five orders of magnitude higher than typical background Mo concentrations found in streams and lakes across B.C.

The updated chronic long-term and acute short-term WQGs for the protection of freshwater aquatic life are increased to 7.6 and 46 mg/L, respectively (Table E.1) and are applicable in all regions of B.C. No WQG for the protection of marine aquatic life is recommended at this time.

For livestock and wildlife, three interim guidelines are proposed based on differences in sensitivity to Mo exposure: 1) ruminant livestock, 0.016 mg/L; 2) ruminant wildlife, 0.034 mg/L; and 3) non-ruminant livestock and wildlife: 0.284 mg/L.

The proposed interim B.C. WQG for irrigation of non-forage crops is 0.028 mg/L; the Mo WQGs for forage crops have not been updated and the 1986 WQGs still apply (ENV 1986).

Table E.1. Summary of recommended water quality guidelines for total molybdenum.

Designated Use	2021 WQGS - Total Mo (mg/L)		1986 WQGS - Total Mo (mg/L)	
	Long-Term Chronic WQG	Short-Term Acute WQG	Long-Term Chronic WQG	Short-Term Acute WQG
Freshwater Aquatic Life	7.6	46	1	2
Livestock (ruminant)	0.016	--	--	0.05
Livestock (non-ruminant)	0.284	--	--	0.05
Wildlife (ruminant)	0.034	--	--	0.05
Wildlife (non-ruminant)	0.284	--	--	0.05
Irrigation (non-forage crops)	0.028*	--	0.03	--
Irrigation (forage crops-poorly drained soil)	0.01	0.05	0.01	0.05
Irrigation (forage crops-well- drained soil)	0.02	0.05	0.02	0.05

*Note: this guideline is intended to be protective of terrestrial plants and is not necessarily protective of livestock consuming these plants.

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AAS: Atomic Absorption Spectrophotometry

AES: Atomic Emission Spectroscopy

AF: Assessment Factor

AFS: Atomic Fluorescence Spectroscopy

Al: Aluminium

ASC: Acceptable Soil Concentration

ASTM: American Society for Testing and Materials

BAF: Bioaccumulation Factor

B.C.: British Columbia

BCF: Bioconcentration Factor

CABIN: Canadian Aquatic Biomonitoring Network

CON: Control

Cu: Copper

DOC: Dissolved Organic Carbon

EC_{10}: 10% Effective Concentration

EC_{50}: Median Lethal Concentration

ECL: Environmental Concern Level

EMS: Environmental Management System

ENV: British Columbia Ministry of Environment and Climate Change Strategy

FAV: Final Acute Value

FCV: Final Chronic Value

Fe: Iron

HC_5: Median Hazardous Concentration Affecting 5% of the species

ICP-OES: Inductively Coupled Plasma-Optical Emission Spectroscopy

ICP-MS: Inductively Coupled Plasma-Mass Spectrometry

LC_{50}: Median Lethal Concentration

LOAEL: Lowest Observed Adverse Effect Level

LOEC: Lowest Observed Effect Concentration

LTV: Long-term Trigger Value

MDL: Method Detection Limit

Mn: Manganese

Mo: Molybdenum

NWQMS: National Water Quality Management Strategy

Na: Sodium

Ni: Nickel

NOAEL: No observed adverse effect level

NOEC: No Observed Effect Concentration

PQL: practical quantitation limit

PNEC: Predicted No-Effect Concentration

PWQO: Provincial Water Quality Objectives

S: Sulfur

SMATC: Species Maximum Acceptable Toxicant Concentration

STV: Short-term Trigger Value

TGV: Trigger Value

TDI: Tolerable Daily Intake

UF: Uncertainty Factor

USEPA: United States Environmental Protection Agency

W: Tungsten

WQG: Water Quality Guideline

1. INTRODUCTION

The British Columbia Ministry of Environment and Climate Change Strategy (ENV) develops province-wide ambient Water Quality Guidelines (WQGs) for substances or physical attributes that are important for managing both fresh and marine surface waters of British Columbia (B.C.). The ENV defines a WQG as a scientifically derived numerical concentration or narrative statement considered to be protective of designated values in ambient conditions. WQGs provide a basis for water quality assessments and inform decision-making in the natural resource sector and may be derived for the protection of designated values, including aquatic life, wildlife, agriculture (livestock watering and irrigation), drinking water sources, and recreation.

In B.C., WQGs are developed to protect the most sensitive endpoint associated with a given value (e.g., aquatic life, wildlife, livestock). For substances with sufficient toxicological data, both short-term acute and long-term chronic guidelines are developed. Interim guidelines are developed when the available toxicological data are insufficient (CCME, 1999a; ENV, 2019a).

WQGs are typically based on toxicological studies conducted under laboratory conditions. There are several uncertainties associated with applying WQGs to field conditions, including:

- Laboratory to field differences in exposure conditions;
- Single contaminant tests in laboratories vs exposure to multiple contaminants in the field that may demonstrate additive, synergistic, or antagonistic effects;
- Toxicity of metabolites;
- Intra- and inter-specific differences between test species used to derive the WQG and those found in the field;
- Indirect effects (e.g., behavioral responses, food web dynamics);
- Laboratory studies conducted on partial life cycle studies which may not include the most sensitive life stage;
- Delayed effects which may not occur within the life stage tested, or may occur across generations; and,
- Cumulative effects of the various stressors, such as habitat loss and climate change, that organisms in the field are faced with.

Given these uncertainties, WQGs are considered an estimate of a no-effect concentration (i.e., no effects are expected if exposure concentrations are below the WQG). An exceedance of the WQGs presented in this document, however, does not imply that unacceptable risks are present, but that the potential for adverse effects is increased and additional investigation and monitoring may be warranted. To that end, ongoing ecological monitoring is encouraged to ensure the WQG is indeed protective under field conditions.

In 1986, B.C. derived WQGs for molybdenum (Mo) for the protection of freshwater aquatic life, wildlife, and agriculture (ENV, 1986). Since that time, additional studies have improved the understanding of Mo toxicity. This report provides the scientific evidence and rationale for the updated B.C. Mo WQGs for aquatic life, livestock, wildlife, and terrestrial vegetation (via irrigation). The WQGs for freshwater aquatic life were derived following the guidance provided in B.C.'s aquatic life derivation protocol (ENV, 2019a) and agriculture and wildlife WQGs were derived following the guidance provided in the Canadian Council of Ministers of the Environment's agriculture derivation protocol (CCME, 1999a).

2. PHYSICAL AND CHEMICAL PROPERTIES OF MOLYBDENUM

Molybdenum is a hard, silvery-white or dark-grey transition metal that is essential for life (Regoli et al., 2012; ATSDR, 2017; Smedley and Kinniburgh, 2017). Molybdenum does not occur as a free metal in the environment (Smedley and Kinniburgh, 2017) but occurs naturally in mineral matrices including molybdenite (MoS_2; molybdenum disulfide), powellite ($CaMoO_4$; calcium molybdate), wulfenite ($PbMoO_4$; lead molybdate), ferrimolybdate ($Fe_2(MoO_4)_3$; hydrous iron molybdate), and various ilsemannites (Mo_3O_8) (Anjum et al., 2015; ATSDR, 2017).

Molybdenum can exist in various oxidation states (i.e., -II to VI), but typically exists as Mo (IV) through Mo (VI) in the environment (Smedley and Kinniburgh, 2017). In oxygenated freshwater environments with pH above 5 and 6, Mo exists as MO(VI) and exclusively as the molybdate anion $[MoO_4]^{2-}$ (Smedley and Kinniburgh, 2017). Molybdate is readily taken up into plant and animal cells (Stiefel, 2002; Oorts et al., 2016). Protonated (Smedley and Kinniburgh, 2017) or polymer (Parker, 1986) forms may occur when Mo concentrations are elevated well above ambient concentrations and exposed to lower pH levels (i.e., < 5). An in-depth review of the geochemical controls on Mo speciation in aquatic environments can be found in Smedley and Kinniburgh (2017). Table 2.1 provides information on Physical-chemical properties of molybdenum and selected molybdenum compounds.

Table 2.1. Physical-chemical properties of molybdenum and select molybdenum compounds.

Property	Molybdenum	Sodium Molybdate	Molybdenite	Molybdite
Chemical Formula	Mo	Na_2MoO_4	MoS_2	MoO_3
CAS Registry Number	7439-98-7	7632-95-0	1309-56-4	1313-27-5
Molecular Weight (g/mol)	95.94	205.92	160.07	143.95
Physical State at 25 ºC	Solid	Solid	Solid	Solid
Melting Point (ºC)	2622	687	--	795
Boiling Point (ºC)	4639	--	450	1155
Density (g/cm^3)	10.2	3.78	5.06	4.69
Water Solubility (g/100mL)	Insoluble	84	Insoluble	0.049

Reference: ATSDR (2017)

3. INDUSTRIAL AND ECONOMICAL IMPORTANCE OF MOLYBDENUM

Molybdenite (MoS_2) is the form of primary commercial importance (Jones, 1999) and is typically obtained as a by-product of copper (Cu) mining. Other commercially important forms include molybdite (MoO_3), wulfenite, powellite and the ilsemannites (Jones, 1999). The majority of mined Mo is used in the production of steel alloys (IMOA, 2008). Molybdenum is also used in petroleum desulphurisation catalysts, and in the production of adhesives, lubricants, corrosion inhibitors, flame-retardants and medical isotopes (IMOA 2008; Smedley and Kinniburgh, 2017). The radioisotopes of Mo, including Mo-99, a beta-emitting isotope used to produce technetium-99m, are globally important for medical scans (Smedley and Kinniburgh, 2017). Molybdite is being used increasingly in nanotechnology applications for batteries and fuel cells to power portable electronic devices and electric vehicles (Avila-Arias et al., 2019).

The global demand for Mo is increasing, mainly from use in China and Europe (IMOA, 2008). All of Canada's Mo reservoirs (235,00 tonnes) are in B.C. (NRCan, 2014). Between 2013 and 2018, Canada mined over 32,000 tonnes of Mo with a total value over $800 million (The Mining Association of Canada, 2019).

4. ENVIRONMENTAL FATE AND TRANSPORT OF MOLYBDENUM

Molybdenum is the least abundant trace element in soil (Anjum et al., 2015) and is the 54th most abundant element in the earth's crust (Lasheen et al., 2015) at concentrations ranging from 0.6 to 1.5 mg/kg in the upper crust to about 0.6 mg/kg in the lower crust (Smedley and Kinniburgh, 2017). The highest Mo concentrations occur in organic rich sediment (2.0-2.6 mg/kg), shale (0.7-2.6 mg/kg) and felsic rock (1-2 mg/kg) with lower concentrations in limestone (0.16-0.40 mg/kg; Kabata-Pendias and Pendias, 1992).

A significant source of Mo to aquatic environments is effluent generated from mining operations (Eisler et al., 1989; Smedley and Kinniburgh, 2017). Other sources include Mo smelting, uranium mining and milling, steel and Cu milling, oil refining, shale oil production, claypit mining, and aluminium (Al) refinery waste (Phillips and Ruso, 1978; McNeely et al., 1979; Eisler et al., 1989; Jones, 1999; van Dam et al., 2018) and runoff (Phillips and Ruso, 1978). Molybdenum sources to terrestrial environments include fertilizer and biosolids applications (McNeely et al., 1979; Avila Arias et al., 2019) and minor inputs from the burning of fossil fuels (Phillips and Ruso, 1978).

Molybdenum is naturally present at very low levels (see Section 6); however, water downstream of mine discharges can become elevated in areas with large-grained igneous (i.e., porphyry) Cu or Mo deposits (Jones, 1999; Smedley and Kinniburgh, 2017). The distribution and speciation of Mo in aquatic environments is affected by pH, redox potential, water hardness, alkalinity, sulphate concentration, and total organic carbon content (Lucas et al., 2017; Smedley and Kinniburgh, 2017). In oxygenated freshwater environments with a pH > 6, Mo is present exclusively as the bioavailable molybdate anion (Lucas et al., 2017; Smedley and Kinniburgh, 2017). Molybdenum can adsorb to Al, iron (Fe), and manganese (Mn) oxides (LeGendre and Runnells, 1975; Smedley and Kinniburgh, 2017) and Al hydroxides, which partition into sediments. In addition, Mo may adsorb to fine-grained particulate matter or organic matter (Smedley and Kinniburgh, 2017).

Molybdenum is present in soil in various forms, including molybdenite and ferrimolybdite, and molybdate (Andresen et al., 2018). The anion, molybdate is the dominant form of Mo in soil and is potentially toxic to living organisms (Anjum et al., 2015; Oorts et al., 2016). Molybdate adsorbs to clay surfaces and the oxides and hydroxides of Fe and Al (Black and Batten, 2017). The availability of Mo in soil is highly dependent on the soil pH, clay content (McGrath et al., 2010b; Oorts et al., 2012; Jiang et al., 2015), and redox potential (Andresen et al., 2018). The retention of Mo is greatest in acidic soils as adsorption decreases with increasing pH (ATSDR, 2017).

5. ANALYSIS OF MOLYBDENUM IN ENVIRONMENTAL SAMPLES

Both total and dissolved fractions of Mo can be analysed in water samples. Dissolved Mo analysis measures only the fraction that passes through a 0.45 µm filter, while total Mo analysis includes the dissolved fraction plus any Mo associated with particulate material (e.g., suspended sediments). The B.C. Laboratory Manual describes sample digestion using a mixture of nitric and hydrochloric acids and analysis by graphite furnace Atomic Absorption Spectrophotometry (AAS) or direct aspiration AAS (ENV, 2015). Graphite furnace AAS provides a method detection limit (MDL) of 0.001 mg/L while the MDL for direct aspiration AAS is 0.1 mg/L (ENV, 2015). AAS converts an analyte solution into a gaseous state within a flame, and the concentration of the analyte is measured by assessing the absorption of a light source at frequencies characteristics of particular elements (Harris, 2003).

Inductively coupled plasma-mass spectrometry (ICP-MS) is another method for Mo analysis (USEPA, 1998; ENV, 2015). The analyte is converted into its ionic form by passing it through a plasma source, then directing ions into a magnetic field that deflects their path onto a detector, which identifies and quantifies

the chemical species in the analyte solution (Harris, 2003). CCME (2016) notes Mo analysis can also be performed with inductively coupled plasma-optical emission spectroscopy (ICP-OES), atomic emission spectroscopy (AES), or atomic fluorescence spectroscopy (AFS), and recommends that analytical standards be matrix-matched to the samples.

6. BACKGROUND CONCENTRATIONS OF MOLYBDENUM IN BRITISH COLUMBIA

Molybdenum is a naturally occurring element in aquatic and terrestrial ecosystems, therefore, background concentrations must be considered when deriving provincial Mo WQGs (ENV, 2019a).

6.1 Background Concentrations of Molybdenum in British Columbia Surface Waters

Background (i.e., from non-impacted sites) Mo concentrations vary across B.C. as a function of local geology and hydrology, therefore, a regional approach was used to estimate background Mo concentrations in aquatic environments following methods used in recent WQG derivation documents (e.g., ENV, 2019b).

6.1.1 Methods for Estimating Background Concentrations of Molybdenum in British Columbia Surface Waters

Data to characterize background Mo concentrations in B.C. surface waters were taken from two sources: the B.C. Environmental Management System (EMS) database and the Canadian Aquatic Biomonitoring Network (CABIN) database. EMS does not identify reference stations, so the database was screened to create a sub-set of water quality stations known to be minimally impacted. To do this, "background" water quality sampling stations that were sampled at least three times over the last 22 years for any water quality parameter (1998/01/01 to 2020/01/15) were extracted. Next, the list of stations with location information was given to ENV environmental impact assessment biologists to identify sites that they considered minimally impacted by human activities. No strict definition of 'minimally impacted' was given to the biologists and station selection was left to their professional judgement. The list of minimally impacted stations was then used to extract Mo water quality data from the EMS database.

The dataset underwent several additional automated and manual data cleaning steps summarized below:

- For lakes' samples, if samples were available at multiple depths, only samples from the surface were included;
- non-detect results with a MDL of 5 µg/L or higher were removed as these would influence the results of the analysis;
- samples were excluded where results were missing or reported as 0; and
- data were visually inspected, and samples were removed where results appeared to be obvious errors, assumed to be attributed to either data entry or analytical errors.

Arithmetic means were calculated for laboratory replicates (analytical replicates taken from one field sample) with the MDL substituted for values below detection. All field replicates were included as independent samples.

The resultant data set was augmented with samples collected by ENV and Environment and Climate Change Canada (ECCC) at B.C. reference stations as part of the CABIN program. CABIN reference stations are located on stream reaches minimally impacted by anthropogenic activities and are generally sampled once during the late summer/early fall low flow period.

The results from each station were given equal weight within an ENV administrative region by calculating the mean Mo concentrations for each station. Station means were calculated using four different approaches depending on the number of samples above (detects) and below (non-detects) the MDL (Table 6.1). A value of ½ the minimum MDL was used to represent station means when all samples were below the MDL (Group 1). The minimum MDL was chosen to account for decreasing MDLs over time. For stations with less than three detects, ½ of the MDL was substituted for non-detect values and the arithmetic mean of all station results was calculated (Group 2). Regression on order statistics (ROS) was used to calculate an estimate of the mean for stations that had a mixture of non-detects and detects with at least three detected values (Huston and Juarez-Colunga, 2009; Group 3). Although Huston and Juarez-Colunga (2009) state that ROS can be used on sample sizes >0, a minimum of three detects is required to calculate a valid regression using the NADA package (Lee, 2017) in R (R Core Team, 2018). The arithmetic mean was calculated for stations where all samples were above the MDL (Group 4). Statistics to summarize the distribution of station means (median, the 10^{th} and 90^{th} percentile) were calculated for each ENV region.

Table 6.1. Statistical approach used to calculate station means.

Group	Conditions	Approach	Total Stations	Total Samples
1	% non-detects = 100	½ of minimum station MDL	18	46
2	0 < % non-detects < 100 AND # detects < 3	Substitute ½ MDL for non-detects and calculate arithmetic mean for all samples	22	173
3	0 < % non-detects < 100 AND # detects ≥ 3	Regression on order statistics	51	2,081
4	% non-detects = 0	Arithmetic mean	556	3,300

6.1.2 Background Concentration Results

Data from 647 EMS and CABIN stations with a total of 5,600 results were used to characterize background Mo concentrations across B.C. (Appendix 1). The distribution of total Mo concentrations by ENV administrative region is summarized in Table 6.2 and Figure 6.1. The median of station means ranged from 0.072 µg/L (Vancouver Island) to 0.820 µg/L (Thompson Region) (Table 6.2).

Of the 647 stations, 81 stations were on lakes and 566 were on rivers. The median of the distribution of station means in rivers (0.58 µg/L) was very close to that of lakes (0.48 µg/L) (see Figure 6.2).

Table 6.2. Summary statistics for station mean total molybdenum at selected minimally impacted stations in British Columbia by region.

Region	Number of Stations	Sample No.	Date Range	Concentration Range Across all Samples (µg/L)	MDL Range Across all Samples	% Samples < MDL	Distribution of Station Means (µg/L)		
							Median	10th Percentile	90th Percentile
Cariboo	64	1,600	1998 – 2020	0.005 – 22	0.005 – 1	3.7	0.735	0.251	1.879
Kootenay	48	291	1998 – 2019	0.09 – 23.2	1	32.0	0.522	0.223	1.236
Lower Mainland	7	43	2002 – 2019	0.05 – 3.68	0.05 – 1	4.7	0.190	0.099	1.436
Okanagan	78	561	1998 – 2020	0.05 – 38.4	0.05	0.4	0.650	0.220	6.649
Omineca	48	651	1998 – 2019	0.006 – 21.5	0.05 – 0.1	2.2	0.392	0.115	1.585
Peace	113	188	2008 – 2019	0.025 – 11.9	0.05 – 0.06	1.6	0.621	0.204	2.532
Skeena	154	1,189	1998 – 2019	0.025 – 36.7	0.005 – 1	1.8	0.340	0.072	1.580
Thompson	50	394	1999 – 2018	0.080 – 17	1 - 2	23.6	0.820	0.349	1.966
Vancouver Island	84	683	1998 – 2019	0.025 – 20	0.05	34.4	0.072	0.025	0.243

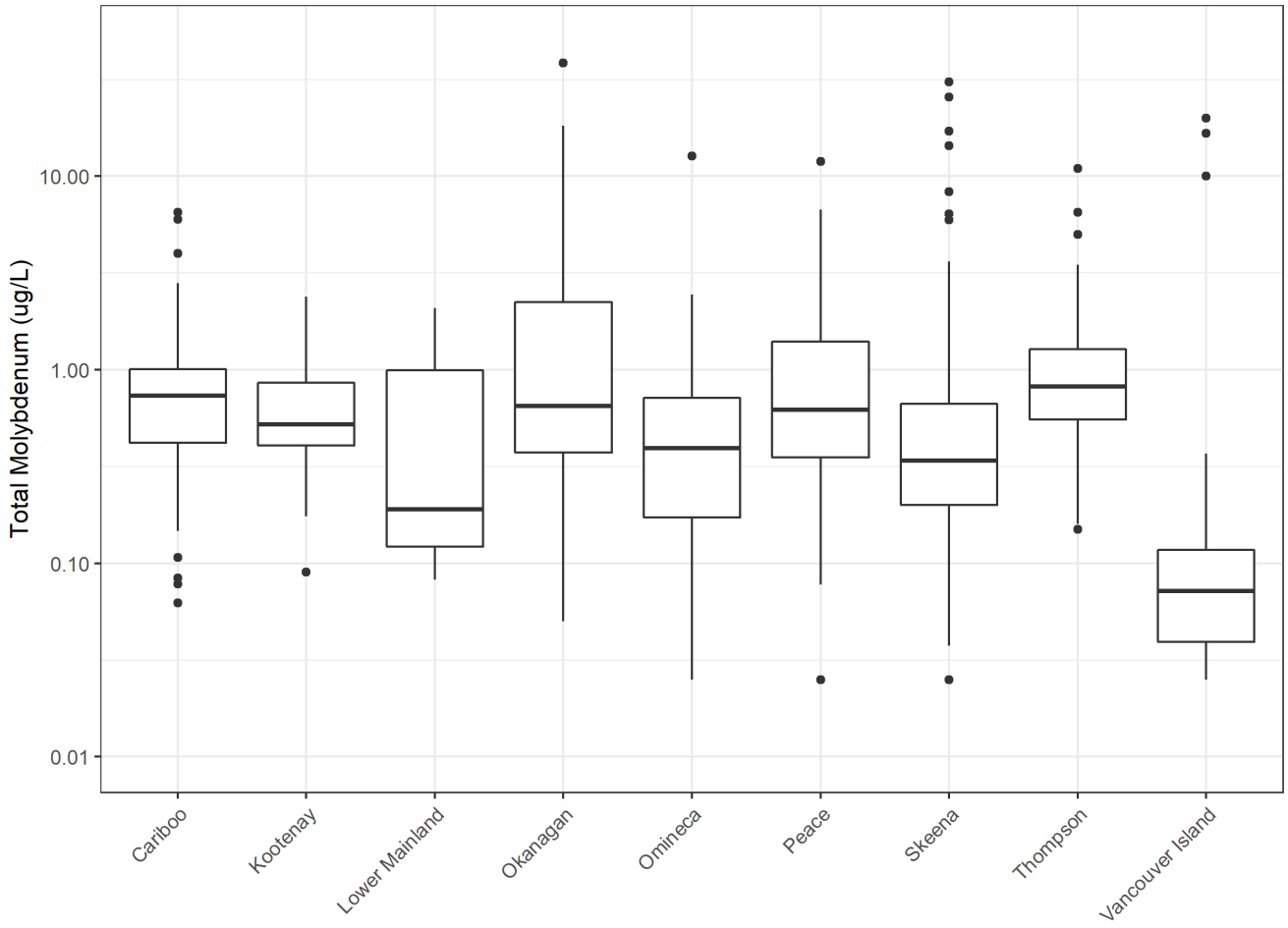

Figure 6.1. Distribution of station mean total molybdenum at selected minimally impacted stations in British Columbia by region.

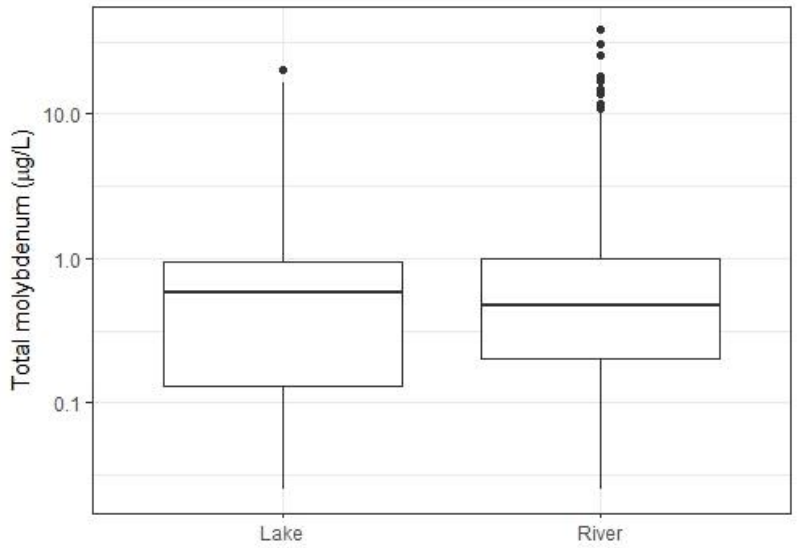

Figure 6.2. Distribution of station means for lakes and rivers for total Mo.

6.2 Background Concentrations of Molybdenum in British Columbia Soils

Background soil Mo concentrations are reported in Protocol 4 of the Contaminated Site Regulation (ENV, 2019c) and represent concentrations found in surface soil samples obtained at ENV background sites. Table 6.3 lists the 95th percentile Mo concentration for each region, ranging from <1 to 6 mg/kg (data taken from Table 1 in ENV 2019c).

Molybdenum concentrations in nine Canadian provinces and territories ranged from <1 to 206 mg/kg (Grunsky et al., 2012) with more than 50% of data below the MDL of 1 mg/kg. The mean concentration was 1.4 mg/kg with a standard deviation of 3.7 mg/kg; Mo values above the mean occurred within survey areas located in central B.C. (Grunsky et al., 2012).

Table 6.3. Background molybdenum concentrations in British Columbia soils.

Region	**95th Percentile (mg/kg)**
Vancouver Island	(1)
Lower Mainland	4
Metro Vancouver	6
Thompson/Nicola/Okanagan	2
Kootenay	(1)
Cariboo	(1)
Skeena	3
Omineca-Peace	3

Note: Values in brackets indicate that more than 50% of values were less than the mean detection concentration (MDC) for Mo and consequently the regional estimate is one-half the MDC.

7. TOXICITY OF MOLYBDENUM

Molybdenum is an essential micronutrient required for growth and development in all organisms (Mendel, 2007; Regoli et al., 2012; ATSDR, 2017). There are roughly 50 major enzymes, including nitrate reductases, sulphite oxidases, xanthine dehydrogenases and oxidases, and aldehyde oxidases (Mendel, 2007), which depend on a Mo-cofactor (Hille, 1999; Mendel, 2007) to drive various metabolic functions. At elevated concentrations, however, Mo can cause adverse effects in humans, animals, and plants (ATSDR, 2017; Smedley and Kinniburgh, 2017).

7.1 Toxicity of Molybdenum to Freshwater Aquatic Organisms

In general, aquatic organisms are relatively tolerant of Mo. Adverse effects on survival (McConnell, 1977; Hamilton and Buhl, 1997; Diamantino et al., 2000; Davies et al., 2005; GEI, 2009; Heijerick and Carey 2017; Lucas et al., 2017), growth (Diamantino et al., 2000 GEI, 2009; De Schamphelaere et al., 2010; Heijerick and Carey, 2017; Lucas et al., 2017) and reproduction (Naddy et al., 1995; Diamantino et al., 2000; GEI, 2009; De Schamphelaere et al., 2010) have only been observed at high Mo concentrations.

The mode of toxicity for Mo to aquatic life is not yet understood. Increased metabolic cost associated with increased respiratory rate was suggested by Reid (2002) as a potential mechanism of Mo toxicity in kokanee (*Oncorhynchus nerka*). However, no increase in oxygen consumption was observed. In addition, later studies did not report increase in respiratory rate after Mo exposure (Kennedy, 2019). In a recent attempt to determine mode of toxic action in fish, Ricketts et al. (2015) investigated physiological and cellular stress responses in rainbow trout (O. mykiss) exposed to Mo. However, no stress response was observed and the mechanism of Mo toxicity in fish and other aquatic biota remains unknown.

7.1.1 Effects on Algae

Molybdenum appears to be relatively less toxic to algae than other metals. Comparative studies on copper (Cu), nickel (Ni), and Mo toxicity to the freshwater algae species *Scenedesmus quadricauda* reported EC_{50} estimates for growth inhibition that were up to 10 times greater for Mo than the other tested metals (Fargašová et al., 1998; Andresen et al., 2018). The green alga *Pseudokirchneriella subcapitata* showed EC_{10} estimates for growth inhibition ranging from 61.2 to 88.7 mg/L during a 72-hour exposure (De Schamphelaere et al., 2010).

7.1.2 Effects on Macrophytes

Macrophytes appear to be tolerant to elevated Mo concentrations. A 7-day exposure of Mo on the common duckweed (*Lemna minor*) resulted in an EC_{10} of 241.5 mg/L for growth inhibition and was the least sensitive species in a study that included fish, invertebrates, and algae (De Schamphelaere et al., 2010). Lower net photosynthesis relative to controls was demonstrated in the European water chestnut (*Trapa natans*) when exposed to 4,797 mg/L of Mo for 10 days (Baldisserotto et al., 2013; Andresen et al., 2018).

7.1.3 Effects on Invertebrates

Freshwater invertebrates vary in their sensitivity to Mo. In short-term tests, Wang et al. (2016) reported a 48-hour LC_{50} of 243.1 mg/L for *Daphnia magna* while Lucas et al. (2017) reported a 96-hour LC_{50} of 2,782 mg/L for the oligochaete *Tubifex tubifex*.

In long-term tests, reproductive endpoints were most sensitive. In 21-day exposures with *D. magna*, EC_{20} estimates ranged between 368 and 396 mg /L for mortality and 116 and 147 mg/L for reproduction (GEI, 2009). *Ceriodaphnia dubia* was more sensitive to Mo exposure in 7-day exposures with EC_{20} estimates

ranging between 166 and 222 mg/L for mortality and 74 to 80 mg/L for reproduction. Naddy et al. (1995) reported a 7-day reproduction IC_{25} for *C. dubia* of 47.5 mg/L. Heijerick and Carey (2017) reported an EC_{10} estimate of 44.6 mg/L for *Hyalella azteca* reproduction after a 42-d exposure.

De Schamphelaere et al. (2010) investigated the effects of Mo on invertebrate growth rates. They reported a 14-day LOEC of 794 mg/L for the midge *Chironomus riparius* based on biomass. A 48-hour exposure resulted in a LOEC of 508 mg/L for the population growth rate of the rotifer *Brachionus calyciflorus*, while a 28-day LOEC of 288 mg/L for biomass was measured in the snail *Lymnaea stagnalis*.

Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa are generally sensitive to chronic metal exposures (Brix et al., 2011), however no studies examining the effect of Mo on sensitive aquatic insects were found.

7.1.4 Effects on Fish

Fish demonstrate a relatively high tolerance to Mo exposure. Studies have reported 96-hour LC_{50} values for coho salmon (*O. kisutch*) and chinook salmon (*O. tshawytscha*) at >1,000 mg/L (Hamilton and Buhl, 1997; Davies et al., 2003). Similarly, Reid (2002) showed kokanee are relatively tolerant to Mo exposure with a 96-hour LC_{50} estimate of >2,000 mg/L. Ricketts et al. (2015) reported no effects to physiological or cellular endpoints (plasma cortisol, blood glucose, hematocrit levels) in juvenile rainbow trout during a 96-hour exposure of up to 1,000 mg/L. McConnell (1977) reported two 96-hour LC_{50} estimates for rainbow trout fry of 800 and 1,320 mg/L depending on the weight of fish used in the tests. Two studies by Birge (1978 and 1980) reported LC_{50} concentrations below 1.0 mg/L, suggesting Mo is quite toxic to fish, however, researchers have since attempted to replicate these results with no success (e.g., Davies et al.,2005). Therefore, these studies (i.e., Birge, 1978 and 1980) are considered unacceptable for further use in informing Mo WQGs (Davies et al., 2005; De Shamphelaere et al., 2010; Lucas et al., 2017).

The chronic effects of Mo on fish have also been studied. Lucas et al. (2017) reported an EC_{10} of 202 mg/L for growth of brown trout (*Salmo trutta*) after an 85-day exposure. Growth endpoints were the most sensitive for rainbow trout with a 78-day exposure LOEC of 121 and 152.7 mg /L for biomass (De Shamphelaere et al., 2010). Fathead minnows (*Pimephales promelas*) were slightly less sensitive with a 32-day exposure resulting in EC_{20} estimates between 162 and 165.1 mg/L (GEI, 2009) and a 34-day exposure LOEC of 53 mg/L (De Shamphelaere et al. 2010). In another study, Reid (2002) found that a 7-day exposure of 25 mg/L produced a 1.7-fold increase in the ventilation rate of juvenile kokanee salmon; however, Kennedy (2019) found no increase in ventilation rates in rainbow trout exposed to concentrations of Mo up to 500 mg/L for 21 days.

7.1.5 Effects on Amphibians

There is very little known about the toxic effects of Mo on amphibians. Only one study was found that tested the effects of Mo on amphibians and this was for a species not found in Canada. De Schamphelaere et al. (2010) conducted a 4-day exposure with the African clawed frog, *Xenopus laevis*, that yielded a LOEC of 369 mg/L for larval survival and a LOEC of 44.6 mg/L for larval malformation; however, the EC_{10} estimate for malformation was 115.9 mg/L.

7.1.6 Bioaccumulation and Biomagnification of Molybdenum in the Aquatic Environment

The available information indicates that Mo bioaccumulation is negligible, and biomagnification does not occur. A review of measured tissue concentrations in aquatic organisms (i.e., fish, molluscs, and algae) found whole-body Mo concentrations to be typically <1 mg/kg dry weight in organisms exposed to background Mo levels (e.g., 0.46 µg/L) and <10 mg/kg dry weight in organisms exposed to water with anthropogenically enriched Mo (up to 766 µg/L) (Regoli et al., 2012). Ward (1978) reported that Mo tissue concentrations in rainbow trout increased minimally when exposed to increasing Mo concentrations,

suggesting there may be a threshold at which excess Mo is efficiently excreted. As with other essential metals, organisms regulate Mo to facilitate metabolic functions at low environmental concentrations and reduce accumulation at higher exposure concentrations to prevent adverse effects (Regoli et al., 2012). Lucas et al. (2017) reported an inverse relationship between Mo bioaccumulation factors (BAF) for brown trout and exposure concentrations, ranging from 0.13 at 20 mg/L to 0.04 at 1,247 mg/L. The same relationship has also been reported for invertebrates, with the BAF for crustaceans exposed to background levels of Mo 10 times greater than for those exposed to high concentrations (Regoli et al., 2012). These findings are consistent with other similar investigations that found an inverse relationship between Mo concentration and BAF (e.g., DeForest et al., 2007).

7.2 Toxicity of Molybdenum to Livestock and Wildlife

Livestock and herbivorous wildlife are exposed to Mo through their diet of vegetation, drinking water, and incidental soil ingestion. While pulmonary uptake via inhalation has been confirmed in several human epidemiological and mammalian laboratory exposure studies, there is little, if any, information available on dermal uptake (MAK, 2000). For mammalian and avian species, the dermal exposure pathway for most contaminants of potential concern is generally assumed to be negligible given the presence of fur and feathers over most of the body surface.

7.2.1 Ruminants

The characteristics of the receptor strongly influence the likelihood and extent of toxic effect from Mo exposure. Ruminants (including cattle, sheep, goats, deer, and moose) are generally recognized to be ten-fold more susceptible to Mo toxicity than non-ruminant mammals given the unique physiology of their digestive tract (Blakley, 2017).

Ruminant wildlife species, such as deer and moose, may be less susceptible to Mo toxicity than livestock, due to lower levels of potential exposure. Generally, wildlife are less likely to be exposed to high Mo concentrations, as they are not confined to one area and exposure to Mo is amortized through grazing (and incidental soil ingestion) and drinking surface water over a wider geographic area. The animal's age also plays a role in Mo sensitivity, since young animals are more sensitive than adult animals and nursing calves may be exposed to Mo via milk, which may result in toxicosis (Blakley, 2017).

7.2.1.1 Mode of toxicity to ruminants

Exposure of ruminants to high concentrations of molybdenum can result in molybdenosis or tert disease. In ruminants, interactions between Mo, copper (Cu) and sulfur (S) metabolism influence the bioavailability, kinetics and toxicity of Mo. Ruminants are particularly susceptible to Mo toxicity due to their unique, four-stomach physiology. The anaerobic ruminant stomachs contain reduced forms of sulphur that combine with ingested Mo to form thiomolybdates which subsequently bind to copper producing cupric thiomolybdate complexes. The copper, bound in this form, is no longer bioavailable and the animal suffers from copper deficiency. Molybdenosis is directly tied to the dietary intake of molybdenum, copper and sulfur (Suttle, 1991; Barceloux and Barceloux, 1999; Blakley, 2017).

Sulfur (S) interacts with Cu and Mo differently in ruminants than in non-ruminants because SO_4^{2-} and S-containing amino acids, such as methionine and cysteine, are readily converted to sulphide (S^{2-}) in the rumen (Ward, 1978). High dietary concentrations of sulphate resulted in signs of Cu deficiency in cattle and sheep (Pitt, 1976) and are assumed to also occur in wildlife but have not been reported. Higher sulphur concentrations in the rumen increase the production of thiomolybdates, increasing the potential for binding Cu (Suttle, 1991), in turn increasing the potential for molybdenosis.

When in systemic circulation, thiomolybdates interfere with a host of biochemical processes via reversible binding with oxidase enzymes, including caeruloplasmin, cytochrome oxidase, superoxide dismutase, ascorbate oxidase, and tyrosinase, for which Cu is an important co-factor, and intake inhibition occurs at levels that are physiologically relevant (Gould and Kendall 2011).

Within the published literature there are proposed Cu:Mo ratios in livestock feed above which the onset of molybdenosis is more probable, based on empirical observations (as discussed in more detail in Section 8.2); however, the expected complexity of interactions between Mo, S, Cu and other elements within the rumen, intestines, and tissue of ruminants suggests that use of simple Cu:Mo ratios on soil, water, or feed as a basis of defining thresholds of potential effects is overly simplistic.

Gould and Kendall (2011) note that the symptoms routinely associated with clinical Cu deficiency (and molybdenosis) in ruminants are usually field-based observations, non-specific, and sub-clinical. These include reduced weight gain, decreased food intake, reduced efficiency of food conversion, alteration in hair/wool texture and pigmentation (spectacles around eyes), delayed puberty, reduced conception rate, inhibition of estrus, and swayback.

7.2.2 Non-Ruminants

Non-ruminant mammals and birds are generally considered to be less sensitive to Mo toxicosis than ruminants (Ward 1978; Barceloux and Barceloux, 1999; NRC, 2005). However, there is some evidence of adverse effects of Mo on non-ruminants at concentrations higher than those that cause effects in ruminants (Ward, 1978; O'Connor, 2001; Eisler, 1989; Millennium EMS Solutions Ltd., 2014). In non-ruminant species, the primary mode of toxic action for Mo involves negative interactions with Cu and S^{2-}. Elevated concentrations of Mo can reduce Cu levels, through the Cu being bound to the Mo and its cofactors and then excreted. Low concentrations of Cu and S^{2-} in the diet can enhance Mo toxicity, and present similarly to Cu deficiency (Barceloux and Barceloux, 1999). Inorganic SO_4^{2-} supplementation seems to have a protective effect against Mo toxicity (Ward, 1978). Also, a high protein diet can partially elevate Mo toxicity, possibly due to the metabolism of S-containing amino acids (NRC, 2005). Toxicity studies in rats have suggested that Mo can induce testicular damage (Pandey and Singh, 2002; Zhai et al., 2013; Murray et al., 2014a; Murray et al., 2014b), due to significant alterations in testicular enzymes and histopathological changes. As well, high concentrations of Mo may lead to morphologically abnormal ovarian mitochondria in mice (Zhang et al., 2013) however these results were not repeated in later studies on rats (Murray et al., 2014a; Murray et al., 2014b). Generally, naturally occurring concentrations of Mo are non-toxic to non-ruminant species, and hence investigation into the mechanism of toxicity to this group is limited.

7.3 Toxicity of Molybdenum to Terrestrial Plants

Molybdenum is an essential plant nutrient required for nitrate assimilation, sulfite detoxification, and hormone synthesis (Gupta and Lipsett, 1982; Chatterjee and Nautiyal, 2001; Baxter et al., 2008). Molybdenum is a cofactor (molybdopterin cofactor) of more than 60 metalloenzymes and proteins in plants, including two major enzymes which enable plant uptake of nitrogen (N) from soil (i.e., nitrogenase and nitrate reductase) (Anjum et al., 2015). Molybdenum deficiency or excess can inhibit plant growth (Kaiser et al., 2005).

Mo toxicity rarely occurs under field conditions (Mengel and Kirby, 2001; Anjum et al., 2015). Plants require molybdate in the 0.1 to 1.0 mg/kg plant (dry weight) range although some plants can tolerate much higher levels (up to 1,000 mg/kg). Molybdenum is often added to soil as a micro-nutrient to ensure

the effectiveness of N assimilation, essential for plant growth (Gupta and Lipsett, 1982). A positive effect on plant growth at low doses of Mo may indicate Mo deficiency in the soil (McGrath et al., 2010a).

Plants exposed to high Mo concentrations may exhibit limited plant growth and yield; reduction in root and shoot length; alteration of leaf, root and stem anatomy; interference with metabolic processes resulting in physiological disorders; increased accumulation of chlorophyll and anthocyanin; and deficiencies in mineral nutrients such as manganese and magnesium (McGrath et al., 2010a; Oorts et al., 2012; Anjum et al., 2015; Oorts et al., 2016).

The effect of soil sodium molybdate (Na_2MoO_4) concentrations on shoot yield is largely dependent upon soil type. For example, McGrath et al. (2010a) tested the effects of sodium molybdate on four plant species (i.e., rapeseed, red clover, ryegrass, and tomato) in ten different soil types, covering a wide variation in chemical and physical properties, from eight European countries and found that the effective concentration for 10% inhibition of shoot yield (EC_{10}) based on measured Mo concentrations in soil varied widely across plant species and within plant species tested in different soils (i.e., from 4 to 2,844 mg/kg for rapeseed, 4 to 1,502 mg/kg for red clover, 14 to 3,476 mg/kg for ryegrass, and 3 to 1,575 mg/kg for tomato). The variation in toxicity between soil types was somewhat narrowed when Mo concentrations in soil solution were considered, indicating that solubility affects bioavailability to the plant. The molybdate anion (MoO_4^{2-}) was the predominant form of Mo in soil solutions and unlike cationic metals (e.g., Cu, Ni, cobalt), less toxic conditions were observed in acidic (i.e., pH <6.5) versus neutral and basic soils.

8. MOLYBDENUM TOXICITY-MODIFYING FACTORS

Both aquatic and terrestrial environmental factors may interact with Mo, affecting its uptake or bioavailability and therefore its toxicity to organisms.

8.1 Aquatic Organisms

The speciation of Mo in oxygenated waters under varying pH conditions is well understood (Smedley and Kinniburgh, 2017). In waters with pH above 5 the molybdate anion predominates and exists exclusively in waters with pH above 6. Therefore, in typical freshwaters observed in B.C. which are generally above pH of 6, it is unlikely that variations in pH will alter the bioavailability of Mo to aquatic life (ENV, 1991).

Water hardness is a well-known toxicity-modifying factor for metals such as cadmium (Cd), Cu, and nickel (Ni) due to the competition between the metal cations and calcium (Ca^{2+}) or, to a lesser extent, magnesium (Mg^{2+}) at the biotic ligands (Niyogi, 2015). Competition for binding sites is not likely to contribute to variations in Mo toxicity as Mo exists as an anion (molybdate) in typical freshwaters and, therefore, is not expected to be influenced by varying water hardness (Heijerick and Carey, 2017).

No information on the binding of Mo with DOC to form organic complexes was found; however, Bibak et. al (1994) observed the binding of Mo with humic acids in soils, while Gustafsson and Tiberg (2015) showed Mo binding with fulvic acids in soils at low pH. However, no studies were found that tested the toxicity modifying potential of humic or fulvic acids for aquatic organisms. Due to the limited data and information available, no toxicity-modifying factors were considered in the derivation of the Mo WQG for the protection of aquatic life.

8.2 Livestock and Wildlife

It is expected that animals with a Cu deficient diet will be at a greater risk of developing molybdenosis than those with adequate Cu. Observations that symptoms of molybdenosis can be reversed or avoided

in livestock and experimental animals through supplementation with Cu further suggests that livestock or wildlife receiving Cu in excess of basic needs will be less susceptible to symptoms of molybdenosis at lower levels of Mo exposure (Raisbeck et al., 2006). However, there is a great deal of debate regarding the protective ratio of Cu:Mo in livestock diets and even whether Cu can mitigate the effects of exposure to high concentrations of Mo.

Gould and Kendall (2011) argued that molybdenosis involves the direct effects of absorbed thiomolybdate via interference with Cu-containing oxidase enzymes, and that decreased Cu absorption efficiency might secondarily exacerbate this process. They also note, however, that thiomolybdate interactions with Cu-containing enzymes is reversible and, at least in some instances, may involve competitive interactions between Cu and Mo. Whether Cu-Mo interactions in ruminants primarily involves altered Cu availability or the interactions are more complex, various empirical observations have led researchers to define proportional concentrations of Cu and Mo in ruminant diets associated with the onset of molybdenosis.

Blakley (2017) reported that a Cu:Mo ratio of 6:1 in cattle ratios is optimal to avoid molybdenosis. Alary et al. (1981) observed mild symptoms of molybdenosis in cattle herds near a steelworks factory grazing on contaminated vegetation (via fly ash fallout deposits) with Cu:Mo ratios of less than 3:1.

Miltimore and Mason (1971) reported that molybdenosis can occur when the Cu:Mo dietary ratio is less than 2:1. They presented ratios based on Cu and Mo concentrations with low Cu concentrations (average 5.7 mg/kg). The authors found that molybenosis-induced scouring (i.e., diarrhea) occurred when the Cu:Mo ratio was 2.3 and severe scouring occurred when the Cu:Mo ratios averaged 1. ENV (1986) and Olkowski (2009) both indicate that effects to the metabolism of ruminants (including Cu deficiency) may occur at Cu:Mo ratios near 2:1.

Suttle (1991) found that the tolerable ratio declines from 5:1 to 2:1 as concentrations of Mo in the pasture forage increase from 2 to 10 mg/kg dry weight (with concurrent Cu increases from 0.4 mg/kg to 5 mg/kg). Conversely, Gardner et al. (2003) found no evidence of Mo toxicity, as measured by weight gain, in herds grazing on forage containing between 13-19 mg/kg of Cu and 21-44 mg/kg of Mo (Cu:Mo ratios ranged from 0.35:1 to 0.62:1) in the health of herds grazing on reclaimed mining areas in British Columbia. Gardner et al. (2003) postulated that the Cu concentrations in the forage were sufficiently elevated to overcome any effects of elevated Mo, or alternatively that the Mo present in the forage was not bioavailable to the cattle.

Raisbeck et al. (2006) found that pregnant cows showed no ill effects from grazing on forage with an average Mo concentration of 13 mg/kg while receiving a Cu supplement of 17 mg/kg (a Cu:Mo ratio of 1.3:1). They noted that trichloracetic acid (TCA)-soluble serum Cu was high in cattle exposed to very high Mo in their forage and concluded that there may be some undefined physiological factors at very high dietary Mo intakes that result in increased TCA-soluble serum Cu which mitigates the potential for molybdenosis.

Currently, there is no consensus on the safe Cu:Mo ratios for ruminants and the concept of a ‘safe’ Cu:Mo threshold in ingested food (and soil) should be re-visited in light of the molybdenosis mechanistic model proposed by Gould and Kendall (2011). A more formalized investigation of potentially important co-variates, such as sulfur species, iron, tungsten, and other trace elements, is also warranted. Finally, studies are also needed to evaluate the interspecific differences in critical dietary proportions of Mo, Cu, and other substances. In conclusion, Cu:Mo ratios in exposure media should not be used to predict the risk of molybdenosis in the absence of other lines of evidence.

8.3 Terrestrial Plants

Leaf and above ground biomass Mo concentrations are generally correlated with Mo concentrations in soil (Tyler, 2000), however, the bioavailability of Mo to plants is affected by soil properties (Zimmer and Mendel, 1999; Connick et al., 2010; McGrath et al., 2010b). Several studies have reported the effects of soil properties on Mo toxicity to plants (McGrath et al., 2010b; Oorts et al., 2012; van Gestel et al., 2011; Oorts et al., 2016). Regression analyses (log-log basis) revealed that molybdate toxicity to terrestrial plants (EC_{50} estimates) was correlated with soil pH, effective cation exchange capacity and content of clay, ammonium oxalate-extractable Fe oxides, and organic carbon in the soil (Oorts et al., 2012). Single linear and multivariate regressions between soil toxicity thresholds (EC_{50} estimates) and soil properties were analysed. Multiple regressions with a combination of pH and clay content showed a better regression fit (i.e., high R^2 value) compared to single linear regressions. The multiple regression models developed by Oorts et al. (2012) resulted in species-specific slope factors for pH and clay content that accounted for 78 to 91% of the variance in the toxicity of soil Mo to five plant species (i.e., rapeseed, red clover, ryegrass, tomato and barley) tested in ten different soil types.

8.3.1 *pH*

The predominant form of soluble Mo at neutral soil pH is the molybdate anion (MoO_4^{2-}), which is the bioavailable form for plants (Kaiser et al., 2005; McGrath et al., 2010a; Anjum et al., 2015; Oorts et al., 2016). The adsorption of the molybdate anion to soil is maximized under acidic conditions and decreases with increasing soil pH (ATSDR 2017) becoming more bioavailable for uptake to vegetation under non-acidic conditions. Mo deficiency is common in acidic soils and can be ameliorated by soil liming to increase pH (Brennan, 2006; BC MOA, 2015). Table 8.1 illustrates the significance of soil pH on Mo uptake by plants. McGrath et al. (2010a) measured the effect of a greater range of soil pH on the uptake of Mo by rapeseed shoots (Table 8.2; adopted from Oorts et al., 2012)

Table 8.1. Effect of soil pH on molybdenum content in plants.

Soil pH	Molybdenum in soil (ppm)	Molybdenum in plant (ppm)
5.5 – 5.7	24	<1
5.9 – 7.9	<24	60

Table 8.2. Effect of soil pH on molybdenum uptake by plants (rapeseed shoots).

Molybdenum Exposure concentration (added) mg/kg soil	Mean molybdenum shoot concentration (mg/kg dry matter)			
	pH 5	pH 6	pH 7	pH 8
0 (Control)	7.08	7.02	9.56	16.53
10	939	963	1,506	2,284

8.3.2 *Soil Clay Content*

A key factor controlling the bioavailability of the molybdate anion in soil is its binding to positively charged soil components, such as clay, oxides, and organic matter. Binding these components results in molybdate partitioning out of soil solution, towards the soil solid phase, thus reducing Mo mobilisation and uptake by plants (McGrath et al., 2010b). Jiang et al. (2015) also reported that the availability of Mo is lower in soils with higher contents of soil organic carbon. Oorts et al. (2012) used the clay content as surrogate for the actual binding surfaces present on clays, including oxides and organic matter. They used multiple

regression to compare clay content and soil pH to Mo toxicity in plants and found the EC50 values for five plant species could be predicted using this information.

9. MOLYBDENUM WATER QUALITY GUIDELINES FROM BRITISH COLUMBIA AND OTHER JURISDICTIONS

9.1 British Columbia

The B.C. Ministry of Environment and Parks established Mo WQGs in 1986 (ENV, 1986) for freshwater aquatic life at < 1.0 mg/L (long-term chronic) and 2.0 mg/L (short-term acute) (Table 9.1). The WQGs for both livestock and wildlife were 0.05 mg/L (short-term acute).

The long-term chronic WQGs for irrigation water were 0.01 mg/L for poorly-drained soils and 0.02 mg/L for well-drained soils and were developed to protect ruminants consuming crops. The lower WQG for poorly-drained soils is based on the potential for the accumulation of Mo in the root zone. A WQG of 0.03 mg/L for irrigation water to be applied for non-forage crops was also established. The short-term acute WQG for all soil types and crop types was 0.05 mg/L.

9.2 Canadian Council of Ministers of the Environment (CCME)

The Mo WQG for the protection of freshwater aquatic life is 0.073 mg/L. This WQG was derived by multiplying the lowest chronic toxicity value for rainbow trout by a safety factor of 0.1 (CCME, 1999b). This is an interim guideline, as the available toxicity data was limited at the time of derivation (CCME, 1999b). The CCME protocol requires long-term exposure data for at least three fish species, including one salmonid and one non-salmonid; three aquatic or semi-aquatic invertebrates, including one planktonic crustacean; and one aquatic plant. The interim WQG was derived in 1999; there are now sufficient toxicity data for Mo to meet the CCME minimum data requirements for a long-term WQG.

The CCME (1987) Mo WQG for livestock is 0.5 mg/L. For irrigation water, the Mo WQG is <0.01 mg/L for continuous use on all soils, or 0.05 mg/L for short-term use on acidic soils (CCME, 1987). These guidelines are recommended for irrigation of forage crops for the protection of livestock consuming feed with high levels of Mo, rather than the protection of plants, which can tolerate up to several hundred mg Mo/kg plant tissue (dry weight) without adverse effects (CCME, 1987).

9.3 Other Provincial Water Quality Guidelines

Canadian provinces develop their own WQGs or adopt WQGs from another jurisdiction (e.g., CCME). The Ontario Ministry of Environment develops Provincial Water Quality Objectives (PWQOs) for surface water to protect aquatic life (OMOE, 1994); their current interim PWQO for total Mo is **0.04 mg/L** (OMOE, 1999). Alberta has adopted the CCME guidelines for the protection of aquatic life and agriculture (GoA, 2018). Quebec has adopted the Michigan Department of Environmental Quality's short-term water quality criteria for aquatic life of **29 mg/L** (MDDEFP, 2002). Saskatchewan has recently developed a long-term Water Quality Objective WQO for the protection of aquatic life (Saskatchewan Water Security Agency, 2018) of **31 mg/L**; no short-term WQO was developed due to a lack of available data.

Table 9.1. Summary of water quality guidelines for total molybdenum by jurisdiction.

Jurisdiction	Aquatic life			Livestock (mg/L)	Wildlife (mg/L)	Irrigation (mg/L)			Year published
	Chronic (mg/L)	Acute (mg/L)	Marine (mg/L)						
CCME	0.073	NA	NA	0.5	NA	Continuous use on all soils Short-term use on acidic soils	0.01 0.05		1986
USEPA	NA	NA	NA	NA	NA	Continuous use on all soils Up to 20 years use on acid fine-textured soils	0.01 0.05		1972
Australia/ New Zealand	0.034	NA	0.023	0.15	NA	Long-term Short-term	0.01 0.05		2000
European Union	12.7	NA	1.92	NA	NA	NA			NA
Food and Agriculture Organization	NA	NA	NA	NA	NA	0.01			1994
British Columbia	1	2	NA	0.05	0.05		**Chronic**	**Acute**	1986
						Forage crops - poorly-drained soils – Cu:Mo ratio < 1:2	0.01	0.05	
						Forage crops - poorly-drained soils – Cu:Mo ratio > 1:2	0.02	0.05	
						Forage crops – well-drained soils	0.02	0.05	
						Non-forage crops	0.03	NA	
Ontario	0.04	NA	NA	NA	NA	NA			1999
Quebec	NA	29	NA	NA	NA	NA			2002
Saskatchewan	31	NA	NA	NA	NA	NA			2018

9.4 United States Environmental Protect Agency

The United States Environmental Protection Agency (USEPA) derives safe concentrations for freshwater environments using the Final Chronic Value (FCV) and Final Acute Value (FAV) methods (Heijerick and Carey, 2017). To derive the FCV, a dataset that includes chronic values for at least eight taxonomic families is required. Heijerick and Carey (2017) proposed an aquatic life chronic value for Mo of **36.1 mg/L** that met USEPA criteria but has yet to be officially accepted by the USEPA. Previously, the USEPA recommended a Mo limit of 0.010 mg/L for waters used continuously on all soils and a Mo limit of 0.050 mg/L for use up to 20 years on acid fine- textured soils (USEPA, 1972).

9.5 Australia and New Zealand

Australia and New Zealand have joint WQGs defined as trigger values (TGVs) that elicit a management response if exceeded (ANZECC 2000a; 2000b). Trigger values have not been calculated for Mo for the protection of aquatic life due to insufficient data. However, a low reliability TGV of **0.034 mg/L** was derived from applying an assessment factor (AF) of 20 to a *D. magna* NOEC for reproduction (ANZECC 2000b). The Australia and New Zealand trigger value for livestock watering is **0.15 mg/L**. The Australian National Water Quality Management Strategy (NWQMS) recommends a long-term (100 year) trigger value for agricultural irrigation water of **0.01 mg/L**. A short-term (up to 20 years) trigger value for irrigation water of **0.05 mg/L** (ANZECC 2000a).

Due to the lack of Mo toxicity data for marine environments, WQG values for Mo are currently based on a conservative threshold with a large safety factor (ANZECC, 2000b). The current Australian and New Zealand guideline has a low reliability TGV of **0.023 mg/L** that was derived with an AF of 200 (ANZECC, 2000b). Recently, a revised WQG for Mo in temperate and marine environments of **3.9 mg/L** has been recommended (van Dam et al., 2018).

9.6 European Union

The European Union (EU) introduced the Registration, Evaluation, Authorization and Restriction of Chemical substances (REACH) legislation in 2006, which requires risk assessments, based on toxicity data, for chemicals manufactured in and imported to Europe (Heijerick and Carey, 2017). Predicted no-effect concentrations (PNECs) are required for each substance of concern (Heijerick et al., 2012). The EU employs a statistical methodology for deriving safe freshwater concentrations based on a Species Sensitivity Distribution (SSD) of chronic toxicity data on a minimum of 10 organisms from 8 different taxonomic groups (De Schamphelaere et al., 2010). Using this method, Mo HC_5 (hazardous concentration affecting 5% of the species) values of **38.2 mg/L** in freshwater environments and **5.75 mg/L** for marine environments (De Schamphelaere et al., 2010; Heijerick et al., 2012) were calculated. Using an AF of three, a REACH freshwater PNEC was established at **12.7 mg/L** and a marine PNEC was set at **1.92 mg/L** (Heijerick et al., 2012).

9.7 Food and Agriculture Organization of the United Nations

The Food and Agriculture Organization (FAO) of the United Nations recommended an irrigation guideline for Mo of **0.01 mg/L** for the protection of livestock (Ayers and Westcot, 1994).

10. WATER QUALITY GUIDELINES FOR FRESHWATER AQUATIC LIFE

WQGs for the protection of freshwater aquatic life were derived using the guidance in *Derivation of Water Quality Guidelines for the Protection of Aquatic Life in British Columbia* (ENV, 2019a). A search of the current scientific literature for studies on Mo toxicity to freshwater aquatic organisms in water-only exposures under laboratory conditions was conducted and selected studies were evaluated to determine if they were scientifically sound and of high-quality (ENV, 2019a). Information on the test species, test conditions, experimental design, chemical and physical properties of the test water, statistical analyses, and negative control performance were reviewed. Studies were then classified as primary, secondary, or unacceptable based on the criteria given in ENV (2019a). A summary of all short-term and long-term primary and secondary data, and the studies classified as unacceptable, is provided in Appendix 2.

The data points were further classified as chronic long-term or acute short-term, in accordance with published protocols (ENV, 2019a; CCME, 2007). In total, 13 studies were classified as primary, four as secondary, and 22 as unacceptable (Appendix 2). From the primary studies, 10 short-term and 110 long-term data points were selected, and three short-term and 10 long-term data points were selected from the secondary studies (Table 10.1). Some studies investigated effects for both short- and long-term durations and therefore included both data types. In addition, some studies investigated the toxic effects of Mo on multiple species belonging to one or more taxonomic group (Appendix 2). Table 10.2 lists all aquatic species represented in the toxicity database.

Table 10.1. Distribution of primary and secondary data points between different taxonomic groups.

Taxonomic group	Total number of studies	Short-term data points	Long-term data points			
			Growth	Reproduction	Survival	Total
Primary studies						
Algae	1	0	4	0	0	4
Macrophytes	1	0	3	0	0	3
Invertebrates	5	7	11	47	11	69
Fish	6	2	23	0	11	34
Amphibians	0	0	0	0	0	0
Total	13	9	41	47	22	110
Secondary studies						
Algae	0	0	0	0	0	0
Macrophytes	0	0	0	0	0	0
Invertebrates	1	0	3	3	3	9
Fish	3	3	0	0	1	1
Amphibians	0	0	0	0	0	0
Total	4	3	3	3	4	10

Table 10.2. Aquatic species included in the molybdenum toxicity dataset.

Taxonomic Group	Common Name	Species	Primary/Secondary
Algae	Green Algae	*Pseudokirchneriella subcapitata*	P
Macrophytes	Duckweed	*Lemna minor*	P
Invertebrates	Amphipod	*Hyalella azteca*	P
	Great Pond Snail	*Lymnaea stagnalis*	P
	Midge	*Chironomus riparius*	P
	Midge	*Chironomus tentans*	P
	Oligichaete	*Tubifex tubifex*	P
	Rotifer	*Brachionus calyciflorus*	P
	Waterflea	*Ceriodaphnia dubia*	P
	Waterflea	*Daphnia magna*	P & S
Fish	Brown Trout	*Salmo trutta*	P
	Fathead Minnow	*Pimephales promelas*	P
	Flannelmouth Sucker	*Catostomus latipinnis*	S
	Rainbow Trout	*Oncorhynchus mykiss*	P & S
	White Sucker	*Catostomus commersoni*	P

The final database includes four primary long-term growth inhibition data points from one algae species, *P. subcapitata* (De Schamphelaere et al., 2010). De Schamphelaere et al. (2010) was also the only study that addressed Mo toxicity in macrophytes (*L. minor*) providing three primary chronic data points for growth inhibition.

The database includes eight invertebrate species with 69 primary long-term data points from five studies and seven primary short-term data points from two studies. Nine secondary long-term data points were included from one study (Diamantino et al., 2000). Eight invertebrate studies were classified as unacceptable (Appendix 2).

There are 34 primary long-term data points and one secondary long-term data point, and two primary short-term and three secondary short-term data points for fish. Eleven fish studies were classified as unacceptable (Appendix 2).

No acceptable studies on Mo toxicity in amphibians were found.

Most unacceptable studies were missing water chemistry data, insufficient data analysis information, or a lack of mortality data.

10.1 Water Quality Guideline Derivation

In total long-term data on 11 species and short-term data on seven species were classified as acceptable. Neither chronic or acute datasets meet the requirement for derivation of a type A1 WQG due to lack of data on amphibian and EPT (ENV, 2019a).

The toxicity datasets meet the minimum requirements for developing the next desired type of WQG (i.e., type A2 long-term chronic and a type A2 short-term acute WQGs) as outlined in ENV (2019a).

10.1.1 *Long-Term Chronic Water Quality Guidelines*

The primary long-term studies included data on one B.C. resident aquatic plant species, one resident algal species, six resident invertebrate species, and three resident fish species (including two salmonid species) (Table 10.3). These studies provide a total of 128 data points and include multiple endpoints and effect levels for different life-stages and test durations. The data were sorted and only the endpoint-effect level combinations that captured the lowest effects concentrations (i.e., most sensitive) from each study were selected for further use in the guideline derivation. If there was more than one comparable record (i.e., same species, same life stage, same endpoint, same exposure duration), the geometric mean of the effect concentrations were used. From this process, no-effect/low-effect estimates on 11 species were selected for deriving the WQG (Table 10.3). The only data available for white sucker (*Catostomus commersonii*), was reported by Pyle (2000). This data point was an unbounded NOEC (i.e., highest concentration did not result in any observed effects) and was considerably lower compared to other effect concentrations. Therefore, it was excluded from the dataset used to derive the chronic WQG.

The R package, *ssdtools* version 0.3.4 (Thorley and Schwarz, 2018) was used to estimate an HC_5 value using maximum likelihood estimation (MLE) and model averaging of six distributions ((i.e., log logistic, log normal, gamma, log Gumbel, Weibull and Gompertz). The calculated HC_5 value is 30.2 mg/L (Figure 10.1).

To account for the sources of uncertainty associated with WQG derivation, an AF must be applied to the calculated HC_5 (ENV, 2019a). The AF begins with a default value of five that may be reduced or increased depending upon the residual uncertainty of the WQG (ENV, 2019a). The minimum AF to be applied to Type A WQGs is 2 to account for the extrapolation of laboratory testing to field conditions. The chronic data set fulfills the minimum number of species required for a type A2 guideline, but multiple uncertainties remain (Table 10.4). There lacks a working hypothesis for the mode of toxic action and there are no data for EPT, B.C. resident amphibians or reproduction in fish. Furthermore, there are no studies of the long-term effects of elevated Mo on aquatic ecosystems. Although most research results suggest that aquatic life are relatively unaffected by Mo (GEI, 2009; De Schamphelaere et al., 2010), the data gaps described above warrant a precautionary approach. For this reason, the AF of 4 was selected and applied to the calculated HC_5 resulting in a WQG of 7.6 mg/L.

The recommended long-term chronic WQG for Mo of 7.6 mg/L is directly applicable to all B.C. waters as background concentrations are typically four orders of magnitude lower than this value (0.48-0.58 µg/L, Section 6.1).

Table 10.3. Data points used to develop the molybdenum long-term water quality guideline for the protection of freshwater aquatic life.

Receptor Group / Species	Selected toxicity test endpoint	Exposure duration	Effect value (mg/L)	Reference
Plants/Algae				
Lemna minor	EC_{10}; Growth	7-d	241.5	De Schamphelaere et al., 2010
Pseudokirchneriella subcapitata	EC_{10}; Growth	72-h	110.4*	De Schamphelaereet al., 2010
Invertebrates				
Brachionus calyciflorus	EC_{10}; Reproduction	48-h	193.6	De Schamphelaere et al., 2010
Ceriodaphnia dubia	$IC_{10/12.5}$; Reproduction	7-8-d	51.3	Naddy et al., 1995; GEI, 2009; and De Schamphelaere et al., 2010
Chironomus riparius	EC_{10}; Growth	14-d	121.4	De Schamphelaere et al., 2010
Daphnia magna	EC_{10}; Reproduction	21-d	93.7*	GEI, 2009; De Schamphelaere et al., 2010
Hyalella azteca	EC_{10}; Reproduction	42-d	44.6	Heijerick and Carey, 2017 (originally from Ziese et al., 2016)
Lymnaea stagnalis	EC_{10}; Growth (length)	28-d	211.3	De Schamphelaere et al., 2010
Fish – non-salmonid species				
Pimephales promelas	EC_{10}; Growth	34-d	39.3	De Schamphelaere et al., 2010
Fish – salmonid species				
Oncorhynchus mykiss	EC_{10}; Biomass	78-d	43.2	De Schamphelaere et al., 2010
Salmo trutta	EC_{10}; Growth	85-d	202	Lucas et al., 2017

EC = effective concentration; d=days; and h=hours.

IC = inhibitory concentration; LC = lethal concentration;

* The reported effect concentrations are geometric means of similar data points (i.e., same species, same life stage, same endpoint and same exposure duration).

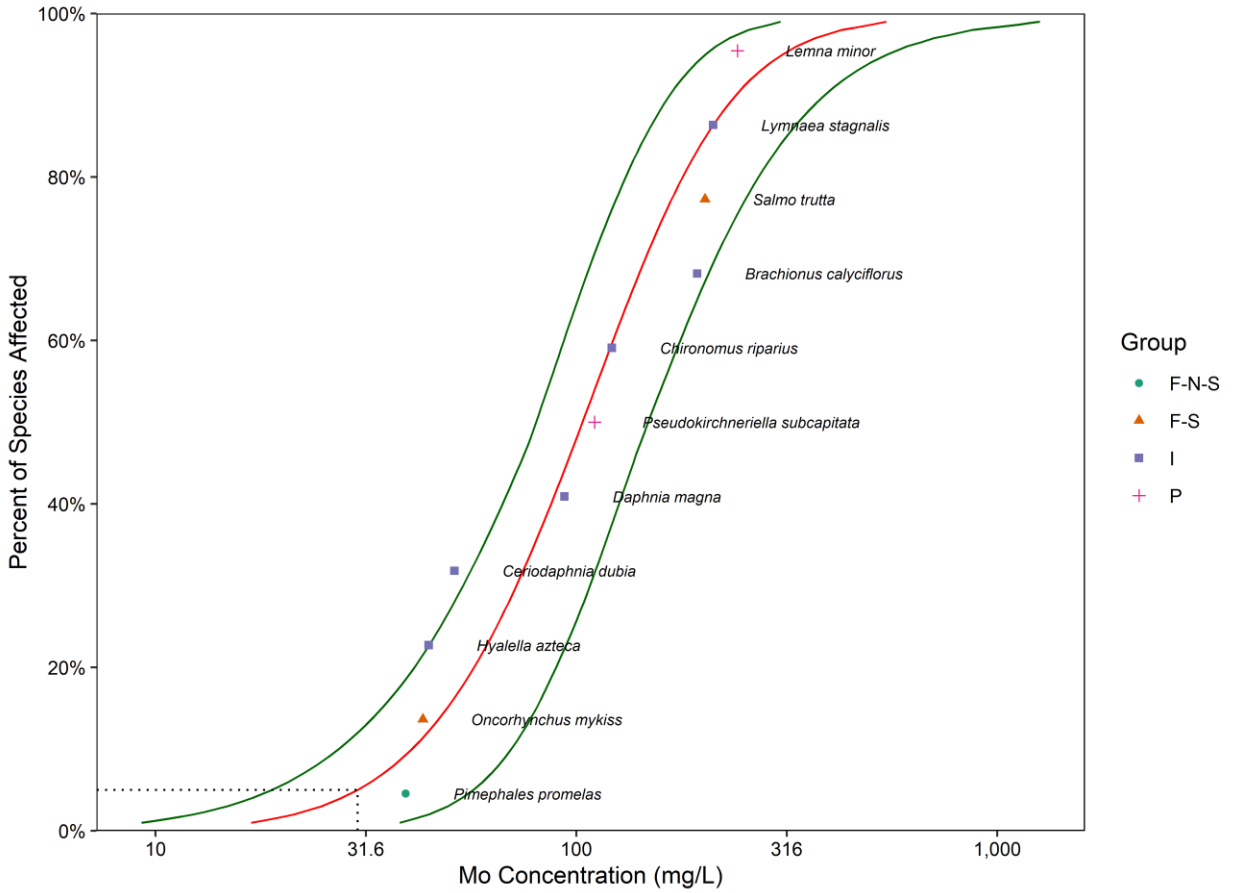

Figure 10.1. The distribution of no-effect/low-effect estimates from the primary studies used to derive the molybdenum type A2 long-term water quality guideline for the protection of freshwater aquatic life.
The red line represents the fit of the averaged distribution and the green lines represent 95% confidence intervals of the fit. Dashed line denotes the HC_5 at the concentration of 30.2 mg/L. F-N-S = non-salmonid fish; F-S = salmonid fish; I = invertebrate; P = plant.

Table 10.4. Considerations for determining the assessment factor for chronic long-term WQGs to protect freshwater aquatic life (ENV, 2019a).

Consideration	Evaluation
The taxonomic and life stage representativity of the database.	No intergenerational information. No information on amphibians or EPT. No information on reproduction in fish.
Knowledge of the toxicity modifying factors and mode of action of the substance.	There is limited information on the potential toxicity modifying factors of Mo and no working hypothesis for mode of action (see Sections 7 and 8).
Whether or not the SSD dataset includes no effect and low effect levels and/or lethal and non-lethal endpoints.	The SSD dataset consists of no-effect data (i.e., EC_{10} and $EC_{12.5}$). All the endpoints are non-lethal endpoints.
Statistical uncertainties of the HC_5 estimate.	The HC_5 estimation is 30.2 with lower and upper CLs of the HC5 are 12.7, and 64.3 respectively. The average model has a poor fit based on visual inspection and the four most sensitive species have a near vertical curve.
The level of agreement between the estimated HC_5 and mesocosm and/or field studies	No information is available on mesocosm and/or field studies.

10.1.2 *Short-Term Acute Water Quality Guidelines*

The primary and secondary short-term studies contained short-term acute data on four B.C. resident invertebrate species and two B.C. resident fish species (Appendix 2). Toxicity data on flannelmouth sucker (*Catostomus latipinnis*) was also included in the acute toxicity dataset. Flannelmouth sucker is a North American species and is used as a surrogate for five B.C. resident sucker species from the same genus:

- bridgelip sucker (*Catostomus columbianus*);
- largescale sucker (*Catostomus macrocheilus*);
- mountain sucker (*Catostomus platyrhynchus*);
- longnose sucker (*Catostomus catostomus*); and
- white sucker *(Castostomus commersonii).*

The available short-term acute toxicity data met the minimum data requirements for the development of a type A2 short-term acute WQG.

The primary and secondary studies included 13 LC_{50} values for B.C. resident species (Appendix 2). In cases where data were available for different life-stages, the LC_{50} that captured the lowest effect concentrations (i.e., most sensitive) was selected for further use in the guideline derivation. If there was more than one comparable study (i.e., same species, same life stage), the geometric mean of effect concentrations was calculated and used instead of the individual values. This resulted in a total of seven LC_{50} estimates to derive the short-term acute WQG (Table 10.5).

Table 10.5. Data points used to develop the short-term acute molybdenum water quality guideline for the protection of freshwater aquatic life.

Group	Species	Exposure duration	LC_{50} (mg/L)	Rank	Reference
Invertebrates					
	Ceriodaphnia dubia	48-h	1,015*	Primary	GEI, 2009
	Chironomus tentans	48-h	7,533*	Primary	GEI, 2009
	Daphnia magna	48-h	1,727*	Primary	GEI, 2009
	Tubifex tubifex	96-h	2,782	Primary	Lucas et al., 2017
Fish - non-salmonid species					
	Pimephales promelas	96-h	643*	Primary	GEI, 2009
	Catostomus latipinnis	96-h	1,940	Secondary	Hamilton and Buhl, 1997
Fish - Salmonid Species					
	Oncorhynchus mykiss	96-h	800	Secondary	McConnell, 1977

LC = lethal concentration; -h = hour.

*The reported effect concentrations are geometric means of results from comparable studies (i.e., same species, same life stage)

LC_{50} estimates of seven species were used to derive the WQG. The R package, *ssdtools* version 0.2.0 (Thorley and Schwarz, 2018) was used to plot the toxicity data using MLE. Three distributions, (i.e., log normal, log logistic and gamma) were fitted to the toxicity data and a model averaging approach was taken to estimate an HC_5 value of 460 mg/L (Figure 10.2) (ENV, 2019a).

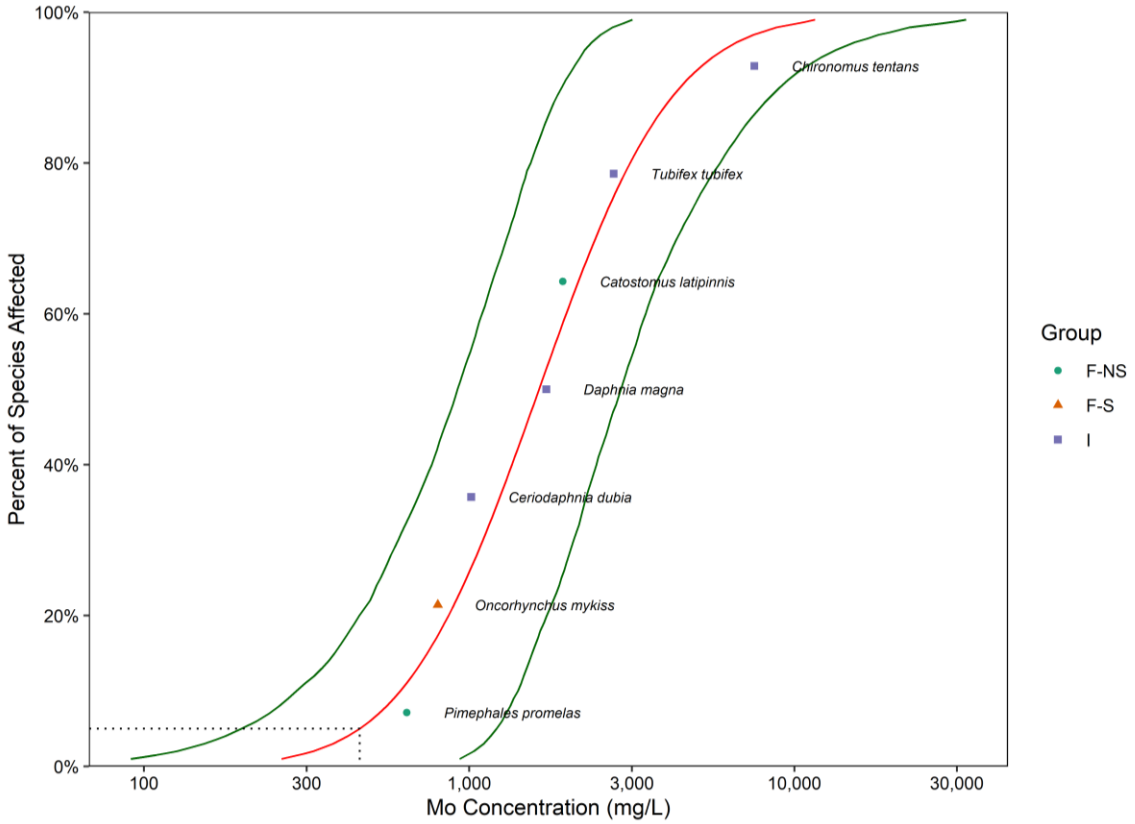

Figure 10.2. The distribution of LC_{50} estimates from the primary and secondary studies used to derive the molybdenum type A2 short-term acute water quality guideline for the protection of freshwater aquatic life.
The dashed line denotes a HC_5 value of 460 mg/L. The red line represents the fit of the averaged distribution and the green lines represent 95% confidence intervals of the fit. F-N-S = non-salmonid fish; F-S = salmonid fish; and I = invertebrate.

There are several uncertainties that need to be considered when assigning the AF for the acute WQG. As with the chronic WQG, there are no data for amphibians or EPT. De Schamphelaere et al. (2010) reported a 96-hour LC_{10} estimate of 415.4 mg/L and a survival LOEC of 369 mg/L for the African clawed frog (*Xenopus laevis*) which suggests amphibians may not be sensitive to Mo, but further information is required for resident B.C. amphibians. Although the toxicological data suggest that aquatic species are not sensitive, further information on EPT is required to ensure the recommended WQG is in fact a low risk benchmark for these taxa. This is especially important given that EPT taxa, a keystone aquatic group known to be sensitive to metals (Brix et al. 2011), provide essential ecological services and are a major food supply for fish in these ecosystems. In addition to the limited data for EPT taxa and amphibians, the mode of toxic action of Mo on aquatic species is unknown and the information on toxicity modifying factors is very limited. Without this information it is difficult to speculate on the possibility for interactions across multiple metals or under changing water chemistry conditions. These uncertainties are further emphasized by the sparse dataset (n=7). Though this suggests that aquatic species are not sensitive to Mo, it also implies a larger risk if additional data reveal sensitivity of an important taxa or unexpected interactions.

Based on the considerations provided in Table 10.6 and those described above, an AF of 10 was applied to the estimated HC_5 which gave an acute short-term WQG of 46 mg/L. The short-term acute WQG for

Mo of 46 mg/L is appropriate for freshwater ecosystems in B.C. as background Mo concentrations are approximately five orders of magnitude lower throughout the province.

Table 10.6. Considerations for determining the assessment factor for acute short-term WQGs.

Consideration	Evaluation
The taxonomic and life stage representativity of the database.	No available information on amphibians, EPT, aquatic plants or algae.
Knowledge of the toxicity modifying factors and mode of action of the substance	There is limited information on the potential toxicity modifying factors of Mo and no working hypothesis for mode of action (see Sections 7 and 8).
Statistical uncertainties of the HC_5 estimate	The HC_5 estimation is 460 and the lower and upper CLs of the HC_5 are 203, and 1240 respectively.

10.1.2.1 Protectiveness of B.C. acute molybdenum guidelines against short-term effects on survival

The WQG derivation protocol characterizes the protection of aquatic life by protecting individual organisms, resulting in the overall protection of populations (ENV, 2019a). However, the most abundant effect level in short-term toxicity studies is the LC_{50} (i.e., the concentration that causes lethality of half the test population). Although the application of an AF offers further protection, acute WQGs based on LC_{50}s may not be protective of sensitive species. To test this, the acute WQG was compared against no-effect concentrations for sensitive species.

Following B.C. protocol (ENV, 2019a), LC_{10} values for the three lowest effect concentrations were calculated from raw data provided in the individual studies and compared against the acute WQG. LC_{10} values are generally considered as no-effect thresholds (CCME, 2007). Fathead minnow was the most sensitive species to short-term Mo exposure, with a primary LC_{50} of 643 µg/L (GEI, 2009) and a calculated LC_{10} of 272 mg/L. Rainbow trout was the second most sensitive species with an LC_{50} of 800 mg/L (McConnell 1997) and a calculated LC_{10} of 326 mg/L. The third most sensitive species is *C. dubia* with the LC_{50} of 1,015 mg/L (GEI 2009) and a calculated LC_{10} of 294 mg/L. Therefore, the recommended acute WQG of 46 mg/L should protect sensitive species against short-term effects on survival.

10.2 Application of Water Quality Guidelines for Freshwater Aquatic Life

The Mo WQGs for the protection of freshwater aquatic life represent predicted no-effect concentrations for the most sensitive life-stage of the most sensitive species. The long-term chronic WQG represents a level which is predicted to protect all aquatic species from negative sub-lethal effects of Mo over indefinite exposures. The short-term acute guideline is designed to protect aquatic species from severe effects, such as lethality, and represents a level that should not be exceeded at any given time.

The long-term chronic and short-term acute Mo WQGs for the protection of freshwater aquatic life are based on the total concentration of Mo in water. While some laboratory studies used in the derivation process reported the effect concentration as the dissolved fraction of Mo (e.g., De Schamphelaere et al., 2010), others reported total concentration of Mo (e.g., GEI, 2009). In addition, evidence of the mechanism of toxicity of Mo is inconclusive; there is no evidence to suggest only the dissolved fraction of Mo in water is toxic to biota and there are uncertainties regarding the role of potential toxicity modifying factors. Therefore, the derived short-term acute and long-term chronic WQGs apply to the total Mo fraction measured in environmental samples.

Molybdenum concentrations are variable in natural waters, therefore, an averaging period approach is used to compare environmental conditions to the WQG. Average concentrations are calculated from a minimum of five weekly samples collected over a 30-day period. Only 20% of the samples (e.g., 1 in 5 samples) can exceed the chronic Mo WQG, provided that the short-term acute WQG is never exceeded. In cases where less than five samples are available, each Mo concentration is compared individually against the chronic long-term WQG.

The Mo short-term acute WQG is a concentration that should not be exceeded at any time to meet the intended protection of the most sensitive species and life stage against severe effects. Short-term maximum WQGs are intended to assess risks associated with infrequent and transient exposure events such as spills.

The long-term chronic and short-term acute WQGs developed in this document do not allow for the direct evaluation of the toxic effects of Mo in combination with other substances (e.g., the possible synergistic or antagonistic interactions). Rather, the application of an AF is meant to account for various uncertainties in extrapolating laboratory data to field conditions. Additional investigation may be needed at sites with multiple contaminants to ensure the protection of freshwater aquatic life.

11. WATER QUALITY GUIDELINES FOR LIVESTOCK AND WILDLIFE

The derivation of WQGs for livestock drinking water follows the CCME publication *Protocols for Deriving Water Quality Guidelines for Agricultural Water Uses (Irrigation and Livestock Water)* (CCME 1999a). This protocol was also used to inform the derivation of WQGs for the protection of wildlife since B.C. and CCME do not have a specific protocol for this application.

11.1 Tolerable Daily Intake Calculation

To develop the livestock and wildlife WQG, a threshold level of total environmental Mo exposure (i.e., the tolerable daily intake (TDI)) that meets the required ecological protection goals described in CCME (1999a) was identified. The TDI is the substance concentration which is not anticipated to result in any adverse health effects following chronic exposure to a population of livestock species, including sensitive subgroups such as nursing calves. Adverse effects are considered as functional impairment or pathological lesions which may affect the performance of the organism or reduce its ability to respond to additional stressors (CCME, 1999a). Unacceptable effects are not expected to occur at contaminant concentrations below the TDI, while there is a potential for toxic effects to occur at concentrations above the TDI.

In deriving TDIs, an uncertainty factor (UF) was applied to the geometric mean of the Lowest Observed Adverse Effect Level (LOAEL) and the No Observed Adverse Effect Level (NOAEL) values. This was done for each species and the resulting lowest value was selected as the species-specific TDI. The UF accounts for a range of uncertainties including: differences in sensitivity associated with genetic variability within the species; sex; life stage; duration of exposure (i.e., to extrapolate to life-time exposures); nature and severity of the effect measured; exposure route; and lab versus field conditions (CCME 1999a).

11.1.1 *Acquisition of Toxicological Data*

To determine the TDI, data on the toxicity of Mo to livestock and wildlife species were compiled from published literature. Given that the mechanism of toxicity in ruminants is different from non-ruminants, and ruminants are significantly more sensitive to Mo toxicity than non-ruminants, separate data were collected for ruminants, non-ruminant mammals, and birds. The B.C. Water Quality Criteria for Molybdenum (ENV, 1986) included a review of studies on the effects of Mo on livestock and wildlife available at that time. Therefore, the search for additional Mo toxicological and epidemiological studies

for this update focused on papers and reports published since 1986 (see Millennium EMS Solutions 2014). The studies obtained for further evaluation included goats (Kusum et al., 2010), rabbits (Bersenyi et al., 2008), cattle (Kessler et al., 2012), chickens (Yang et al., 2011; Xiao et al., 2011a,b), and red deer (Grace et al., 2005).

11.1.2 *Evaluation and Classification of Toxicological Data*

Studies and toxicological data were classified as primary, secondary, and unacceptable, as per CCME (1999a) with the below exception. CCME (1999a) suggests that a study is classified primary if the dosage rates are reported and secondary if the exposure concentrations, the ingestion rate and the body weight of the test organisms are presented (i.e., reported all the components required to calculate dose). However, for this WQG, studies that did not report all needed components for dose calculation were still considered secondary if reliable estimates were available. The studies were also evaluated to determine if the exposure duration was chronic (i.e., exposure duration equal or more than half of the organisms' life cycle) or sub-chronic (exposures duration less than half of organisms life cycle) (Sample, 1996).

The dose to which the test animals were exposed was calculated as mg/kg body weight (BW)/day (see Section 11.1.4 for more details). Effect levels were categorized as either a no-observed adverse effect level (NOAEL), determined within each individual study as the highest dose at which there was not an observed adverse effect, or a lowest observed adverse effect levels (LOAEL), determined within each individual study as the lowest dose at which there was an observed adverse effect.

11.1.3 *Selected Toxicity Data*

Seventy-six studies were screened and 27 were acceptable (10 primary and 17 secondary) for use in WQG derivation (Appendix 2). Of these, only 20 provided the necessary LOAEL data (Appendix 2) needed for dose and TDI calculations (see sections 11.1.4). These included 12 ruminant studies, nine non-ruminant mammal studies, and three bird studies. Further details are provided in Appendix 2.

The toxicological dataset did not meet the minimum requirements for a full WQG (i.e., primary data on three or more mammalian species and two or more avian species), however, the minimum requirement for an interim WQG (i.e., primary or secondary data on two or more mammalian species and one or more avian species) was met (CCME, 1999a). More conservatism is applied towards the derivation of interim WQGs compared to full WQGs and interim WQGs are ideally replaced by full WQGs when knowledge gaps are filled (CCME, 1999a).

11.1.4 *Methodology for Dose Calculation*

Results expressed in terms of exposure concentrations were converted to dose values (Appendix 3) for use in TDI calculations. Molybdenum concentrations reported as wet weight (WW) (typically grain or forage) were standardized to dry weight (DW), using the following conversion:

Equation 1: Food Dry Weight Conversion from Wet Weight

$$\text{DW (kg)} = \text{WW (kg)} \times \frac{100 - \text{percent moisture}}{100}$$

Equation 2: Molybdenum (Mo) Concentration Conversion to Food Dry Weight from Food Wet Weight

$$\text{Mo Concentration}\left(\frac{\text{mg}}{\text{kg DW Food}}\right) = \text{Mo Concentration}\left(\frac{\text{mg}}{\text{kg WW Food}}\right) \times \left[\frac{100}{100 - \text{percent moisture}}\right]\}$$

Where percent moisture was not reported, wet food was assumed to consist of 8.67% moisture, based on values reported in Quinton et al. (1993), and representative of herbivore diets. Where ingestion rates were not reported, the rates provided in ENV (1996) were used. For studies that did not include Mo exposure as a dose, in mg/kg BW/day, the dose was calculated using Equation 3.

Equation 3: Dose Calculation

$$\text{Mo Dose}\left[\text{mg}\frac{\text{Mo}}{\text{day} * \text{BW kg}}\right] = \text{Mo Concentration}\left(\frac{\text{mg}}{\text{kg DW food}}\right) \times \text{Food Ingestion Rate}\left(\frac{\left(\frac{\text{kg DWfood}}{\text{day}}\right)}{\text{Body weight (kg BW)}}\right)$$

The collated data for each study was categorized to the four following groups: ruminants; non-ruminant mammals; and birds (Tables 11.1 to 11.3). Graphical summaries of toxicity endpoints are provided in Figures 11.1 to 11.3.

Table 11.1. Studies selected for tolerable daily intake derivation – ruminant livestock.

Test species	Life stage	Exposure duration	Endpoint	Dose (mg/kg BW/day)	Effect Level	Classification/ dose reported or calculated	Reference
Cattle	Juvenile (male)	Sub-chronic (100 days)	Body weight	0 1.5	CON LOAEL	Primary (reported)	Cook et al., 1966
Cattle	Juvenile (male)	Sub-chronic (56 weeks)	Food intake & Molybdenosis symptoms	0 4.98	CON LOAEL	Primary (calculated)	Kessler et al., 2012
Cattle	Juvenile (female)	Sub-chronic (336 days)	Body weight & Blood factor-hematocrit	0 3.38	CON LOAEL	Primary (calculated)	Lesperance et al., 1985
Cattle	Adult (female)	Sub-chronic (1 year)	Symptoms of molybdenosis	0.30 5.29	NOAEL LOAEL	Secondary (calculated)	Raisbeck et al., 2006
Sheep	Adult (female)	Sub-chronic (9 weeks)	Body weight & reproduction behavior - silent heats	0 1.61	CON LOAEL	Secondary (calculated)	du Plessis et al., 1999
	Juvenile (female)	Sub-chronic (18 weeks)	Body weight & Reproduction - early anoestrus	0 2.08	CON LOAEL		
Sheep	Juvenile (male and female)	Sub-chronic (108 days)	Physical condition & wool characteristics	0 0.86	CON LOAEL	Secondary (calculated)	Mills and Fell, 1960
Mule deer	Adult (female)	Sub-chronic (25 days)	Body weight	11.11 27.78	NOAEL LOAEL	Secondary (calculated)	Nagy et al., 1975
Red deer	Juvenile (female)	Sub-chronic (100 days)	Body weight	0.12 0.33	NOAEL LOAEL	Secondary (calculated)	Grace et al., 2005

Table 11.2. Studies selected for tolerable daily intake derivation – non-ruminant wildlife (mammals).

Test species	Life stage	Exposure duration	Endpoint	Dose (mg/kg BW/day)	Effect level	Classification/dose reported or calculated	References
Mouse	Adult (male)	Sub-chronic (100 days)	Reproduction - sperm motility and abnormality	0 84.2	Control LOAEL	Secondary (calculated)	Wang et al., 2016
Mouse	Adult (male)	Sub-chronic (14 days)	Reproduction - sperm normality	10.5 21.1	NOAEL LOAEL	Secondary (calculated)	Zhai et al., 2013
Mouse	Adult (female)	Sub-chronic (14 days)	Reproduction - ovulation and oocyte morphology Reproduction, ovarian hyperemia and abnormal mitochondria	4.2 8.4 2.1 4.2	NOAEL LOAEL NOAEL LOAEL	Secondary (calculated)	Zhang et al., 2013
Rabbit	Juvenile (male and female)	Sub-chronic (17 weeks)	Body weight, abnormality and survival	16.9 33.9	NOAEL LOAEL	Secondary (calculated)	Arrington and Davis, 1953
Rat	Juvenile (male and female)	Sub-chronic (7 weeks)	Growth - body weight	5.7 11.4	NOAEL LOAEL	Secondary (calculated)	Gray and Ellis, 1950
Rat	Juvenile (male)	Sub-chronic (4 weeks)	Growth - body weight	0 71	Control LOAEL	Secondary	Neilands et al. 1948
Rat	Adult (male and female)	Sub-chronic (90 days)	Growth - body Weight	17 60	NOAEL LOAEL	Primary (reported)	Murray et al., 2014b
Rat	All life stages (male and female)	Chronic (two generations)	Growth - body Weight, food and water consumption	17 40	NOAEL LOAEL	Primary (reported)	Murray et al., 2019
Rat	Adult (male)	Sub-chronic (60 days)	Reproduction - sperm motility and count, weight of several organs	7.1 21.4	NOAEL LOAEL	Primary (reported)	Pandey and Singh, 2002

Table 11.3. Studies selected for tolerable daily intake derivation – non-ruminant livestock and wildlife (birds).

Test Species	Life stage	Exposure duration	Endpoint	Dose (mg/kg BW/day)	Effect level	Classification/dose reported or calculated	References
Chicken	Juvenile (male and female)	Sub-chronic (4 weeks)	Growth - body weight	27.2 34.0	NOAEL LOAEL	Secondary (calculated)	Davies et al., 1960
Chicken	Adult (female)	Sub-chronic (21 days)	Growth - body weight	0 28.4	CON LOAEL	Secondary (calculated)	Lepore and Miller, 1965
		Sub-chronic (19 days)	Reproduction - egg production	0 28.4	CON LOAEL		
		Sub-chronic (19 days)	Reproduction - embryo survival	0 28.4	CON LOAEL		
Quail	Juvenile (male and female)	Sub-chronic (30 days)	Growth - body weight	134 253	NOAEL LOAEL	Primary (reported)	Stafford et al., 2016

CON = control

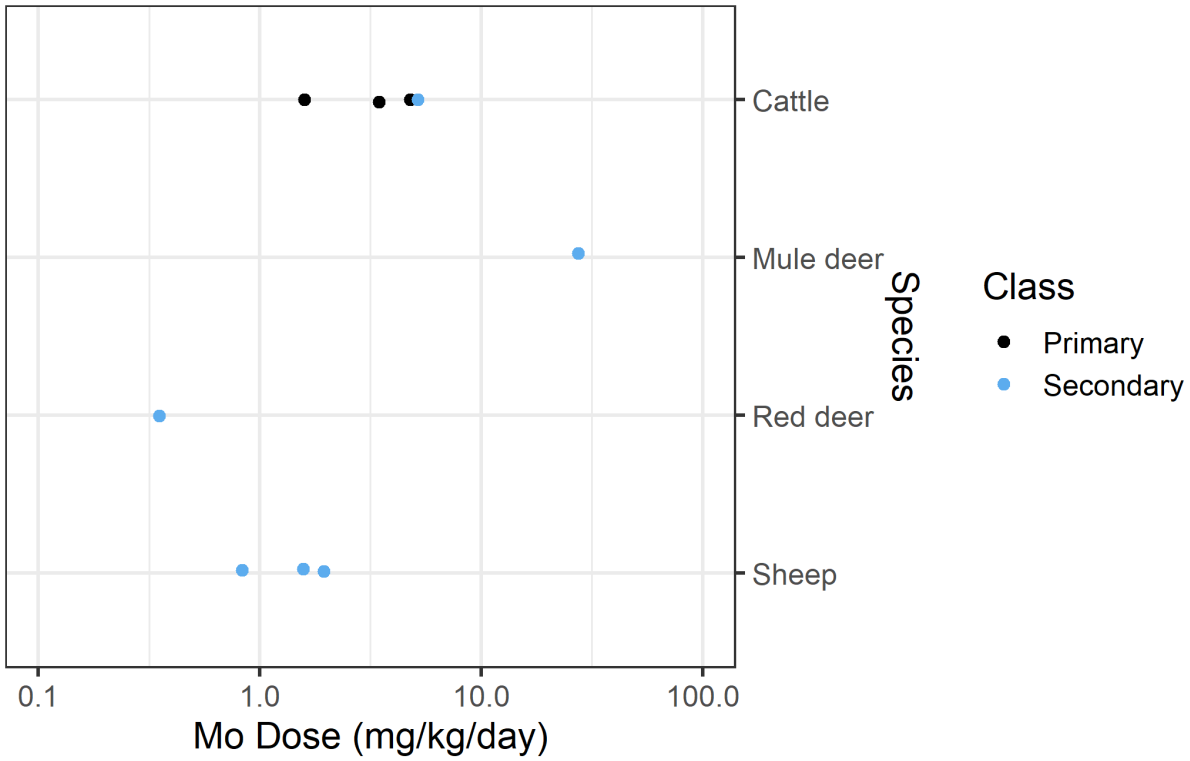

Figure 11.1 Distribution of LOAEL data from primary and secondary studies used to determine TDIs for ruminants.

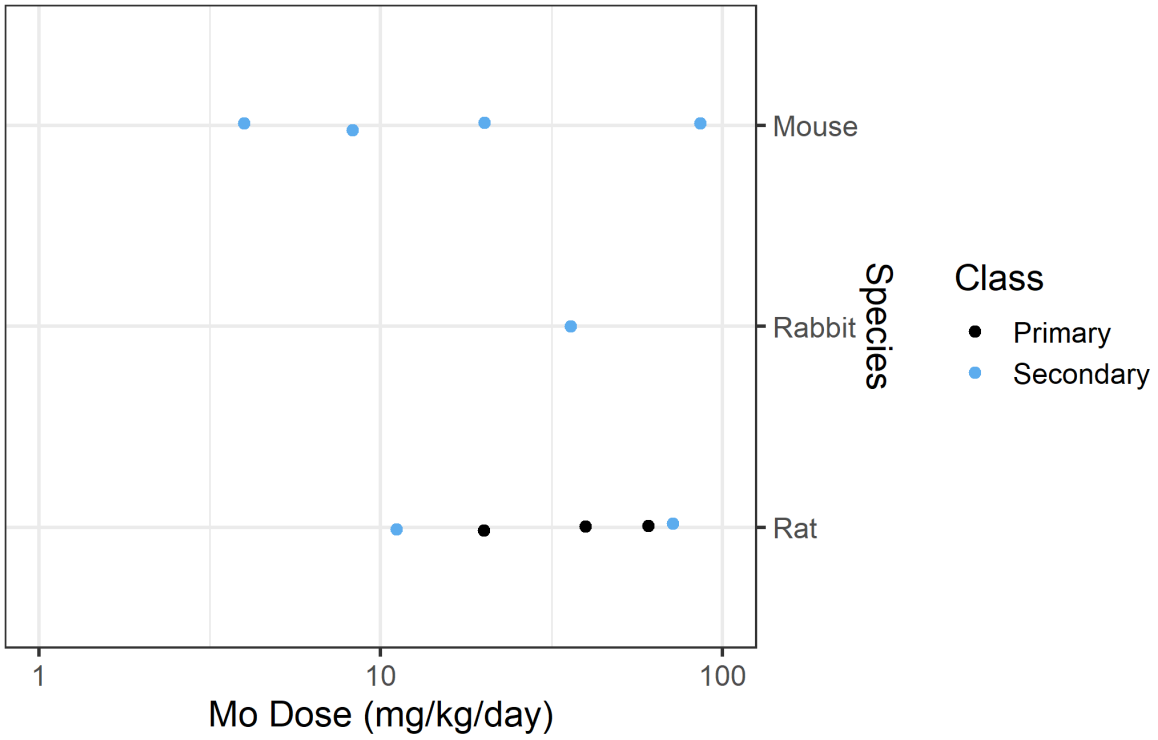

Figure 11.2 Distribution of LOAEL data from primary and secondary studies used to determine TDIs for non-ruminant mammals.

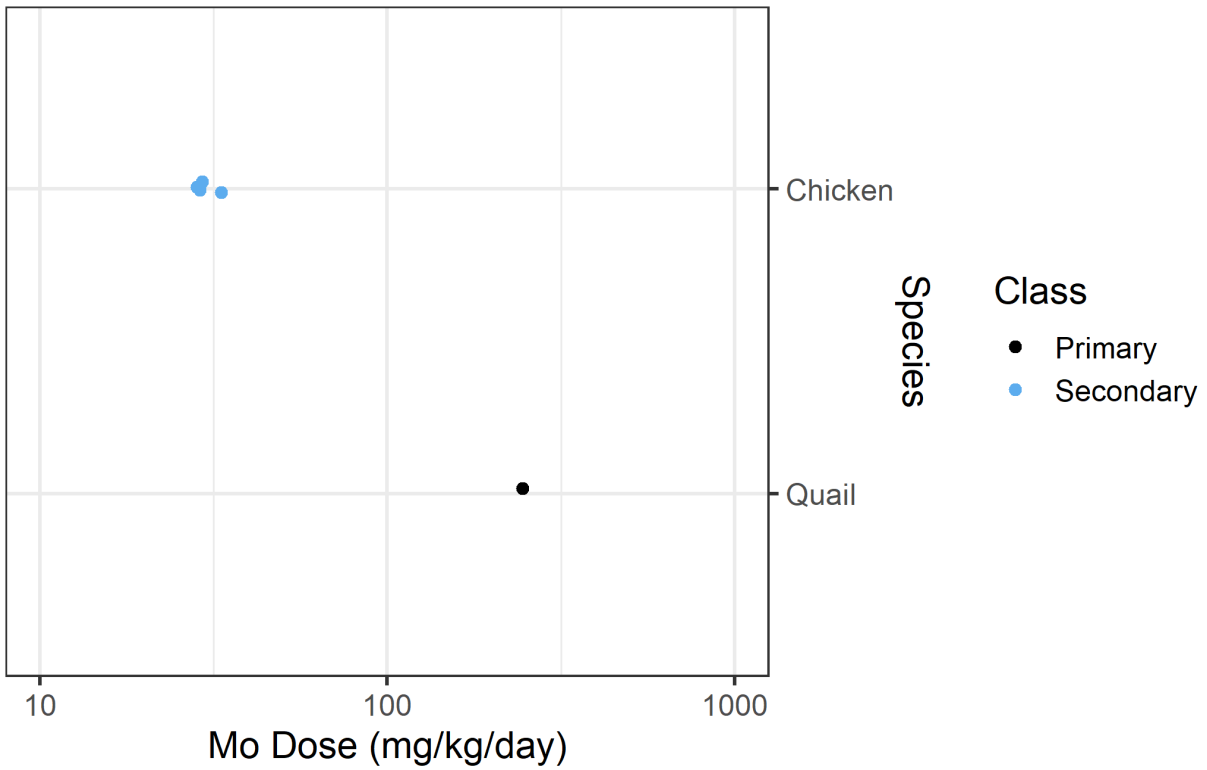

Figure 11.3. Distribution of LOAEL data from primary and secondary studies used to determine TDIs for birds.

11.1.5 *TDI Calculation*

As per the CCME (1999a) protocol, the lowest geometric mean of doses associated with LOAEL and NOAEL for each species was selected and an appropriate UF was applied to calculate species-specific TDI values (Table 11.5) using Equation 4.

Equation 4: TDI Calculation

$$TDI = (LOAEL \cdot NOAEL)^{0.5} \div UF$$

Whenever the NOAEL equals 0, the NOAEL was estimated using Equation 5 (CCME, 1999a).

Equation 5: NOAEL Calculation

$$NOAEL = LOAEL \div 5.6$$

Since the lowest LOAEL for all species were from sub-chronic studies an UF of 20 was applied instead of the minimum UF of 10 to account for additional uncertainty of extrapolation from sub-chronic to life-time exposures (CCME 1999a). The only exceptions were for the TDIs calculated from the data presented on mice in Zhang et al. (2013). The Zhang et al. (2013) study is technically defined as sub-chronic, however, the minimum UF of 10 was applied because this study investigated the effects of Mo on very sensitive endpoints (i.e., ovarian hyperemia and abnormal mitochondria).

Table 11.4. Tolerable daily intakes calculated for each species. Bolded values are the minimum values for each group.

Species	Exposure duration	LOAEL	NOAEL	Effect	Uncertainty factor*	TDI (mg/kg BW/day)	Source
Ruminants							
Cattle	Sub-chronic	1.5	0.26**	Growth	20	0.031	Cook et al., 1966
Sheep	Sub-chronic	0.86	0.15**	Physical condition	20	0.018	Mills and Fell, 1960
Mule deer	Sub-chronic	27.8	11.1	Growth	20	0.878	Nagy et al., 1975
Red deer	Sub-chronic	0.33	0.12	Growth	20	**0.010**	Grace et al., 2005
Non-Ruminant mammals							
Mouse	Sub-chronic	4.2	2.1	Reproduction	10	**0.296**	Zhang et al., 2013
Rabbit	Sub-chronic	33.9	16.9	Growth	20	1.196	Arrington and Davis, 1953
Rat	Sub-chronic	21.4	7.1	Growth	20	0.616	Pandey and Singh, 2002
Birds							
Chicken	Sub-chronic	31.3	5.59*	Growth and reproduction	20	**0.600**	Lepore and Miller, 1965
Quail	Sub-chronic	253	134	Growth	20	9.206	Stafford et al., 2016

Note: * Uncertainty factors of 20 were applied to the geometric mean of LOAEL and NOAEL (reported or estimated) to the data from sub-chronic exposures except for Zhang et el. (2013) where an UF of 10 was applied.
** Since the NOAEL was not available for these studies, an estimation of NOAEL was calculated by dividing the LOAEL by 5.6 (CCME, 1999a).

11.2 Guideline Derivation

The TDI values were used to calculate reference concentrations in livestock and wildlife drinking water. Since only the minimum data set for an interim guideline is fulfilled, the most conservative livestock BW/daily water intake (WIR) ratio should be used to determine the reference concentration (RC), regardless of what animal was the most sensitive species, to provide an additional uncertainty factor to compensate for the added uncertainty (CCME, 1999a). However, considering the significant difference in sensitivity of different receptors (i.e., ruminants, non-ruminant mammals, and birds) the lowest TDI was identified for each group and used for RC calculation of receptors in that group (Table11.5).

The following equation is used to calculate the RC using the receptor specific TDI (Equation 6):

Equation 6: Calculate Reference Concentration

$$\mathrm{RC} = \mathrm{TDI} * \frac{(\mathrm{BW})}{\mathrm{WIR}}$$

Where:

RC = reference concentration (mg/L)
TDI = the lowest tolerable daily intake rate in the receptor group (mg/kg/day)
BW = body weight (kg)
WIR = daily water intake (L/day)

By default, the CCME (1999a) allocates 20% of the allowable internal dose to the livestock drinking water pathway as one of five routes of exposure for receptors. The other four routes are food ingestion, dermal exposure, inhalation of dust, and inhalation of vapours.

The RC is used in Equation 7 to calculate the threshold concentration (TC):

Equation 7: Water Quality Guideline Calculation

$$\mathrm{TC} = \mathrm{RC} * \mathrm{PDWC}$$

Where:

TC = threshold concentration (mg/L)
RC = reference concentration (mg/L)
PDWC = percentage drinking water contribution (20%).

The Mo TC was calculated for all livestock and/or wildlife species with available water intake ration data in CCME (1999a) and ECCC (2012) and are presented in Table 11.5. In each receptor group, the species with the lowest BW/WIR was identified and the TC of that species was used to derive WQGs.

The CCME (1999a) specifies that:

> "If only the interim guideline dataset is fulfilled, then the water quality guideline is based on the most sensitive animal, livestock or non-livestock."

However, considering the extreme sensitivity of ruminants compared to other livestock and wildlife recipients (Section 7.2), three separate WQGs for: 1) ruminant livestock; 2) ruminant wildlife; and 3) non-ruminant livestock and wildlife recipients are derived.

Table 11.5. The threshold molybdenum concentrations for drinking water for livestock and wildlife (the lowest value by receptor group is shown in bold).

Receptor	TDI (mg Mo/kg BW/day)	BW/WIR	Mo TC from drinking water (mg/L)
Ruminant livestock			
Beef cattle[1]	0.010	10.6[2]	0.021
Lactating dairy cattle[1]	0.010	10.3[2]	0.020
Goat (lactating) [1]	0.010	10.1[2]	0.020
Goat (maintenance) [1]	0.010	18[2]	0.036
Sheep[1]	0.010	**8.0**	**0.016**
Ruminant wildlife			
Moose[3]	0.010	20	0.040
White-tailed deer[3]	0.010	**16.7**	**0.034**
Non-ruminant mammals			
American mink[3]	0.296	33.3	1.971
Black bear[3]	0.296	16.7	0.989
Common shrew[3]	0.296	5.9	0.349
Deer mouse[3]	0.296	5.3	0.314
Horse[1]	0.296	11.7[2]	0.693
Meadow vole[3]	0.296	**4.8**	**0.284**
Mink[1]	0.296	11.2[2]	0.663
Mouse[1]	0.296	**4.8**[2]	**0.284**
Muskrat[3]	0.296	10	0.592
Northern river otter[3]	0.296	12.5	0.740
Pig dry sow, boars, and replacement[1]	0.296	11.5[2]	0.681
Pig grower[1]	0.296	10.2[2]	0.604
Pig finisher[1]	0.296	8.7[2]	0.515
Pig lactating sow[1]	0.296	8.7[2]	0.515
Pig weaner[1]	0.296	11[2]	0.651
Rabbit[1]	0.296	9.6[2]	0.568
Rat[1]	0.296	11.8[2]	0.699
Red fox[3]	0.296	11.1	0.657
Short-tailed weasel[3]	0.296	9.1	0.539
Snowshoe hare[3]	0.296	10	1.125
Birds			
American robin[3]	0.600	7.1	0.852
Bald eagle[3]	0.600	25	3.000
Barn swallow[3]	0.600	4.5	**0.540**
Chicken (white Leghorn)[1]	0.600	8.42	1.008
Chicken (Ross Boiler)[1]	0.600	12.32	1.476
Common loon[3]	0.600	33.3	3.996

Receptor	TDI (mg Mo/kg BW/day)	BW/WIR	Mo TC from drinking water (mg/L)
Common merganser[3]	0.600	20	2.400
Duck[1]	0.600	5.72	0.684
Goose[1]	0.600	9.82	1.176
Great blue heron[3]	0.600	25	3.000
Lesser scaup[3]	0.600	14.3	1.716
Mallard[3]	0.600	16.7	2.004
Peregrine falcon[3]	0.600	16.7	2.004
Red-tailed hawk[3]	0.600	16.7	2.004
Ruffed grouse[3]	0.600	14.3	1.716
Spotted sandpiper[3]	0.600	5.9	0.708
Spruce grouse[3]	0.600	14.3	1.716
Turkey[1]	0.600	5.92	0.710

[1]: CCME (1999a) estimates were used for body weight and daily water intake ratios.

[2]: This value is the average of the two values provided by CCME (1999a).

[3]: ECCC (2012) estimates were used for body weight and daily water intake ratios.

11.2.1 *Water Quality Guidelines for Ruminant Livestock*

For ruminant livestock, the lowest calculated ruminant TDI (calculated for red deer) was used (Table 11.4). The red deer TDI is from a secondary study (Grace et al. 2005) in which six-month-old hinds grazed on pastures containing between 10 and 13.1 mg Mo/kg dry matter (LOAEL) for 102 days and demonstrated reduced weight gain compared to controls grazing on pastures containing between 1.5 and 2.4 Mo/kg. This study is well designed, statistically strong, and has the required survival in the control group. Hence the LOAEL reported in this study was selected to calculate TDI for derivation of the ruminants. The maximum food intake of 1.8 kg/head/day for winter (the season that the experiment was conducted in) (Tuckwell, 2003) was divided by the average body weight of the deer during the experiment (63 kg) to calculate food intake ratio of 0.03 kg food/kg BW/ day. The Mo dose of 0.33 mg/kg BW/day was then calculated by multiplication 0.03 kg food/kg BW/day by the average of the Mo concentrations (10 and 13.1 mg/kg) in the LOAEL group. A same approach was taken to calculate the does of 0.12 mg/kg BW/day as NOAEL for this study.

Since the endpoint presented in the Grace et al. (2005) study is a sub-chronic study (102 days) the UF of 20 was applied to the geometric mean of the LOAEL and the NOAEL. Hence the TDI selected for use in guideline derivation is 0.010 mg/kg BW/day.

Based on a TDI of 0.010 mg/kg BW/day and using equations 6 and 7, the lowest threshold for Mo exposure from drinking water was calculated for sheep (**0.016 mg/L**). All other ruminant livestock species have higher Mo thresholds (Table 11.5) and are protected by the threshold value calculated for sheep. Therefore, the calculated WQG protective of ruminant livestock is **0.016 mg/L (16 µg/L)** and applies to all ruminant livestock.

11.2.2 *Water Quality Guidelines for Ruminant Wildlife*

The ruminant wildlife WQG is based on the TDI calculated for red deer (0.010 mg Mo/kg BW/day). Using the water intake ratios (Table 11.5), threshold concentrations were calculated for two ruminant wildlife

species (i.e., moose and white-tailed deer). The lowest threshold belongs to white-tailed deer (**0.034 mg/L; 34 µg/L)** and applies to all ruminant wildlife.

11.2.3 *Water Quality Guidelines for Non-ruminant Livestock and Wildlife*

For non-ruminant livestock and wildlife, the lowest threshold concentration for non-ruminant mammals and birds was selected **0.284 mg/L** (**284 µg/L**). This threshold belongs to mice and meadow vole and is applicable for all non-ruminant wildlife and livestock animals. This WQG was calculated using the TDI from a sub-chronic study (Zhang et al., 2013) for mice in which the mice were exposed to Mo via their drinking water for 14 days, and sensitive reproductive endpoints were measured (e.g., ovarian hyperemia). The Zhang et al. (2013) study is technically defined as a sub-chronic duration, however, an UF of 10 was applied for TDI calculation given the sensitivity of the endpoint (see the rational provided in Section 11.1.5).

11.3 Uncertainty Analysis

The TDIs used in the derivation of the WQG were generally based on single compound tests, while metals in the environment are most likely present as a mixture. Metals in mixtures can have agonistic, antagonistic or synergist effects, hence the uncertainty associated with WQGs based on single compound tests is unknown. For example, it is known that exposure to Cu and SO_4^{2-}, concurrent with Mo exposure, can mitigate or exacerbate the effects of Mo exposure and molybdenosis (Section 8.2).

TDIs are developed based on studies that use chemical formulations that are likely to be far more bioavailable to plants and animals than those forms that are present in the environment. Consequently, the TDIs used in this assessment are expected to overestimate the potential for adverse effects.

Uncertainties are present in the extrapolation of TDIs from controlled studies to conditions generally experienced by livestock and wildlife. Generally, this uncertainty is minimized by using the most sensitive receptor to develop the WQG for ruminant livestock and ruminant wildlife. Extrapolating from lab rats/mice and poultry to wildlife non-ruminant mammals and birds, respectively, may result in some uncertainty, but generally this uncertainty is mitigated by the selection of conservative TDIs and the use of an UF when calculating the TDI.

11.4 Application of Water Quality Guidelines for Livestock and Wildlife

While three WQGs are provided, consideration should be given to using the most sensitive animal receptor drinking the water. The livestock watering WQG (**0.016 mg/L**) is protective of ruminant livestock, ruminant wildlife, and non-ruminant livestock and wildlife. If the water is not used to water ruminant livestock, but is used by ruminant wildlife, then the ruminant wildlife WQG (**0.034 mg/L**) can be applied. If it can be conclusively proven that the water will not be used for ruminant livestock watering or by ruminant wildlife, then the non-ruminant livestock and wildlife WQG (**0.284 mg/L**) can be applied.

When the toxic endpoint due to Mo exposure is molybdenosis, the exposure concentration of Cu also plays a role in the severity of symptoms. The livestock watering guideline is based on a TDI from a study in which red deer were exposed to 10 - 13.1 mg/kg of Mo and 9 - 59 mg/kg of Cu and growth impairment was noted. While previously it was considered that a Cu:Mo ratio of 2:1 would be sufficient to prevent molybdenosis (ENV, 1986), more recent studies have found that ratios with lower relative Cu concentrations did not result in toxicity, and that the concentration of Cu, as well as the Cu:Mo ratio, play a role in the development of molybdenosis (Gardner et al. 2003, Raisbeck et al.,2006). Unfortunately, the available data were insufficient to model the relationship between concentrations of Cu, Cu:Mo ratios, and toxic effects (as represented by effect sizes). Furthermore, contemporary theoretical models explaining molybdenosis symptoms (Gould and Kendall, 2011) suggest not all toxicological effects are a result of Cu deficiency due to reduced gastrointestinal bioavailability, and that the Cu:Mo ratio in forage

or water may not be a good predictor for the risk of molybdenosis. Hence, the WQG is not easily modified to address the effect of Cu:Mo ratios on molybdenosis and is not readily amendable to site-specific changes where Cu and Mo co-occur (such as in mining). A reasonable basis for a Mo WQG that is protective against molybdenosis would be the lowest Mo dose documented that is associated with significant effects, regardless of the estimated ratio of ingested Cu:Mo.

12. WATER QUALITY GUIDELINES FOR IRRIGATION OF FORAGE CROPS

No information was found to support an update of the current Mo WQGs for the protection of forage crops. The 1986 WQGs (ENV, 1986) designed to protect ruminant animals feeding on irrigated forage crops still apply with some modifications (Table 12.1).

The 1986 WQG was based on the criteria proposed by the USEPA (1972) and takes soil drainage into consideration. Since poorly drained fields allow the added Mo in the form of irrigated water to stay within the root zone, the amount of Mo taken up by plants will increase. Whereas in well drained soils, plants contain relatively lower levels of Mo. Therefore, the chronic WQG value recommended for well-drained soils is double the value recommended for poorly drained soils. Given the lack of short-term toxicity data, the maximum WQG (i.e., acute short-term) is the same for both poorly drained and well drained soils.

The 1986 WQG also considered Cu:Mo ratios based on an understanding at the time of the relationship between Cu:Mo ratio and the likelihood of an animal to suffer from molybdenosis. However, more recent data have demonstrated that Cu:Mo ratios cannot be used to predict molybdenosis in the absence of other lines of evidence (Section 8.2). Therefore, the consideration of Cu:Mo ratios has been removed from the WQGs (Table 12.1).

Table 12.1. A summary of molybdenum WQGs for the protection of forage crops to protect livestock.

Water Use	**Average Chronic Long-Term) (mg/L total Mo)**	**Maximum (Acute Short-Term) (mg/L total Mo)**
Poorly drained soil	0.01 mg/L	0.05 mg/L
Well-drained soil	0.02 mg/L	0.05 mg/L

13. WATER QUALITY GUIDELINES FOR IRRIGATION OF NON-FORAGE CROPS

A Mo WQG for irrigation water for non-forage crops was derived using *A Protocol for Deriving Water Quality Guidelines for Irrigation Water* (CCME, 1999a). This protocol is for individual chemicals and does not account for chemical mixtures in soil or irrigation water. It also does not directly consider the potential for persistent substances, such as metals, to concentrate in soils and the resultant adverse effects on crops.

13.1 Acquisition, Evaluation and Classification of Toxicological Data

A literature search was conducted to identify studies on the toxicity of Mo to agricultural plants grown in Canada. A total of 31 toxicity studies were identified on several plant species (Appendix 2).

Studies and toxicological data were classified as primary, secondary, or unacceptable, following the CCME (1999a) protocol, to ensure acceptable laboratory practices were followed in the design and delivery of the experiment. From the 31 studies, four studies were classified as primary, one as secondary, and 26 as unacceptable (Appendix 2).

13.2 Selected Toxicity Data

Seventy-one acceptable EC_{10} estimates were identified from the five studies, however, data were further excluded if the soils were outside the typical conditions found in B.C. (e.g., pH <4), or if the studies involved sewage sludge and/or mixtures of toxicants. This left 46 EC_{10} estimates on six plant species in up to ten different soil types for the derivation of the non-forage crops WQGs. Data were available for wheat, rapeseed, red clover, ryegrass, barley and tomato (Buekers et al., 2010; McGrath et al., 2010a; McGrath et al., 2010b; van Gestel et al., 2011; and Oorts et al., 2012). These studies used shoot yield, root elongation, or plant yield as the toxicity endpoint. A geometric mean of normalized EC_{10} estimates was calculated where multiple data were available for the same response in the same species. All 46 EC_{10} estimates were classified as primary data and resulted from standardised long-term plant toxicity tests conducted in accordance with International Organization for Standardization (ISO) Protocol 11269-1 and 11269-2.

13.3 Derivation Approach

The CCME (1999a) protocol requires a minimum of eight Canadian crop species to derive a full irrigation WQG or four Canadian crop species to derive an interim guideline. Crop species must include two or more species of cereals, tame hays, or pastures, and two or more plant species from the following families: Leguminosae (e.g., soybeans, peas), Compositae (e.g., lettuce, sunflower), Cruciferae (or Brassicaceae; e.g., cabbage, rapeseed), Cucurbitaceae (e.g., cucumber), Liliaceae (e.g., onion), Solanaceae (e.g., tomato), Umbelliferae (e.g., carrot), and Chenopodiaceaeon (e.g., sugar beet). The resulting toxicological dataset met the minimum requirements for an interim guideline, and included four species of cereals, tame hays and pastures (i.e., red clover, ryegrass, barley and wheat) and two species from the latter group of families (i.e., rapeseed and tomato).

All selected toxicological data were from soil studies; no acceptable irrigation studies were found. Therefore, WQG derivation followed the alternative method recommended by CCME (1999a). This method consists of three main steps:

1) calculation of the acceptable soil concentration (ASC) based on the available toxicological data;
2) determination of species maximum acceptable toxicant concentrations (SMATC); and
3) derivation of the WQG based on the lowest SMATC.

These steps are described in the sections below.

13.4 Acceptable Soil Concentration

A stepwise approach was used to develop ASC values for Mo in B.C. soils. Since soil pH and clay content are strongly correlated with Mo toxicity to plants (see discussion in section 8.3), soil pH and clay content (%) data were used to characterize soil conditions for B.C. (i.e., reference soil). The species-specific slope factors for pH and clay content recommended by Oorts et al. (2016) were used to normalize the EC_{10} estimates to account for differences in Mo bioavailability resulting from the pH and clay content of the tested soil versus a reference soil for B.C. The lowest normalised EC_{10} estimate from all plant species evaluated was selected as the ASC.

13.4.1 *Reference Soil Characterization for B.C.*

The pH ranges for optimum growing conditions for crops in B.C. are dependent on soil type, ranging from 5.5 to 8.0 for mineral soils and from 4.5 to 6.0 for organic soils (BC MOA, 2015).

Background soil quality conditions across B.C. regions are provided in Table 13.1 (ENV, 2017) and represent the average of concentrations at two depths: 0 – 10 cm and 50 – 60 cm. Soil pH was determined

in all seven regions: Vancouver Island, Metro Vancouver, South Interior, Kootenay, Cariboo, Skeena, and Omineca Peace. Soil clay content data was determined for three regions: Vancouver Island, Metro Vancouver and the Cariboo.

Table 13.1. Summary of average background soil pH and clay content for seven regions of British Columbia (source: ENV, 2017).

Statistic	Vancouver Island	Metro Vancouver	South Interior	Kootenay	Cariboo	Skeena	Omineca-Peace	All Regions
Soil pH								
n	39	28	39	39	12	24	30	210
Minimum	3.4	2.9	4.6	3.6	5.6	2.9	3.5	2.9
Maximum	6.8	5.6	8.0	8.1	7.9	6.8	7.4	8.1
Mean	4.9	4.1	6.8	5.8	6.8	4.9	5.5	5.5
95th Percentile	6.6	5.2	7.7	7.9	7.8	6.4	7.2	7.7
Soil Clay Content (%)								
n	43	26	--	--	--	16	--	85
Minimum	2.4	3.4	--	--	--	5.2	--	2.4
Maximum	38.1	39.8	--	--	--	52.7	--	52.7
Mean	21.3	15.1	--	--	--	17.3	--	18.6
5th Percentile	3.2	3.5	--	--	--	6.7	--	3.4

Molybdenum toxicity to plants increases as soil pH increases and soil clay content decreases and the high end of the soil pH range (i.e., 95th percentile) and the low end of the soil clay content range (i.e., 5th percentile) were conservatively selected to represent reference soil conditions in B.C. EC_{10} estimates were normalized using a soil pH of 7.7 and clay content of 3.4%.

13.4.2 *Normalization of Toxicity Data to Reference Soil Conditions*

Species-specific slope factors based on regression analyses of the effect of soil pH and clay content on plant toxicity were selected from Oorts et al. (2012) (Table 13.2) and used to normalize EC_{10} estimates. In the absence of species-specific slope values for wheat, the lowest pH slope value (-0.61) and highest clay slope value (1.08) were used to produce the most conservative normalized EC_{10} estimate.

The normalization of study EC_{10} estimates followed the approach developed by Smolders et al. (2009) and adopted by Oorts et al. (2012; 2016):

Table 13.2. Species-specific slope factors for molybdenum toxicity to plants.

Plant Species	pH Slope	Clay Slope
Rapeseed/canola (*Bassica napus*)	-0.61	1.08
Red Clover (*Trifolium pretense*)	-0.50	0.77
Ryegrass (*Lolium perenne*)	-0.35	0.90
Tomato (*Lycopersicon esculentum*)	-0.45	0.93
Barley (*Hordeum vulgare*)	-0.28	0.56
Wheat (*Triticum aestivum L.*)[1]	-0.61	1.08

Source: Oorts et al. 2012

[1]Assumed lowest pH slope and highest clay slope values

Equation 7: Normalization of Toxicity Data to Soil Parameters

$$EC_{10\ ref} = \left(EC_{10\ study}\right) \times \left[\left[\frac{10^{-pH_{ref}}}{10^{-pH_{study}}}\right]^{-pH\ slope} \times \left[\frac{Clay_{ref}}{Clay_{study}}\right]^{Clay\ slope}\right]$$

Where:

EC_{10}ref (mg/kg)	= EC_{10} study normalized for soil pH and clay content
EC_{10}study (mg/kg)	= EC_{10} reported in toxicity study
pH_{ref}	= reference soil pH = 7.7
pH_{study}	= pH of study soil
$clay_{ref}$	= reference soil clay content (%) = 3.4
$clay_{study}$	= clay content (%) of study soil
pH_{slope}	= slope from log-log based regression for plant toxicity vs soil pH
$clay_{slope}$	= slope from log-log based regression for plant toxicity vs soil % clay

Detailed information on the individual original and normalized EC_{10} estimates, soil type, species-specific slope factors, and reference pH and clay content are provided in Appendix 3. Study EC_{10} estimates for a toxicity endpoint in a single plant species varied widely among the various soils tested (i.e., 16-fold to > 700-fold for different species). This range in species response was significantly reduced following normalization of study EC_{10} estimates to standard soil pH and clay content (5-fold to 14-fold). The normalized effect concentrations and ASC for different species are given in Table 13.3.

Table 13.3. A summary of normalized EC_{10} and acceptable soil concentration (ASC) for different crop species with available toxicity data.

Plant Species	Endpoint	Normalized EC_{10} (mg/kg)	Uncertainty factor	ASC (mg/kg)
Rapeseed/canola	Shoot yield	1.12*	5	0.22
Red Clover	Shoot yield	2.42*	5	0.48
Ryegrass	Shoot yield	7.48*	5	1.63

Tomato	Shoot yield	2.28*	5	0.46
Barley	Root elongation	18.85*	5	3.77
Wheat	Plant yield	0.84	5	0.17

* The reported effect concentrations are geometric means of similar data points (i.e., same species, same life stage, same endpoint and same exposure duration).

The CCME (1999a) protocol prescribes the application of an UF of 10 to the geometric mean of NOEC and LOEC data points. All effect concentrations used in deriving this WQG were EC_{10} values, which are generally considered to be "no-effect concentrations". Therefore, an UF of 5, rather than 10, was applied to all normalized EC_{10} values. The lowest value listed in Table 13.3 is 0.17 mg/kg for wheat and is used as the ASC for Mo in B.C. reference soils.

13.5 Species Maximum Acceptable Toxicant Concentration

A SMATC was calculated using the ASC and default values for the bulk density of agricultural soils, soil bulk volume, and irrigation rate suggested by CCME (1999a). Crop irrigation rates in B.C. vary according to region and were reported to range from 141 to 1,058 mm /m^2 (Tam and Petersen, 2014). Therefore, the irrigation rate of 1,200 mm/m^2 (CCME, 1999) was assumed to simulate a worst-case plant exposure scenario and ensure the irrigation guideline is protective of all areas.

Equation 8: Species Maximum Acceptable Toxicant Concentration

$$SMATC = \left(\frac{ASC\ x\ soil\ bulk\ density\ x\ \ soil\ bulk\ volume}{irrigation\ rate}\right)$$

Where:

SMATC (mg/L)	= species maximum acceptable toxicant concentration
ASC (mg/kg)	= acceptable soil concentration
Soil bulk density (kg/m^3)	= 1,300
Soil bulk volume (m^3)	= 1,500
Irrigation rate per year (L/ha per annum)	= $1.2x10^7$

A summary of SMATCs calculated for different crop species is provided in Table 13.4.

Table 13.4. Species maximum acceptable toxicant concentration for different species.

Plant Species	SMATC (mg/L)
Rapeseed/canola	0.036
Red Clover	0.078
Ryegrass	0.265
Tomato	0.074

Barley	0.612
Wheat	**0.028***

* This value is the lowest SMATC and is adopted as interim WQG for Mo

13.6 Guideline Derivation

13.6.1 *Interim Irrigation Guideline*

The lowest SMATC was for wheat **0.028 mg/L (28 µg/L)**. Considering the toxicity dataset only met the minimum requirement for an interim WQG, this value is adopted as B.C.'s interim Mo irrigation WQG in B.C. Interim WQGs are ideally replaced by full WQGs when knowledge gaps are filled.

13.6.2 *Accumulation of Molybdenum in Soil as a Result of Irrigation*

Irrigating with water containing 28 µg Mo/L at an irrigation rate of $1.2x10^7$ L/ha would result in an annual accumulation of 0.17 mg Mo/kg within the top 15 cm of a hectare of agricultural soil (i.e., equal to the ASC). The Mo concentration in soil would be reduced over time as a result of uptake by plants and leaching and aging processes in soil. However, the exact reduction rate of Mo depends on several factors including soil properties. Therefore, ongoing monitoring of Mo concentration in the soil is warranted.

13.6.3 *Considerations Other than Plant Toxicity*

The recommended irrigation WQG provides a predicted no-effect concentration and concentrations below the WQG value are expected to be of low risk to non-forage crop species. However, the CCME protocol for irrigation guideline development does not account for the potential accumulation of contaminants in forage crops, which could be toxic to livestock. The WGQ for irrigation of forage crops (Section 12.1) should be used in cases where livestock will be ingesting the crops.

14. DATA GAPS AND RESEARCH NEEDS

14.1 Freshwater Aquatic Life

More research is needed on the mechanism of toxicity and the rate of Mo uptake in aquatic organisms to further assess the risks of Mo to freshwater aquatic life. Specifically, the following information would reduce the uncertainty of a WQG for freshwater aquatic life:

- mechanism of toxicity to freshwater aquatic life;
- toxicity testing on resident amphibian species;
- toxicity testing on resident EPT;
- further research into toxicity modifying factors, especially the role of humic and fulvic acids; and
- field or mesocosm studies of long-term exposures of freshwater aquatic life to elevated Mo concentrations.

14.2 Livestock and Wildlife

To develop full WQGs to protect livestock and wildlife, primary Mo toxicity studies are required for livestock (ruminant and non-ruminant species), ruminant wildlife, and birds. Additional research to quantify the interactions of Cu and Mo other potential toxicity modifying factors is also needed.

14.3 Terrestrial plants and soils

Primary research on Mo toxicity for three additional Canadian crop species including at least two from the following families: Leguminosae, Compositae, Cruciferae, Cucurbitaceae; Liliaceae, Solanaceae, Umbelliferae and Chenopodiaceae, is needed to develop a full irrigation WQG using the CCME (1999a) protocol for the protection of non-forage plant species. Also, a model for predicting the potential for Mo accumulation in soils of different characteristics would be helpful for application of the WQG.

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